PEDIATRIC ENDOCRINOLOGY

FOURTH EDITION

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PEDIATRIC ENDOCRINOLOGY, FOURTH EDITION ISBN: 978-1-4557-4858-7

Cover image courtesy Professor Michael J. Waters, PhD, DSc; NHMRC Senior Principal Research Fellow; Institute for Molecular Bioscience and School of Biomedical Sciences; University of Queensland; St. Lucia, Queensland, Australia

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Library of Congress Cataloging-in-Publication Data

Pediatric endocrinology (2014)
Pediatric endocrinology / [edited by] Mark A. Sperling. — Fourth edition.
p. ; cm.
Includes bibliographical references.
ISBN 978-1-4557-4858-7 (hardcover : alk. paper)
I. Sperling, M., editor of compilation. II. Title.
[DNLM: 1. Endocrine System Diseases. 2. Child. 3. Infant. WS 330]
RJ418
618.92′4—dc23

2013021244

Senior Content Strategist: Stefanie Jewell-Thomas
Developmental Editor: Jennifer Shreiner
Publishing Services Manager: Anne Altepeter
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Designer: Louis Forgione

Printed in the United States of America

Last digit is the print number: 9 8 7 6 5 4 3 2 1

To my parents, who gave and sustained my life; to my wife, Vera, "Woman of Valor"; children, Lisa and Steven; Jonathan and Shoshana; and grandchildren, Jacob, Benjamin, Tzvi, Sydney, Rebecca, Julian, and David, who provide meaning, joy, and continuity to our lives

Foreword to the First Edition

The aim of the editor and contributors to this volume is to establish an effective bridge between the surging progress in biomedical science and the clinical practice of pediatric endocrinology. Half a century ago the biochemical elucidation of the structure and subsequent synthesis of steroid hormones provided the basis for a revolution in the diagnosis and treatment of a number of endocrine and nonendocrine disorders; that era was soon followed by a succession of fundamental discoveries: structure of peptide hormones, identification of releasing hormones from the brain, rapid and precise assay methods, and synthesis of peptide hormones by molecular biological techniques, to name but a few.

In no field has laboratory science been more effectively translated into clinical progress than in pediatric endocrinology. A glance at the roster of contributors to this volume may well provide insight into why; many who are responsible for the dramatic advances in the laboratory also pursue active clinical careers.

This volume includes many new sections that were not presented in previous texts devoted to clinical pediatric endocrinology. It will serve as a valuable reference for family physicians, internists, pediatricians, and other health professionals, covering as it does the gamut of information from basic molecular biology to practical considerations in the diagnosis and treatment of pediatric endocrine disorders.

Solomon A. Kaplan, MD

Preface

The fourth edition of *Pediatric Endocrinology* aims to build on the models of excellence in basic and clinical research in endocrinology applied to newborns, infants, and children as they evolved in the first three editions. Now, close to 20 years since its inception, the current issue provides perspective on how much has been learned and applied for the benefit of those children referred to pediatric endocrinologists for evaluation and management. Each new edition has attempted to incorporate advances in the field that occurred in the preceding five years; the fourth edition is no exception. To achieve these aims, we involved those at the forefront of their field to share their expertise and to transmit new knowledge in the context of contemporary developments. This should be evident in the reorganization and increased number of contributors to this volume. In terms of organization, three sections are delineated. The first deals with principles and methods in pediatric endocrinology, beginning with an overview and historical evolution of the field, and its unique pediatric aspects. Three succeeding chapters review principles of molecular endocrinology and genetics, receptor signal transduction, and laboratory methods in pediatric endocrinology; together they form the nucleus of our discipline and provide a framework for the methods used to understand, investigate, and treat our patients. The second section contains five chapters dealing specifically with the most common endocrine-related disorders encountered in the newborn nursery and the NICU; ambiguous genitalia, hypoglycemia, disorders of the thyroid and abnormal thyroid function tests, disorders in calcium and phosphorous metabolism, and neonatal diabetes mellitus (NDM). Although rare, NDM has been disproportionately of value in understanding the role of the same defects, albeit to a milder degree, in the much larger problem of T2DM. The third section is organized on the traditional organ approach; here, several new authors have taken over a chapter or enlarged the list of contributors to an existing chapter, in each case bringing in new insights and perspectives in the process.

Dramatic changes have occurred in the past five years. Whole genome and exon sequencing is increasingly being applied as the cost of sequencing declines, yielding discovery of spectacular new findings. This might be best exemplified by the increasing complexity of the genes regulating puberty and reproduction, especially hypogonadotropic hypogonadism, and the recent description of a gene responsible for precocious puberty, *MKRN3*. In diabetes research, the discovery of betatrophin, a hormone that regulates β-cell mass and insulin secretion in response to insulin resistance; Irisin, which regulates fat metabolism and mediates improved glucose homeostasis in response to exercise; and the imminent application of closed-loop systems, as an "artificial pancreas", for treating children with T1DM requiring insulin treatment, portend exciting developments in the near future. Similar exciting discoveries are to be found in virtually every chapter. Altogether this edition covers a broad, but contemporary canvas of the field of pediatric endocrinology. We trust it is suitable to inform the serious student, and update the experienced practitioner or investigator in the field. We have introduced questions for each chapter and an online version supplemented by rich, in full color illustrations.

There undoubtedly will be errors and possibly omissions for which, as editor, I take responsibility and extend my apologies. I welcome any comments and advice on where and how to improve this book so as to make it a "must have" on the shelf of every NICU and every endocrinologist's office. I extend sincere and warm-hearted thanks to all contributors; each of your contributions is highly respected and highly valued.

As I look back on this and previous editions, I reflect on the enormous effort involved and yet consider it a labor of love and a great privilege.

Mark A. Sperling
Pittsburgh, Pennsylvania
Fall 2013

Acknowledgments

An enterprise of this nature requires the contribution and support of numerous individuals. I gratefully acknowledge the contributions of each of the authors, whose friendship and collegiality were—and continue to be—indispensable to bring this work to publication. Constructive critique was provided by colleagues, post-doctoral trainees, residents, and visiting scholars to our departmental program in endocrinology. Ms. Anna Marie Rompala provided invaluable secretarial assistance. A special thanks to Ms. Jennifer Shreiner of Elsevier, whose experience, diligence, and patience enabled this project to move to timely conclusion. I am very grateful to Professor Michael Waters for permission to use his figure of the Growth Hormone Receptor as the cover image and to Elsevier for giving me the privilege of editing this book.

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SECTION I

PRINCIPLES AND METHODS OF PEDIATRIC ENDOCRINOLOGY

CHAPTER 1

Overview and Principles of Pediatric Endocrinology

Mark A. Sperling, MD

CHAPTER OUTLINE

HISTORICAL BACKGROUND

Endocrinology is a discipline of science that seeks to understand how chemical signals secreted by cells regulate the function of distant (endocrine) or local (paracrine) tissues, or even their own function (autocrine), in order to integrate vital processes of life such as growth, reproduction, and metabolism (Figure 1-1). Classical endocrinology derived from careful clinical observation such as, for example, the gigantism associated with pituitary tumors or the characteristic bodily changes now known as Cushing disease which is also associated with pituitary tumors; histology indicated the former was likely the result of a product made by "acidophilic cells," whereas the latter was associated with the expansion of "basophilic cells." Unlike the chemical substances secreted into ducts leading to a target tissue ("exocrine"), the products of these acidophilic or basophilic cells had to traverse the bloodstream in order to reach their distant and often multiple targets. Hence, these were internal ("endocrine") secretions. Cushing disease was associated with hypertrophy of the adrenal cortex and certain tumors of these tissues mimicked the features of Cushing disease.[1] Hence, it was readily postulated that the pituitary secretes a substance that affects the adrenal glands and function; this substance was named adrenocorticotrophic hormone (ACTH) and it was deduced that the features of Cushing disease/syndrome were the result of a product or products from the adrenal gland. The destruction of adrenal tissue by tuberculosis or tumor was identified by Thomas Addison in 1855 and treatment of this entity with adrenal extracts, resulting in marked improvement, was first undertaken by William Osler in 1896.[2] However, the purification of these "internal secretions" began in earnest only at the turn of the 20th century with spectacular success as reviewed by Dr. Delbert Fisher.[2] In the first quarter of the 20th century, epinephrine, thyroxin, insulin, and parathyroid hormone (PTH) were purified, followed by the purification of the sex steroids from the ovary and testes as well as the pituitary and placental gonadotropins that stimulated the gonads to secrete these substances. These purifications required laborious chemical methods and the elucidation or measurement of their function required costly and cumbersome bioassays. For example, the assay of insulin potency, discovered in 1921, required the use of rabbits; the definition of 1 international unit (IU) of insulin was assigned to be the amount of insulin that lowers the blood glucose of a healthy 2-kg rabbit, fasted for 24 hours, to 45 mg/dL within 5 hours of injection. Clearly, such potency estimates reflected the relative crudeness of the purification; today's recombinant human insulin possesses approximately 29 IU/mg, whereas the potency of porcine insulin in the early 1980s was ~23 IU/mg and likely less at the dawn of insulin therapy for diabetes. Moreover, the lack of sensitivity in such assays prohibited the ability to measure this substance in normal blood or other biologic fluids; refinements such as measuring the incorporation of labeled glucose into the fat pad or diaphragm of a rat represented only an incremental improvement.[3] Growth hormone (GH), isolated in 1944, was assayed by its ability to increase the width of the tibia growth plate in rats after a defined period of injections and by comparing the unknown relative to a dose response of known concentrations administered in vivo.[4] Attempts to improve sensitivity and specificity led to the "sulfation

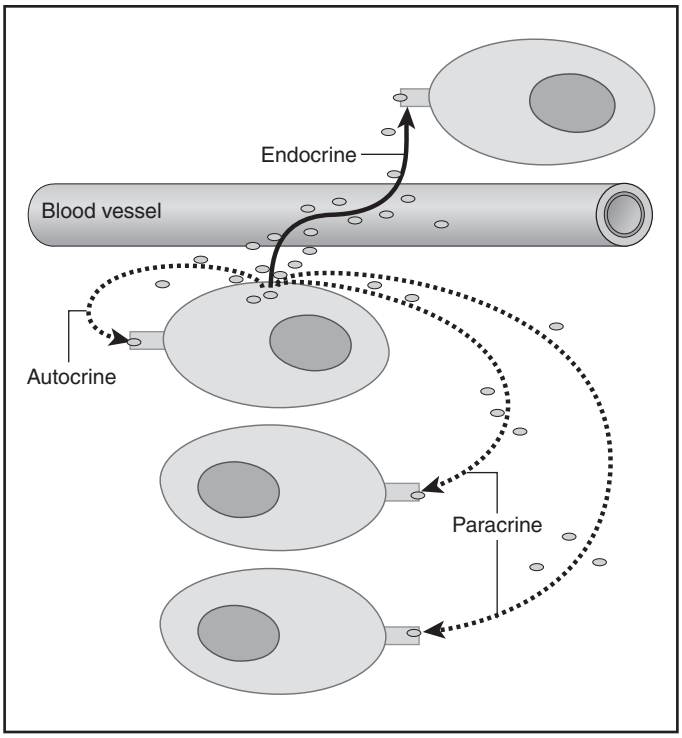

FIGURE 1-1 ■ Cellular signaling. Chemical signals synthesized and secreted by cells, may be released into the bloodstream to be distributed to target cells with specific capability to respond to the signal. These blood-borne chemicals constitute classical endocrine signals, also known as "internal secretions," to distinguish them from the chemicals secreted into a duct that leads directly to another organ (e.g., pancreatic enzymes destined for the duodenum via the pancreatic duct ["exocrine"]). However, the same cell may release the chemical that then affects nearby cells without traversing the bloodstream (these are known as paracrine effects) or act on a receptor on its own surface to modify the cells own functions (autocrine). (From King TC [2006]. *Elsevier's integrated pathology.* Philadelphia: Mosby, Figure 3-6.)

factor-somatomedin hypothesis" (Figure 1-2), in which it was postulated that GH leads to the generation of a second substance, derived from the liver, which mediates the growth-promoting (somatotropic) effects and hence was named "somatomedin."[4,5] Subsequent studies demonstrated that this substance was identical to a factor in serum, which had insulin-like properties in vitro, that was retained even after all insulin was "quenched" by an excess of antibodies specific for insulin. The convergence of these two pathways eventually led to the discovery of the factor now known as insulin-like growth factor (IGF)-I.[5] Despite these limitations, the scientific curiosity of these chemical substances that regulated functions as diverse as blood pressure (epinephrine, cortisol), water metabolism (arginine vasopressin [AVP], cortisol), growth (GH), glucose (insulin, cortisol), and reproduction (sex steroids, follicle-stimulating hormone [FSH], luteinizing hormone [LH]) spurred the formation of medical societies focused on endocrine diseases. As detailed in the article by Fisher from which the following historical aspects are quoted,[2] the Association for the Study of Internal Secretions was established in 1918 in the United States and renamed the Endocrine Society in 1952.

Pediatric endocrinology began as a subspecialty only in the 1940s with the establishment of endocrine clinics at the Massachusetts General Hospital and Johns Hopkins. These programs attracted postdoctoral trainees who then established their own pediatric endocrine units in the burgeoning growth of academic medical centers in the 1950s and 1960s. In the United States, the Pediatric Endocrine Society, first named the Lawson Wilkins Pediatric Endocrine Society (LWPES), was formed in 1972 and established as a subspecialty by the American Board of Pediatrics with its first certification examination in 1978; there are more than 1000 board-certified pediatric endocrinologists today in the United States. The European Society for Pediatric Endocrinology was formed in 1966, followed by the Japanese Society for Pediatric Endocrinology in 1967 and the British Pediatric Endocrine Group in 1972, all preceding the LWPES in the United States. Several other regional pediatric endocrine groups were formed, including the Australian Pediatric Endocrine Group, the Sociedad Latino Americana de Endocrinologia Pediatrica, and the Asia Pacific Pediatric Endocrine Society. All of these groups now meet jointly every 4 years at an International Pediatric Endocrine Congress.[2]

IMPACT OF HORMONAL ASSAYS AND MOLECULAR BIOLOGY

Two discoveries revolutionized the field of endocrinology and led to an explosion of basic, clinically relevant therapeutic knowledge in the second half of the 20th century. The first was the development of radioimmunoassay by Yalow and Berson, reported for insulin in 1960.[6] Here was a method for measuring the low concentrations of a hormone using as little as 10 to 50 μL of a biological fluid in an accurate, reproducible way with precision and sensitivity adequate for in vivo studies in humans or other species, as well as in vitro studies, such as the regulation of insulin secretion by nutrients, hormones, ions, and pharmaceutical agents in whole animals including humans, in vivo, or in isolated perfused pancreas, or in isolated islets. This was followed by the rapid development of assays for various hormones and an explosion of discovery, including the distinction between absolute and relative insulin deficiency as the difference between "juvenile" and "maturity onset" diabetes, the regulation of GH secretion in normal individuals at different ages and in clinical disorders of growth, the changes in thyroid function at birth and the possibility of screening for neonatal hypothyroidism, and the changes in gonadotropins and sex hormones during the process of normal and abnormal puberty. The discovery and purification of the hypothalamic releasing hormones for TSH, FSH/LH, GH, and ACTH were made possible by these precise assays using (rat) pituitary cells perfused by protein fractions derived from the hypothalami of animals.[7] The discovery that a hormone produced in a cell could affect the function of its neighboring cell(s), without traveling through the bloodstream (paracrine action) or even its own function (autocrine), was also enabled by the use of these sensitive and precise tools, expanding our concepts of a hormone

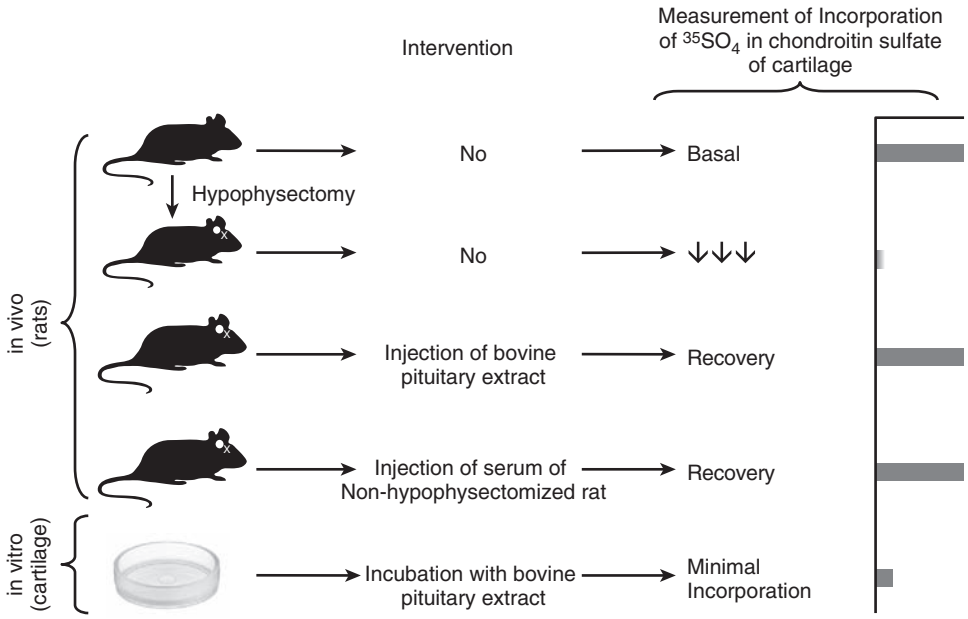

FIGURE 1-2 ■ "Sulfation factor" assay of growth hormone. The bioassay of growth hormone (GH) consisted of administering graded doses of bovine GH with potency approximately 1.25U/mg via daily subcutaneous (SC) injections for 4 days to young, growing, prepubertal rats (approximately 31 days) that had been hypophysectomized 10 days previously. Approximately 5 animals per group and about 5 doses (0 plus 4 graded increments) were used to construct the "dose-response curve" of the increase in the width of the tibia growth plate; the unknown was 2 to 3 mL of plasma also administered SC to approximately three to five animals per test dose. The sulfation assay was an attempt to refine the technique by examining the dose-response relationship between the incorporation of $^{35}SO_4$ into chondroitin sulfate in vivo, or in vitro into uniformly prepared cartilage rings obtained from young rats. As shown in the figure, the in vivo component examined basal activity of the animal's serum on the amount of radioactivity incorporated in vivo. Hypophysectomy almost completely abolished this ability to stimulate $^{35}SO_4$ incorporation, but injection of bovine pituitary extract or serum from a nonhypophysectomized animal restored this activity. However, in vitro incubation of cartilage rings with bovine pituitary extract resulted in only minimal incorporation. Thus, it was proposed that GH acted on an internal organ to produce the "sulfation factor." The sensitivity of these assays were, at best, in the 1 to 10 μg/mL range, and precision and reproducibility were poor (Tweed DC, McCullagh EP [1962]. Assay of growth hormone-like activity in blood plasma: a comparison of two methods. Clin Chem 8:141-150; also see references 4 and 5). Today's immunoassays permit measurement of plasma concentrations of GH with 1000-fold greater sensitivity than these early bioassays (ng/mL compared to μg/mL above) with high degrees of precision and reproducibility (Chapter 4). (The author is indebted to Oscar Escobar, MD, Associate Professor, Department of Pediatrics, University of Pittsburgh School of Medicine, Division of Pediatric Endocrinology, Children's Hospital of Pittsburgh, for creating this figure and granting permission for its use in this chapter.)

as a chemical messenger that influences, directs, and coordinates cellular functions throughout the body (see Figure 1-1). Similar principles enabled the identification of the receptor molecules at the cell surface or in its cytoplasm that permit the hormone signal to be transduced to a message for turning biological processes on, or off, in specific tissues.[8] Refinements using the principles of radioimmunoassay (RIA) but without radioactivity are the bases of modern laboratory methodologies for hormone measurement as well as for other chemical substances such as drugs; examples of modern application of these methods as well as the pitfalls are reviewed in Chapter 4. Signal transduction pathways and their relevance to pediatric endocrinology are discussed in Chapter 3. The notion that a hormone may not be capable of eliciting a response despite high concentrations was implicit in the entity labeled "pseudohypoparathyroidism" by Dr. Fuller Albright[9] but receptors and their signal transduction pathways were only systematically investigated beginning in the 1970s. These systematic studies, still ongoing,[8] continue to identify the pathways by which the hormone, after binding to its receptor, may elicit a response in one tissue but not in another. There may be other reasons why a hormone does not elicit an appropriate tissue response despite apparent high concentrations in the circulation. For example, an abnormal sequence in a hormone may prevent its full action at the receptor, and feedback control increases the hormone's secretion leading to high concentrations of partially functioning hormone with only minor or moderate impairment of function. Examples of such abnormalities include disorders of pro-insulin conversion to insulin.[10] Depending on the site of the abnormality in cleavage of pro-insulin, both the measurement of insulin and pro-insulin or its cleavage product C-peptide may demonstrate higher than normal values that could be interpreted as "insulin resistance." These studies also recognized that an activating mutation in a receptor will mimic the action(s) of a hormone, although the hormone concentration may be barely detectable, as exemplified by the precocious puberty in MCune-Albright syndrome (Chapter 3), whereas loss of function mutations result in the same clinical syndrome as hormone deficiency, even though the hormone

concentration is markedly increased compared to normal, as exemplified in Laron syndrome with poor growth and low IGF-I concentrations despite high circulating growth hormone concentrations (Chapter 10).Thus, the concentration of a hormone may not be directly related to its action. In summary, it is the ability to measure hormones at low concentrations (e.g., picomolar to nanomolar) in small volumes of biological fluids that enabled the rapid proliferation and understanding of endocrine regulation and function. It may be difficult for any reader who was not brought up in the era of bioassays to fully appreciate the impact of the application of the tool of RIA and its modifications to modern endocrine concepts and practice.

The second revolution built on the discovery of the double helix by Crick, Watson, and Wilkins, a discovery for which they received the Nobel Prize in 1962. This discovery and its offspring propelled the ability to identify the molecular basis of cell function, the genes that regulate these processes, and the genetic mutations that underlie congenital or acquired disorders, including those of the endocrine system. Pediatric medicine is a particular beneficiary of these techniques because congenital malformations of the endocrine glands, the abnormalities of hormone signaling as a result of defective hormone synthesis, and processing or receptor function to recognize and act on the hormonal signal are at the core of pediatric endocrinology, as reflected in this book. Chapter 2 provides an overview of the molecular and genetic methodologies applied in practice and research and also discusses the pitfalls in interpreting the results of genetic mutational analyses. This is a rapidly evolving field powered by the declining cost of gene sequencing and the ability to store and analyze huge data sets via computers. Whole genome sequencing of an individual is already being applied and likely will become part of established medical diagnostic practice in the near future. Therapeutic applications, such as, for example, choice of drug for maximum effectiveness while avoiding drug interactions or excluding drug sensitivity, are likely to follow.[11]

UNIQUE ASPECTS OF PEDIATRIC ENDOCRINOLOGY

Fetal Origins of Adult Disease

Pediatrics is all about growth and development of each aspect of human life—physical, emotional, cognitive, and sexual—from conception through birth, neonatal adaptation, infancy, childhood, puberty, and young adulthood. Changes in the mature organism continue, but the tempo is considerably slower than the rapid changes of early life. The endocrine system plays a central role in these adaptations and changes. It has long been known that environmental insults such as viral infections (rubella, herpes, human immunodeficiency virus [HIV], cytomegalovirus) or drugs (thalidomide, phenytoin), particularly in the first trimester, can result in distinct patterns of embryopathy. Only recently has it been appreciated that developmental plasticity is influenced by the intrauterine nutritional environment that may predispose to the later development of diseases such as type 2 diabetes. As a hypothesis, the "fetal origins of adult disease" is now well supported by epidemiologic and experimental data, including the epigenetic modification of gene expression via patterns of methylation or other modifications, some affecting the expression of genes regulating insulin secretion, cortisol, and other hormones.[12,13] These interactions are not restricted to early development—they may occur in the third trimester or beyond— but once "imprinted" they may be passed on to succeeding generations. Thus, the intrauterine environment may permanently modify the expression of genes, including those of the endocrine system.[14]

Acquisition of Patterns of Hormone Secretion and Action

At any given time, the plasma concentration of a hormone reflects its synthesis, secretion, and clearance, a concept that implies removal from the circulation in units of time. Indeed, the relationship between the production rate (PR) of a hormone (units/time), the serum or plasma concentration (C = units/mL), and its metabolic clearance rate (MCR) from the plasma (mL/time) is defined by the formula $PR = MCR \times C$, so knowing any two of these three variables permits one to solve the third. However, the concentration of a hormone may vary depending on such factors as time of day, stages of sleep, stage of puberty, renal or hepatic function, and the ingestion of other drugs. Hence, each of these aspects represents complex interactions. For example, the secretion of ACTH and the chief product of its action, cortisol, are related in phase, the former preceding the latter and being highest in the early morning and lowest toward midnight. ACTH synthesis may have a basal rate determined by the impulses from its hypothalamic releasing hormone, known as corticotropin releasing hormone (CRH), but both display a diurnal rhythm entrained by the light/dark cycle and relayed to the suprachiasmatic nucleus of the hypothalamus. As the fetus is not exposed to light/dark cycles and the newborn spends a majority of time asleep, it is important to know when the diurnal rhythm becomes established.[15,16] This has importance for undertaking measurements of cortisol in the newborn to determine if the hormone is deficient, as, for example, in evaluating newborn hypoglycemia. Also, stress results in a rapid increase in the CRH-ACTH-cortisol axis and the secretion of cortisol. Such rapid adaptation is essential for the appropriate adjustments to physiologic stress resulting in the "fight or flight response." It is known that the hypothalamic pituitary adrenal axis is established in utero and functional because inborn errors of metabolism such as those found in congenital adrenal hyperplasia as a result of defects in enzymes responsible for cortisol synthesis result in markedly elevated ACTH in utero, with hypertrophy of the adrenal cortex. However, after birth, there is a brief period during which adrenal secretion of cortisol is low as reflected in the plasma levels,[17] and it is not known how rapidly the newborn can adjust to a stressful situation by appropriate increments in the ACTH-cortisol secretion rates.[18] Some of the hormonal

cycles, such as GH secretion and FSH/LH secretion at the onset of puberty, are related to sleep rather than the light-dark cycle, as discussed in the relevant chapters of this book. But it is not known if these sleep-regulated patterns are operative in the newborn. Following birth, some inhibitory pathways of hormone secretion are not yet established, resulting in high hormone concentrations whose functional significance is not known. For example, inhibition of GH secretion by somatotropin release-inhibiting factor (SRIF) appears to develop only after birth, so that GH concentrations in serum are quite high in the newborn (averaging approximately 40 ng/ml), values that would be consistent with acromegaly in an adult. On the other hand, the expression of GH receptors on tissues and their linkage to postreceptor events is delayed and become fully operational only after several months, so that the effects of the high GH concentrations are muted.[19,20] The practical implications of these findings are that GH deficiency in a full-term newborn will not be characterized by discernible small size. Indeed, GH deficiency does not become manifest in a delayed growth velocity until after 3 to 6 months when the GH–growth hormone receptor (GHR) axis becomes established. Yet GH plays an important role in maintaining glucose homeostasis in the newborn, because GH deficiency may be associated with hypoglycemia, and this deficiency can be diagnosed without the need for stimulation tests if the GH concentration is lower than 10 ng/mL in the first week of life. Another hormone whose concentrations remain very high at birth and for several weeks thereafter is prolactin, presumably because the neurologic pathways responsible for dopamine secretion are not yet fully developed.[21] The physiologic implications of these adaptive processes in the secretion and action of prolactin in the newborn are not fully understood.

Adaptations in Endocrine Function at Birth

Separation of the newborn baby from its maternal blood supply after birth imposes a near-instantaneous need for adaptations in functions such as the requirements of oxygen, maintenance of body temperature, and sources of nutrients. The endocrine system plays a vital role in several of these adaptations; three are briefly described here and are explored in more detail in relevant chapters in this book.

Maintenance of Body Temperature

The hypothalamic-pituitary-thyroid axis is intimately involved in the adaptations of body temperature regulation. The fetus, bathed in amniotic fluid and supplied by maternal blood, maintains a temperature of 37° C in utero. Delivery into an ambient temperature of approximately 20° to 25° C in a modern delivery room represents a significant fall in ambient temperature that activates thyroid function. The concentration of TSH increases approximately 10-fold between birth and 15 to 30 minutes after cord cutting, to values of approximately 100 μU/mL. Simultaneously, type 2 deiodinase is activated, converting T_4 to T_3 rather than the fetal pattern of type 3 deiodinase that converts T_4 to reverse T_3. These rapid changes result in a surge of T_3 concentration, a decline in reverse T_3 concentration, and a somewhat later rise of T_4 concentration in the newborn's blood. Together, these coordinated changes in thyroid function enable T_3 to act on brown adipose tissue to activate nonshivering thermogenesis. Although TSH concentrations decline to values below 10 μU/mL by day 2 to day 3 of life, T_3 and T_4 values remain elevated for days to weeks at concentrations that would be consistent with thyrotoxicosis in older children and adults (see Chapter 7 for details). Hence, values of thyroid function reported for full-term or premature newborns must be reported as age specific and may often be labeled erroneously as "hyperthyroid" in laboratories that only list reference values suitable for adults.

Glucose Homeostasis

The fetus obtains all of its glucose via placental transfer from the mother, with little, if any, endogenous glucose production until delivery. Following cord cutting, epinephrine and glucagon each rise approximately three- to fivefold, growth hormone is high at approximately 40 ng per dl (as mentioned previously) as are cortisol values, which are highest at about 2 hours after birth and, on average, remain in the range of 2.7 to 7.6 μg/dL, mostly in the free form, in the first week of life.[17] The coordinated effects of these four classic "counterregulatory" hormones, together with a small fall in insulin, stem the initial decline in blood glucose concentration, activate glycogen breakdown and gluconeogenesis, and initiate lipolysis with later activation of ketone body production by day 2 to day 3 of life. Understanding these critical adaptations is essential for the appropriate management of hypoglycemia in a newborn, as detailed in Chapter 6.

Gonadotropins and Sex Hormones

In males, testosterone concentrations on day 1 of life are high, ranging from 75 to 400 ng/dL, values that are consistent with Tanner stages 3 to 4 of male puberty. These high concentrations decline rapidly within the first few days after delivery but remain elevated at 20 to 50 ng/dL in comparison to males aged 1 to 10 years in whom values are $<$ 10 ng/dL. A second rise in testosterone concentrations occurs between 1 week and 1 to 2 months, the mean values being approximately 200 ng/dl. Both FSH and LH also are relatively high in males at this age of life and decline to prepubertal levels only at about the end of the first year. In females, concentrations of estradiol are markedly elevated after birth and fall rapidly during the first week of life to prepubertal values, with a secondary rise occurring between 30 and 60 days followed by decline to prepubertal concentrations after 1 to 2 years. Values of FSH may range up to 14 mIU/mL in females and decline more slowly than in males, reaching prepubertal values only after 2 to 3 years. Likewise, LH values in females may be in the classical pubertal range in the first few months of life and decline to prepubertal values only after 1 to 2 years. This "minipuberty of infancy" is discussed

in greater detail in Chapters 5 and 15. A precise function for these perinatal changes in sex hormones and gonadotropins is unknown, but it has been proposed that they may have relevance to patterning of male or female neural function. In addition, clinical relevance is related to the common problem of thelarche in newborn-infant females, as discussed in Chapter 15.

In summary, knowledge of the endocrine adaptations following birth is essential for the appropriate evaluation of suspected abnormalities in endocrine function and the interpretation of age- and sex-specific values of circulating hormones.

EVALUATING ENDOCRINE DISORDERS IN INFANCY AND CHILDHOOD

A general principle of pediatric endocrinology is that the earlier the manifestation of either underactivity or excess hormone function, the more likely the cause is to be a genetic disorder with possible structural abnormalities. For example, the entity of septo-optic dysplasia with underactivity of anterior and posterior pituitary function may be associated with typical structural abnormalities of optic nerve hypoplasia, absence of the septum pellucidum or corpus callosum, a small anterior pituitary, interruption or absence of the pituitary stalk, and an ectopic or absent posterior pituitary bright spot on magnetic resonance imaging (MRI) of the brain.[22] Hypothyroidism found on newborn screening is most commonly associated with an ectopic thyroid gland; total absence or goitrous hypothyroidism should suggest a defect in the genes responsible for thyroid gland formation (TTF-1, TTF-2, PAX8) or an "inborn error of thyroid hormone synthesis" (Chapter 7). To be sure, perinatal events and maternal illness or medication must be considered. For example, perinatal asphyxia or a difficult delivery may be associated with later evidence of hypopituitarism. Maternal ingestion of antithyroid medications would result in their transfer across the placenta and may cause transient neonatal hypothyroidism, as these agents affect the fetal thyroid in the same was as they affect the maternal thyroid gland. Similarly, transfer of IgG antibodies that block or stimulate thyroid function will result in newborn hypothyroidism or hyperthyroidism that lasts for several weeks, until maternal antibodies are cleared from the circulation. A newborn with severe hyperthyroidism in the absence of any evidence of autoimmune disease in the mother almost certainly has an activating mutation of the TSH receptor (Chapter 7).

More subtle defects in endocrine function may appear later in childhood but still have a genetic basis (e.g., hypogonadotropic hypogonadism). This entity may not become manifest or discovered until delayed puberty is investigated. Macrosomia in an infant born to a mother with poorly controlled diabetes mellitus reflects secondary hyperinsulinism in the fetus, with hypoglycemia in the newborn when the maternal supply of glucose is curtailed. However, similar features in a baby born to a healthy young mother should immediately suggest the possibility of a genetic form of hyperinsulinism, most commonly the result of an inactivating mutation in the genes regulating the adenosine triphosphate (ATP)–regulated potassium channel (K_{ATP}) (Chapter 6).

Autoimmunity, trauma, and chemo/radiotherapy for a childhood malignancy are the most common causes of acquired endocrine disorders. Thus, as in all of medicine, a careful and thorough history, careful physical examination, and targeted investigation of hormonal measurements (taking into account age, time of day, and the value of obtaining a "free" hormone versus total hormone measurement) form the basis of the diagnostic approach. This may be followed by imaging of the suspected organ involved to diagnose the basis of the suspected endocrine dysfunction in the particular patient being investigated. Increasingly, molecular diagnostics is becoming an integral component of this evaluation, both to establish the cause of the entity and to provide guidance for prognosis, such as MEN2 (Chapter 14).

Definitive diagnosis may require stimulation tests (e.g., for growth hormone deficiency or with ACTH for suspected adrenal insufficiency) because a single random value is not sufficiently informative.

Finally, modern biology has provided pure synthetic compounds to replace what may be missing, such as thyroid hormone, cortisol, administration of pulsatile GnRH for Kallman syndrome, GnRH analogues for suppressing puberty, growth hormone, insulin, and ultra-long-acting somatostatin for childhood acromegaly as occurs in Carney complex or some patients with McCune-Albright syndrome. Hormone replacement must also take into consideration administration via the dermal rather than the oral route in order to avoid "first pass" considerations in bypassing the liver, as, for example, giving sex hormones such as estrogen.

CONCLUDING REMARKS

Endocrinology is the science of cellular communication that enables the biochemical integration of life's vital processes. Pediatric endocrinology is the linchpin for these processes during the developmental epoch from fetus to mature adult. Evolving developments in molecular biology, bioinformatics, pharmacogenetics, and bioimaging will ensure that this specialty remains at the forefront of pediatric research and practice. This chapter is intended as an introduction to this important field, with greater detail to be found in the chapters that follow. Much remains to be learned.

REFERENCES

1. Medvei, V. C. (1991). The history of Cushing's disease: a controversial tale. *J Royal Soc Med*, 84, 363–366.
2. Fisher, D. A. (2004). A short history of pediatric endocrinology in North America. *Pediatr Res*, *55*, 716–726.
3. Randle, P. J. (1954). Assay of plasma insulin activity by the rat-diaphragm assay. *Brit Med J*, *1*, 1237.
4. Salmon, W. D., Jr., & Daughaday, W. H. (1957). A hormonally controlled serum factor which stimulates sulfate incorporation by cartilage in vitro. *J Lab Clin Med*, *49*, 825–836.
5. Kaplan, S. A., & Cohen, P. (2007). Review: the somatomedin hypothesis 2007: 50 years later. *J Clin Endocrinol Metab*, *92*, 4529–4535.
6. Yallow, R. S., & Berson, S. A. (1960). Immunoassay of endogenous plasma insulin in man. *J Clin Invest*, *39*, 1157–1175.

7. Guillemin, R. (2005). Hypothalamic hormones a.k.a. hypothalamic releasing factors. *J Endocrinol*, *184*, 11–28.
8. Rajagopal, S., Rajagopa, K., & Lefkowitz, R. J. (2010). Teaching old receptors new tricks: biasing seven-transmembrane receptors. *Nat Rev Drug Discov*, *9*, 373–386.
9. Kleeman, C. R., Sevine, B. S., & Felsenfeld, A. J. (2009). Fuller Albright: the consummate clinical investigator. *Clin J Am Soc Nephrol*, *4*, 1541–1546.
10. Robbins, D. C., Tager, H. S., & Rubenstein, A. H. (1984). Biologic and clinical importance of proinsulin. *N Engl J Med*, *310*, 1165–1175.
11. Kohane, I. S., Drazen, J. M., & Campion, E. W. (2012). A glimpse of the next 100 years in medicine. *N Engl J Med*, *367*, 2538–2539.
12. Gluckman, P. D., Hanson, M. A., Bateson, P., et al. (2009). Towards a new developmental synthesis: adaptive developmental plasticity and human disease. *Lancet*, *373*, 1654–1657.
13. Lim, A. L., Ng, S., Leow, S. C., et al. (2012). Epigenetic state and expression of imprinted genes in umbilical cord correlates with growth parameters in human pregnancy. *J Med Genet*, *49*, 689–697.
14. Reynolds, R. M., & Seckl, J. R. (2012). Antenatal glucocorticoid treatment: are we doing harm to term babies? *J Clin Endocrinol Metab*, *97*, 3457–3459.
15. Jett, P. L., Samuels, M. H., McDaniel, P. A., et al. (1997). Variability of plasma cortisol levels in extremely low birth weight infants. *J Clin Endocrinol Metab*, *82*, 2921–2925.
16. Vermes, I., Dohanics, J., Toth, G., et al. (1980). Maturation of the circadian rhythm of the adrenocortical functions in human neonates and infants. *Horm Res*, *12*, 237–244.
17. Sippell, W. G., Becker, H., Versmold, H. T., et al. (1978). Longitudinal studies of plasma aldosterone, corticosterone, deoxycorticosterone, progesterone, 17-hydroxyprogesterone, cortisol and cortisone determined simultaneously in mother and child at birth and during the early neonatal period. I. spontaneous delivery. *J Clin Endocrinol Metab*, *46*, 971.
18. Soliman, A. T., Taman, K. H., Rizk, M. H., et al. (2004). Circulating adrenocorticotropic hormone (ACTH) and cortisol concentrations in normal, appropriate-for-gestational-age newborns versus those with sepsis and respiratory distress: cortisol response to low-dose and standard-dose ACTH tests. *Metabolism*, *53*, 209–214.
19. Goodyer, C. G., Figueiredo, R. O., & Krackovitch, S. (2001). Characterization of the growth hormone receptor in human dermal fibroblasts and liver during development. *Am J Physiol Endocrinol Metab*, *281*, E1213–E1220.
20. Romero, G. S., Stephan, D. A., Sperlihng, M. A., et al. (1996). Distinct sexual dimorphism in the effect of hypothyroidism on the expression of the growth hormone receptor and growth hormone-binding protein gene in rat liver. *Horm Res*, *45*, 273–278.
21. Grumbach, M. M., & Gluckman, P. D. (1994). The human fetal hypothalamus and pituitary gland: the maturation of neuroendocrine mechanisms controlling the secretion of fetal pituitary growth hormone, prolacting, gonadotropins, adrenocorticotropin-related peptides, and thyroptropin. In D. Tulchinsky, & A. B. Little (Eds.), *Maternal-fetal endocrinology*, 2nd ed. Philadelphia: W.B. Saunders Company, 193–262.
22. McCabe, M. J., Alatzoglou, K. S., & Dattani, M. T. (2011). Septo-optic dysplasia and other midline defects: the role of transcription factors: HESX1 and beyond. *Best Pract Res Clin Endocrinol Metab*, *25*, 115–124.

QUESTIONS

1. What key methodologies propelled progress in endocrinology in the 20th century?
   a. Radioactive tracers
   b. Radioimmunoassay
   c. Protein purification
   d. Molecular basis of hormone synthesis and action
   e. b and d
   f. All of the above

Answer: e

2. A unit of insulin is the amount of activity in which of the following?
   a. 1 μg of purified hormone
   b. The amount of insulin that will lower blood glucose in an average human adult by 100 mg/dl
   c. The amount of insulin that will lower blood glucose to 45 mg/dl in a 2-kg rabbit fasted for 24 hours
   d. The amount of insulin that can conveniently be packaged as 100 units/ml in 10-ml aliquots

Answer: c

3. Why is the "minipuberty of infancy" so named?
   a. Breast development or pubic hair is frequently observed in the first year of life
   b. Testosterone in males and E_2 in females are in the midpubertal range in the first few weeks of life
   c. LH responds briskly to a pulse of GnRH in infants at 3 months of age
   d. Maternal HCG from the placenta stimulates the development of testes and ovaries at term

Answer: b

4. The fetal origins of adult disease hypothesis postulates which of the following?
   a. All diseases in adults originate in the intrauterine environment.
   b. The intrauterine environment may induce heritable traits via epigenetic mechanisms.
   c. Diseases such as T2DM and metabolic syndrome could be avoided by appropriate nutrition for the mother during pregnancy.
   d. Post-zygotic mutations define the tendency for certain common diseases such as hypertension in adults.

Answer: b

CHAPTER 2

Molecular Endocrinology and Endocrine Genetics

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CHAPTER OUTLINE

INTRODUCTION

The study of the endocrine system has undergone a dramatic evolution since the 1990s, from the traditional physiologic studies that dominated the field for many years to the discoveries of molecular endocrinology and endocrine genetics.[1,2] At the present time the major impact of molecular medicine on the practice of pediatric endocrinology relates to diagnosis and genetic counseling for a variety of inherited endocrine disorders. In contrast, the direct therapeutic application of this new knowledge is still in its infancy, although recently the results of the first successful human gene therapy trials were reported (but not for endocrine diseases). In addition, the new information has led to a host of molecularly targeted therapies mainly in cancer; endocrine oncology has greatly benefited from the application of new drugs that were designed to battle specific mutations in, for example, thyroid cancer. This chapter is an introduction to the basic principles of molecular biology, common laboratory techniques, and some examples of the recent advances made in clinical pediatric endocrinologic disorders with an emphasis on endocrine genetics. Most new diagnostic testing, pharmacogenetics, and molecular therapies are discussed in the disease-specific chapters of this book, and only examples that highlight the principle/strategy under discussion are listed in this chapter.

BASIC MOLECULAR TOOLS

Isolation and Digestion of DNA and Southern Blotting

The human chromosome comprises a long double-stranded helical molecule of DNA associated with different nuclear proteins.[3,4] As DNA forms the starting point of the synthesis of all the protein molecules in the body, molecular techniques using DNA have proven to be crucial

in the development of diagnostic tools to analyze endocrine diseases. DNA can be isolated from any human tissue, including circulating white blood cells. About 200 μg of DNA can be obtained from 10 to 20 mL of whole blood with the efficiency of DNA extraction being dependent on the technique used and the method of anticoagulation employed. The extracted DNA can be stored almost indefinitely at an appropriate temperature. Furthermore, lymphocytes can be transformed with the Epstein-Barr virus (and other means) to propagate indefinitely in cell culture as "immortal" cell lines, thus providing a renewable source of DNA. For performing molecular genetic studies, lymphoid lines are routinely the tissue of choice, because a renewable source of DNA obviates the need to obtain further blood from the family. Fibroblast-derived cultures can also serve as a permanent source of DNA or RNA (once transformed), but they have to be derived from surgical specimens or a biopsy. It should be noted that, because the expression of many genes is tissue specific, immortalized lymphoid or fibroblastoid cell lines cannot be used to anlyze the abundance or composition of messenger RNA (mRNA) for a specific gene. Hence, studies involving mRNA necessitate the analysis of the tissue(s) expressing the gene as outlined in the section on "RNA Analysis" that begins on page 13.

DNA is present in extremely large molecules; the smallest chromosome (chromosome 22) has about 50 million base pairs and the entire haploid human genome is estimated to comprise 3 million to 4 billion base pairs. This extreme size precludes the analysis of DNA in its native form in routine molecular biology techniques. The techniques for identification and analysis of DNA became feasible and readily accessible with the discovery of enzymes termed *restriction endonucleases*. These enzymes, originally isolated from bacteria, cut DNA into smaller sizes on the basis of specific recognition sites that vary from two to eight base pairs in length.[5,6] The term *restriction* refers to the function of these enzymes in bacteria. A restriction endonuclease destroys foreign DNA (such as bacteriophage DNA) by cleaving the DNA at specific sites, thereby "restricting" the entry of foreign DNA in the bacterium. Several hundred restriction enzymes with different recognition sites are now commercially available. Because the recognition site for a given enzyme is fixed, the number and sizes of fragments generated for a particular DNA molecule remain consistent with the number of recognition sites and provide predictable patterns after separation by electrophoresis.

Analysis of the DNA fragments generated after digestion usually employs the technique of electrophoresis.[7] Electrophoresis exploits the property that the phosphate groups in the DNA molecule confer a negative charge to that molecule. Thus, when a mixture of DNA molecules of different sizes is electrophoresed through a sieve (routinely either agarose or acrylamide), the longer DNA molecules migrate more slowly relative to the shorter fragments. Following electrophoresis, the separated DNA molecules can be located by a variety of staining techniques, of which ethidium bromide staining is a commonly used method.

Although staining with ethidium bromide is a versatile technique, analysis of a few hundred base pairs of DNA in the region of interest is difficult when the DNA from all the human chromosomes are cut and separated on the same gel. These limitations are circumvented by the technique of Southern blotting (named after its originator, Edward Southern) and the use of labeled radioactive or more commonly nonradioactive probes. Southern blotting involves digestion of DNA and separation by electrophoresis through agarose.[8] After electrophoresis, the DNA is transferred to a solid support (such as nitrocellulose or nylon membranes), enabling the pattern of separated DNA fragments to be replicated onto the membrane (Figure 2-1). The DNA is then denatured (i.e., the two strands are physically separated), fixed to the membrane, and the dried membrane is mixed with a solution containing the DNA probe. A DNA probe is a fragment of DNA that contains a nucleotide sequence specific for the gene or chromosomal region of interest. For purposes of detection, the DNA probe is labeled with an identifiable tag, such as radioactive phosphorus (e.g., ^{32}P) or a chemiluminescent moiety; the latter has almost exclusively replaced radioactivity. The process of mixing the DNA probe with the denatured DNA fixed to the membrane is called hybridization, the principle being that there are only four nucleic acid bases in DNA—adenine (A), thymidine (T), guanine (G), and cytosine (C)—that always remain complementary on the two strands of DNA, A pairing with T, and G pairing with C. Following hybridization, the membrane is washed to remove the unbound probe and exposed to an x-ray film either in a process called autoradiography

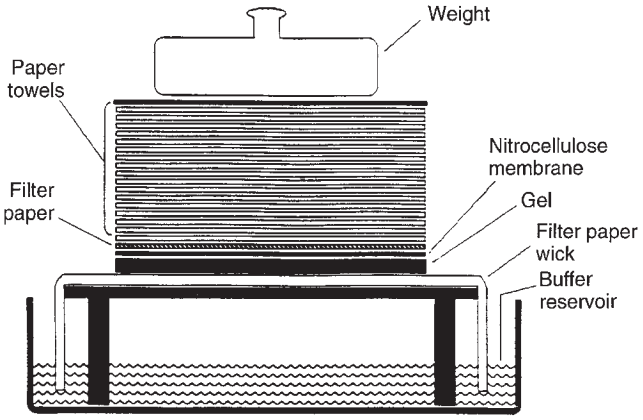

FIGURE 2-1 ■ Southern blot. Fragments of double-stranded DNA are separated by size by agarose gel electrophoresis. To render the DNA single stranded (denatured), the agarose gel is soaked in an acidic solution. After neutralization of the acid, the gel is placed onto filter paper, the ends of which rest in a reservoir of concentrated salt buffer solution. A sheet of nitrocellulose membrane is placed on top of the gel and absorbent paper is stacked on top of the nitrocellulose membrane. The salt solution is drawn up through the gel by the capillary action of the filter paper wick and the absorbent paper towels. As the salt solution moves through the gel, it carries along with it the DNA fragments. Because nitrocellulose binds single-stranded DNA, the DNA fragments are deposited onto the nitrocellulose in the same pattern that they were placed in the agarose gel. The DNA fragments bound to the nitrocellulose are fixed to the membrane by heat or UV irradiation. The nitrocellulose membrane with the bound DNA can then be used for procedures such as hybridization to a labeled DNA probe. Techniques to transfer DNA to other bonding matrices, such as nylon, are similar. (Adapted from Turco E, Fritsch R, Trucco M [1990]. Use of immunologic techniques in gene analysis. In Herberman RB, Mercer DW [eds.], *Immunodiagnosis of cancer*. New York: Marcel Dekker, 205.)

to detect radioactive phosphorus or in a process used to detect the chemiluminescent tag. Only those fragments that are complementary and have bound to the probe containing the DNA of interest will be evident on the x-ray film, enabling the analysis of the size and pattern of these fragments. As routinely performed, the technique of Southern analysis can detect a single copy gene in as little as 5 μg of DNA, the DNA content of about 10^6 cells.

Restriction Fragment Length Polymorphism and Other Polymorphic DNA Studies

The number and size of DNA fragments resulting from the digestion of any particular region of DNA form a recognizable pattern. Small variations in a sequence among unrelated individuals may cause a restriction enzyme recognition site to be present or absent; this results in a variation in the number and size pattern of the DNA fragments produced by digestion with that particular enzyme. Thus this region is said to be polymorphic for the particular enzyme tested—that is, a restriction fragment length polymorphism (RFLP) (Figure 2-2). The value of RFLP is that it can be used as a molecular tag for tracing the inheritance of the maternal and paternal alleles. Furthermore, the polymorphic region analyzed does not need to encode the genetic variation that is the cause of the disease being studied, but only to be located near the gene of interest. When a particular RFLP pattern can be shown to be associated with a disease, the likelihood of an offspring inheriting the disease can be determined by comparing the offspring's RFLP pattern with the RFLP pattern of the affected or carrier parents. The major limitation of the RFLP technique is that its applicability for the analysis of any particular gene is dependent on the prior knowledge of the presence of convenient ("informative") polymorphic restriction sites that flank the gene of interest by at most a few kilobases. Because these criteria may not be fulfilled in any given case, the applicability of RFLP cannot be guaranteed for the analysis of a given gene.

In the early years of the molecular endocrinology era, the RFLP technique was the mainstay of experimental strategies employed for investigating the genetic basis of endocrine diseases. For example, RFLP-based genomic studies were used to identify mutations in the rearranged during transfection (RET) oncogene as the etiology of the multiple endocrine neoplasia type-2 syndrome. However, at present for routine disease mapping, whole genome and even gene-specific association studies, the RFLP technique has been supplanted by more powerful and facile techniques such as microsatellite and single-nucleotide polymorphism studies (discussed later); at present RFLP analysis is only used within the context of a specific gene investigation and is increasingly being replaced by other means (genomic) of investigation.

Polymerase Chain Reaction

The polymerase chain reaction (PCR) is a technique that was developed in the late 1980s and has indeed revolutionized molecular biology (Figure 2-3). PCR allows the selective logarithmic amplification of a desired fragment of DNA from a complex mixture of DNA that theoretically contains at least a single copy of the target fragment. In the typical application of this technique, some knowledge of the DNA sequences in the region to be amplified is necessary, so that a pair of short (approximately 18 to 25 bases in length) specific oligonucleotides ("primers") can be synthesized. The primers are synthesized in such a manner that they define the limits of the region to be amplified. The DNA template containing the segment that is to be amplified is heat denatured such that the strands are separated and then cooled to allow the primers to anneal to the respective complementary regions. The enzyme Taq polymerase, a heat stable enzyme originally isolated from the bacterium *Thermophilus aquaticus*, is then used to initiate synthesis (extension) of DNA. The DNA is repeatedly *denatured*, *annealed*, and *extended* in successive cycles in a machine called the "thermocycler" that permits this process to be automated. In the usual assay, these repeated cycles of denaturing, annealing, and extension result in the synthesis of approximately 1 million copies of the target region in about 2 hours. To establish the veracity of the amplification process, the identity of the amplified DNA can be analyzed by electrophoresis, hybridization to RNA or DNA probes, digestion with informative restriction enzyme(s), or subjected to direct DNA sequencing. The relative simplicity combined with the power of this technique has resulted in widespread use of this procedure and has spawned a wide variety of variations and modifications that have been developed for specific applications.[9,10] From a practical point of view, the major drawback of PCR is the propensity to get cross-contamination of the target DNA. This drawback is the direct result of the extreme sensitivity of the method that permits amplification from one molecule of the starting DNA template. Thus, unintended transfer of amplified sequences to items used in the procedure will amplify DNA in samples that do not contain the target DNA sequence (i.e., a false positive result). Cross-contamination should be suspected when amplification occurs in negative controls that did not contain the target template. One of the most common modes of cross-contamination is via aerosolization of the amplified DNA during routine laboratory procedures such as vortexing, pipetting, and manipulation of microcentrifuge tubes. Meticulous care to experimental technique, proper organization of the PCR workplace, and inclusion of appropriate controls are essential for the successful prevention of cross-contamination during PCR experiments.

In general, PCR applications are either directed toward the identification of a specific DNA sequence in a tissue or body fluid sample or used for the production of relatively large amounts of DNA of a specific sequence, which then are used in further studies. Examples of the first type of application are common in many fields of medicine, such as in microbiology, wherein the PCR technique is used to detect the presence DNA sequences specific for viruses or bacteria in a biological sample. Prototypic examples of such an application in pediatric endocrinology include the use of PCR of the *SRY* gene for detecting Y chromosome

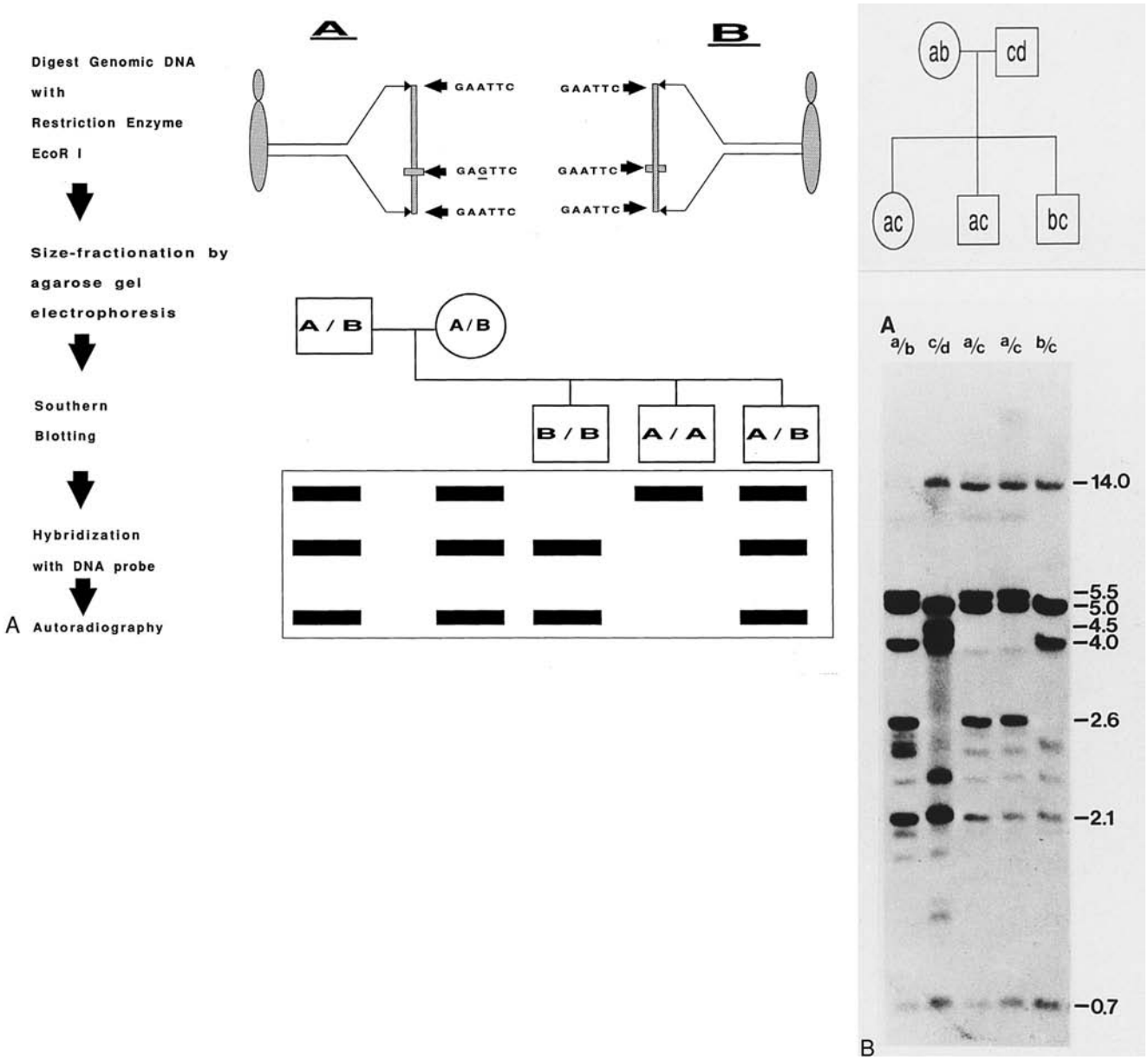

FIGURE 2-2 ■ Restriction fragment length polymorphism. **A,** Schematic illustration. A and B represent two alleles that display a polymorphic site for the restriction enzyme EcoR I. EcoR I will cut DNA with the sequence "GAATTC"; hence, allele B will be cut by EcoR I at three sites to generate two fragments of DNA, whereas allele A will be cut by EcoR I only twice and not at the site (indicated by horizontal bar) where nucleotide G (underlined) replaces the nucleotide A present in allele B. Following digestion, the DNA is size-fractionated by agarose gel electrophoresis and transferred to a membrane by Southern blot technique (see Figure 2-1 for details). The membrane is then hybridized with a labeled DNA probe, which contains the entire sequence spanned by the three EcoR I sites. Autoradiography of the membrane will detect the size of the DNA fragments generated by the restriction enzyme digestion. In this particular illustration, both parents are heterozygous and possess both A and B alleles. Matching the pattern of the DNA bands of the offspring with that of the parents will establish the inheritance pattern of the alleles. For example, if allele A represents the abnormal allele for an autosomal recessive disease, then examination of the Southern blot will establish that (from left to right) the first offspring (B/B) is homozygous for the normal allele, the second offspring (A/A) is homozygous for the abnormal allele, and the third offspring (A/B) is a carrier. **B,** RFLP analysis of the DQ-beta gene of the HLA locus. Genomic DNA from the members of the indicated pedigree was digested with restriction enzyme Pst I, size-fractionated by agarose gel electrophoresis, and transferred to nitrocellulose membrane by Southern blot technique. The membrane was then hybridized with a cDNA probe specific for the DQ-beta gene; the excess probe was removed by washing at appropriate stringency and was analyzed by autoradiography. The sizes of the DNA fragments (in kilobases, kb) are indicated on the right. The pedigree chart indicates the polymorphic alleles (a, b, c, d) and the bands on the Southern blot corresponding to these alleles (a [5.5 kb], b [5.0 kb], c [14.0 kb], d [4.5 kb]) indicate the inheritance pattern of these alleles. (Adapted from Turco E, Fritsch R, Trucco M [1998]. First domain encoding sequence mediates human class II beta-chain gene cross-hybridization. Immunogenetics 28:193.)

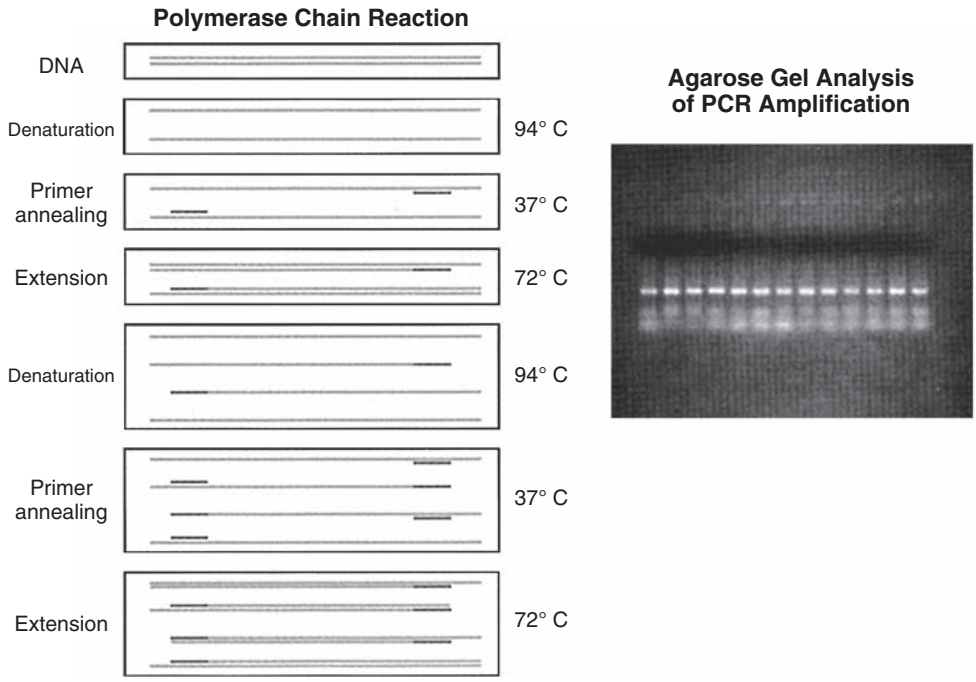

FIGURE 2-3 ■ Polymerase chain reaction (PCR). A pair of oligonucleotide primers (solid bars), complementary to sequences flanking a particular region of interest (shaded, stippled bars), are used to guide DNA synthesis in opposite and overlapping directions. Repeated cycles of DNA denaturation, primer annealing, and DNA synthesis (primer extension) by DNA polymerase enzyme result in an exponential increase in the target DNA (i.e., the DNA sequence located between the two primers) such that this DNA segment can be amplified 1 × 106-7 times after 30 such cycles. The use of a thermostable DNA polymerase (i.e., Taq polymerase) allows for this procedure to be automated. Inset: The amplified DNA can be used for subsequent analysis (i.e., size-fractionation by agarose gel electrophoresis). (Adapted from Trucco M [1992]. To be or not to be ASP 57, that is the question. Diabetes Care 15:705.)

material in patients with karyotypically defined Turner syndrome and the rapid identification of chromosomal gender in cases of fetal or neonatal sexual ambiguity[11] (Figure 2-4).

Most PCR applications, both as research tools and for clinical use, are directed toward the production of a target DNA or the complementary DNA of a target RNA sequence. The DNA that is made ("amplified") is then analyzed by other techniques such as RFLP analysis, allele-specific oligonucleotide hybridization, or DNA sequencing.

RNA Analysis

The majority (> 95%) of the chromosomal DNA represents noncoding sequences. These sequences harbor regulatory elements, serve as sites for alternate splicing, and are subject to methylation and other epigenetic changes that affect gene function. However, at present most disease-associated mutations in human gene have been identified in coding sequences. An alternate strategy to analyze mutations in a given gene is to study its messenger RNA (mRNA), which is the product (via transcription) of the remaining 5% of chromosomal DNA that encodes for proteins. In addition, because the mRNA repertoire is cell and tissue specific, the analyses of the mRNA sequences provide unique information about tissue-specific proteins produced in a particular organ/tissue.

There are many techniques for analyzing mRNA. Northern blotting (so named because it is based on the same principle as the Southern blot) is one of the original methods used for mRNA analysis. In Northern blotting, RNA is denatured by treating it with an agent such as formaldehyde to ensure that the RNA remains unfolded and in the linear form.[12,13] The denatured RNA is then electrophoresed and transferred onto a solid support (such as nitrocellulose membrane) in a manner similar to that described for the Southern blot.[8] The membrane with the RNA molecules separated by size is probed with the gene-specific DNA probe labeled with an identifiable tag that, as in the case of Southern blotting, is either a radioactive label (e.g., ^{32}P) or more commonly a chemiluminescent moiety. The nucleotide sequence of the DNA probe is complementary to the mRNA sequence of the gene and is hence called complementary DNA (cDNA). It is customary to use labeled cDNA (and not labeled mRNA) to probe Northern blots because DNA molecules are much more stable and easier to manipulate and propagate (usually in bacterial plasmids) than mRNA molecules. The Northern blot provides information regarding the amount (estimated by the intensity of the signal on autoradiography) and the size (estimated by the position of the signal on the gel in comparison to concurrently electrophoresed standards) of the specific mRNA. Although the Northern blot technique represents a versatile and straightforward method to analyze mRNA, it has major drawbacks. Northern analysis is a relatively

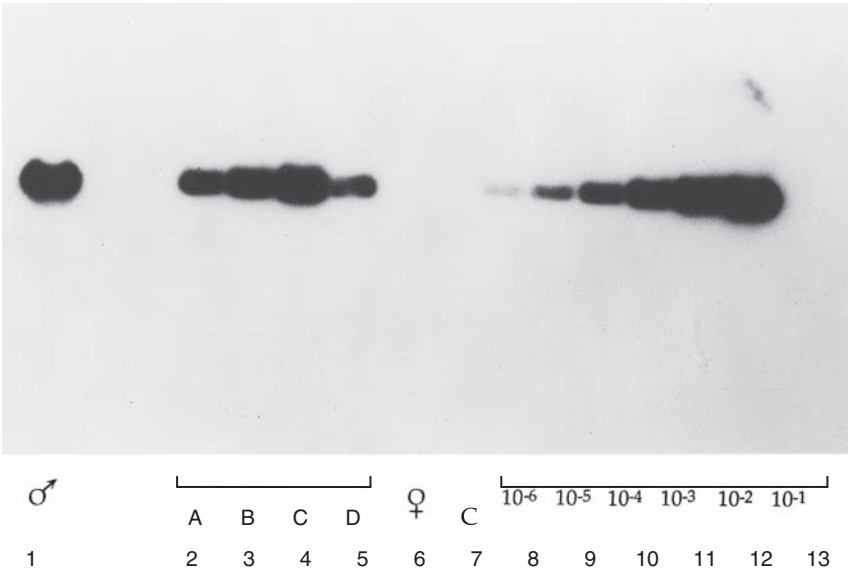

FIGURE 2-4 ■ Detection of *SRY* gene–specific sequence in Turner syndrome by polymerase chain reaction (PCR) amplification and Southern blot. *SRY*-specific primers were used in PCR to amplify DNA from patients with 45X karyotype. The amplified DNA was size-fractionated by agarose gel-electrophoresis and transferred to membrane by Southern blotting. The membrane was then hybridized to labeled *SRY*-specific DNA and autoradiographed. From left to right: amplified male DNA (lane 1); amplified DNA from patients with 45X karyotype (lanes 2-5); amplified female DNA (lane 6); negative control with no DNA (lane 7); serial dilution of male DNA (lanes 8-13). (Adapted from Kocova M, Siegel SF, Wenger SL, et al. [1993]. Detection of Y chromosome sequence in Turner's syndrome by Southern blot analysis of amplified DNA. Lancet 342:140. © Copyright by the Lancet Ltd.)

insensitive technique, both in terms of the concentration of mRNA that can be detected and in terms of the fine structure. This technique cannot detect small changes in size, nucleotide composition, or the abundance of the mRNA being analyzed. At present, reverse transcriptase-PCR (RT-PCR) has become the technique of choice for the routine analysis of mRNA.

One of the most sensitive methods for the detection and quantitation of mRNA currently available is the technique of quantitative RT-PCR (qRT-PCR).[9] This technique combines the unique function of the enzyme reverse transcriptase with the power of PCR. qRT-PCR is exquisitely sensitive, permitting analysis of gene expression from very small amounts of RNA. Furthermore, this technique can be applied to a large number of samples or many genes (multiplex) in the same experiment. These two critical features endow this technique with a measure of flexibility unavailable in more traditional methods such as Northern blot or solution hybridization analysis. Whereas the detection of a specific mRNA by this technique is relatively straightforward, the precise quantitation of the mRNA in a given sample is more complicated. The first step in qRT-PCR analysis is the production of DNA complementary (cDNA) to the mRNA of interest. This is done by using the enzymes with RNA-dependent DNA polymerase activity that belong to the reverse transcriptase (RT) group of enzymes (e.g., Moloney murine leukemia virus [MMLV], avian myeloblastosis virus [AMV] reverse transcriptase, an RNA dependent DNA polymerase). The RT enzyme, in the presence of an appropriate primer, will synthesize DNA complementary to RNA. The second step in the qRT-PCR analysis is the amplification of the target DNA, in this case the cDNA synthesized by the reverse transcriptase enzyme. The specificity of the amplification is determined by the specificity of the primer pair used for the PCR amplification. To establish the veracity of the amplification process, the identity of the amplified DNA can be analyzed by electrophoresis, hybridization to RNA or DNA probes, digestion with informative restriction enzyme(s), or subjected to direct DNA sequencing

Whereas the detection of a specific mRNA by this technique is relatively straightforward, the precise quantitation of the mRNA in a given sample is more complicated. Because the production of DNA by PCR involves an exponential increase in the amount of DNA synthesized, relatively minor differences in any of the variables controlling the rate of amplification will cause a marked difference in the yield of the amplified DNA. In addition to the amount of template DNA, the variables that can affect the yield of the PCR include the concentration of the polymerase enzyme, magnesium, nucleotides (dNTPs), and primers. The specifics of the amplification procedure including cycle length, cycle number, annealing, extension and denaturing temperatures also affect the yield of DNA. Because of the multitude of variables involved, routine RT-PCR is unsuitable for performing a quantitative analysis of mRNA. To circumvent these pitfalls alternate strategies have been developed. One technique for determining the concentration of a particular mRNA in a biological sample is a modification of the basic PCR technique called competitive RT-PCR.[9,14,15] This method is based on the co-amplification of a mutant DNA that can be amplified with the same pair of primers being used for the target DNA. The mutant DNA is engineered in such a way that it can be distinguished from the DNA of interest by either size or the inclusion

of a restriction enzyme site unique to the mutant DNA. The addition of equivalent amounts of this mutant DNA to all the PCR reaction tubes serves as an internal control for the efficiency of the PCR process, and the yield of the mutant DNA in the various tubes can be used for the equalization of the yield of the DNA by PCR. It is important to ensure for accurate quantitation of the DNA of interest that the concentrations of the mutant and target template should be nearly equivalent. Because the use of mutated DNA for normalization does not account for the variability in the efficiency of the reverse transcriptase enzyme, a variation of the original method has been developed. In this modification, competitive mutated RNA transcribed from a suitably engineered RNA expression vector is substituted for the mutant DNA in the reaction prior to initiating the synthesis of the cDNA. Competitive RT-PCR can be used to detect changes of the order of two- to threefold of even very rare mRNA species. The major drawback of this method is the propensity to get inaccurate results because of the contamination of samples with the mRNA of interest. In theory, as the technique is based on PCR, contamination by even one molecule of mRNA of interest can invalidate the results. Hence, scrupulous attention to laboratory technique and set up is essential for the successful application of this technique.

In general, two types of methods are used for the detection and quantitation of PCR products: the traditional "end-point" measurements of products and the newer "real-time" techniques. End-point determinations (e.g., the competitive RT-PCR technique described earlier) analyze the reaction after it is completed, whereas real-time determinations are made during the progression of the amplification process. Overall the real-time approach is more accurate and is currently the preferred method. Advances in fluorescence detection technologies have made the use of real-time measurement possible for routine use in the laboratory. One of the popular techniques that takes advantage of real-time measurements is the TaqMan (fluorescent 5′ nuclease) assay (Figure 2-5).[16,17]

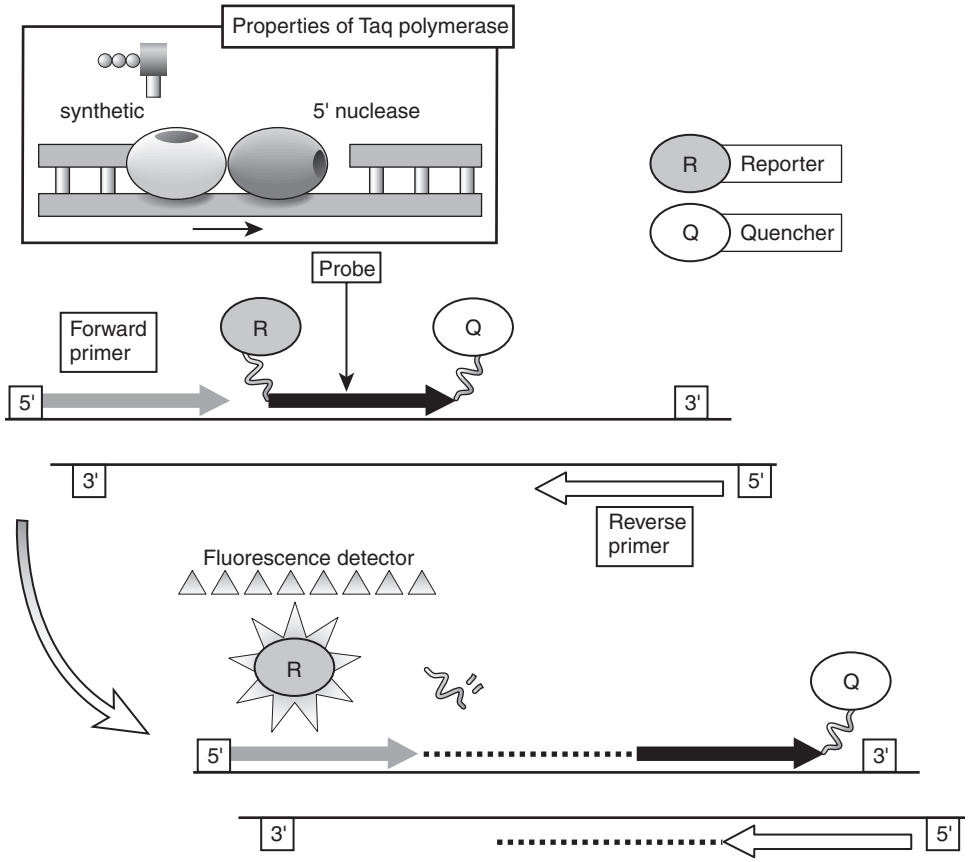

FIGURE 2-5 ■ Fluorescent 5′ nuclease (TaqMan) assay. Three synthetic oligonucleotides are utilized in a fluorescent 5′ nuclease assay. Two oligonucleotides function as "forward" and "reverse" primers in a conventional polymerase chain reaction (PCR) amplification protocol. The third oligonucleotide, termed the TaqMan probe, consists of an oligonucleotide synthesized with a 5′-reporter dye (e.g., FAM; 6-carboxy-fluorescein) and a downstream, 3′-quencher dye (e.g., TAMRA; 6-carboxy-tetramethyl-rhodamine). When the probe is intact, the proximity of the reporter dye to the quencher dye results in suppression of the reporter fluorescence, primarily by Forster-type energy transfer. During PCR, forward and reverse primers hybridize to a specific sequence of the target DNA. The TaqMan probe hybridizes to a target sequence within the PCR product. The Taq polymerase enzyme, because of its 5′-3′ exonuclease activity, subsequently cleaves the TaqMan probe. The reporter dye and the quencher dye are separated by cleavage, resulting in increased fluorescence of the reporter dye as a direct consequence of target amplification during PCR. Both primers and probe must hybridize to the target for amplification and cleavage to occur. Hence, the fluorescence signal is generated only if the target sequence for the probe is amplified during PCR. Fluorescent detection takes place through fiberoptic lines positioned above the caps of the reaction wells. Inset: The two distinct functions of the enzyme Taq polymerase: the 5′-3′ synthetic polymerase activity and the 5′-3′ polymerase-dependent exonuclease activity.

The unique design of TaqMan probes, combined with the 5′ nuclease activity of the PCR enzyme (Taq polymerase), allows direct detection of PCR product by the release of fluorescent reporter during the PCR amplification by using specially designed machines (ABI Prism 5700/7700). The TaqMan probe consists of an oligonucleotide synthesized with a 5′-reporter dye (e.g., FAM; 6-carboxy-fluorescein) and a downstream, 3′-quencher dye (e.g., TAMRA; 6-carboxy-tetramethyl-rhodamine). When the probe is intact, the proximity of the reporter dye to the quencher dye results in suppression of the reporter fluorescence, primarily by Forster-type energy transfer. During PCR, forward and reverse primers hybridize to a specific sequence of the target DNA. The TaqMan probe hybridizes to a target sequence within the PCR product. The Taq polymerase enzyme, because of its 5′-3′ nuclease activity, subsequently cleaves the TaqMan probe. The reporter dye and the quencher dye are separated by cleavage, resulting in increased laser-stimulated fluorescence of the reporter dye as a direct consequence of target amplification during PCR. This process occurs in every cycle and does not interfere with the exponential accumulation of product. Both primer and probe must hybridize to the target for amplification and cleavage to occur. The fluorescence signal is generated only if the target sequence for the probe is amplified during PCR. Because of these stringent requirements, nonspecific amplification is not detected. Fluorescent detection takes place through fiber optic lines positioned above optically nondistorting tube caps. Quantitative data are derived from a determination of the cycle at which the amplification product signal crosses a preset detection threshold. This cycle number is proportional to the amount of starting material, thus allowing for a measurement of the level of specific mRNA in the sample. An alternate machine (Light Cycler) also uses fluorogenic hydrolysis or fluorogenic hybridization probes for quantification in a manner similar to the ABI system.

MicroRNA

One of significant advances in the early 2000s in the field of RNA biology is the discovery of small (20 to 30 nucleotide) noncoding RNAs. In general there are two categories of small noncoding RNAs: microRNA (miRNA) and Small interfering RNA (siRNA). miRNAs are expressed products of an organism's own genome, whereas siRNAs are synthesized in the cells from foreign double-stranded RNA (e.g., from viruses or transposons or from synthetic DNA introduced into the cell to study the function of a particular gene/process). Additionally, there are differences in the biogenesis of these two classes of small nucleotide RNAs. These differences notwithstanding, the overall biological effect of these small nucleotide RNAs is translational repression or target degradation and gene silencing by binding to complementary sequences on the 3′ untranslated region of target messenger RNA; positive regulation of gene expression via such a mechanism is distinctly uncommon. The complexity of the phenomenon is increased by the fact that in a cell- or tissue-specific context a single miRNA can target multiple RNAs and more than one miRNA can recognize the same mRNA target to amplify and strengthen the translational repression of the target gene. It is estimated that this phenomenon is present in several cell types and the human genome codes for more than 1000 miRNAs that could target 60% to 70% of mammalian genes. miRNA-mediated events have been implicated in regulation of cell growth and differentiation, cell growth, apoptosis, and other cellular processes. To date, the major impact of the discovery of miRNA has been in the fields of developmental biology, organogenesis, and cancer. miRNA and miRNA-related events (e.g., proteins involved in miRNA processing) have been directly implicated in only a small number of nonneoplastic endocrine disorders (e.g., diGeorge syndrome and X-linked mental retardation). It is predicted that as we learn more about the basic biology of this process, small nucleotide noncoding RNAs will be implicated in the pathogenesis of a wider spectrum of endocrine diseases.

DETECTION OF MUTATIONS IN GENES

Changes in the structural organization of a gene that impact its function involve deletions, insertions, or transpositions of relatively large stretches of DNA, or more frequently single-base substitutions in functionally critical regions. Southern blotting and RFLP analysis can usually detect the deletion or insertion of large stretches of DNA. However, these analytic methods can be used for detecting point mutations only if the mutation involves the recognition site for a particular restriction enzyme, such that the absence of a normally present restriction site or the appearance of a novel site unmasks the presence of the point mutation. More commonly these techniques cannot be used for such an analysis, necessitating alternate procedures. High throughput or next-generation sequencing has revolutionized the identification of mutations in genes.[48]

Direct Methods

DNA sequencing is the current gold standard for obtaining unequivocal proof of a point mutation. However, DNA sequencing has its limitations and drawbacks. A clinically relevant problem is that current DNA sequencing methods do not reliably and consistently detect all mutations. For example, in many cases where the mutation affects only one allele (heterozygous), the heights of the peaks of the bases on the fluorescent readout corresponding to the wild-type and mutant allele are not always present in the predicted (1:1) ratio. This limits the discerning power of "base calling" computer protocols and results in inconsistent or erroneous assignment of DNA sequence to individual alleles.[18] Because of this limitation, clinical laboratories routinely determine the DNA sequence of both the alleles to provide independent confirmation of the absence/presence of a putative mutation. DNA sequencing can be labor intensive and expensive, although advances in pyrosequencing (discussed later), for example, have made it technically easier and cheaper.

Although the first DNA sequences were determined with a method that chemically cleaved the DNA at each

of the four nucleotides,[19] the enzymatic or dideoxy method developed by Sanger and colleagues in 1977 is the one most commonly used for routine purposes[20] (Figure 2-6). This method uses the enzyme DNA polymerase to synthesize a complementary copy of the single-stranded DNA ("template") whose sequence is being determined. Single-stranded DNA can be obtained directly from viral or plasmid vectors that support the generation of single-stranded DNA or by partial denaturing of double-stranded DNA by treatment with alkali or heat.[21] The enzyme DNA polymerase cannot initiate synthesis of a DNA chain de novo but can only extend a fragment of DNA. Hence, the second requirement for the dideoxy method of sequencing is the presence of a "primer." A primer is a synthetic oligonucleotide, 15 to 30 bases long, whose sequence is complementary to the sequence of the short corresponding segment of the single-stranded DNA template. The dideoxy method exploits the observation that DNA polymerase can use both 2′-deoxynucleoside triphosphate (dNTP) and 2′,3′-dideoxynucleoside triphosphates (ddNTPs) as substrates during elongation of the primer. Whereas DNA polymerase can use dNTP for continued synthesis of the complementary strand of DNA, the chain cannot elongate any further after addition of the first ddNTP because ddNTPs lack the crucial 3′-hydroxyl group. To identify the nucleotide at the end of the chain, four reactions are carried out for each sequence analysis, with only one of the four possible ddNTPs included in any one reaction. The ratio of the ddNTP and dNTP in each reaction is adjusted so that these chain terminations occur at each of the positions in the template where the nucleotide occurs. To enable detection by autoradiography, the newly synthesized DNA is labeled, usually by including in the reaction mixture radioactively labeled dATP (for the older manual methods) or, most commonly, currently fluorescent dye terminators in the reaction mixture (now in use in automated techniques). The separation of the newly synthesized DNA strands manually is done via high-resolution denaturing polyacrylamide electrophoresis or with capillary electrophoresis in automatic sequencers. Fluorescent detection methods have enabled automation and enhanced throughput. In capillary electrophoresis, DNA molecules are driven to migrate through a viscous polymer by a high electric field to be separated on the basis of charge and size. Though this technique is based on the same principle as that used in slab gel electrophoresis, the separation is done in

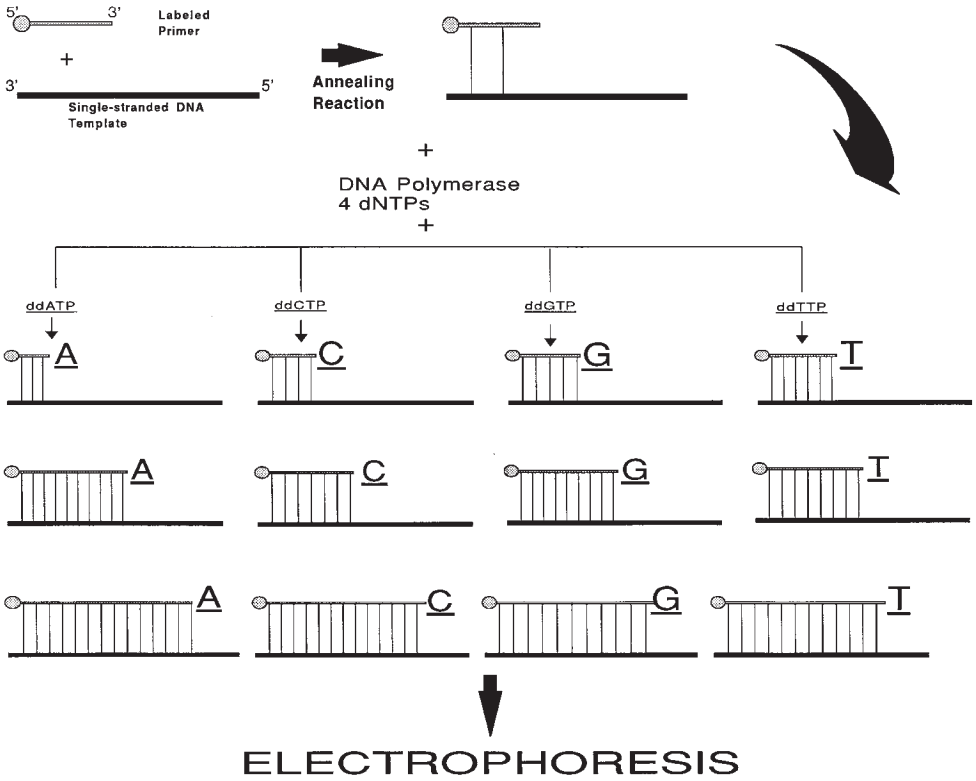

FIGURE 2-6 ■ DNA sequencing by the dideoxy (Sanger) method. A 5′-end-labeled oligonucleotide primer with sequence complementarity to the DNA that is to be sequenced (DNA template) is annealed to a single-strand of the template DNA. This primer is elongated by DNA synthesis initiated by the addition of the enzyme DNA polymerase in the presence of the four dNTPs (2′-deoxynucleoside triphosphates) and one of the ddNTPs (2′,3′-dideoxynucleoside triphosphates); four such reaction tubes are assembled to use all the four ddNTPs. The DNA polymerase enzyme will elongate the primer using the dNTPs and the individual ddNTP present in that particular tube. Because ddNTPs are devoid of 3′ hydroxyl group, no elongation of the chain is possible when such a residue is added to the chain. Thus, each reaction tube will contain prematurely terminated chains ending at the occurrence of the particular ddNTP present in the reaction tube. The concentrations of the dNTPs and the individual ddNTP present in the reaction tubes are adjusted so that the chain termination takes place at every occurrence of the ddNTP. Following the chain elongation-termination reaction, the DNA strands synthesized are size-separated by acrylamide gel electrophoresis and the bands visualized by autoradiography.

individual glass capillaries rather than gel slabs, facilitating loading of samples and other aspects of automation. Whereas manual methods allow the detection of about 300 nucleotide of sequence information with one set of sequencing reactions, automated methods using fluorescent dyes and laser technology can analyze 7500 or more bases per reaction. To sequence larger stretches of DNA it is necessary to divide the large piece of DNA into smaller fragments that can be individually sequenced. Alternatively, additional sequencing primers can be chosen near the end of the previous sequencing results, allowing the initiation point of new sequence data to be moved progressively along the larger DNA fragment.

One of the seminal technological advances has been the introduction of microarray-based methods for detection, and analysis of nucleic acids (discussed later). For purposes of detection of mutations, the oligonucleotides fixed to the slide/membrane are complementary to all possible base substitutions or a subset of small deletions and insertions. Fluorescent labeled PCR probes derived from the patient and representing the genes to be tested are then hybridized to the microarrays. Following appropriate washing protocols, the retention of particular probes on the slide will provide information regarding the presence/absence of a given mutation/deletion/insertion. There are limitations of microarray-based techniques; for example, similar to direct DNA sequencing methods, microarray-based methods also suffer from the disadvantage of not being able to reliably and consistently detect heterozygous mutations. Furthermore, microarrays cannot be used to detect insertions of multiple nucleotides without exponentially increasing the number of oligonucleotides that must be immobilized on the glass slides.

The most exciting new technique in mutation identification is pyrosequencing, which is based on an enzymatic real-time monitoring of DNA synthesis by bioluminescence; this read-as-you-go method uses nucleotide incorporation that leads to a detectable light signal from the pyrophosphate released when a nucleotide is introduced in the DNA strand.[22] The rapidity and reliability of this method far exceed other contemporary DNA sequencing techniques. However, the major limitation of this technique is that it can only be used to analyze short stretches of DNA sequence.

Pyrosequencing, introduced in the early 2000s, provided the background for the explosion of new techniques collectively known as high-throughput or next-generation sequencing (NGS). NGS provides longer read length and cheaper price per base of sequencing compared to Sanger sequencing. NGS is based on the uncoupling of the traditional nucleotide-identifying enzymatic reaction and the image capture and doing so in an ever-speedier way allows for essentially unlimited capacity. Currently, two systems are being used for NGS: the SOLiD (by Applied Biosystems, Inc.) and the Illumina (Solexa) systems. The first discoveries of gene mutations for endocrine diseases exploiting NGS were published in 2011.[21]

Semiconductor and nanotechnology-based systems are currently under use in massive sequencing efforts and promise an even cheaper and faster way of determining mutations and other abnormalities of the human genome; however, these technologies are currently not available for use in clinical pediatric endocrinology.

Indirect Methods

In the mid-1980s, the need for rapid, high-throughput, accurate, and economical mutation analysis systems led to the development of several technologies, as an alternative to analysis by direct sequencing, that allowed detection of single mutations in long stretches of DNA (200 to 600 bp). However, screening for mutations by indirect methods has fallen out of favor as NGS and Sanger sequencing have become faster and cheaper methods for identifying gene mutations. We only refer to them here briefly and for historical purposes, because some of these techniques are still in use sporadically, and understanding the literature published since the 1980s would be impossible without knowledge of the principles underlying these techniques.

Indirect methods of mutation identification included restriction endonuclease digestion of PCR products (PCR-RFLP), denaturing-gradient gel electrophoresis (DGGE), single-strand conformation polymorphism (SSCP), dideoxy fingerprinting (ddF), and heteroduplex mobility assay (HMA). Most of these methods utilized PCR to amplify a region of the DNA, a physical or chemical treatment of amplified DNA (e.g., by denaturation or restriction enzyme digestion), separation of the amplicons by gel electrophoresis (by denaturing or nondenaturing), and visualization of the separated sequence strands (by autoradiography or fluorescence-based detection). Most modifications in some of these techniques allowed simultaneous separation and detection of DNA fragments with the use of sophisticated equipment such HPLC and capillary electrophoresis.

Originally described in 1989, SSCP analysis had been a widely used method for the detection of mutations because of its simplicity and efficiency. In SSCP, DNA regions with potential mutations were first amplified by PCR in the presence of a radiolabeled dNTP (Figures 2-7 and 8). Single-stranded DNA fragments were then generated by denaturation of the PCR products and separated on a native polyacrylamide gel. As the denatured PCR product moved through the gel and away from the denaturant, it regained a secondary structure in a sequence-dependent manner. The electrophoretic migration of single stranded DNA is a function of its secondary structure. Therefore, PCR products that contained substitution differences, as well as insertions and deletions, had different mobility when compared with wild-type DNA. Overall, SSCP, depending on factors such as the specific sequence, amplicon size, and location of the mutation, was successful in 60% to 90% of instances in detecting previously identified sequence changes.

Dideoxy-fingerprinting (ddF) was essentially a hybrid of SSCP and dideoxy-sequencing in which primer extension products were generated in the presence of one dideoxynucleotide and subjected to chemical denaturation and electrophoresis on a non-denaturing polyacrylamide gel

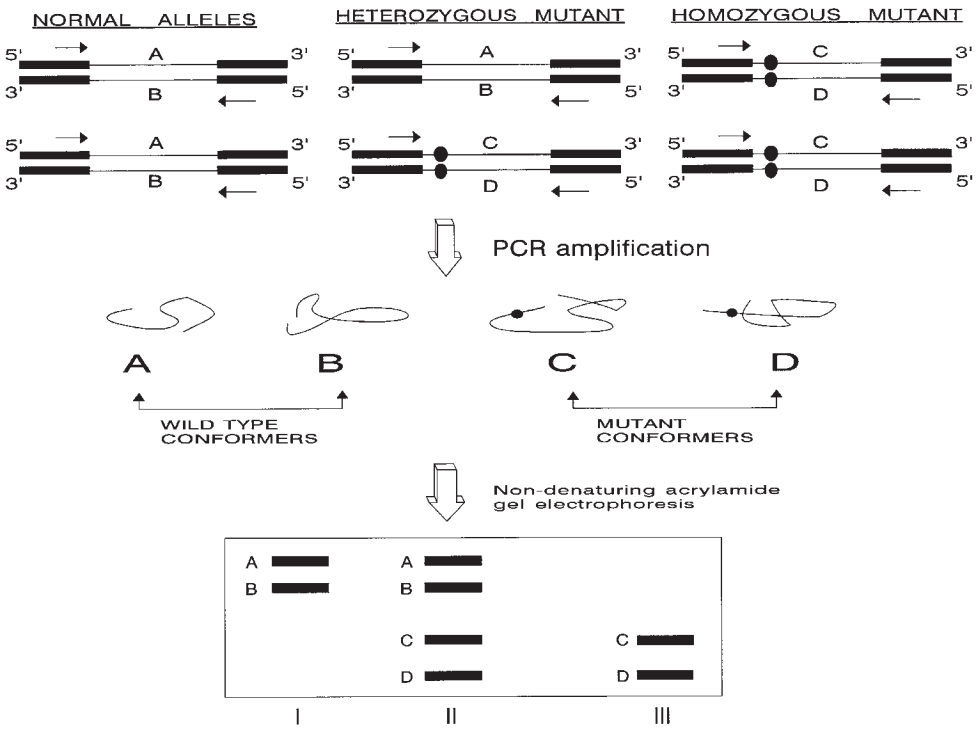

FIGURE 2-7 ■ Single-stranded conformational polymorphism (SSCP). Schematic representation of an experiment designed to use SSCP to detect the presence of heterozygous and homozygous single-base pair mutation (represented as a filled circle). The segment of DNA is amplified using PCR with flanking primers (represented by arrows). ^{32}P is incorporated into the newly synthesized DNA by either end-labeling the primers or by the addition of ^{32}P-dATP to the PCR reaction. Theoretically, four different types of conformers can be formed: A and B from the sense and antisense strand of the wild-type (normal) DNA, C, and D from the sense and antisense strands containing the single-base pair mutation. Following PCR the DNA is denatured and analyzed by nondenaturing gel electrophoresis: Lane I represents the wild-type conformers, Lane II represents the wild-type and mutant conformers from a heterozygous patient, and Lane III represents the presence of mutant conformers with the absence of wild-type conformers from a homozygous patient.

to exploit differences in secondary structure of single stranded conformers. The resulting electrophoretic pattern resembled sequencing gels in which the mobility of extension products was determined by both size and conformation. Modifications of the original procedure resulted in new and improved variants of ddF. Bi-directional ddF (Bi-ddF), in which the dideoxy termination reaction was performed simultaneously with two opposing primers, has allowed larger fragments (~ 600 bp) to be screened with almost 100% sensitivity. RNA ddF (R-ddF), in which RNA was used as starting material, enabled identification of mutations that resulted in splicing errors and allowed screening of genes with large intronic regions. Denaturing fingerprinting (DnF), a modification of Bi-ddF in which fingerprints were generated by performing denaturing gel electrophoresis on bidirectional "cycle-sequencing" reactions with each of two dideoxy terminators, allowed screening of DNA regions with high GC content avoiding the generation of "smearing" bands and, thus, increasing the sensitivity of the technique in identifying heterozygous mutations.

DGGE is a method that was used to detect single-base pair substitutions (or small insertions or deletions) in genes.[23-25] Like SSCP, DGGE is also a PCR-based method and exploited the observation that when double stranded DNA migrates through a polyacrylamide gel incorporating a gradient of chemical denaturing agents, the mobility of the partially denatured ("melted") DNA molecule is extremely sensitive to its base composition. Thus, even single-base changes in the nucleotide composition of the DNA will result in altered mobility of the partially denatured DNA. G-C clamp is a modified DGGE procedure wherein the sensitivity of the procedure is enhanced by the incorporation of a G- and C-rich region at one end of the DNA.[24,26-28] This manipulation is most readily done by modifying one of the primers used for the PCR. The addition of the G-C rich region increases the melting point of the DNA fragment and makes it easier to detect changes in the nucleotide composition. Depending on the relative orientation of the chemical gradient and the electrical field during electrophoresis, DGGE could either be parallel or perpendicular. The relative sensitivity of these two protocols has to be determined empirically for each application of this method. Denaturing high-performance liquid chromatography (DHPLC) is a related technique that detects a variation in the DNA sequence but uses high-performance liquid chromatography (HPLC) instead of gel electrophoresis for separation of DNA fragments. The incorporation of HPLC allows for automation of this technique and significantly enhances improves throughput by this method.

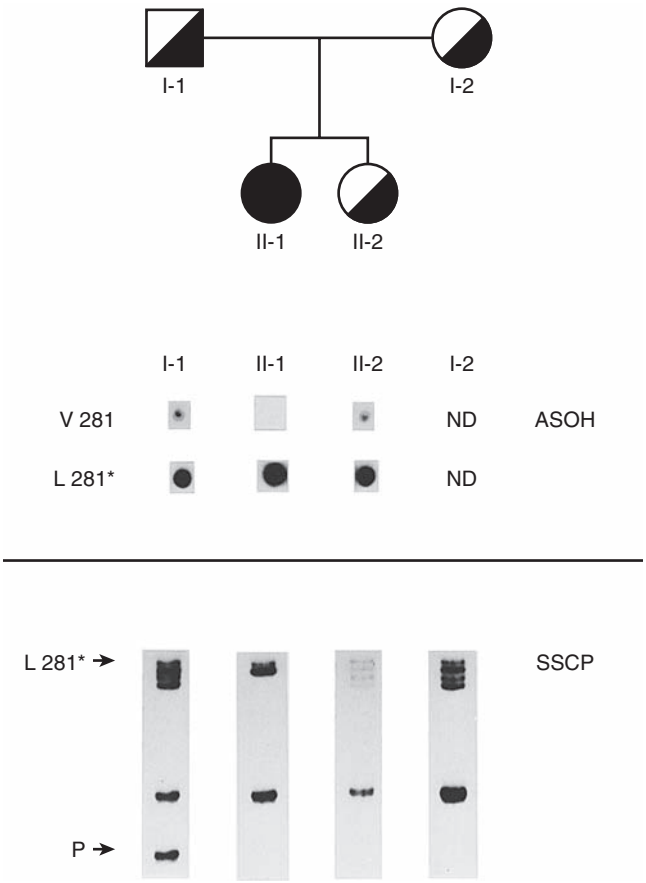

FIGURE 2-8 ■ Application of SSCP and ASOH to the analysis of the 21-hydroxylase gene in congenital adrenal hyperplasia. Detection of a mutation of codon 281 from valine to leucine (V281L). Upper panel: Pedigree of a family with proband II-1, who was referred for evaluation of hirsutism and secondary amenorrhea. Middle panel: ASOH results from both mutant and normal alleles at codon 281 shows both the father and sister of the proband to be carriers of the V281L mutation, while the patient is homozygous. ASOH was not performed on the mother. The asterisk indicated the mutant allele (L281). Lower panel: SSCP analysis revealed that the two additional conformers, representing the L281 conformer, were detected at the top of the gel in this patient and her family. The greater intensity of the conformers in the proband compared to her family members and the disappearance of the normal V281 conformer (adjacent to the L281 conformers) are consistent with her being homozygous for this mutation. L281 indicates the mutant conformer; P, normal polymorphism. (Adapted from Siegel SF, Hoffman EP, Trucco M [1994]. Molecular diagnosis of 21-hydroxylase deficiency: detection of four mutations on a single gel. Biochem Med Metab Biol 51:66.)

Heteroduplex analysis was a variation of the SSCP method and is used to detect single pair mismatches in double-stranded DNA.[29-31] Allele-specific oligonucleotide hybridization (ASOH) and reverse blot technique analyze DNA after amplification by PCR and detect sequence variations by the success or failure of hybridization of short oligonucleotide probes, which either exactly match or mismatch the sequence being tested (Figure 2-9). The amplified, target DNA was first denatured and applied to a nylon membrane in the form of a small dot. Once this target DNA was anchored to the membrane either by heating or by brief ultraviolet irradiation, the DNA was hybridized with a labeled (usually with ^{32}P) oligonucleotide, which encompasses the variable nucleotides of the DNA sequence of interest. The membrane is then washed with a salt solution where the salt concentration and the temperature control the specificity or "stringency" of the procedure. Following the wash, the probe remaining on the membrane was detected by autoradiography. When several nucleotide variations (i.e., alleles) are known to exist in the same target sequence, several identical membranes are prepared and each is hybridized with a different oligonucleotide probe that is complementary to one of the known sequence variations. The major disadvantage of this method was that it required prior knowledge of the base changes involved in the mutation and the precise stringency parameters for hybridization and washing of the membrane. A variation of the original method to perform ASOH is called the reverse blot technique[32] (see Figure 2-9). The difference when compared to ASOH is that the amplified target DNA is labeled and then hybridized to an anchored unlabeled probe. Because the length of the DNA molecules is an important factor that facilitates binding to the membrane, a key to the design of this method was the development of a relatively simple way to synthesize, using the PCR, a stretch of DNA that contained multiple copies (i.e., a polymer) of the relatively short allele-specific oligonucleotide probes.[33] In practice, the amplified DNA is nonradioactively labeled by previously tagging the PCR primers with either fluorescein or biotin. After hybridization of the denatured PCR product in the presence of a membrane containing all of the relevant polymeric probes, and washing as described earlier, the retained PCR products are revealed by detection via enzyme activity linked to either fluorescein antibodies or to streptavidin.

Certain classes of mutations are inherently difficult to detect using the traditional methods of detection outlined here,[18] and thus as NGS and other genomic methods become cheaper and more widely available they are expected to replace all of the preceding techniques. Mutations that are difficult to detect with any of the preceding methods include promoter region, 3′ untranslated region (UTR) or intronic region mutations affecting levels of transcript of mRNA as well as deletions of entire genes or of contiguous exons. Thus, if the genomic region examined is deleted from the mutant allele, PCR-based methods are unable to detect this mutation because the PCR product obtained from the genomic DNA will be exclusively derived from the wild-type allele and thus appear to be normal. Promoter regions, 3′ UTR, and intronic regions usually span genomic segments several orders larger than the coding exons and are thus not easily accessible for analysis with the methods outlined here. Different strategies need to be implemented for the analysis and detection of such mutations. Thus, deletions of one or more exons can be detected by quantitative hybridizations, quantitative PCR, Southern blotting, or fluorescence in situ hybridization with the combined use of such methods enhancing the sensitivity of the testing protocol. A technique termed "conversion" exploits the

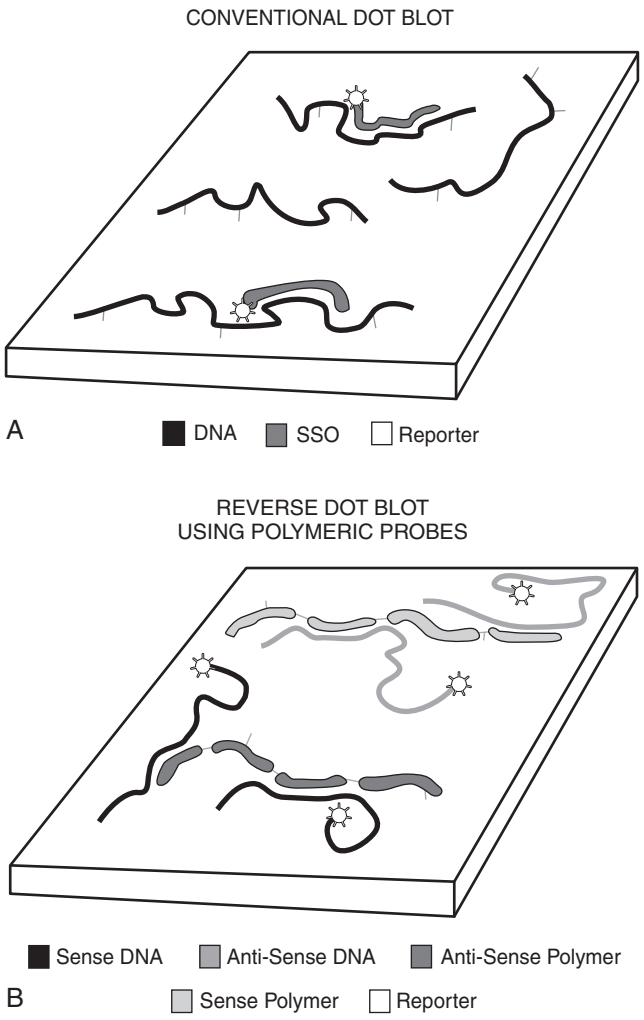

FIGURE 2-9 ■ **A,** Allele specific oligonucleotide hybridization (ASOH). The denatured (single-stranded) target DNA is anchored to a membrane, which is then treated (hybridized) with a solution of a short DNA segment of the gene of interest (sequence-specific oligonucleotide [SSO]). The SSO is tagged with a reporter molecule, such as ^{32}P. Unbound probe DNA is removed by washing with buffer solutions. Appropriate stringency conditions of hybridization and washes limit the hybridization of the probe specifically to its complementary segment in the target DNA molecule. Depending on the manner in which the DNA is spotted on to the membrane, this procedure is referred to as either a dot or a slot blot. **B,** Reverse dot blot. In this variation of the conventional ASOH procedure, the DNA probes (sense and antisense polymer) tagged with the reporter molecule are fixed to a membrane and the denatured target DNA (sense and antisense DNA) is then hybridized to the immobilized probe. Similar to the ASOH procedure, appropriate stringency conditions of hybridization and washes limit the hybridization of target DNA to those that contain the complementary segment to the immobilized probe. The advantages of this method include the increased sensitivity derived from the ability to fix multiple copies (polymer) of the probe to the membrane and that both complementary strands of the probe sequence are present on the membrane. (Adapted from Trucco M [1992]. To be or not to be ASP 57, that is the question. Diabetes Care 15:705.)

principle that the diploid state of the human genome is converted to a haploid state, which is then analyzed by one of the traditional methods.[34] The critical advantage of this manipulation is that heterozygous mutations are easier to detect in the haploid state because of the absence of the normal wild-type sequence.

POSITIONAL GENETICS IN ENDOCRINOLOGY

The Principles of Positional Genetics

For the purpose of disease gene identification, the candidate gene approach relies on partial knowledge of the genetic basis of the disease under investigation. This process was successful in identifying disease genes whose function was obvious. For example, the genetic defects of most of the hereditary enzymatic disorders, including congenital adrenal hyperplasia (CAH) syndromes, became known in the late 1980s, when the introduction of PCR made the tools of molecular biology widely available to the medical and genetic research community. However, at about the same time, research on diseases without any obvious candidate genes (e.g., the multiple endocrine neoplasia [MEN] syndromes) or diseases in which the screening of obvious candidate genes failed to reveal mutations was ongoing. It was in these diseases that the application of "reverse genetics," or more appropriately termed "positional cloning,"[35,36] yielded information regarding the genetic basis of these disease states. Positional cloning is complemented by the Human Genome Project (HGP) and the worldwide web in making available, in a fast and controlled manner, information that would otherwise be inaccessible.[37]

The process of positional genetics is outlined in Figure 2-10. The first step is the collection of clinical information from families with affected members, the determination of the mode of inheritance of the defect (autosomal dominant or recessive, X-linked, complex inheritance), and the phenotyping of subjects (or tissues) following well-established criteria for the diagnosis of the disorder. If inheritance is not known, formal segregation analysis needs to be performed to determine the autosomal, or X-linked, and the recessive or dominant nature of the inheritance.[38] Once this determination is made and the penetrance of the disorder is known, appropriate linkage software may be used.[39] For more information on currently available computer software packages, the reader may check http://darwin.case.edu and other related links.

Linkage is examined with polymorphic markers that span the entire human genome[36]; any marker that shows polymorphism and is known to lie close to or within a putative disease gene may be used. Genetic linkage can be defined as the tendency for alleles close together on the same chromosome to be transmitted together as an intact unit though meiosis. The strength of linkage can then be used as a unit of measurement to find out how close genetically different loci are to each other. This unit of map distance is an approximation of physical distance but is also highly dependent on other factors (for example, the frequency of recombination is not the same in both genders, differing along the length of chromosomes and through the various chromosomes). The likelihood (logarithm of odds [or LOD] score) method is widely used for linkage analysis.

Once a locus on a chromosome has been identified, the region (which is usually several thousands of base pairs in length) is narrowed by analyzing informative recombinations in the cohort of patients and families available for study. The disease region may harbor

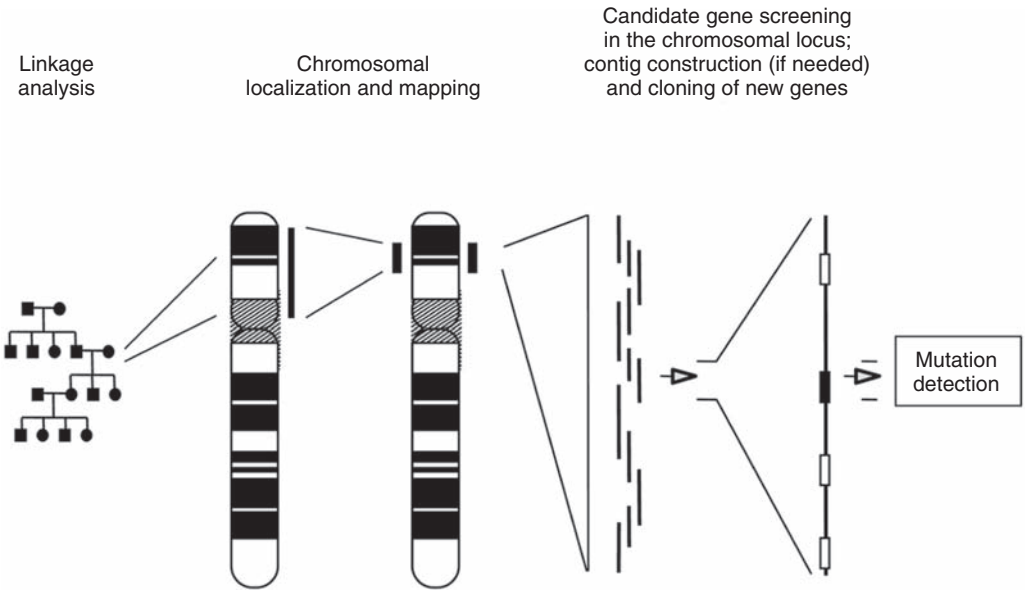

FIGURE 2-10 ■ The steps of positional cloning (see text).

already mapped genes. Online databases such as Gen Bank, ENSEMBL (www.ensembl.org), and others, and especially for clinicians the Online Mendelian Inheritance in Man (OMIM),[40] may provide all the necessary information. If a transcript is a reasonable candidate, mutation screening may identify the disease gene. If, however, these steps fail to identify the disease gene, screening new sequences from the area may be needed; today this is done typically by NGS followed by Sanger sequencing for confirmation. Chromosomal maps are linked by sequence-tagged sites (STSs) that are present in more than one genomic clone, thus providing critical information that allows for the proper aligning of DNA in a given locus. Polymorphic markers (including those that were used for the linkage part of the process) are the most useful STSs because they provide a direct link between the genetic and the physical mapping data. Individual clones can be sequenced; genes are identified in this process through their unique sequence features or through in vitro translation. In the past, expressed sequence tags (ESTs) provided information for what gene sequences are expressed from this area. Today, almost all the genes are fully sequenced; however, ESTs are still useful to look at especially when one is trying to identify a new gene for a given disease-linked locus. Each one of the newly identified genes may be screened for mutations, as long as the expression profile of the identified transcript matches the spectrum of the tissues affected by the disease under investigation. Although this is helpful for most diseases, for others the expression profile may even be misleading; thus, the presence of a transcript in an affected tissue is not always necessary. Complete segregation of the disease with an identified mutation, functional proof, or mutations in two or more families with the same disease are usually required as supportive evidence that the cloned sequence is the disease gene.[41]

Genomic Identification of "Endocrine" Genes

In the 1990s, positional cloning was used to identify a number of genes relevant to endocrinology; today this is being achieved by NGS and other genome-wide methods of identification of new disease genes. It is worth noting that endocrine tumor syndromes, despite their rarity and small overall impact on everyday clinical endocrine practice, are seminal examples of diseases whose molecular etiology was elucidated by positional cloning. Positional cloning played an essential role in unraveling the etiology of these syndromes because for most endocrine tumor syndromes there were no obvious candidate genes. Furthermore, positional cloning of genes responsible for familial tumor syndromes was greatly assisted by the use of neoplastic tissue for studies such as loss-of-heterozygosity (LOH), comparative genomic hybridization (CGH), and fluorescent in situ hybridization (FISH) applications. These techniques narrowed the genetically defined chromosomal regions and thus facilitated the identification of the responsible genes; LOH studies were critical in the identification of von Hippel–Lindau disease (VHL-elongin),[42] MEN 1 (menin),[43] Cowden disease (PTEN),[44] Peutz-Jeghers syndrome (STK11/LKB1),[45,46] and Carney complex (PRKAR1A)[47] genes.

NGS is used today to sequence the entire genome (whole-genome sequencing [WGS]) or only the expressed genes (whole exome sequencing [WES]) of a proband (or his family members) with the intention to identify the mutations responsible for a disease after the methods described here have positionally identified a locus of interest. In the absence of linkage or other positioning information (such as, for example, in the absence of family history, tumor material, or additional DNA samples) an individual's genomic DNA (or tissue derived from the individual) may be sequenced by WGS or WES.

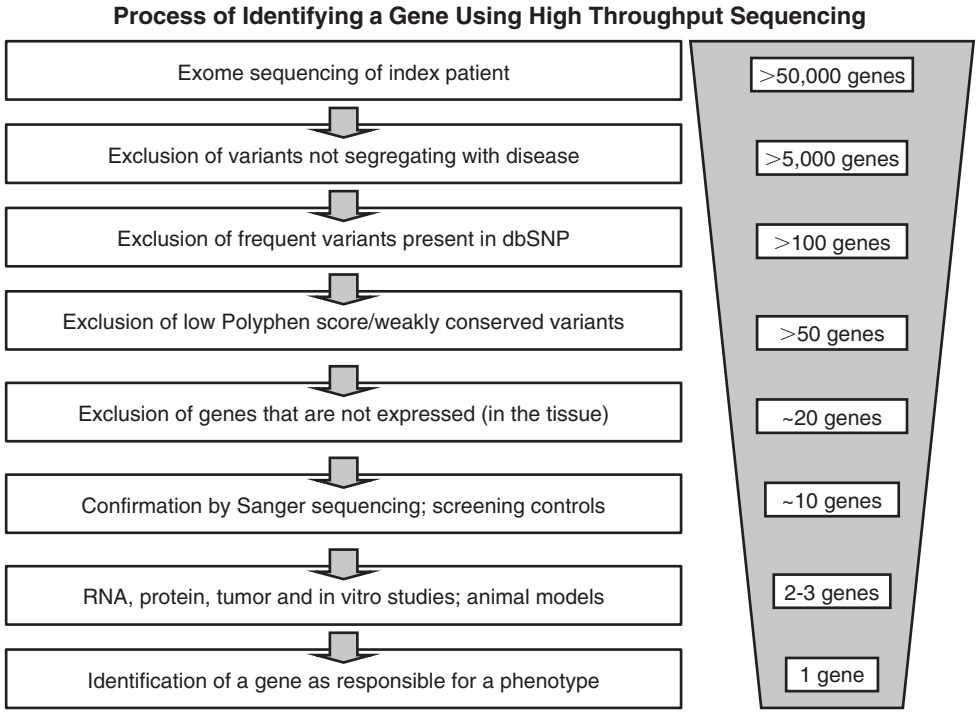

FIGURE 2-11 ■ Whole genome or exome sequencing is the currently preferred DNA sequencing technology for gene identification. However, even in the genome of phenotypically normal human subjects, these sequencing techniques yield a multitude of sequencing variants, including bona fide protein-truncating mutations, hence the need to use a systematic and careful filtering process to identify the causative gene(s). The steps in the analysis of gene mutations identified by high throughput sequencing techniques that culminate in the assignment of a gene to a specific phenotype are illustrated.

This, however, leads to the identification of many mutations and other variants that need to be excluded to identify the disease-specific gene(s). A typical flowchart for something like this is shown in Figure 2-11.

Impact of Modern Sequencing in Clinical Practice

Today genome-sequencing studies indicate that each human subject carries as many as 100 loss-of-function mutations with more than 20 genes being completely inactivated.[49] Many genetic variants are found to have previously unsuspected functional redundancies,[50,51] thus a patient may have many genetic variants within a singular signaling pathway leading to graded phenotypes.

An example of the application of the techniques of modern DNA sequencing in pediatric endocrinology that resulted in the elucidation of the disease mechanism is in the so-called white Addison disease (WAD) phenotype.[52,53] It has been recognized that patients presenting with primary adrenal insufficiency (pAI) are not always pigmented; this variant of pAI was called WAD. Increased pigmentation in pAI is attributed to the binding of high levels of adrenocorticotropin (ACTH) to the melanocortin (MC) 1 receptor (MC1R). MC1R, a molecule with high degree of sequence similarity to the MC2R, the ACTH receptor, binds to its regular ligand, α-melanocyte-stimulating hormone (α-MSH) and ACTH with an almost equal affinity.[54] α-MSH stimulates melanogenesis in cultured human melanocytes and acts specifically to increase the synthesis of eumelanin. Both α-MSH and ACTH are splice products of the same precursor pituitary protein, proopiomelanocortin (POMC), and play a role in determining pigmentation in humans, as homozygous or compound heterozygote mutations in the POMC gene are associated with hypopigmentation and red hair. In pAI that is not appropriately treated with glucocorticoids, POMC expression increases, and both α-MSH and ACTH increase. The important role of MC1R in the determination of human skin and hair pigmentation is beyond doubt: reduced function MC1R alleles lead to red hair, freckling, sun sensitivity, and an increased risk of cutaneous cancers, including melanoma.[54,55]

Familial glucocorticoid deficiency (FGD) due to ACTH resistance consists of at least three distinct genetic syndromes that are all inherited as autosomal recessive (AR) traits: inactivating mutations of the ACTH receptor (the MC2R gene) and its accessory protein (MRAP); isolated ACTH resistance (iACTHR) without MC2R, MRAP, or any other known mutation; and Allgrove syndrome (AS).[56,57] Turan and colleagues[52] described a patient with FGD without pigmentation: she was born with red hair, which gradually darkened during early childhood; despite repeated episodes of hypoglycemia, she was not diagnosed with pAI and was thus exposed to high ACTH levels until she was 6 years old. MC2R sequencing showed a homozygous T152K mutation that is known to affect trafficking of the receptor, like most ACTH receptor defects causing FGD. MC1R sequencing showed a homozygous R160W mutation that is

among the most common genetic variants of the receptor in red-haired individuals. In a fascinating confirmation of the roles of ACTH (and possibly other POMC-derived peptides) and MC1R in determining not only skin but also hair pigmentation, the patient's hair lightened and "reverted to a reddish color" following proper replacement with hydrocortisone and a decrease of serum ACTH levels.

This is the first demonstration of MC1R genetic variants affecting the phenotype in pAI. It is possible that MC1R, the major freckle gene, modifies the phenotype in Carney complex and other disorders associated with skin pigmentation and endocrine disease (e.g., McCune-Albright syndrome, neurofibromatosis syndromes, and others). Beyond the obvious diagnostic implications, MC1R variants also predispose to nevi and cancer. Could, for example, the nevi observed in Turner syndrome and their response to growth hormone (GH) depend on MC1R function? And how might variant MC1R signaling affect the function of other melanocortin receptors that to some extent interact and coordinate their cAMP responses? Would the clinical presentation of an MRAP defect causing FGD or an AAAS mutation causing AS be affected equally by MC1R variants?

With genetic variants responsible for even subtler effects (than, for example, red hair), DNA sequencing data challenge the practicing clinician to incorporate systems biology information in clinical practice. Clearly not all sequence defects identified by WGS or WES cause diseases; quite the opposite. The evidence is that there is redundancy and an exquisitely delicate and complex molecular balance in human biology. But clinicians have to incorporate genetics in their daily practice, and educators have to introduce molecular pathways and their genetic variability in classic physiology and pathophysiology lectures.[58]

EXPRESSION STUDIES (MICROARRAYS, SAGE)

Advances in biotechnology, instrumentation, robotics, computer sciences, and the completion of genome sequencing initiatives for several organisms, including the human, have resulted in the development of novel and powerful techniques. A seminal example of such a technique is the development of the microarray technology. Microarrays contain thousands of oligonucleotides deposited or synthesized in situ on a solid support, typically a coated glass slide or a membrane. In this technique, a robotic device is used to print DNA sequences onto the solid support. The DNA probes immobilized on the microarray slide as spots can either be cloned cDNA or gene fragments (ESTs, expressed sequence-tags), or oligonucleotides corresponding to known genes or putative open reading frames. The arrays are hybridized with fluorescent targets prepared from RNA extracted from tissue/cells of interest; the RNA is labeled with fluorescent tags such as Cy3 and Cy5. The prototypic microarray experimental paradigm consists of comparing mRNA abundance in two different samples. One fluorescent target is prepared from control mRNA and the second target with a different fluorescent label is prepared from mRNA isolated from the treated cells or tissue under investigation. Both targets are mixed and hybridized to the microarray slide, resulting in target gene sequences hybridizing to their complementary sequences on the microarray slide. The microarray is then excited by laser, and the fluorescent intensity of each spot is determined with the relative intensities of the two colored signals on individual spots being proportional to the amounts of specific mRNA transcripts in each sample (Figure 2-12). Analysis of the fluorescent intensity data yields an estimation of the

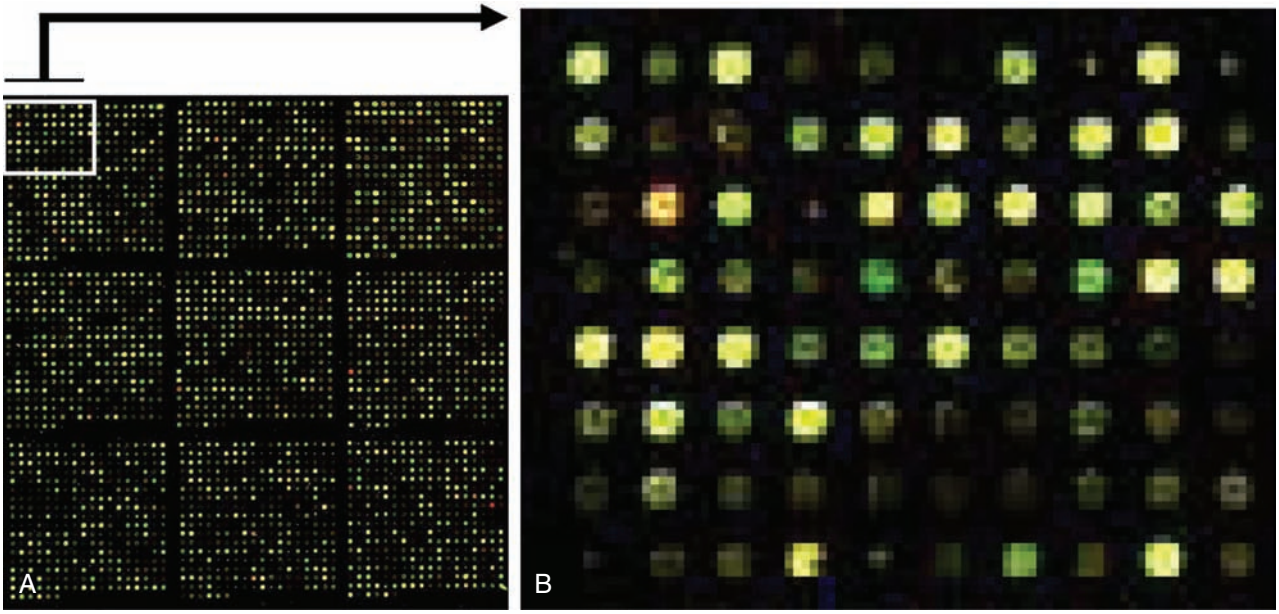

FIGURE 2-12 ■ **A,** cDNA microarray, fluorescent labeled cDNA targets, ACTH-independent bilateral macronodular adrenal hyperplasia (Cy3), and ACTH-dependent hyperplasia (Cy5) were hybridized to glass slides containing genes involved in oncogenesis. Following laser activation of the fluorescent tags, fluorescent signals from each of the DNA "spots" are captured and subjected to analysis. **B,** Magnified view of the microarray platform displaying the fluorescent signals; green (Cy3) and red (Cy5) with yellow representing overlap of these two colors. *(This image can be viewed in full color online at ExpertConsult.)*

relative expression levels of the genes in the sample and control sample. Microarrays enable individual investigators to perform large-scale analyses of model organisms and to customize arrays for special genome applications.

The method of choice for global expression profiling depends on several factors including technical aspects, labor, price, time and effort involved, and, most important, the type of information that is sought. Technical advances in the development of expression arrays, their abundance and commercial availability, and the relative speed with which analysis can be done are all factors that make arrays more useful in routine applications. In addition, array content can now be readily customized to cover from gene clusters and pathways of interest to the entire genome: some studies examine series of tissue-specific transcripts or genes known to be involved in particular pathology; others directly use arrays covering the whole genome. Another factor that needs to be considered prior to embarking on any high-throughput approach is whether individual or pooled samples will be investigated. Series of pooled samples reduce the price, the time spent, and the number of the experiments down to the most affordable. Investigating individual samples, however, is important for identifying unique expression ratios in a given type of tissue or cell.

A requirement of all high-throughput screening approaches is confirmation of findings (expression level of a given gene/sequence) by other independent methods. A select group of genes are tested usually; these genes are picked from the series of sequences that were analyzed either because they were found to have significant changes or because of their particular interest with regard to their expression in the studied tissue or their previously identified relationship to pathology or developmental stage. The confirmation process attempts to support the findings on three different levels: (1) reliability of the high-throughput experiment (for this purpose the same samples examined by the microarrays are used); (2) trustfulness of the observations in general (to achieve that, larger number of samples are examined, assessment of which by high-throughput approaches is often unaffordable price- or labor-wise); and (3) verification of the expression changes at the protein level.[58] A commonly used confirmatory technique is quantitative real-time reverse transcription PCR (qRT-PCR). For verification at the protein level, immunohistochemistry (IHC) and Western blot are the two most commonly chosen techniques. IHC is not quantitative but has the advantage of allowing for the observation of the exact localization of a signal within a cell (cytoplasmic versus nuclear) and the tissue (identifying histologically the tissue that is stained). Modern Western blot methods require a smaller amount of protein lysate than older techniques and have the advantage of offering high-resolution quantitation of expression without the use of radioactivity.

CHROMOSOME ANALYSIS AND MOLECULAR CYTOGENETICS

Chromosomes represent the most condensed state in metamorphosis of the genome during a cell cycle. Condensation of the genetic material at metaphase stage is a crucial event that provides precise and equal segregation of chromosomes between the two daughter's newborn cells during the next step, anaphase. This is followed by relaxation of the genetic content after cell division. This ability of the genome to transform from a molecular level (DNA) to a materialistic submicroscopic stage (chromosome) provides a unique opportunity to visualize the genome of an individual cell of an organism. Different chromosomal abnormalities related to particular diseases or syndromes can be detected at this stage by karyotyping chromosomes.

Chromosomes can be individually recognized and classified by size, by shape (ratio of the short/long arm), and by using differential staining techniques. In the past, identification of chromosomes was restricted to chromosome groups only. The introduction of chromosome banding technique revolutionized cytogenetic analysis.[59] The banding patterns are named by the following abbreviations: G for Giemsa, R for reverse, Q for quinacrine, and DAPI for 4′6′-diamino-2-phenylindole; the last two give a pattern similar to G-banding. Further development of high-resolution banding techniques[60] enabled the study of chromosomes at earlier stages of mitosis, prophase, and prometaphase. Chromosomes are longer and have an enriched banding pattern at those stages, providing great details for the identification of chromosomal aberrations.

Outline of Methods

Preparation of good-quality chromosomes is an art. Many different methods for chromosome isolation have been developed in cytogenetics since the 1960s. The main principle behind all methods is to arrest cells at metaphase by disruption of the cell spindle. Metaphase spindle is a structure composed of tubular fibers formed in the cell to which the chromosomes are attached by kinetochores (centrosomes). The spindle separates the chromosomes into the two daughter cells. The agent commonly used for spindle disruption is Colcemid. The exposure time to Colcemid varies depending on the proliferative activity of cells. Cells with a high proliferative index need a shorter time of exposure to a high concentration of Colcemid, 0.1 to 0.07 μg/mL for 10 to 20 minutes. Slow-growing cells require longer exposure, 1 to 4 hours or overnight with a lower concentration, 0.01 to 0.05 μg/mL. Prolonged exposure to Colcemid or the use of high concentrations increases the proportion of chromosomes at late metaphase, resulting in shortening of the chromosomes. Conversely, a short exposure with a high concentration of Colcemid reduces the total yield of metaphases. The optimum strikes a balance of these parameters. There are some additional modifications that allow for the enrichment of long (prometaphase) chromosomes by using agents that prevent DNA condensation, such as actinomycin D, ethidium bromide, or BrDU. Cell synchronization techniques can also significantly increase the total yield of metaphase chromosomes.

Applications

Chromosomes are invaluable material for the evaluation of the genome integrity and its preservation at the microscopic chromosomal level. The areas of application

include prenatal diagnostics, genetic testing of multiple familial syndromes including cancer, positional cloning of the genes, and physical mapping (assignment of the genes on chromosomes and subchromosomal regions). The number and morphology of all 23 chromosome pairs in humans can be examined using G-banding differential staining of chromosomes obtained from a peripheral blood sample. Aberrations in the number of chromosomes or visible chromosomal alterations, such as translocations, deletions, and inversions involving extended regions, can be detected by this method. Advances such as spectral karyotyping (SKY) allow for better visualization of aneuploidy and translocations between different chromosomes.[61] Subtle rearrangements, such as submicroscopic deletions or cryptic translocations (an exchange of the small distal telomeric regions between the two nonhomologous chromosomes), can be visualized using specific probes in the FISH technique[62] (Figure 2-13).

Future Developments

Chromosome analysis will remain a powerful analytic tool in clinical and research fields for the foreseeable future. Possible strategies to improve existing methods include automatization and linearization of the genetic content by increasing resolution to visualize at the level of the chromosome, chromatin, DNA, and gene. Another possible direction of development is functional analysis of the genome using constitutional chromosomes and labeled expressed sequences from particular tissues mapped directly to their original positions on chromosomes.

MOLECULAR BASIS OF PEDIATRIC ENDOCRINOPATHIES

Since the 1990s, the application of recombinant DNA technology to the understanding of the pathogenesis of endocrinopathies has increased. Although this new approach to endocrinologic disorders has resulted in the delineation of new syndromes, its major impact has been in facilitating the diagnosis of these disorders. Genetic counseling including anticipatory surveillance, such as in multiple endocrine neoplasia (MEN) syndromes (Chapter 14), is also one of the areas of clinical pediatric endocrinology with major impact of this "new" knowledge. For example, it is becoming increasingly clear that phenotypically homogenous clinical syndromes may result from different genotypical abnormalities and that similar genetic abnormalities may have very different clinical manifestations. In contrast, therapeutic implications of such knowledge are still limited. Hence, the earlier hopes of spectacular gains from gene therapy have not been realized and significant problems need to be addressed before gene therapy becomes a reality in routine patient care. However, targeted pharmacotherapy exploiting knowledge gained regarding molecular mechanisms and pathogenesis has been successfully employed in the treatment of some diseases such as androgen insensitivity and thyroid cancer. This section demonstrates a couple of seminal examples of clinical endocrine disorders whose molecular basis has been elucidated. Details of the specific disorders are elaborated in the respective chapters of this book.

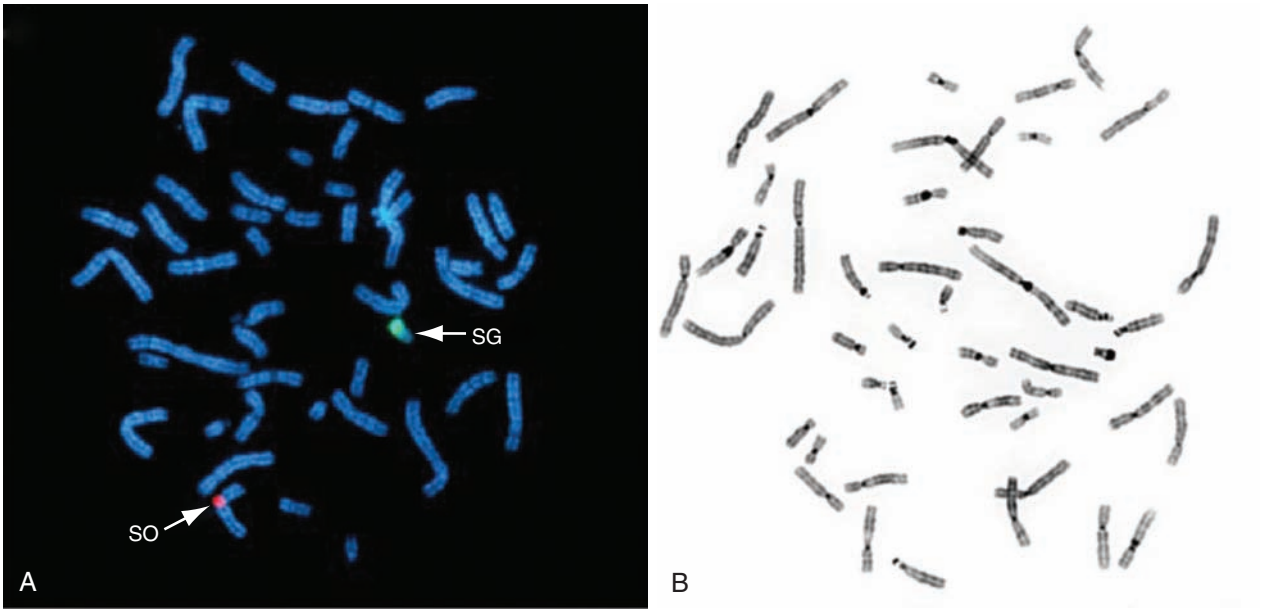

FIGURE 2-13 ■ Human metaphase chromosomes (A) after FISH using the chromosome X-specific centromeric probe labeled with Spectrum Orange (SO) and chromosome Y-specific heterochromatin labeled with Spectrum Green (SG); and (B) with the inverted DAPI banding (similar to G-banding) allowing chromosomal identification. *(This image can be viewed in full color online at ExpertConsult.)*

Defects in Peptide Hormones

Genomic Deletions Causing Human Endocrine Disease

One of the early discoveries regarding the molecular basis of endocrinopathies was the absence of the gene coding for a particular peptide hormone; the entire gene could be missing or only a part of the gene could be deleted. In either case, this resulted in the inability to synthesize a functional peptide so that the patient presents with clinical features indicative of deficiency of the hormone. A classical example of this type of endocrinopathy relevant to pediatric endocrinology is isolated growth hormone deficiency, type 1A (IGHD 1A).[63] In this syndrome the gene for human growth hormone-N (hGH-N) is deleted; disease results when both alleles of hGH-N are absent (indicative of an autosomal recessive inheritance). In the human there are two hGH genes (hGH-N and hGH-V) and both of them, along with the three placental lactogen (chorionic somatomammotropin) genes, are clustered along a 48-kilobase region of DNA. hGH-N is expressed in the pituitary gland and is the source of circulating GH in the human, whereas hGH-V is expressed only in the placenta and its biological function is not clear at this time. In autosomal recessive, type 1A isolated GH deficiency, although hGH-V is intact, the absence of the active gene, hGH-N, results in deficiency of circulating GH and the GH-deficient phenotype. In the original case description, the infants were of relatively normal size at birth but developed severe growth failure during the first year of life. A distinctive feature of these infants was the propensity to develop antibodies against GH in response to exogenously administered GH. This feature, although common, is not invariably present in patients with IGHD 1A. The state of the art method for screening for an hGH-N deletion is based on the PCR amplification of the highly homologous regions of DNA on the long arm of chromosome 17 that flank the *hGH-N* gene.[64,65] The presence of convenient restriction enzyme sites, such as SmaI, in the amplified DNA is exploited to ascertain the presence or absence of deletions in this region of the chromosome. Although this PCR-based approach is useful in most cases, it does not identify all cases with abnormalities in the *hGH-N* gene. In particular more rigorous methods are required for the identification of the less common causes of IGHD-1A such as point mutations. The study of the hGH-hPL gene cluster has also shed light on the function of prolactin, as several studies have identified subjects with hPL deletions. These deletions do not result in overt clinical abnormalities and specifically do not cause clinical effects in pregnant patients, suggesting that, despite high levels of hPL found during pregnancy, hPL serves no essential function in the human.[66]

Point Mutations

Peptide hormones act by binding to a specific receptor, which then results in the biological actions attributed to that particular hormone. The binding of the peptide hormone to its receptor, classically described as the lock and key mechanism, is a precise mechanism dependent on the complementary structures of the receptor and the site on the hormone that is involved in binding to the receptor. A change in the nucleotide sequence of the gene coding for a peptide hormone that results in altering an amino acid residue of the hormone can affect the function of the hormone if this change interferes with the hormone's ability to either bind or activate the receptor. Historically, point mutations resulting in aberrant protein production were well described with hemoglobin. This was possible because hemoglobin is present in abundant quantities in the blood, enabling purification and analysis of hemoglobin from patients suspected to have hemoglobinopathies. On the other hand, peptide hormones are present in infinitesimally lesser amounts in circulation, making their direct purification and analysis from blood a more daunting task. With the advent of recombinant DNA technology, it became feasible to clone and analyze the gene coding for the hormone directly, without having to resort to purification of the protein from blood or tissue. This approach has resulted in the identification of several clinical syndromes due to mutant hormones. One of the classical examples of this type of molecular pathology is noninsulin diabetes mellitus (NIDDM) due to mutant insulins.[67] A number of patients have been described with point mutations in the insulin gene. In general these patients present with hyperglycemia, hyperinsulinemia, and normal insulin sensitivity, a clinical picture that is attributed to the production of an abnormal insulin molecule with reduced biological activity. Thus, Insulin Chicago is characterized by a single nucleotide change, TTC to TTG, which results in the substitution of a leucine for phenylalanine residue at position 25 of the B chain (Phe-B25-Leu). Similarly, the other two well-characterized point mutations are characterized by the change of a single amino acid residue and result in the formation of Insulin Wakayama (Val-A3-Leu) and Insulin Los Angeles (Phe-B24-Ser). These mutations are located within the putative receptor-binding region of the insulin molecule, and the insulin molecules transcribed from these mutant genes are characterized by low binding potency ($< 5\%$ compared to normal) for the insulin receptor. A separate class of mutations in the insulin gene gives rise to the syndrome of hyperproinsulinemia with or without clinically significant carbohydrate intolerance. Thus, two mutations described involve substitution of a histidine for an arginine at position 65, and in the other case histidine at position 10 of the B chain is changed to aspartic acid. Although the original descriptions of these mutant insulins relied on the purification and analysis of the abnormal insulin molecule per se, the current availability of PCR-based methods to screen for these mutations has greatly simplified the laboratory diagnosis of this syndrome.

Another example of point mutations resulting in a phenotype that is especially relevant to pediatric endocrinologists is hypopituitarism secondary to abnormalities in transcription factors that orchestrate embryologic development of the anterior pituitary gland.[68,69] POU1F1 (also known as Pit1 or GHF-1) was the first transcription factor that was identified to play a specific role in

pituitary development. The *POU1F1/PIT-1* gene encodes a 291 amino acid POU homeodomain DNA binding nuclear protein that is present in somatotrophs, lactotrophs, and thyrotrophs. *POU1F1/PIT-1* is necessary for the normal development of these pituitary cell types. The first indication that abnormalities in *POU1F1/PIT-1* may result in a phenotypic change was derived from studies on strains of mice with genetic forms of dwarfism. The Snell and Jackson strains of dwarf mice are characterized by a deficiency of GH, prolactin, and thyroid-stimulating hormone (TSH). In 1990 Li and colleagues reported that Snell phenotype was caused by a missense mutation and that the Jackson phenotype was caused by a rearrangement in the *POU1F1/PIT-1* gene.[70] Since this landmark study, several recessive and dominant types of *POU1F1/PIT-1* abnormality have been recognized in sporadic cases and multiplex families with hypopituitarism.[71-73] *POU1F1/PIT-1* activates GH and prolactin gene expression, and can bind to and transactivate the TSH-B promoter. Accordingly, patients with *POU1F1/PIT-1* mutations demonstrate GH, prolactin, and variable TSH deficiencies.[71] It can be inherited in an autosomal dominant or recessive manner, but *POU1F1/PIT-1* mutations are not a common cause of combined pituitary hormone deficiencies.

HESX1 is a paired-like homeodomain transcription factor expressed in the developing pituitary gland. Mutations in this gene leading to decreased activity have been found in two siblings with panhypopituitarism, absent septum pellucidum, optic nerve hypoplasia, and agenesis of the corpus callosum, implicating a role for HESX1 in mediating forebrain development.[74,75] Another mutation in HESX1 was recently discovered in a patient with septo-optic dysplasia and isolated growth hormone deficiency.[76] Experimental evidence suggests that this particular mutation results in the production of an altered HESX1 protein with enhanced DNA binding activity that abrogates the transcriptional activity of PROP1, another transcription factor necessary for pituitary development.

PROP1 is thought to be involved in the differentiation of somatotropes, thyrotropes, lactotropes, and gonadotropes. Mutations in PROP1 leading to reduced DNA-binding and transcriptional activity have been identified in patients with combined pituitary hormone deficiency. These patients have a deficiency of TSH, GH, prolactin, luteinizing hormone (LH), and follicle-stimulating hormone (FSH).[77] Patients with a demonstrated gonadotropin deficiency may present with failure to enter puberty spontaneously, whereas other patients do enter puberty but have a subsequent loss of gonadotropin secretion. Although PROP1 is directly implicated in the differentiation of only four out of the five anterior pituitary cell types, some patients have also been described with ACTH deficiency. PROP1 mutations are believed to be relatively common (32% to 50%) genetic causes of combined pituitary hormone deficiency (PTH).[78]

Rathke's pouch initially forms but fails to grow in LHX3-knockout mice.[79] Humans have been found to have mutations in the *LHX3* gene, a LIM-type homeodomain protein, and demonstrate complete deficits of GH, prolactin (PRL), TSH, and gonadotropins in addition to a rigid cervical spine leading to limited head rotation. LHX4 is a related protein, which similar to LHX3 regulates proliferation and differentiation of pituitary lineages. A patient has been identified with a dominant mutation in this protein demonstrating deficiencies of GH, TSH, and ACTH, a small sella turcica, a hypoplastic anterior hypophysis, an ectopic posterior hypophysis, and a deformation of the cerebellar tonsil into a pointed configuration.[80]

Finally, Rieger syndrome is a condition with abnormal development of the anterior chamber of the eye, dental hypoplasia, and a protuberant umbilicus associated with growth hormone deficiency. All mutations in RIEG (Pitx2) found thus far have been heterozygous, with an autosomal dominant inheritance.[81]

Defects in Peptide Hormone Receptors

Molecular defects resulting in phenotypic abnormalities in humans have been described in a variety of peptide hormone receptors including GH, LH, FSH, TSH, ACTH, and insulin, and it is expected that this list will continue to expand in the future. Mutations in receptors for peptide hormone interfere in the actions of the hormone by altering the binding of the hormone, by altering the number of the receptors available for binding to the hormone, by interfering with the synthesis or intracellular processing of the receptor, or by disrupting the activation of the postreceptor signaling pathways. In general, mutations in the receptor result in decreased actions of the cognate hormone. However, mutations involving G-protein linked receptors are exceptions to this generalization and can result in a phenotype characterized by "overactivity" of the particular hormone system. Examples of these "gain of function" mutations include mutations in the LH receptor responsible for familial testotoxicosis[82] and mutations in the TSH receptor causing thyrotoxicosis[83] (refer to Chapter 7).

Insulin Receptor

Following the initial reports in 1988, a variety of mutations have been identified in the insulin receptor gene, with the majority of them being in patients with genetic syndromes associated with acanthosis nigricans and insulin resistance.[84] Patients with leprechaunism, a congenital syndrome characterized by extreme insulin resistance, fasting hypoglycemia, and intrauterine growth retardation, have two mutant alleles of the insulin receptor gene. Another syndrome associated with acanthosis nigricans and extreme insulin resistance, the Rabson-Mendenhall syndrome, has been linked to two different mutations within the insulin receptor gene existing in a compound heterozygous state. The syndrome of type A insulin resistance is a heterogeneous collection of conditions defined by the presence of insulin resistance, acanthosis nigricans, and hyperandrogenism in the absence of lipoatrophy or obesity. Molecular analysis of the insulin receptor gene has revealed that several of these patients have mutations in either one or both alleles of the insulin receptor gene. The initial expectation of mutations in the

insulin receptor providing the molecular basis for the common type of type 2 (NIDDM) diabetes mellitus has not been justified. Hence, no alterations in the insulin receptor gene were identified in a study of Pima Indians, an ethnic group with a greater than 50% incidence of type 2 diabetes mellitus. It is noteworthy, however, that studies have advanced the search for genetic variations that influence the propensity to develop type 2 diabetes mellitus.[85,86] Positional cloning-based analysis suggests that specific polymorphisms in the *CAPN10* gene are associated with type 2 diabetes mellitus in the Finnish and Mexican-American (Pima Indian) populations. Whether these genetic variations in the *CAPN10* gene, located on chromosome 2, which encodes a widely expressed calpain-like cysteine protease, are causal factors for type 2 diabetes mellitus or merely co-segregating markers remains to be established. However, current studies have not excluded the possibility that polymorphisms in the insulin receptor gene may confer a genetic predisposition for the precipitation of the development of NIDDM by obesity or hypertension.

GH Receptor

Genetic abnormalities in the GH receptor result in the primary form of the syndrome of GH insensitivity, also called Laron syndrome.[87] The human GH receptor gene, located on the proximal part of the short arm of chromosome 5, spans approximately 90 kilobases and includes nine exons, numbered 2 through 10, that encode the receptor protein, and additional exons in the 5′ untranslated region of the gene. The GH receptor protein contains an open reading frame of 638 amino acids, which predicts a 246 amino acid-long extracellular ligand-binding domain, a single membrane spanning domain and a cytoplasmic domain of 350 amino acids. In the human, the extracellular portion of the receptor exists in circulation as the GH-binding protein (GHBP). Exon 2 encodes a signal sequence, exons 3 through 7 the extracellular GH-binding domain, exon 8 the transmembrane domain, and exons 9 and 10 the cytoplasmic domain and the 3′ untranslated region. The mutations that have been described in the GH receptor gene include large deletions, nonsense mutations, splice mutations, and frameshift mutations.[88] The diagnosis of growth hormone insensitivity resulting from mutations in the growth hormone receptor gene is considered when patients demonstrate elevated GH levels and low IGF-1 levels. Because the growth hormone binding protein (GHBP) is the cleaved extracellular portion of the growth hormone receptor, patients with mutations in the growth hormone receptor that result in decreased synthesis of the receptor protein can demonstrate low GHBP levels in circulation. However, mutations in the growth hormone receptor gene that selectively involve the transmembrane or intracellular domains may demonstrate normal or even enhanced circulating levels of GHBP. For example, a patient with a mutation inhibiting dimerization of the receptor had normal GHBP levels because the receptor had a normal GH-binding site.[89] Another set of affected individuals had a mutation of the transmembrane domain of the receptor, leading to a truncated growth hormone receptor product that is postulated to be more easily released from the cell membrane and elevated GHBP levels were noted.[90]

PRINCIPLES OF INTERPRETATION OF GENETIC TESTS IN THE DIAGNOSIS AND MANAGEMENT OF PEDIATRIC ENDOCRINE DISEASES

In the past, genetic tests were usually carried out by research laboratories that had a specific interest in the disease/syndrome under investigation. Under this scenario the medical provider could generally rely on the expertise of the research laboratory to help with the identification of the appropriate test/test panel and interpretation of the results of the tests. However, with the increasing use of commercial laboratories for these tests, these responsibilities are being transferred to the medical providers. Hence, it is now essential for medical providers to become familiar with issues such as choosing of the appropriate test, the potential usefulness of the information provided by the test including false-positive and false-negative results, available preventive or treatment options, and social and behavioral issues related to genetic testing. The following are some of the points that should be taken into account when ordering or interpreting a genetic test for a pediatric endocrine disorder:

Limitations of commonly used PCR-based assays. In general routine PCR-based assays (whether they involve electrophoresis or DNA sequencing of the product) cannot reliably differentiate between the two alleles of an individual gene. Hence, in a given instance the detection of a mutation in an autosomal recessive disease could be either due to the mutation being present in only one of the alleles (and thus not likely to have clinical manifestation) or due to the mutation being present in a single allele with the other allele being absent (e.g., due to gene deletion) in which case the mutation in the one allele present would clinically manifest. In a similar manner the inability to amplify a loss-of-function mutant allele by PCR could either be due to the presence of two normal alleles (hence excluding the genetic defect) or because of the absence of both the alleles (due to gene deletion) in which case the genetic defect would be symptomatic. In many cases the latter scenario can be excluded by testing using alternative techniques such as Southern blotting.

Germline versus somatic mutation. Germline mutations are present in every cell descended from the zygote to which that mutant gamete (ova/sperm) contributed. In contrast, somatic mutations occur in a somatic cell (e.g., liver or bone marrow or skin) and hence are not present in other cell types in the body. Most common examples of inherited mutations are germline mutations. Examples of somatic mutations causing endocrine disorders are more rare—for example, in McCune-Albright syndrome the mutation in the GNAS gene may only be detectable in the skin (café au lait) or the bone (fibrous dysplasia) lesion.

Disorders of imprinting. In many instances, the genetic basis of the endocrine disorder is not due to a mutation but

rather is due to an abnormality in genomic imprinting. Genomic imprinting is the modification of gene expression dependent on whether the genetic material is inherited from the mother or the father. Classic examples are Prader-Willi syndrome (chromosome 15q12 deletion inherited from father) and Angelman syndrome (chromosome 15q12 deletion inherited from mother). Other examples of endocrine disorders in which imprinting is implicated are pseudohypoparathyroidism and Albright hereditary osteodystrophy, Russell-Silver syndrome, Beckwith-Wiedemann syndrome, the focal form of persistent hyperinsulinemic hypoglycemia of infancy (PHHI), and transient neonatal diabetes.

Penetrance and expressivity. Penetrance is defined as the percentage of people who have the gene and who develop the cognate phenotype. Expressivity is the extent to which a gene is expressed in one person. For example, when a gene has 50% expressivity, only half the features are present or the disease severity is only half of what can occur with full expression. The variable penetrance of many of the neoplastic components of the MEN syndrome is an example of this phenomenon.

One gene, multiple diseases. Examples of mutations in the same gene causing different disease (e.g., lamin A/C gene [LMNA] mutation causing Emery-Dreifuss muscular dystrophy, Hutchinson-Gilford Progeria, Charcot-Marie-Tooth type 2, familial partial lipodystrophy syndrome, and dilated cardiomyopathy). In many such instances, the variability can be attributed to specific mutation(s) for each of the different clinical manifestations or sets of mutations within different domains/regions of the gene. A similar example is mutations in the RET oncogene implicated in MEN2 syndromes, nonsyndromic paraganglioma, and in Hirschsprung disease.

One disease, multiple genes (phenocopy). Phenocopy refers to the development of disease manifestations that are usually associated with mutations of a particular gene but instead are due to another gene/etiology. Such a scenario could confound clinical diagnosis and management of a suspected hereditary endocrine disorder. For example, it has been reported that MEN1 syndrome which is usually caused by mutation in the *MEN1* gene can be mimicked by familial hypocalciuric hypercalcemia (FHH) due to an inactivating mutation in the calcium-sensing receptor, and the hyperparathyroidism-jaw tumor (HPT-JT) syndrome due to a mutation in the gene responsible for hyperparathyroidism type 2 (HRPT2).

RECOMBINANT DNA TECHNOLOGY AND THERAPY OF PEDIATRIC ENDOCRINE DISEASES

From a therapeutic point of view, recombinant DNA technology can be exploited to either tailor pharmacotherapy according to the genotype of a patient (i.e., targeted pharmacotherapy), manipulate genes within the human body (gene therapy), or engineer prokaryotic or eukaryotic cells to produce proteins such as hormones, which can then be administered for therapy or diagnosis. Whereas targeted pharmacotherapy and gene therapy are mostly restricted to the research arena, the use of hormones produced by recombinant DNA technology is well established in clinical endocrinology. Historically, insulin was the first hormone synthesized by recombinant DNA technology to be approved for clinical use.[91,92] At present, a variety of recombinant hormones including GH, LH, FSH, TSH, PTH, and erythropoietin are being used clinically or are in advanced stages of clinical trials.

On a theoretic basis it should be possible to synthesize any protein hormone whose gene has been cloned and DNA sequence determined. Thus, recombinant DNA technology makes it possible to insert the gene coding for a particular protein hormone into a host cell such that the protein is produced by the host cell's protein-synthesizing machinery. The synthesized protein is then separated from the rest of the host cell proteins to obtain the pure form of the hormone of interest. Both prokaryotic and eukaryotic cells can serve as the host cell for the production of proteins by this technology. Because post-translational modifications such as glycosylation may be essential for the optimal action of a protein hormone, the choice of the specific cell system utilized for the production of a particular protein hormone is critical. Prokaryotic cell systems such as *Escherichia coli* are suitable for the production of protein hormones that do not need posttranslational modifications, such as GH.[93] Eukaryotic cell systems such as Chinese hamster ovary (CHO) cells that are capable of post-translational modification of the protein are useful for the production of hormones such as TSH that require glycosylation for optimal bioefficacy.[94] In addition, eukaryotic cells are capable of synthesizing proteins that undergo the appropriate folding, a step that is not carried out by prokaryotic cells. The advantages of the use of recombinant DNA for the production of these proteins include the possibility of a limitless supply of a highly pure form of a protein and the absence of the risk of contamination with biological pathogens associated with the extraction of proteins from human or animal tissue. In addition, this technology permits the development of hormone analog and antagonists with much greater ease than conventional protein synthesis protocols.

The influence of genetic factors on the metabolism of various drugs is a well-established phenomenon with the effect of various iso-enzymes of cytochrome p450 on the circulating half-life of drugs such as anticonvulsants being a classic example of this interaction. Another example of the role of genotype on the choice of pharmacotherapy is the phenomenon of drug-induced hemolytic anemia in patients with G6PD deficiency. The genomic revolution has allowed for the exploitation of computational approaches to identify polymorphisms of known genes encoding proteins with different functional characteristics. These single nucleotide variations (also called single nucleotide polymorphisms, or SNPs) occur with varying frequencies in different ethnic populations and are the focus of intense scrutiny at this particular time. The promise that these SNPs hold out is that analysis of these SNPs for a given gene will allow the investigator to predict the response of the particular individual to a class of drugs/chemicals. This pharmacogenomic approach to

clinical therapeutics has already been successful in demonstrating an association between specific polymorphisms in the β-adrenergic receptor and response to β-agonists in patients with bronchial asthma, and polymorphisms in hydroxytryptamine receptors and response to neuroleptic drugs. The widespread application of the tools of molecular biology to unravel the molecular basis of action of hormones has also yielded benefits by allowing for customization of the pharmacotherapy of endocrine diseases and syndromes based on the specific individual genetic defect. One such example is the report of directed pharmacologic therapy of an infant with ambiguous genitalia resulting from a mutation in the androgen receptor.[95] In this infant with a M807T mutation in the androgen receptor, in vitro functional studies had indicated that the mutant receptor exhibited loss of binding capacity for testosterone with retention of binding for dihydrotestosterone. Furthermore, this differential binding was also reflected in the better preservation of the transactivation potential of dihydrotestosterone (DHT) compared to testosterone. These in vitro findings were exploited to treat the infant with DHT, resulting in restoration of male genital development. This case illustrates that in selected cases, in vitro functional assays can help identify subsets of patients with ambiguous genitalia and androgen insensitivity who would respond to targeted androgen therapy. It can be anticipated that in the coming years, more examples of such innovative therapeutic strategies and "customized" hormonal treatment protocols will become routine and implemented in the practice of clinical pediatric endocrinology.

CONCLUDING REMARKS

The application of recombinant DNA technology has resulted in a tremendous increase in our understanding of physiologic processes and pathologic conditions. Achievements such as the full sequence of the human genome in normal subjects[49] have resulted in a paradigm shift in the way that we think about genetic causes and predisposition to disease[58] and in the analysis of the function of hormones and related proteins.[96] In the traditional paradigm, investigators seeking to discover new genes or to analyze the function of known proteins needed to devote a significant part of their time to conducting "bench research" in "wet laboratories." The new approach in this "postgenomic" era takes advantage of the unprecedented power of computational biology to "mine" nucleotide, protein sequence, and other related databases. In the future, most researchers will deal with abstract models and data sets stored in computer databases. Hence, initial discoveries of novel genes or novel interactions between known proteins or intracellular signaling pathways could be made using the analytic power of computational software tools (functional genomics); these initial insights can then be verified and expanded upon by traditional laboratory bench methods. The advantages of this new paradigm are obvious with computational approaches taking a significantly shorter time, with less demand on manpower, and can easily expand the scope of the search to include multiple molecules and organisms (phylogenetic profiling). Several public-domain web-accessible databases are currently serving as the major repositories for this information. GenBank is the major repository for sequence information and is currently supported by the National Institutes of Health. One of the main sources of the physical location, clinical features, inheritance patterns, and other related information of specific gene defects is the Online Mendelian Inheritance in Man (OMIM) operated by Johns Hopkins University in Baltimore, Maryland. Johns Hopkins University also operates the online Genome Data Base (GDB), which allows scientists to identify polymorphisms and identify contacts for gene probes and other related research tools. The ever-expanding number of endocrine (and other) disorders that can be attributed to changes in the nucleotide sequence of specific genes has also increased the necessity for the availability of accurate, reliable, and timely genetic tests such as mutation detection. One source for such information is a collaborative website (www.genetests.org) that maintains an up-to-date catalog of commercially available and research-based tests for inherited disorders.

With the ubiquitous use of these powerful tools in laboratories around the world, genes are being cloned and genetic diseases are being mapped at a rapid pace. In all of these exciting developments, one still needs to keep in mind that whereas this "new" science has allowed for hitherto inaccessible areas of human biology to be probed and studied, a lot remains to be understood with respect to individual disease processes. Hence, at the present time, we have only a rudimentary understanding of the correlations between phenotype and genotype in many of the common genetic diseases such as congenital adrenal hyperplasia. These lacunae in our knowledge dictate that clinicians should be cautious about basing therapeutic decisions solely on the basis of molecular and genetic studies. This is especially true in the area of prenatal diagnosis and recommendation for termination of pregnancy based on genetic analysis. As we improve our understanding of the molecular and genetic basis of disease and translate this knowledge into gains at the bedside, it behooves us, both as individuals and as a society, to be cognizant of critical issues relating to the privacy of health data and to remain vigilant against misuse by inappropriate disclosure of this powerful knowledge.

REFERENCES

1. Weintraub, B. D. (1994). *Molecular endocrinology: basic concepts and clinical correlations.* New York: Raven Press.
2. Shupnik, M. (2000). *Gene engineering in endocrinology.* Totowa, NJ: Humana Press.
3. Alberts, B., Johnson, A., Lewis, J., et al. (2002). *Molecular biology of the cell.* New York: Garland Science.
4. Lodish, H., Scott, M., Matsudaira, P., et al. (2003). *Molecular cell biology* (5th ed.). New York: W. H. Freeman.
5. Roberts, R. (1989). Restriction enzymes and their isoschizomers. *Nucleic Acids Res, 17*(suppl), R347.
6. Nathans, D., & Smith, H. (1975). Restriction endonucleases in the analysis and restructuring of DNA molecules. *Annu Rev Biochem, 44*, 273–293.
7. Sambrook, J., & Russell, D. (2001). *Molecular cloning: a laboratory manual* (3rd ed.). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

8. Southern, E. (1975). Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J Mol Biol*, *98*, 503–517.
9. Innis, M., Gelfand, D., & Sninsky, J. (1999). *PCR applications: protocols for functional genomics*. San Diego, CA: Academic Press.
10. Innis, M., Gelfand, D., Sninsky, J., & White T. (1990). *PCR protocols: a guide to methods and applications*. San Diego, CA: Academic Press.
11. Kocova, M., Siegel, S., Wenger, S., Lee, P., & Trucco, M. (1993). Detection of Y chromosome sequence in Turner's Syndrome by Southern blot analysis of amplified DNA. *Lancet*, *342*, 140–143.
12. Lehrach, H., Diamond, D., Wozney, J., & Boedtker, H. (1977). RNA molecular weight determinations by gel electrophoresis under denaturing conditions. *Biochemistry*, *16*, 4743–4751.
13. Thomas, P. (1980). Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. *Proc Natl Acad Sci USA*, *77*, 5201–5205.
14. Rudert, W., Kocova, M., Rao, A., & Trucco, M. (1994). Fine quantitation by competitive PCR of circulating donor cells in posttransplant chimeric recipients. *Transplantation*, *58*, 964–965.
15. Gilliland, G., Perrin, S., Blanchard, K., & Bunn, H. (1990). Analysis of cytokine mRNA and DNA: detection and quantitation by competitive polymerase chain reaction. *Proc Natl Acad Sci USA*, *87*, 2725–2729.
16. Biosystems PA (1997). User Bulletin 2: ABI Prism 7700 Sequence Detection System, Relative Quantitation of Gene Expression: PE Applied Biosystems.
17. Biosystems PA (1998). User Bulletin 5: ABI Prism 7700 Sequence Detection System, Multiplex PCR with Taqman Probes: PE Applied Biosystems.
18. Yan, H., Kinzler, K., & Vogelstein, B. (2000). Genetic testing: present and future. *Science*, *289*, 1890–1892.
19. Maxam, A., & Gilbert, W. (1980). Sequencing end-labeled DNA with base-specific chemical cleavages. *Methods Enzymol*, *65*, 499–560.
20. Sanger, F., Nicklen, S., & Coulson, A. (1977). DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci USA*, *74*, 5463–5467.
21. Choi, M., Scholl, U. I., Yue, P., et al. (2011). K+ channel mutations in adrenal aldosterone-producing adenomas and hereditary hypertension. *Science*, *331*, 768–772.
22. Ahmadian, A., Ehn, M., & Hober, S. (2006). Pyrosequencing: history, biochemistry and future. *Clin Chim Acta*, *363*, 83–94.
23. Myers, R., Maniatus, T., & Lerman, L. (1985). Detection and localization of single base changes by denaturing gradient gel electrophoresis. In R.Wu (Ed.), *Methods in enzymology*. San Diego, CA: Academic Press.
24. Abrams, E., Murdaugh, S., & Lerman, L. (1990). Comprehensive detection of single base changes in human genomic DNA using denaturing gradient gel electrophoresis and a GC clamp. *Genomics*, *7*, 463–475.
25. Gejman, P., & Weinstein, L. (1994). *Detection of mutations and polymorphisms of the Gs alpha subunit gene by denaturing gradient gel electrophoresis*. Orlando, FL: Academic Press.
26. Myers, R., Fischer, S., Maniatus, T., & Lerman, L. (1985). Modification of the melting properties of duplex DNA by attachment of a GC-rich DNA sequence as determined by denaturing gradient gel electrophoresis. *Nucl Acids Res*, *13*, 3111–3129.
27. Myers, R., Fischer, S., Lerman, L., & Maniatus, T. (1985). Nearly all single base substitutions in DNA fragments joined to a GC-clamp can be detected by denaturing gradient gel electrophoresis. *Nucl Acids Res*, *13*, 3145.
28. Sheffield, V., Cox, D., Lerman, L., & Myers, R. (1989). Attachment of a 40-base pair G + C rich sequence (GC clamp) to genomic DNA fragments by the polymerase chain reaction results in improved detection of single-base changes. *Proc Natl Acad Sci USA*, *86*, 232–236.
29. Nagamine, C., Chan, K., & Lau, Y.-F. (1989). A PCR artifact: generation of heteroduplexes. *Am J Hum Genet*, *45*, 337–339.
30. White, M., Carvalho, M., Derse, D., et al. (1992). Detecting single base substitutions as heteroduplex polymorphisms. *Genomics*, *12*, 301–306.
31. Keen, J., Lester, D., Inglehearn, C., et al. (1991). Rapid detection of single base mismatches as heteroduplexes on Hydrolink gels. *Trends Genet*, *7*, 5.
32. Rudert, W., & Trucco, M. (1992). Rapid detection of sequence variations using polymers of specific oligonucleotides. *Nucl Acids Res*, *20*, 1146.
33. Rudert, W., & Trucco, M. (1990). DNA polymers of protein binding sequences generated by PCR. *Nucl Acids Res*, *18*, 6460.
34. Yan, H., Papadopoulos, N., Marra, G., et al. (2000). Conversion of diploidy to haploidy. *Nature*, *403*, 723–724.
35. Collins, F. (1992). Positional cloning: let's not call it reverse anymore. *Nat Genet*, *1*, 3–6.
36. Collins, F. (1995). Positional cloning moves from perditional to traditional. *Nat Genet*, *9*, 347–350.
37. Borsani, G., Ballabio, A., & Banfi, S. (1998). A practical guide to orient yourself in the labyrinth of genome databases. *Hum Mol Genet*, *7*, 1641–1648.
38. Khoury, M., Beaty, T., & Cohen, B. (1993). *Fundamentals of genetic epidemiology*. New York: Oxford University Press.
39. Terwillinger, J., & Ott, J. (1994). *Handbook of human genetic linkage*. Baltimore: The Johns Hopkins University Press.
40. 2007 Online Mendelian Inheritance in Man, OMIM. (2007). In National Library of Medicine: McKusick-Nathans Institute for Genetic Medicine, Johns Hopkins University (Baltimore, MD) and National Center for Biotechnology Information, National Library of Medicine (Bethesda, MD), Center for Medical Genetics, Johns Hopkins University and National Center for Biotechnology Information.
41. Bishop, M. (1997). Gene mapping and isolation: access to databases. *Methods Mol Biol*, *68*, 237–259.
42. Linehan, W., Lerman, M., & Zbar, B. (1995). Identification of the von Hippel-Lindau (VHL) gene. *JAMA*, *273*, 564–570.
43. Chandrasekharappa, S., Guru, S., Manickam, P., et al. (1997). Positional cloning of the gene for multiple endocrine neoplasia-type 1. *Science*, *276*, 404–407.
44. Liaw, D., Marsh, D., Li, J., et al. (1997). Germline mutations of the PTEN gene in Cowden disease, an inherited breast and thyroid cancer syndrome. *Nat Genet*, *16*, 64–67.
45. Hemminki, A., Markie, D., Tomlinson, I., et al. (1998). A serine/threonine kinase gene defective in Peutz-Jeghers syndrome. *Nature*, *391*, 184–187.
46. Jenne, D., Reimann, H., Nezu, J., et al. (1998). Peutz-Jeghers syndrome is caused by mutations in a novel serine threonine kinase. *Nat Genet*, *18*, 38–43.
47. Kirschner, L., Carney, J., Pack, S., et al. (2000). Mutations of the gene encoding the protein kinase A type I-alpha regulatory subunit in patients with the Carney complex. *Nat Genet*, *26*, 89–92.
48. Ng, S. B., Nickerson, D. A., Bamshad, M. J., & Shendure, J. (2010). Massively parallel sequencing and rare disease. *Hum Mol Genet*, *19*, R119–R124.
49. MacArthur, D. G., Balasubramanian, S., Frankish, A., et al. (2012). A systematic survey of loss-of-function variants in human protein-coding genes. *Science*, *335*, 823–828.
50. Gundry, M., & Vijg, J. (2012). Direct mutation analysis by high-throughput sequencing: from germline to low-abundant, somatic variants. *Mutat Res*, *729*, 1–15.
51. Gullapalli, R. R., Desai, K.V., Santana-Santos, L., et al. (2012). Next generation sequencing in clinical medicine: challenges and lessons for pathology and biomedical informatics. *J Pathol Inform*, *3*, 40. doi: 10.4103/2153-3539.103013.
52. Turan, S., Hughes, C., Atay, Z., et al. (2012). An atypical case of familial glucocorticoid deficiency without pigmentation caused by coexistent homozygous mutations in MC2R (T152K) and MC1R (R160W). *J Clin Endocrinol Metab*, *97*, E771–E774.
53. Kendereski, A., Mici, D., Sumarac, M., et al. (1999). White Addison's disease: what is the possible cause? *J Endocrinol Invest*, *22*, 395–400.
54. Millington, G. W. (2006). Proopiomelanocortin (POMC): the cutaneous roles of its melanocortin products and receptors. *Clin Exp Dermatol*, *31*, 407–412.
55. Rees, J. L., & Harding, R. M. (2012). Understanding the evolution of human pigmentation: recent contributions from population genetics. *J Invest Dermatol*, *132*, 846–853.
56. Clark, A. J., Chan, L. F., Chung, T. T., & Metherell, L. A. (2009). The genetics of familial glucocorticoid deficiency. *Best Pract Res Clin Endocrinol Metab*, *23*, 159–165.
57. Brooks, B. P., Kleta, R., Stuart, C., et al. (2005). Genotypic heterogeneity and clinical phenotype in triple A syndrome: a review of the NIH experience 2000-2005. *Clin Genet*, *68*, 215–221.
58. Stratakis, C. A. (2012). Patients can have as many gene variants as they damn well please: why contemporary genetics presents us daily with a version of Hickam's dictum. *J Clin Endocrinol Metab*, *97*, E802–E804.

59. Caspersson, T., Zech, L., & Johansson, C. (1970). Differential binding of alkylating fluorochromes in human chromosomes. *Exp Cell Res*, *60*, 315–319.
60. Yunis, J. (1976). High resolution of human chromosomes. *Science*, *191*, 1268–1270.
61. Schrock, E., du Manoir, S., Veldman, T., et al. (1996). Multicolor spectral karyotyping of human chromosomes. *Science*, *273*, 494–497.
62. Cremer, T., Lichter, P., Borden, J., et al. (1988). Detection of chromosome aberrations in metaphase and interphase tumor cells by in situ hybridization using chromosome specific library probes. *Hum Genet*, *80*, 235–246.
63. Phillips, J., Hjelle, B., Seeburg, P., & Zachmann, M. (1981). Molecular basis for familial isolated growth hormone deficiency. *Proc Natl Acad Sci USA*, *78*, 6372–6375.
64. Vnencak-Jones, C., Phillips, J., & De Fen, W. (1990). Use of polymerase chain reaction in detection of growth hormone gene deletions. *J Clin Endocrinol Metab*, *70*, 1550–1553.
65. Kamijo, T., & Phillips, J. (1992). Detection of molecular heterogeneity in GH-1 gene deletions by analysis of polymerase chain reaction amplification products. *J Clin Endocrinol Metab*, *74*, 786–789.
66. Hill, D. (1992). What is the role of growth hormone and related peptides in implantation and the development of the embryo and fetus? *Horm Res*, *38*, 28–34.
67. Steiner, D., Tager, H., Chan, S., et al. (1990). Lessons learned from molecular biology of insulin-gene mutations. *Diabetes Care*, *13*, 600–609.
68. Rosenfeld, M., Briata, P., Dasen, J., et al. (2000). Multistep signaling and transcriptional requirements for pituitary organogenesis in vivo. *Recent Prog Horm Res*, *55*, 1–13, discussion 13-14.
69. Cushman, L., & Camper, S. (2001). Molecular basis of pituitary dysfunction in mouse and human. *Mamm Genome*, *12*, 485–494.
70. Li, S., Crenshaw, E., Rawson, E., et al. (1990). Dwarf locus mutants lacking three pituitary cell types result from mutations in the POU-domain gene Pit-1. *Nature*, *347*, 528–533.
71. Hendriks-Stegeman, B., Augustijn, K., Bakker, B., et al. (2001). Combined pituitary hormone deficiency caused by compound heterozygosity for two novel mutations in the POU domain of the Pit1/POU1F1 gene. *J Clin Endocrinol Metab*, *86*, 1545–1550.
72. Drouin, J. (2006). Molecular mechanisms of pituitary differentiation and regulation: implications for hormone deficiencies and hormone resistance syndromes. *Front Horm Res*, *35*, 74–87.
73. Quentien, M., Barlier, A., Franc, J., et al. (2006). Pituitary transcription factors: from congenital deficiencies to gene therapy. *J Neuroendocrinol*, *18*, 633–642.
74. Dattani, M., Martinez-Barbera, J., Thomas, P., et al. (1998). Mutations in the homeobox gene HESX1/Hesx1 associated with septo-optic dysplasia in human and mouse. *Nat Genet*, *19*, 125–133.
75. Thomas, P., Dattani, M., Brickman, J., et al. (2001). Heterozygous HESX1 mutations associated with isolated congenital pituitary hypoplasia and septo-optic dysplasia. *Hum Mol Genet*, *10*, 39–45.
76. Cohen, R., Cohen, L., Botero, D., et al. (2003). Enhanced repression by HESX1 as a cause of hypopituitarism and septooptic dysplasia. *J Clin Endocrinol Metab*, *88*, 4832–4839.
77. Wu, W., Cogan, J., Pfaffle, R., et al. (1998). Mutations in PROP1 cause familial combined pituitary hormone deficiency. *Nat Genet*, 18, 147–149.
78. Deladoey, J., Fluck, C., Buyukgebiz, A., et al. (1999). "Hot spot" in the PROP1 gene responsible for combined pituitary hormone deficiency. *J Clin Endocrinol Metab*, *84*, 1645–1650.
79. Zhao, Y., Morales, D., Hermesz, E., et al. (2006). Reduced expression of the LIM-homeobox gene Lhx3 impairs growth and differentiation of Rathke's pouch and increases cell apoptosis during mouse pituitary development. *Mech Dev*, *123*, 605–613.
80. Machinis, K., Pantel, J., Netchine, I., et al. (2001). Syndromic short stature in patients with a germline mutation in the LIM homeobox LHX4. *Am J Hum Genet*, *69*, 961–968.
81. Sadeghi-Nejad, A., & Senior, B. (1974). Autosomal dominant transmission of isolated growth hormone deficiency in iris-dental dysplasia (Rieger's syndrome). *J Pediatr*, *85*, 644–648.
82. Shenker, A., Laue, L., Kosugi, S., et al. (1993). A constitutively activating mutation of the lutenizing hormone receptor in familial male precocious puberty. *Nature*, *365*, 652–654.
83. Duprez, L., Parma, J., Van, Sande, J., et al. (1999). Pathology of the TSH receptor. *J Pediatr Endocrinol Metab*, *12*, 295–302.
84. Taylor, S., & Arioglu, E. (1998). Syndromes associated with insulin resistance and acanthosis nigricans. *J Basic Clin Physiol Pharmacol*, *9*, 419–439.
85. MT M. (2005). Genetics of type 2 diabetes mellitus. *Diabetes Res Clin Pract*, *68*, S10–S21.
86. Mercado, M., McLenithan, J., Silver, K., & Shuldiner, A. (2002). Genetics of insulin resistance. *Curr Diab Rep*, *2*, 83–95.
87. Laron, Z., Pertzelan, A., & Mannheimer, S. (1966). Genetic pituitary dwarfism with high serum concentrations of growth hormone: a new inborn error of metabolism. *Isr J Med Sci*, *4*, 883–894.
88. Rosenbloom, A. (1999). Growth hormone insensitivity: physiologic and genetic basis, phenotype, and treatment. *J Pediatr Endocrinol Metab*, *135*, 280–289.
89. Duquesnoy, P., Sobrier, M., Duriez, B., et al. (1994). A single amino acid substitution in the exoplasmic domain of the human growth hormone (GH) receptor confers familial GH resistance (Laron syndrome) with positive GH-binding activity by abolishing receptor homodimerization. *Embo J*, *13*, 1386–1395.
90. Woods, K., Fraser, N., Postel-Vinay, M., et al. (1996). A homozygous splice site mutation affecting the intracellular domain of the growth hormone (GH) receptor resulting in Laron syndrome with elevated GH-binding protein. *J Clin Endocrinol Metab*, *81*, 1686–1690.
91. Goeddel, D., Kleid, D., Bolivar, F., et al. (1979). Expression in Escherichia coli of chemically synthesized genes for human insulin. *Proc Natl Acad Sci USA*, *76*, 106–110.
92. Riggs, A. (1981). Bacterial production of human insulin. *Diabetes Care*, *4*, 64–68.
93. Goeddel, D., Heyneker, H., Hozumi, T., et al. (1979). Direct expression in *Escherichia coli* of a DNA sequence coding for human growth hormone. *Nature*, *281*, 544–548.
94. Gesundheit, N., & Weintraub, B. (1986). Mechanisms and regulation of TSH glycosylation. *Adv Exp Med Biol*, *205*, 87–105.
95. Ong, Y., Wong, H., Adaikan, G., & Yong, E. (1999). Directed pharmacological therapy of ambiguous genitalia due to an androgen receptor gene mutation. *Lancet*, *354*, 1444–1445.
96. Hsu, S., & Hsueh, A. (2000). Discovering new hormones, receptors, and signaling mediators in the genomic era. *Mol Endocrinol*, *14*, 594–604.

QUESTIONS

1. Which of the following is the correct statement with regard to miRNA?
   a. A single miRNA can target only one RNA.
   b. A single mRNA target can be recognized by only one miRNA.
   c. miRNAs act by binding to complementary sequences on the 5′ untranslated region of target mRNAs.
   d. One of the mechanisms by which miRNAs exert biological effect is by enhancing degradation of the target mRNA.
   e. Positive regulation of gene expression is the most common biological effect of miRNAs.

Correct answer: d

2. You are evaluating an infant who is suspected of having isolated growth hormone deficiency, type 1A (IGHD 1A). Which of the following statements is correct with regard to IGHD 1A?
   a. The gene that is deleted is the hGH-V gene expressed in the pituitary gland and is the source of circulating GH in the human.
   b. The classical inheritance pattern of this condition is autosomal dominant.
   c. These infants are usually small at birth.
   d. These infants have a propensity to develop antibodies against GH in response to exogenously administered GH.
   e. Measurement of random GH levels is not useful to make a diagnosis of GH deficiency in the newborn period.

Correct answer: d

3. Which of the following statements is correct with regard to commonly used PCR-based assays?
   a. In general, PCR-based assays can routinely differentiate between the two alleles of an individual gene.
   b. The inability to amplify a loss-of-function mutant allele by PCR is diagnostic of a gene deletion.
   c. DNA contamination is not a significant concern with PCR-based assays.
   d. The PCR technique can be used to directly amplify products of protein degradation.
   e. PCR-based assays use the enzyme Taq polymerase, a heat-stable enzyme originally isolated from the bacterium *Thermophilus aquaticus*.

Correct answer: e

4. Prader-Willi syndrome is an example of a disorder of imprinting. Which of the following statements is true about genomic imprinting?
   a. Genomic imprinting is caused by a mutation in genomic DNA.
   b. Genomic imprinting is more relevant for diseases inherited from the mother.
   c. Genomic imprinting is more likely to occur in the firstborn child.
   d. Genomic imprinting is almost always caused by endocrine disruptors.
   e. The focal form of persistent hyperinsulinemic hypoglycemia of infancy (PHHI) is an example of a disorder of imprinting.

Correct answer: e

5. Positional cloning (or "reverse" genetics) resulted in the identification of the genetic basis of several endocrine disorders. Which of the following statements is true about positional cloning?
   a. The positional cloning strategy requires at least a partial knowledge of the genetic basis of the disease under investigation.
   b. The positional cloning strategy is useful for identifying the genetic basis of diseases without any obvious candidate gene(s).
   c. Positional cloning can only be applied to diseases with an autosomal dominant inheritance pattern.
   d. Informative recombination interferes with positional cloning.
   e. The Human Genome Project (HGP) did not facilitate the strategy of positional cloning.

Correct answer: b

CHAPTER 3

Receptor Transduction Pathways Mediating Hormone Action

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CHAPTER OUTLINE

INTRODUCTION

Hormones exert their actions by binding to specific receptor proteins, a process that induces conformational changes or compartmental redistribution of these proteins. The activated receptor is now capable of inducing positive (or negative) intracellular effects that ultimately are recognized as a physiologic response. The specificity of hormone action is determined by the affinity of hormones for different receptors, the cell-specific expression

TABLE 3-1 **Major Types of Hormone Receptors**

Receptor Class	Hormone Receptors
G protein–coupled receptors	ACTH and other melanocortins, V2 vasopressin, LH, FSH, TSH, GnRH, TRH, GHRH, corticotropin releasing factor, somatostatin, glucagon, oxytocin, gastric inhibitory peptide, type 1 PTH, free fatty acid, GPR54, orexin, ghrelin, melanin-concentrating, calcitonin, glucagon-like peptide-1, and calcium-sensing receptors
Type 1 cytokine receptors	Growth hormone, prolactin, and leptin receptors
Receptor tyrosine kinases	Insulin, IGF-1, and fibroblast growth factor receptors
Nuclear receptors	Thyroid hormone, vitamin D_3, PPARγ, HNF-4α, glucocorticoid, androgen, estrogen, mineralocorticoid, and DAX1 receptors

of the receptor, and the unique responses induced by ligand occupancy.

Since the early 2000s, our understanding of hormone action has advanced rapidly with the success of genomics and advanced molecular biologic techniques. This combined approach has led to the discovery and classification of an unexpectedly large number of receptors, some quite novel and others even unanticipated, that are members of large families of genetically conserved proteins. Moreover, our understanding of receptor action has been clarified by the identification and detailed characterization of post-receptor signaling proteins and signaling mechanisms. Four major receptor superfamilies have been identified that are distinguished by protein structure, cellular localization, and effector systems. These families include the G protein–coupled receptors (GPCRs), cytokine receptors, tyrosine kinase receptors (RTKs), and nuclear receptors (Table 3-1). This chapter reviews major features of these important receptor families. Mutations influencing receptor function leading to endocrine disorders are also highlighted.

G PROTEIN–COUPLED RECEPTORS

More than 1% of the genome of vertebrates encodes a large protein family of receptors that sense molecules outside the cell and activate inside signal transduction pathways and, ultimately, cellular responses. These receptor proteins are embedded in the plasma membrane and are coupled to intracellular signal generating systems by heterotrimeric G proteins (i.e., G protein coupled receptors [GPCRs]).[1] GPCRs are also known as seven-transmembrane domain receptors, 7TM receptors, heptahelical receptors, and serpentine receptors. They are called transmembrane receptors because they pass through the cell membrane, and they are called seven-transmembrane receptors because they pass through the cell membrane seven times. The human genome encodes roughly 950 G protein–coupled receptors, which detect photons (light), hormones, growth factors, drugs, and other endogenous ligands. Approximately 150 of the GPCRs found in the human genome have unknown functions. Most GPCRs are odorant and pheromone receptors.[1] Also important to note is that most hormones bind to GPCRs, and hence G protein–dependent signal transduction represents the most common mechanism for hormone action (Table 3-2).

G protein–coupled receptors are involved in many diseases and are also the target of approximately 40% of all modern medicinal drugs.[2] G proteins were discovered when Alfred G. Gilman and Martin Rodbell investigated stimulation of cells by adrenaline. These investigators discovered that when adrenaline binds to a receptor, the receptor does not stimulate enzymes directly. Instead, the receptor stimulates a GTP binding protein, which stimulates an enzyme. An example is adenylate cyclase (AC), which produces the second messenger cyclic AMP. For this discovery, they won the 1994 Nobel Prize in physiology or medicine. The 2012 Nobel Prize in chemistry was awarded to Brian Kobilka and Robert Lefkowitz for their work that was "crucial for understanding how G protein–coupled receptors function."

The GPCR superfamily is divided into eight major classes.[1,3] These receptors contain an N-terminal extracellular domain that is frequently called the ectodomain or exodomain.[4] These receptors also contain seven putative transmembrane spanning alpha helices (TM-I to TM-VII). The alpha helices are connected by three intracellular (i1 through i3) and three extracellular (e1 through e3) loops that are often collectively called the *serpentine region* (Figure 3-1).[4,5] The C-terminal intracellular region is usually referred to as the *endodomain*.[4]

GPCRs are activated by a wide variety of signals, including proteins, nucleotides, amino acid residues, Ca^{2+}, light photons, and odorants (see Figure 3-1).[1] It is postulated that ligand binding alters the conformation of transmembrane domains and intracellular loops, increasing the affinity of the receptor for specific heterotrimeric guanosine nucleotide binding proteins (G proteins) (see Figure 3-1).[6,7] G proteins share a common heterotrimeric structure consisting of an α subunit and a tightly coupled βγ dimer. The α subunit interacts with detector and effector molecules, binds guanosine 5′-triphosphate (GTP), and possesses intrinsic GTPase activity. There are 16 genes in mammals that encode some 20 different α chains. The Gα subunits are categorized in four classes and include Gsα (G stimulatory), Giα (G inhibitory) and Goα (G other), Gq/11α, and G12/13α. They behave differently in the recognition of the effector but share similar structures and mechanism of activation. The Gα subunits consist of two domains: a GTP-binding domain and a helical insertion domain. The GTP-binding domain is homologous to Ras-like small GTPases and includes switch regions I and II, which change conformation during activation. The switch regions are loops of

TABLE 3-2 **G Protein–Coupled Receptors and Clinical Conditions Associated with Receptor Mutations**

Receptor	Germline Mutation	Endocrine Disorder
ACTH/melanocortin-2 receptor	Inactivating mutations (homozygous, compound heterozygous)	Familial glucocorticoid deficiency type 1
Melanocortin-4 receptor	Inactivating mutations (most heterozygous, some homozygous)	Obesity
V2 vasopressin receptor	Inactivating mutations (most X-linked recessive, rarely X-linked dominant)	X-linked nephrogenic diabetes insipidus
LH receptor	Inactivating mutations (homozygous, compound heterozygous) Activating mutations (heterozygous)	Males: types I and II Leydig cell hypoplasia Females: asymptomatic or hypergonadotropic hypogonadism with primary amenorrhea Males: male limited precocious puberty
FSH receptor	Inactivating mutations (homozygous, compound heterozygous)	Females: autosomal recessive hypergonadotropic ovarian dysgenesis or milder hypergonadotropic hypogonadism Males: variable impairment of spermatogenesis
TSH receptor	Inactivating mutations (most homozygous or compound heterozygous, rarely heterozygous) Activating mutations (heterozygous)	Resistance to TSH Autosomal-dominant inherited non-autoimmune hyperthyroidism/toxic adenomas
GnRH receptor	Inactivating mutations (homozygous or compound heterozygous)	Isolated hypogonadotropic hypogonadism
TRH receptor	Inactivating mutations (compound heterozygous)	Central hypothyroidism
GPR54	Inactivating mutations (homozygous, compound heterozygous)	Normosmic isolated hypogonadotropic hypogonadism
Ghrelin	Inactivating mutations (homozygous, possible heterozygous)	Short stature due to decreased growth hormone secretion
GHRH receptor	Inactivating mutations (homozygous/compound heterozygous)	Isolated growth hormone deficiency
Type 1 PTH receptor	Inactivating mutations (homozygous, heterozygous) Activating mutations (heterozygous)	Blomstrand's chondrodysplasia if homozygous and rarely if heterozygous; enchondromatosis if heterozygous Jansen's metaphyseal chondrodysplasia
Calcium-sensing receptor	Inactivating mutations (heterozygous, homozygous) Activating mutations (heterozygous)	Familial benign hypocalciuric hypercalcemia typical if heterozygous, neonatal severe hyperparathyroidism rarely if heterozygous, typical if homozygous Autosomal-dominant hypocalcemic hypocalciuria, Bartter syndrome type V

alpha helices with conformations sensitive to guanine nucleotides. The helical insertion domain is inserted into the GTP-binding domain before switch region I and is unique to heterotrimeric G proteins. This helical insertion domain sequesters the guanine nucleotide at the interface with the GTP-binding domain and must be displaced to enable nucleotide dissociation.

The α subunits associate with a smaller group of β(5) and γ(12) subunits.[8] Combinatorial specificity in the associations between various G protein subunits provides the potential for enormous diversity and may allow distinct heterotrimers to interact selectively with only a limited number of G protein–coupled receptors and effector proteins.[6,7,9]

There are two principal signal transduction pathways involving the G protein–coupled receptors: the cyclic AMP signal pathway and the phosphatidylinositol signal pathway. G protein–induced signal generation is regulated by a "molecular timer" that is determined by the rate of GTP exchange and hydrolysis. In the inactive state, G proteins exist in the heterotrimeric form with guanosine 5′-diphosphate (GDP) bound to the α chain. Interaction of a ligand-bound receptor with a G protein leads to release of GDP, with subsequent binding of GTP to the α chain. The binding of GTP to the α chain leads to dissociation of the α chain from the βγ dimer, allowing the now free α-GTP chain to interact with target enzymes and ion channels. The βγ dimers also participate in downstream signaling events through interaction with an ever-widening array of targets, including certain forms of AC and phospholipase C, potassium channels, and G protein–coupled receptor kinases.

G protein signaling is terminated by the hydrolysis of α-GTP to α-GDP by an intrinsic GTPase. A group of proteins, called regulator of G protein signaling (RGSs), acts as GTPase-activating proteins (GAPs), specific for Gα subunits. These proteins accelerate hydrolysis of GTP to GDP and terminate the transduced signal. In some cases, the effector itself may possess intrinsic GAP activity, which helps deactivate the pathway. This is true in the case of phospholipase C β, which possesses GAP activity within its C-terminal region. This is an alternate

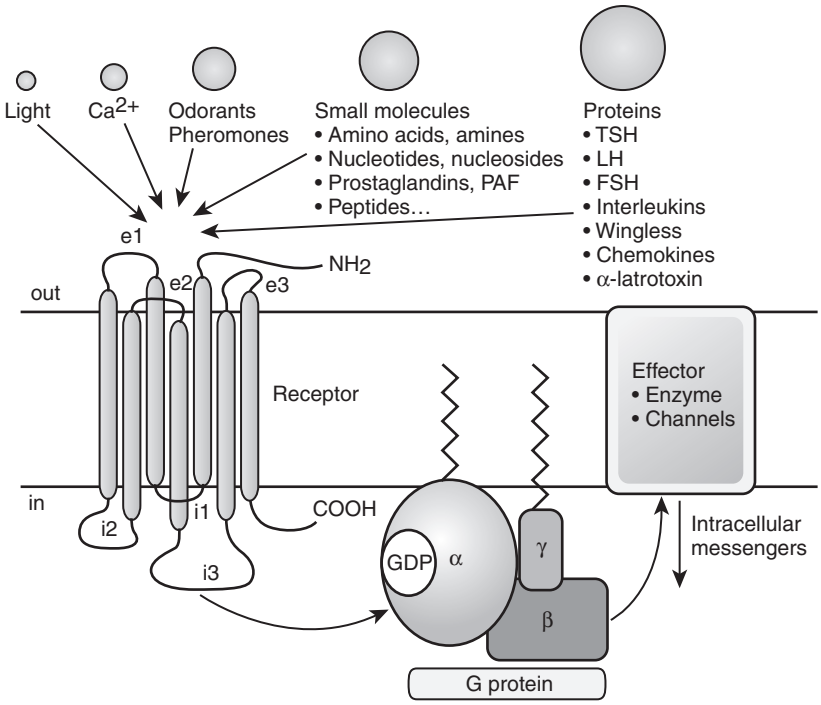


FIGURE 3-1 ■ GPCR structure and function. GPCRs have an N-terminal extracellular domain, seven putative transmembrane domains separated by three extracellular loops (e1-e3) and three intracellular loops (i1-i3), and a C-terminal intracellular domain. Ligand binding results in the exchange of GTP for GDP, which induces dissociation of the G protein into a GTPα subunit and a βγ subunit. Then these subunits alter the activity of intracellular effector enzymes and transmembrane channels, resulting in the alteration of intracellular levels of second messengers that can include cAMP and calcium. (Adapted with permission from Bockaert, J., & Pin, J. P. (1999). Molecular tinkering of G-protein–coupled receptors: an evolutionary success. *EMBO J, 18,* 1724.)

form of regulation for the Gα subunit. However, it should be noted that the GAPs do not have catalytic residues to activate the Gα protein. Rather, GAPs reduce the required activation energy for the reaction to take place. After hydrolysis of GTP, the Gα-GDP chain reassociates with the βγ dimer; the reassociated heterotrimeric G protein is now capable of participating in another cycle of receptor-activated signaling.[1,6,7,9]

Specificity in ligand binding is conferred by variations in the primary structures of the extracellular and intracellular domains.[1] Specificity of effector responses is conferred by the variations in the primary structure of intracellular domains and isoforms of the Gα subunits of G proteins.[10,11] Some GPCRs couple predominantly with $G\alpha_i$ /$G\alpha_o$ subunits that act primarily to decrease adenylyl cyclase activity.[11-14] Other GPCRs couple predominantly with $G\alpha_s$ subunits that increase adenylyl cyclase activity or $G\alpha_q$/Gα[13,16-18] subunits that increase phospholipase C activity.[11,15,16]

Interestingly, data show that cytoskeletal proteins may modulate receptor–G protein coupling. For example, the erythrocyte membrane cytoskeletal protein 4.1G can interfere with A1 adenosine receptor signal transduction.[17] 4.1G also influences metabotropic glutamate receptor 1α-mediated cAMP accumulation, increases the ligand-binding ability of metabotropic glutamate receptor 1α, and alters its cellular distribution.[18] 4.1G may also play a role in receptor-receptor dimerization.

Receptor agonist-independent and agonist-induced homo- and heterodimerization have increasingly been recognized as important determinants of GPCR function.[19] For example, the GPCR somatostatin receptor 5 (SSTR5) primarily exist as monomers in the absence of an agonist. However, they form homodimers in the presence of an agonist.[20] Furthermore, it has been shown that SSTR5 can form heterodimers with type 2 dopamine receptors (DRD2)—another GPCR—in the presence of hsst2 agonist or dopamine.[21] Agonist-induced activation of SSTR5-DRD2 heterodimers in Chinese hamster ovary (CHO) cells expressing SSTR5 and DRD2 is increased when compared to agonist-induced activation of monomers and homodimers in CHO cells expressing only SSTR5 or DRD2.[21] Heterodimerization of receptors may also lead to inactivation of one of the receptors in the complex. For example, heterodimerization of somatostatin receptor 2A (sst2A) with somatostatin receptor 3 (SSTR3) appears to lead to inactivation of the heterodimerized SSTR3 without inactivating the heterodimerized SSTR2.[22]

GPCRs can form heterodimers with nonreceptor transmembrane proteins. Both the calcitonin receptor (CALCR) and the calcitonin receptor-like protein (CALCRL) can form heterodimers with three different accessory proteins that are termed "receptor-activity–modifying proteins (RAMPs)": RAMP1, RAMP2, and RAMP3.[23-25] Whereas CALCRs can be activated by ligand in the absence of heterodimerization with a RAMP, CALCRLs are only activated by ligand if heterodimerized with a RAMP.[23,24] RAMPs alter the ligand specificity of the heterodimerized receptor.

CALCRs that are not in heterodimers with RAMPS are activated by calcitonin and thus constitute the classic

CALCR.[23,24] However, CALCRs heterodimerized with RAMP1, RAMP2, and RAMP3 bind amylin and constitute amylin1, amylin2, and amylin3 receptors, respectively.[23,24] CALCRLs dimerized with RAMP1 bind calcitonin gene–related peptide and constitute the calcitonin gene–related peptide receptor.[23,24] CALCRLs dimerized with RAMP2 and RAMP3 bind adrenomedullin and constitute adrenomedullin1 and adrenomedullin2 receptors, respectively.[23,24] RAMPS alter function of other GPCRs that transduce hormone action. The distribution and function of parathyroid hormone 1 and 2 receptors are altered by binding to RAMP2 and RAMP3, respectively.[26] The distribution and function of the glucagon receptor is altered by binding to RAMP2.[26] Dimerization/heterodimerization may occur in the endoplasmic reticulum (ER) shortly after protein synthesis occurs.[27] The ER plays a role in determining whether or not a protein will be expressed elsewhere in the cell, thus protecting the cell from misfolded and (likely) mutant proteins.[27] The non-heterodimerized CALCRL is an orphan receptor because the CALCRLs cannot leave the ER for the cell membrane unless heterodimerized with RAMPs.[28]

The melanocortin receptors also utilize accessory proteins. Circulating ACTH binds to five different forms of the melanocortin receptor (types 1-5), but only the melanocortin 2 receptor (MC2R) in the adrenal cortex leads to release of adrenal steroids. MC2R interacts with Gs, which leads to activation of adenylyl cyclase and formation of cAMP. The MC2R is the smallest G protein–coupled receptor known to date and belongs to a family of melanocortin receptors (types 1 to 5) that bind to various derivatives of proopiomelanocortin, especially α-MSH. The accessory protein melanocortin 2 receptor-associated protein (MRAP) is required for MC2R function, as it is critical for the translocation of the receptor from the endoplasmatic reticulum to the cell surface.[29] Moreover, MRAP facilitates signaling of the MC2R.[30] Loss of function of MRAP thus prevents membrane expression of MC2R and completely prevents ACTH signaling. Interestingly, MRAP forms a unique antiparallel homodimer in close proximity to the MC2R.[31] The MRAP accessory protein can also interact with other melanocortin receptors, particularly MC5R, but exerts negative effects on their signaling. Expression of MRAP was shown to be predominantly present in the zona fasciculata in the rat adrenal gland, consistent with its facilitating role in glucocorticoid production. Hence, mutations in MC2R[32] or MRAP[29] can lead to familial glucocorticoid deficiency secondary to ACTH resistance. By contrast, MRAP2, a protein with 39% amino acid homology to MRAP, shares the MC2R-trafficking function of MRAP but does not appear to play a major supportive role in adrenocortical ACTH signaling. On the contrary, in vitro studies have shown that overexpression of MRAP2 can suppress MC2R activation.

Failure of the endoplasmic reticulum to export mutant GPCR homodimers and mutant GPCR wild-type GPCR heterodimers to the cell membrane has been found to be the cause of dominant negative endocrine conditions. A dominant negative mutation is a heterozygous mutation that results in a phenotype that would only be expected in the presence of a homozygous mutation. Some heterozygous MC4R mutations cause dominantly inherited obesity due interaction of wild-type MC4R with the mutant receptor, and this specific effect of protein-protein interaction results in a dominant-negative effect.[33,34] In addition, some V2 vasopressin receptor gene mutations that are known to cause nephrogenic diabetes insipidus encode mutant V2 vasopressin receptors that form dimers in the ER that cannot be exported to the cell membrane.[35]

These mutant receptors also interfere with cell surface expression of wild-type receptors by forming heterodimers with the wild-type receptors that cannot be exported from the ER to the cell membrane.[36] This finding explains why females heterozygous for these V2 vasopressin receptor gene mutations do not concentrate their urine with even high doses of desmopressin, a synthetic V2 vasopressin receptor agonist, in spite of being able to produce wild-type V2 vasopressin receptors.[37] A similar phenomenon explains dominant transmission of partial TSH receptor resistance in patients heterozygous for some inactivating TSH receptor mutations.[38] In these patients, mutant TSH receptors form oligomers with wild-type receptors and prevent export of wild-type receptors from the endoplasmic reticulum to the cell membrane.[38]

Similarly, misfolding and misrouting of some mutant gonadotropin-releasing hormone (GnRH) receptors in the endoplasmic reticulum (as well as oligomerization of these mutant GnRH receptors with wild-type GnRH receptors) decrease cell membrane expression of wild-type GnRH receptors.[39-41] This phenomenon, however, has not been found to have clinical implications in relatives of probands homozygous or compound heterozygous for mutations that cause isolated hypogonadotropic hypogonadism (IHH) because individuals heterozygous for these mutations demonstrate an intact GnRH-gonadotropin axis and do not have clinical signs of IHH. Thus, in these individuals enough wild-type GnRH receptors do not oligomerize with mutant GnRH receptors and are transported to the cell membrane to maintain sufficiently normal GnRH-GnRH receptor interactions to avoid development of IHH.[40]

GPCRs activate G proteins at very low levels in the absence of ligand binding, but in some cases genetic mutations that lead to substitution of a single amino acid can greatly increase the interaction rate of the unliganded receptor for its G protein. Hence, GPCRs that are specific for luteinizing hormone, thyroid-stimulating hormone, thyrotropin-releasing hormone, glucagon-like peptide-1, melanocortin, and cannabinoid receptors can activate G proteins in the absence of ligand binding,[42,43] demonstrating constitutive activity that increases linearly with increased cell surface expression of the receptors.[44] It has also been recognized that there are ligands (often called inverse agonists) that decrease the activity of these receptors.[45] Receptor ligands that neither increase nor decrease the activity of receptors are now frequently referred to as neutral antagonists.[43]

The term *antagonists* is applied to these ligands because they block activation and inactivation of receptors by agonists and inverse agonists, respectively.[43] The term

agonist only refers to receptor ligands that increase receptor activity.[43] A scale has been formulated to express the continuity in receptor ligand function—from −1 (representing a full inverse agonist), to 0 (representing a neutral antagonist), to +1 (representing a full agonist).[43,45] It is possible that inverse agonists play a role in treating medical conditions caused by GPCR mutations that lead to increased constitutional activation of the receptor.[43]

Receptor desensitization and resensitization play a role in GPCR activity. Since the early 2000s, mechanisms of GPCR desensitization and resensitization have been elucidated. Three processes for receptor desensitization have been described.[46,47] The first receptor desensitization process is rapid uncoupling of the G protein from GPCRs.[47] This process occurs within seconds to minutes after initiation of the process and occurs as a result of phosphorylation of GPCRs.[47] G protein receptor kinases (GRKs) have been increasingly recognized as playing a major role when this process involves homologous desensitization.[46]

Homologous or agonist-dependent desensitization occurs after agonist activation of the receptor that is desensitized.[47] GRK-mediated phosphorylation of serine and threonine residues in the third intracellular loop, or the C-terminal intracellular domain leads to activation of β-arrestins, which in turn inactivate adenylyl cyclase (Figure 3-2).[46-49] Second-messenger–dependent protein kinases also contribute to receptor desensitization when this process involves homologous desensitization, but they also participate in receptor desensitization when desensitization involves heterologous desensitization. Heterologous or agonist-independent desensitization occurs as a result of activation of a different receptor from the one that is desensitized.[47]

The second receptor desensitization process is internalization/sequestration of GPCRs. This process is slower than receptor phosphorylation-induced uncoupling of the G protein from GPCRs and occurs within minutes to hours after initiation of the process. This process is reversible because the receptors can be recycled to the cell surface (see Figure 3-2).[47] GRKs and β-arrestins play a role in initiating internalization/sequestration of β2-adrenergic, LH, FSH, TSH, TRH, vasopressin V2, angiotensin II type 1A, and other G protein–coupled receptors in clathrin-coated vesicles (see Figure 3-2).[46,50-57] Dephosphorylation of the sequestered receptor followed by disassociation of the receptor from β-arrestin is necessary for the receptor to be recycled to the cell membrane and resensitized (see Figure 3-2).[16]

The third receptor desensitization process is down-regulation. With down-regulation, the number of intracellular GPCRs decreases due to increased lysosomal degradation and decreased synthesis of the receptors due to alteration of transcriptional and posttranscriptional regulatory mechanisms (see Figure 3-2).[58,59] Down-regulation is a slow process that occurs within several hours to days after initiation of the processes that lead to its development.[60]

One of the ways the Arg137His V2 vasopressin receptor mutation interferes with mutant receptor function and causes X-linked nephrogenic diabetes insipidus is by

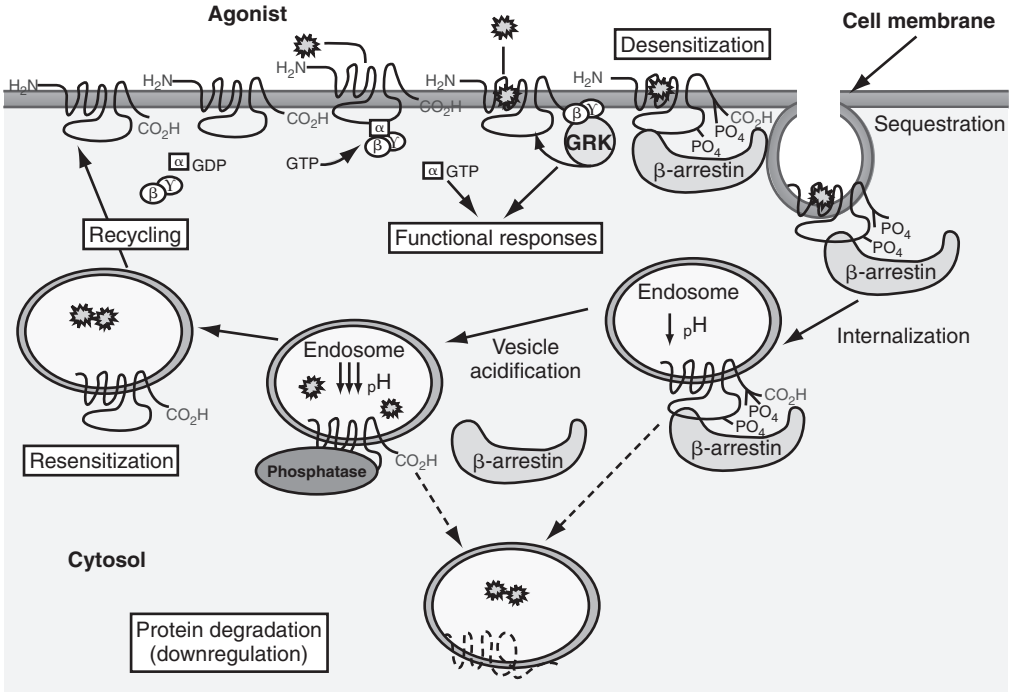


FIGURE 3-2 ■ Desensitization and recycling of GPCRs. Shortly after an agonist binds a GPCR, G-protein–receptor kinases phosphorylation of serine and threonine residues in the third intracellular loop or the C-terminal intracellular domain leads to activation of β-arrestin. Activation of β-arrestin inactivates adenylyl cyclase and initiates sequestration of the GPCR in clathrin-coated vesicles. Dephosphorylation of the sequestered receptor and subsequent disassociation of the receptor from β-arrestin is followed by recycling of the GPCR to the cell membrane. Alternatively, once sequestered the GPCR can be destroyed in lysosomes. (Adapted with permission from Saunders, C., & Limbird, L. E. (1999). Localization and trafficking of α2-adrenergic subtypes in cells and tissues. *Pharmacol Ther, 84,* 200.)

altering desensitization and recycling of the mutant receptor.[61] In vitro studies have revealed that the mutant receptor is constitutively phosphorylated. Thus, even in the absence of ligand binding the mutant receptor is bound by β-arrestin—which in turn leads to sequestration of the mutant receptor within clathrin-coated vesicles. Recycling of the mutant receptor back to the cell membrane requires the mutant receptor to be dephosphorylated and disassociated from β-arrestin. However, the mutant receptor remains constitutively phosphorylated while sequestered and thus cannot be disassociated from β-arrestin and recycled to the cell membrane—thereby reducing cell membrane expression of the mutant receptor.

Some investigators suggest that most GPCR-inactivating mutations can be classified by the effects of the mutations into one of five classes.[62] Class I inactivating mutations interfere with receptor biosynthesis. Class II inactivating mutations interfere with receptor trafficking to the cell surface. Class III inactivating mutations interfere with ligand binding. Class IV inactivating mutations impede receptor activation. Class V inactivating mutations do not cause discernible defects in receptor biosynthesis, trafficking, ligand binding, or activation but may cause medical disorders. There are also inactivating mutations that interfere with receptor function via multiple mechanisms and thus cannot be placed into one class.

Of the eight classes of GPCRs, only classes A, B, and C contain receptors for mammalian hormones (Figure 3-3).[3] Class A receptors contain the rhodopsin-like receptors and are divided into at least 15 groups.[3,63] Four of these groups contain receptors activated by hormones. These are the peptide receptor, hormone protein receptor, GnRH receptor, and the thyrotropin-releasing hormone (TRH) and secretagogue receptor groups.[3]

The peptide receptor group includes the angiotensin, adrenocorticotropin hormone (ACTH)/melanocortin, oxytocin, somatostatin, and vasopressin receptors.[3] The hormone protein receptor group includes the receptors for glycoprotein hormones, including follicle-stimulating hormone (FSH), luteinizing hormone (LH), and thyrotropin (TSH) receptors.[3] These receptors have large extracellular N-terminal domains and ligand-binding sites that include the first and third extracellular loops (see Figure 3-3).[1,3] There is also much similarity in amino acid sequence among these receptors (see Figure 3-3).[1] The GnRH receptor group only contains the GnRH receptor.[2] The TRH and secretagogue receptor group includes the TRH receptor and the growth hormone secretagogue receptor.[3]

Class B GPCRs are structurally similar to members of the hormone protein receptor group (see Figure 3-3).[1] However, unlike the glycoprotein hormone receptors, class B GPCRs do not share similar amino acid sequences.[1] This family contains receptors for high-molecular-weight hormones, including calcitonin, glucagon, gastric inhibitory peptide, parathyroid hormone (PTH), and corticotrophin-releasing factor (CRF).[1,3,64]

Class C receptors have a large extracellular domain with two lobes separated by a hinge region that closes on

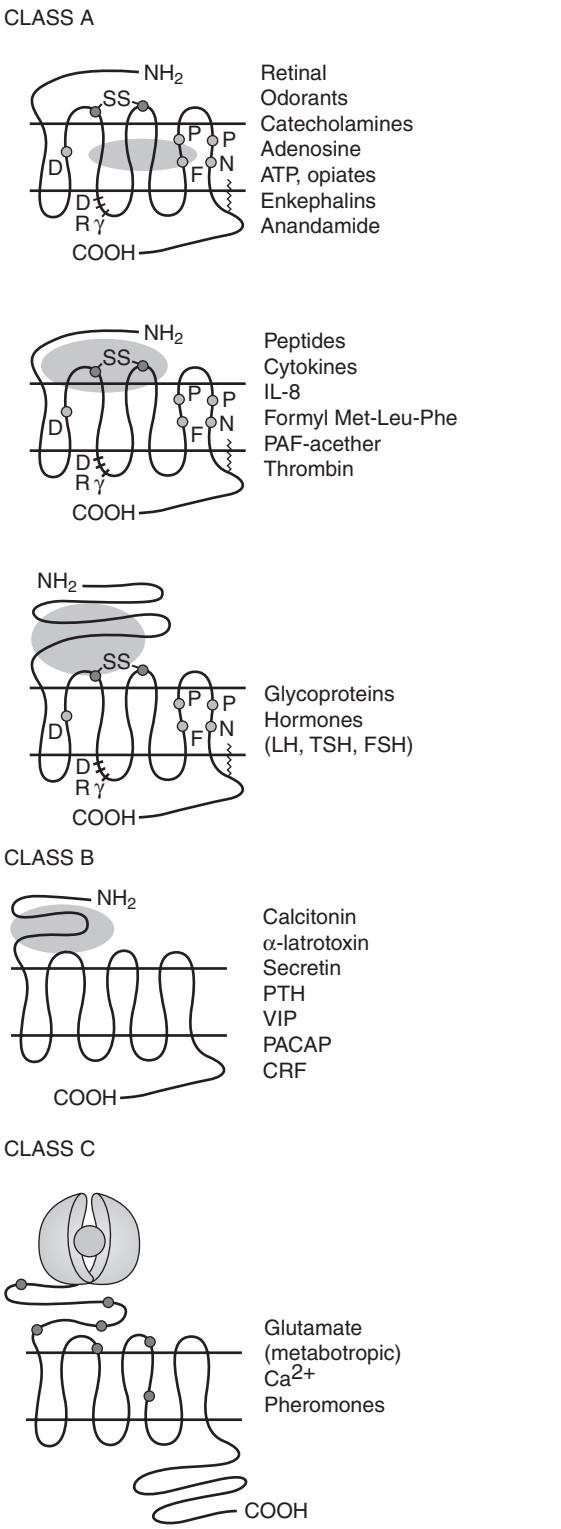


FIGURE 3-3 ■ Examples of class A, B, and C GPCRs. The oval represents the ligand. These receptors can differ in amino acid sequence, in length of the N-terminal extracellular and C-terminal cytoplasmic domains, and in the receptor regions involved with ligand-receptor interactions. (Adapted with permission from Bockaert, J., & Pin, J. P. (1999). Molecular tinkering of G protein-coupled receptors: an evolutionary success. *Embo J, 18*, 1725.)

the ligand (see Figure 3-3).[65] This region has also been called the *Venus flytrap* domain or module due to the trapping mechanism of the hinge region.[66] This family includes the calcium-sensing receptor (CASR).[1,2]

CLASS A RECEPTORS THAT TRANSDUCE HORMONE ACTION

The Peptide Receptor Group

Adrenocorticotropin and Melanocortin-2 Receptors

It is important to note that a newly accepted name for the ACTH receptor is *melanocortin-2 receptor* (MC2R) because the ACTH receptor is one of five members of the melanocortin receptor family of GPCRs.[62] For the purpose of clarity, when discussing interactions between ACTH and its receptor, the older name will be used for the remainder of this chapter. The ACTH receptor gene is located on the short arm of chromosome 18.[67] The ACTH receptor has a small extracellular and intracytoplasmic domain. Adrenocorticotropin-induced activation of the ACTH receptor in the *zona fasciculata* and *zona reticularis* of the adrenal cortex stimulates $G\alpha_s$, resulting in increased intracellular cAMP levels that stimulate steroidogenesis by activating cAMP-dependent kinases.[68-70]

Hereditary isolated glucocorticoid deficiency, resistance to ACTH, and familial glucocorticoid deficiency (FGD) are the same names for an autosomal recessive syndrome that consists of glucocorticoid deficiency accompanied by normal mineralocorticoid secretion. FGD has been classified further as FGD types 1 and 2 and the triple A syndrome.[71] Patients with FGD type 1 are homozygous or compound heterozygous for point mutations, resulting in ACTH receptors with abnormal function and account for 25% of FGD cases.[71-77] In contrast, patients with FGD type 2 have ACTH resistance due to mutations in melanocotin-2 receptor accessory protein (MRAP).[78] Triple A (Allgrove syndrome) is an autosomal recessive syndrome characterized by ACTH-resistant adrenal insufficiency, achalasia, and alacrima—which is caused by mutations in the achalasia-adrenocortical insufficiency-alacrima syndrome (AAAS) gene[79] thought to regulate nuclear pore complexes and nucleocytoplasmic transport.[80]

Patients with FGD type 1 usually present during infancy or early childhood with hypoglycemia.[74,82-86] Less commonly, patients may present with a severe infection, frequent minor infections, or childhood asthma that resolves with treatment with physiologic doses of glucocorticoids.[71,74,82] Hyperpigmentation thought to be due to increased ACTH levels acting on the MC1R may be seen as early as the first month of life, but usually becomes apparent after the fourth month of life.[72-74,82-84,87] There is one reported case of FGD type 1 without hyperpigmentation despite elevated ACTH levels in a patient with homozygous mutations in both the MC2R and the MC1R.[88] The assumption that hyperpigmentation is caused by increased ACTH levels acting on the MC1R (both in FGD type 1 and Addison's disease) was substantiated in this patient whose MC1R mutation had previously been implicated in red hair and pale skin phenotypes. Neonates with FGD type 1 may also suffer from jaundice.[74,84,86,89] Tall stature accompanied by an advanced or dissociated bone age, in spite of normal age of onset of puberty, appears to be common in children with FGD type 1.[71,74,77,82,83,87] Patients with FGD type 2 have normal heights[71,90] and present earlier than patients with FGD type 1. The pathophysiology of tall stature in FGD type 1 is unknown. Both forms of FGD exhibit absent adrenarche confirming the importance of ACTH in the induction and maintenance of adrenarche.[78]

At presentation, plasma cortisol, androstenedione, and dihydroepiandrosterone levels are low or low normal—and plasma ACTH levels are elevated.[71,74,82-86] When supine, patients with FGD type 1 have renin and aldosterone levels that are near normal.[71,83,85,86] Histologically, the *zona fasciculata* and *zona reticularis* are atrophied with FGD.[82] However, demonstrating the lack of an essential role for ACTH in the embryologic development and maintenance of the *zona glomerulosa*, adrenal cortices in patients with all types of FGD contain *zona glomerulosa* cells.[71,81-83,87,91]

Abnormalities in ACTH receptor expression may be seen in other conditions. Evidence suggests that the ACTH receptor-$G\alpha_s$-adenylyl cyclase-cAMP cascade maintains differentiation of adrenocortical cells and that impairment of this cascade leads to dedifferentiation and increased proliferation of adrenocortical cells.[92,93] Adrenocortical carcinomas from some patients have been found to have a loss of heterozygosity (LOH) for the ACTH receptor gene, resulting in markedly decreased ACTH receptor mRNA expression.[93] Growth of the tumors with LOH for the ACTH receptor gene also may be more aggressive than the other tumors. An activating mutation of G_{i2} that constitutively suppresses adenylyl cyclase activity has also been found in adrenocortical tumors.[92] Thus, decreased ACTH receptor activity may be associated with tumorigenesis.

Interestingly, many patients with ACTH-independent macronodular adrenal hyperplasia (AIMAH)—a cause of ACTH-independent Cushing syndrome—exhibit increased glucocorticoid levels in response to noncorticotropin hormones that do not normally induce glucocorticoid release.[94-99] These hormones include gastric inhibitory peptide, exogenous arginine and lysine vasopressin, luteinizing hormone, human chorionic gonadotropin, angiotensin II, catecholamines, leptin, and serotonin receptor agonists.[94-99] Increased expression of the receptors for these ligands in the abnormal adrenal glands has been implicated as a possible explanation for the abnormal induction of glucocorticoid release by these noncorticotropin ligands.[99] However, receptors for some of these ligands are expressed in normal adrenal glands.[99] Thus, the mechanism for this phenomenon remains to be fully elucidated.

Other Melanocortin Receptors

Murine studies reveal that melanocortin-3 receptor (MC3R), another of the five members of the melanocortin receptor family, regulates fat deposition as MC3R

deficient mice have normal body weight with increased fat mass.[62] The role of the MC3R in humans is less clear. More than 24 human MC3R mutations have been identified without evidence of obesity.[100,101] However, patients with these mutations were not phenotyped for fat mass to assess whether they phenocopy the mouse model, which exhibits normal body weight with altered partitioning leading to increased fat mass and reduced lean mass.[101-103] Homozygosity for a pair of single-nucleotide polymorphisms of the MC3R gene that result in production of partially inactive MC3Rs was found to be associated with pediatric-onset obesity in Caucasian American and African American children.[104]

The melanocortin-4 receptor (MC4R) is another member of the melanocortin receptor family and plays a role in controlling appetite and weight.[105] The MC4R has baseline constitutive (i.e., ligand-independent) activity that can be inhibited by the inverse agonist agouti-related peptide (AgRP).[105,106] Activation of the MC4R by its natural agonist α-melanocyte stimulating hormone (α-MSH) produces anorexigenic effects.[105,107,108] More than 150 naturally occurring MC4R mutations have been identified, causing hyperphagic obesity, increased lean body mass, increased bone density, and increased linear growth.[109-114] Patients with homozygous mutations appear to have more severe obesity than their heterozygous relatives, consistent with codominant inheritance.[7,115]

MC4R mutations are thought to be the most common monogenic cause of human obesity. The prevalence of pathogenic MC4R mutations in obese populations varies widely ranging from 0.5% to 5.8% depending on the screening criteria and population.[116-119] AgRP gene polymorphisms appear to be associated with anorexia nervosa.[112,113]

Little is known about melanocortin-5 receptors (MC5Rs) in animals and humans. There is only weak evidence from a single linkage and association study of families in Quebec that suggests that MC5Rs may also play a role in regulating body weight and fat mass.[114]

Another member of the melanocortin receptor family, the melanocortin-1 receptor (MC1R), controls skin and hair pigmentation. Activation of MC1Rs in skin and hair follicle melanocytes by the pro-opiomelanocortin (POMC)-derived peptides α-MSH and ACTH stimulates the synthesis of eumelanin, a brown-black pigment.[120,121] Inhibition of MC1R baseline constitutive activity by agouti protein, or specific mutations, leads to release of pheomelanin, a red-yellow pigment, from the melanocytes.[121]

Inactivating homozygous mutations of the POMC gene cause hypoadrenalism, red hair, fair skin, and early-onset obesity. Hypoadrenalism is characterized by glucocorticoid deficiency due to lack of ACTH production from the POMC precursor. Fair skin and red hair are due to a lack of ACTH and α-MSH-induced melanocyte release of eumelanin that results from activation of MC1Rs. Of note, non-white patients with homozygous POMC mutations do not appear to have the fair skin and red hair phenotype.[118,122] In white individuals eumelanin synthesis appears to be dependent on POMC-derived peptides, whereas in darker individuals other genes may control eumelanin synthesis.[123] Obesity is due to lack of α-MSH–induced anorectic effects, which normally result when α-MSH activates MC4Rs.[124] Heterozygosity for POMC gene mutations has been associated with hyperphagia, early-onset obesity, and increased linear growth.[124-126] Both homozygous[127,128] and heterozygous[129] mutations of prohormone convertase 1 cause obesity in humans. Prohormone convertase 1 acts on POMC, proinsulin, and proglucagon. Patients with prohormone convertase 1 deficiency also have neonatal enteropathy and postprandial hypoglycemia. The cause of enteropathy is unknown but hypothesized to be related to the processing of GLP-2 by prohormone convertase 1. GLP-2 is known to stimulate proliferation and repair of intestinal epithelium.[130]

Vasopressin Receptors

Nephrogenic diabetes insipidus (NDI) results from decreased responsiveness of the renal tubule to arginine vasopressin (AVP), with resulting excessive loss of free water. NDI is characterized by polydipsia and polyuria that is not responsive to vasopressin and vasopressin analogs.[131] Vasopressin binds to the V2 vasopressin receptor (AVPR2), a Gs-coupled receptor, in the basolateral membrane of collecting duct principal cells in the kidney and activates translocation of aquaporin-2 (AQP2) water channels to the apical membrane, thereby inducing water permeability. X-linked NDI is caused by inactivating mutations of the V2 vasopressin receptor (AVPR2) gene located at Xq28 and accounts for about 90% of genetically determined NDI.[132-135] More than 200 AVPR2 mutations have been described including missense, nonsense, insertions, deletions, and complex rearrangements.[136] Mutations have been categorized into five classes based on mechanism, including abnormal transcription, mRNA processing, translation, aberrant folding and intracellular retention, loss of the G protein binding site, loss of the AVP binding site, and defects in intracellular trafficking.[131,137,140] Some patients with X-linked NDI are responsive to high doses of desmopressin. Autosomal recessive NDI (ARNDI) is caused by loss-of-function mutations in the gene for the aquaporin-2 water channel and accounts for about 10% of genetic forms of NDI.[137,139] More than 40 known mutations cause ARNDI. Autosomal dominant forms of NDI are also caused by mutations in AQP2 that are functional but fail to be transported to the apical membrane. They account for $< 1\%$ of genetic forms of NDI and generally have a milder phenotype than ARNDI or X-linked NDI.

Gain-of-function mutations in the V2 vasopressin receptor have also been reported.[141] DNA sequencing of two patients' V2R gene identified heterozygous missense mutations in both, with resultant changes in codon 137 from arginine to cysteine (R137C) or leucine (R137L). These mutations resulted in constitutive activation of the receptor and clinical features of inappropriate antidiuretic hormone secretion (SIADH), which was termed *nephrogenic syndrome of inappropriate antidiuresis (NSIAD)*.[141] The patient with the R137L mutation demonstrated the expected decrease in AVP levels with a water-loading test but urine aquaporin 2 levels remained inappropriately elevated.[142]

The Glycoprotein Hormone Receptor Group

The glycoprotein hormones include TSH, FSH, LH, and HCG. These hormones share common α subunits that dimerize with hormone-specific β subunits. TSH, FSH, and LH bind to the extracellular N-terminal domain of the TSH, FSH, and LH receptors, respectively.[1,3,143,144] The effects of HCG are mediated by the LH receptor, which is also known as the luteinizing hormone/choriogonadotropin receptor (LHCGR).[145]

Glycoprotein hormone receptors have a large (350 to 400 residues) extracellular N-terminal domain, also known as the ectodomain, that participates in ligand binding (see Figure 3-3).[4,145] The ectodomain includes leucine-rich repeats that are highly conserved among the glycoprotein hormone receptors.[4,145] There is 39% to 46% similarity of the ectodomain and 68% to 72% similarity of the transmembrane or serpentine domain among the three glycoprotein hormone receptors.[4]

The glycoprotein hormone receptors are coupled to G_s, and hormone binding stimulates adenylyl cyclase, leading to increased intracellular cAMP levels and protein kinase A (PKA) activation.[145] Mutations leading to endocrine dysfunction have been reported for each of the glycoprotein hormone receptors.

LHCGR Receptors

Both inactivating and activating mutations of the LH receptor have been found in humans.[145] The LH receptor gene is located in chromosome 2 p21 and consists of 11 exons.[146,147] Exon 1 encodes a peptide that directs the LH receptor to the plasma membrane.[145] Exons 2 through 10 encode the ectodomain.[145] The last exon encodes the transmembrane domains that are also known as the serpentine regions.[4,145,146] Single nonsense mutations, amino acid changes, and partial gene deletions have been described that generate LH receptors with decreased activity.[145] Single–amino-acid changes have also been found that lead to activation of G_s in the absence of ligand binding.[145]

Development of LH resistance requires biallelic mutations that inactivate the LH receptor gene, as one normal receptor allele is capable of producing adequate receptor protein to ensure physiologic signaling.[145] In contrast, activating mutations of the LH receptor gene cause endocrine disorders in the heterozygous state.[145]

In the fetus, LH receptors are primarily activated by HCG.[145] Leydig cells begin to express LH receptors shortly after testicular differentiation at 8 weeks of gestation.[145] Thereafter, androgen production due to activation of these receptors by HCG plays an important role in the development of male genitalia and testicular descent.[145] Thus, male infants with inactivating mutations of the LH receptor may present with abnormally developed genitalia—including micropenis, cryptorchidism, and an XY disorder of sexual differentiation.[145]

Males with mutations that completely inactivate the LH receptor exhibit failure of fetal testicular Leydig cell differentiation. This phenotype, which is known as type 1 Leydig cell hypoplasia, includes female external genitalia with a blind-ending vagina, absence of Müllerian derivatives, and inguinal testes with absent or immature Leydig cells.[148-155] In addition, patients have elevated serum LH levels, normal serum FSH levels, and decreased serum testosterone levels that do not increase in response to HCG administration.[148-155] Mutations that lead to this phenotype include a nonsense mutation (Arg545Stop) that results in a receptor that is missing TM4-7, an Ala593Pro change, and a TM7 deletion (ΔLeu608, Val609) that decreases cell surface expression of the LH receptor.[153,154,156] These mutant receptors are unable to couple to G_s.[153,154,156]

Males with mutations that do not completely inactivate the LH receptor present with type 2 Leydig cell hypoplasia, which is characterized by a small phallus and decreased virilization.[152] A mutation that leads to this phenotype includes the insertion of a charged lysine at position 625 of TM7 in place of hydrophobic isoleucine that disrupts signal transduction.[157] Another mutation (Ser616Tyr, found in patients with mild Leydig cell hypoplasia) is associated with decreased cell surface expression of the LH receptor.[154,157] Other deletion and nonsense mutations have also been found to cause mild Leydig cell hypoplasia.[145]

Males with inactivating mutations of the LH receptor may also present with a phenotype intermediate in severity between type 1 and type 2 Leydig cell hypoplasia. A compound heterozygote patient with Ser616Tyr on one allele and an inactivating deletion (Δexon 8) on the other allele presented with Leydig cell hypoplasia, micropenis, and hypospadias.[158] The Cys131Arg mutation has also been found in patients with Leydig cell hypoplasia, small phallus, and hypospadias.[159] This mutation is located in the leucine-rich repeat segment of the LH receptor extracellular domain and interferes with high-affinity ligand binding.[159]

Deletion of exon 10 of the LH receptor gene leads to an LH receptor that binds LH and HCG normally.[160] Interestingly, whereas HCG binding can elicit normal transmembrane signaling, LH binding fails to activate the receptor.[160] Because HCG is the principal in utero LH receptor-activating hormone, and second-messenger response of the mutant receptors to HCG is not impaired, it is not surprising that a male patient found to be homozygous for the mutation was born with normal male genitalia.[62,160] Pubertal progression and later gonadal function, however, are dependent on LH activation of the LH receptor.[62,160]

Because deletion of exon 10 of the LH receptor gene results in a mutant LH receptor with diminished intracellular signaling in response to LH, it is also not surprising that the patient homozygous for this mutation was found to have delayed pubertal development, small testes, and hypergonadotropic hypogonadism when evaluated at the age of 18 years.[160] Prolonged HCG therapy resulted in normalization of testicular testosterone production, increased testicular size, and the appearance of spermatozoa in semen.[160] Similarly, inactivating mutations of the LH β subunit cause abnormal pubertal development, severe testosterone deficiency and azoospermia but normal external genitalia in males. In females inactivating mutations of the LH β subunit are

associated with normal pubertal development and menarche followed by oligomenorrhea, enlarged multicystic ovaries and infertility.[161]

Mutations that constitutively activate LH receptors cause male-limited precocious puberty (MLPP), also known as testotoxicosis—which may be familial or sporadic.[155,162,163] Boys with this condition present with GnRH-independent precocious puberty before the age of 4 years when the Asp578Gly is present and as early as the first year of life when the Asp578Tyr mutation is present.[145,164-166] Patients with this condition may also have an enlarged phallus at birth.[164]

During the first 5 years of life, patients with MLPP have very low LH and FSH levels but have testosterone levels in pubertal range.[167] During adolescence and adult life, testosterone levels do not increase above age-appropriate concentrations and gonadotropin levels normalize.[145,167-169] Thus, adolescents and adults with MLPP do not usually manifest signs of androgen excess (such as hirsutism or severe acne).[145,167] Most mutations that cause MLPP are located in the TM6 and i3, regions that participate in receptor-Gs protein coupling.[145] A milder phenotype was reported in a patient with a heterozygous activating mutation (C617Y) in TM7.[170] This mutation was inherited from the patient's mother who was apparently unaffected. Somatic activating mutations cause sporadic Leydig cell adenomas.[105,171]

Activating mutations of the LH receptor do not appear to have a phenotype in females. In prepubertal girls, this may be due to low or absent LH receptor expression or due to insufficient aromatase expression in prepubertal granulosa cells. During puberty, activation of LH receptors on ovarian theca cells leads to the production of androgens that are converted to estrogens by aromatase in granulosa cells.[145] LH, along with FSH, also plays a role in inducing the differentiation of follicles into Graafian follicles and triggers ovulation and release of the oocyte.[145] Detailed phenotyping of the carrier mother of a MLPP male with the Asp578Gly activating mutation of the LH receptor failed to reveal any abnormalities in her menstrual cycles or fertility. LH dynamics, androgen, and FSH levels as well as response to GnRH agonists were normal.[173]

Females with inactivating mutations of the LH receptor may be asymptomatic or present with primary amenorrhea.[145] Females with complete inactivating LH receptor mutations may present with primary amenorrhea, inability to ovulate, and decreased estrogen and progesterone levels accompanied by elevated LH and FSH levels.[154,174] Affected individuals may have signs of low estrogen levels, including a hypoplastic uterus, a thin-walled vagina, decreased vaginal secretions, and decreased bone mass.[154,174] A homozygous LH receptor mutation (N400S) has been associated with empty follicle syndrome.

FSH Receptors

Inactivating and activating FSH receptor mutations have also been described,[175] but they are far less common than LH receptor mutations.[175] The FSH receptor gene is located in chromosome 2 at p21 and contains 10 exons.[176] The last exon of the FSH receptor gene encodes the transmembrane and intracellular domains.[177]

FSH is required in females for normal follicle maturation and the regulation of estrogen production by ovarian granulosa cells.[175,178,179] FSH is required in pubertal males for Sertoli cell proliferation, testicular growth, and the maintenance of spermatogenesis.[175,180]

The first inactivating mutation of the FSH receptor was found in Finnish females with autosomal recessive inherited hypergonadotropic ovarian dysgenesis (ODG). ODG is characterized by primary amenorrhea, infertility, and streak or hypoplastic ovaries in the presence of a 46XX karyotype and elevated gonadotropin levels.[181] Twenty-two out of 75 Finnish patients with ODG were found to be homozygous for a C566T point mutation in exon 7 of the FSH receptor gene.[182] This mutation leads to the production of an FSH receptor with an Ala189Val substitution in an area of the extracellular ligand- binding domain that is thought to play a role in turnover of the receptor or in directing the receptor to the plasma membrane.[182] The mutated receptor demonstrates normal ligand-binding affinity but has decreased binding capacity and impaired signal transduction when studied in transfected mouse Sertoli cells.[182] Males homozygous for this mutation have variable impairment of spermatogenesis and low to low-normal testicular volume but are not azoospermic and can be fertile.[183] The C556T point mutation is uncommon outside Finland, where the carrier frequency is 0.96%.[184] Other mutations that alter signal transduction but not receptor expression or binding include Ala189Val, Asn191Ile, Ala419Thr, and Phe591Ser. The Ala189Val mutation causes primary hypergonadotropic amenorrhea in women and no spermatogenesis in men in the homozygous state and secondary amenorrhea in the heterozygous state.[185,186] The nearby Asn191Ile mutation also causes hypergonadotropic amenorrhea in the homozygous state but no clinical phenotype in the heterozygous state.[187] The Ala419Thr mutation was identified in a heterozygous woman with primary amenorrhea.[188] The Phe591Ser mutation causes primary amenorrhea and premature ovarian failure (POF) in the homozygous state and a predisposition to sex cord ovarian tumors in the heterozygous state.[189] Primary amenorrhea and POF have been described in women with homozygous mutations that totally impaired receptor binding to FSH[190] or that resulted in reduced expression of the FSH receptor on the cell surface.[191]

Compound heterozygosity for mutations that cause partial loss of FSH receptor function may cause endocrine dysfunction in women.[192,193] Women may present with infertility, secondary amenorrhea, osteoporosis, and a history of delayed onset of puberty accompanied by elevated LH and FSH, low-normal plasma estradiol, low plasma inhibin B levels, slightly enlarged ovaries with immature follicles, and a small uterus.[192] This may be caused by FSH receptor gene mutations that result in an Ile160Thr mutation in the extracellular domain that impairs cell surface expression and an Arg573Cys mutation in e3 that interferes with signal transduction.[192] Other women present with primary amenorrhea and very elevated gonadotropin, low plasma estradiol and inhibin B levels, normal-size ovaries with immature follicles, and a

normal-size uterus.[193] This condition is associated with an Asp224Val substitution in the extracellular domain leading to impaired cell-surface expression and a Leu601Val substitution in e3 impairing signal transduction.[193]

Activating mutations of the FSH receptor have also been described. Surprisingly, a hypophysectomized male was found to be fertile and to have serum testosterone levels above 4.9 nmol/L and normal testis volume in spite of undetectable gonadotropin levels.[194] This patient was found to be heterozygote for an A1700G mutation in exon 10 of the FSH receptor gene that resulted in an Asp567Gly substitution in an area of the third intracytoplasmatic loop that is highly conserved among FSH, LH, and TSH receptors.[194-196] The same substitution in corresponding areas of the LH and TSH receptors also results in constitutively active receptors and is found in MLPP and thyroid adenomas, respectively.[194-196] Other activating mutations have been identified to cause spontaneous ovarian hyperstimulation syndrome (OHSS). Ovarian hyperstimulation syndrome is a common complication of treatment protocols used to induce ova for in vitro fertilization and is characterized by multiple follicular cysts lined by luteinized cells, which can result in abdominal discomfort and distention as well as ovarian enlargement and fluid sequestration. One such mutation is the Asp567Asn, which was found in a woman with recurrent spontaneous OHSS.[197] The Thr449Ile and Thr449Ala mutations cause a conformational change that leads to loss of specificity for FSH leading to sensitivity to HCG[198] and TSH[199] causing spontaneous OHSS during pregnancy or with hypothyroidism. The Ile-545Thr mutation caused spontaneous OHSS in a woman during the first trimester of pregnancy despite a normal HCG level.[200] This mutant receptor displayed detectable constitutive activity as well as promiscuous activation by HCG and TSH.

TSH Receptors

The TSH receptor gene is located on chromosome 14 and contains 10 exons, with the first nine exons encoding the large extracellular domain and the tenth exon coding the remainder of the receptor.[201-204] At low extracellular TSH concentrations, TSH receptor activation leads to stimulation of $G\alpha_s$—which activates adenylyl cyclase, resulting in increased intracellular cAMP levels.[205,206] At higher extracellular TSH concentrations, activation of the TSH receptor also stimulates the G_q and G_{11} proteins—activating phospholipase C and resulting in the production of diacylglycerol and inositol phosphate.[206]

TSH receptors differ from the other glycoprotein hormone receptors in that they exist in two equally active forms.[207,208] These are the single-chain and two-subunit forms of the TSH receptor (Figure 3-4). The single-chain form of the TSH receptor is made up of three contiguous subunits: the A subunit, C peptide, and B subunit.[208-210] The A subunit begins at the N terminal of the extracellular domain and contains most of the extracellular domain.[208-210] The C peptide is connected to the C terminal of the A subunit and continues the extracellular domain.[208-210] The C peptide contains a 50-amino-acid sequence that is only found in TSH receptors.[208-210] The B subunit is connected to the C terminal of the C peptide and contains the TMs and the C-terminal cytoplasmic portion of the receptor.[208-210] The two-subunit form of the receptor is missing the C peptide, which is cleaved from the protein during intracellular processing and consists of the A and B subunits attached by disulfide

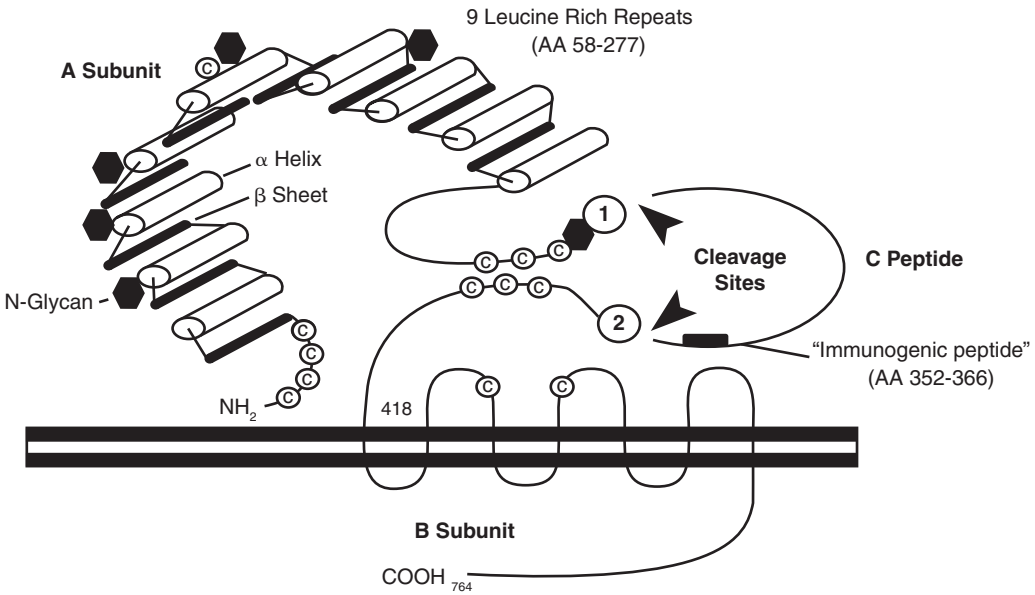


FIGURE 3-4 ■ The TSH receptor. There are two forms of TSH receptors. The single-chain form is made up of an A subunit, C peptide, and B subunit. Posttranslational cleavage of the C peptide from the single chain form results in the two-subunit form. This form consists of the A subunit joined to the B subunit by disulfide bonds between the C-terminal cysteine residues of the A subunit and the N-terminal cysteine residues of the B subunit. (Reproduced with permission from Rapoport, B., Chazenbalk, G. D., Jaume, J. C., & McLachlan, S. M. (1998). The thyrotropin [TSH] receptor: interaction with TSH and autoantibodies. *Endocr Rev, 19*, 676. Copyright 1998, The Endocrine Society.)

bonds.[211-214] It is surprising that both receptor forms are activated equally by TSH because the C peptide and nearby regions of the A and B subunits participate in signal transduction.[207-209,215,216]

Spontaneous single-allele mutations of the TSH receptor gene leading to replacement of Ser-281 (near the C terminal of the A subunit, with Ile, Thr, or Asn) result in a constitutively active TSH receptor that may cause intrauterine or congenital hyperthyroidism, or toxic adenomas.[210,217-219] Activating somatic mutations that cause toxic adenomas have also been found in different transmembrane domains of the TSH receptor.[220-227] More specifically, clusters of mutations are located in the i3 and TM6 regions—found to be involved with signal transduction in all glycoprotein hormone receptors.[220-222,224-226] The prevalence of activating mutations of the TSH receptor in toxic adenomas has been estimated to range from 2.5% in Japan to 86% in Brazil.[223,224,226,228-231]

Activating somatic mutations of the TSH receptor have also been found in multinodular goiters.[232] Interestingly, different activating mutations have been found in separate nodules in the same individual.[232] Some well-differentiated thyroid carcinomas have activating mutations of the TSH receptor.[233-235] Somatic activating mutations of the *GNAS* gene encoding $G\alpha_s$ have also been found in some toxic adenomas and differentiated thyroid carcinomas.[92,236,237] Activating germline mutations of the TSH receptor can cause sporadic or autosomal dominant inherited non-autoimmune hyperthyroidism that presents in utero, during infancy, during childhood, and in some cases in adulthood.[219,238-247] These mutations have been found in the N-terminal extracellular and transmembrane domains.[219,239-248]

Patients with heterozygous mutations that lead to constitutively active TSH receptors typically develop hyperthyroidism.[208] In contrast, biallelic loss of function mutations in the TSH receptor genes is required to cause hypothyroidism.[208] Most known loss-of-function TSH receptor mutations are located in the N-terminal extracellular domain.[249] A spontaneous Asp410Asn substitution, near the carboxy terminus of the C peptide, results in a TSH receptor with normal ligand binding affinity and impaired $G\alpha_s$-mediated signal transduction.[250] Patients homozygous for this type of mutation present with compensated hypothyroidism.[250]

Patients homozygous or compound heterozygous for loss-of-function mutations of the TSH receptor present with the syndrome of resistance to TSH (RTSH). Loss-of-function mutations of the TSH receptor that cause RTSH have been identified in the N-terminal extracellular domain, TM4, TM6,[251] i2, e1, and e3.[252] Clinical severity of RTSH may range from a euthyroid state accompanied by elevated TSH levels (fully compensated RTSH), to mild hypothyroidism unaccompanied by a goiter (partially compensated hypothyroidism), to congenital thyroid hypoplasia accompanied by profound hypothyroidism (uncompensated RTSH).[70,250,253-257] In patients with uncompensated RTSH, a small bilobar thyroid gland is located at the normal site.[70] Loss-of-function mutations of the TSH receptor are a rare cause congenital hypothyroidism[258,259] more common in Japan and Taiwan (up to 7% of children)[260,261] where R450H is particularly frequent. Because the sodium-iodide symporter is TSH dependent,[162] iodine and (99m) pertechnetate uptakes are diminished or absent in patients with RTSH.[70,262] In rare cases, iodine uptake is high-normal.[263] These compound heterozygous mutations of the TSH receptor had some $G\alpha_s$ activity and no G_q activity suggesting that iodine uptake is solely controlled by $G\alpha_s$ activity and not G_q activity. Some families have been found to have an autosomal-dominant form of RTSH that is not caused by a mutation of the TSH receptor.[264,265]

HCG and TSH Receptors during Pregnancy

Due to its structural similarity with TSH, at high concentrations HCG can activate the TSH receptor.[266] During pregnancy, HCG activation of TSH receptors leads to elevation in thyroid hormones seen after the ninth week of gestation—and decreases in TSH levels between the ninth and twelfth weeks of gestation.[267] This phenomenon does not usually result in maternal hyperthyroidism (gestational thyrotoxicosis).[267,268] However, when HCG levels are abnormally elevated due to gestational trophoblastic disease due to a molar pregnancy or choriocarcinoma, hyperthyroidism may occur.[269-273] The prevalence of thyrotoxicosis in gestational trophoblastic disease correlates with HCG levels. In one study of 196 patients treated with chemotherapy for gestational trophoblastic neoplasia, the prevalence of thyrotoxicosis was 7%.[274] Biochemical thyrotoxicosis only occurred in patients with HCG levels $> 10^5$ and clinical thyrotoxicosis only occurred in patients with HCG levels $>10^6$. Serum TSH is consistently suppressed when HCG levels are above 4×10^5.[275]

A mother and daughter were identified with recurrent gestational hyperthyroidism and normal serum HCG levels.[276] These individuals were found to be heterozygous for a point mutation in the TSH receptor gene, resulting in a Lys183Arg substitution in the extracellular domain of the receptor. It is believed that this substitution increases activation of the receptor by HCG, causing gestational hyperthyroidism.

The Gonadotropin-Releasing Hormone Receptor Group

Gonadotropin-Releasing Hormone Receptors

The GnRH receptor gene is located on 4q13 and includes three exons.[280,281] Unlike glycoprotein hormone receptors, GnRH receptors lack an intracellular C-terminal domain.[282,283] By contrast to most GPCRs, the GnRH receptor is coupled to G_q/G_{11} and hence ligand-binding leads to stimulation of phospholipase C and not adenylyl cyclase.[284] Phospholipase C cleaves phosphatidylinositol-4,5-diphosphate (PIP2) to inositol 1,4,5-triphosphate (IP3) and diacylglycerol, leading to increased protein kinase C (PKC) activity.[285,286]

Some patients with idiopathic hypogonadotropic hypogonadism (IHH) are homozygous or compound heterozygous for loss-of-function mutations in the GnRH receptor gene.[287-289] Unlike patients with Kallmann syndrome, they have a normal sense of smell.[287-289] GnRH

receptor mutations that cause IHH result in decreased binding of GnRH or impaired GnRH receptor signal transduction, or decreased GnRH receptor cell membrane expression due to misrouting of GnRH receptor oligomers from the endoplasmic reticulum.[40,41,287-289] Some mutations, including E90K, L266R and S168R, that cause misfolding and retention within the endoplasmic reticulum exhibit a dominant negative effect due to retention of wild-type receptors.[290]

Female patients with mutations that partially compromise GnRH receptor function may present with primary amenorrhea and infertility associated with a normal or small uterus and small ovaries with immature follicles.[287,291] Males with the same mutations may present with incomplete hypogonadotropic hypogonadism (characterized by a delayed and incomplete puberty) or with complete hypogonadotropic hypogonadism (characterized by absent puberty).[287,291]

Some patients with IHH due to mutated GnRH receptors have partial or normal gonadotropin responses to exogenous GnRH.[287,291] However, decreased amplitude in the pulsatile LH secretion can be observed in these patients.[287] Females with a partial or normal gonadotropin response to exogenous GnRH are more likely than nonresponders to become fertile in response to pulsatile exogenous GnRH. [287,292]

Activating mutations of the GnRH receptor have not been described in the germline or in pituitary adenomas.[293]

The Thyrotropin-Releasing Hormone and Secretagogue Receptor Group

Thyrotropin-Releasing Hormone Receptors

Like the GnRH receptor, TRH receptor activation leads to increased phospholipase C activity.[294] To date, only inactivating mutations that cause endocrine dysfunction have been reported for the TRH receptor. One patient was identified with central hypothyroidism due to mutated TRH receptors.[295] He presented during the ninth year of life with short stature (−2.6 SD) accompanied by a delayed bone age (−4.1 SD), a low plasma thyroxine level, and a normal plasma TSH level. Exogenous TRH did not induce an increase in plasma TSH and prolactin levels. He was found to be compound heterozygous for TRH receptor gene mutations, resulting in receptors that failed to bind TRH or induce IP3 production. Another family was identified with complete resistance to TRH due to a nonsense mutation in the TRHR (p.R17X) producing a TRH receptor that lacked the entire transmembrane domain.[296] The proband was homozygous and presented with short stature, growth failure, and fatigue at age 11. He had a low free T4 with a low-normal TSH. TRH stimulation testing failed to stimulate TSH or prolactin. Surprisingly his 33 year-old sister who was also homozygous had escaped detection despite two normal pregnancies brought to term. She had no signs or symptoms of hypothyroidism but exhibited thyroid function tests similar to the proband. She breastfed normally. Both the proband and his sister had normal cognitive function. This report suggested that the TRH receptor is not essential for normal cognitive function or female fertility and lactation. The mouse model corroborates these findings.[297]

Other Class A Receptors That Transduce Hormone Action

Free Fatty Acid Receptor 1

At the time a new GPCR is discovered, the ligand for the newly discovered receptor is often unknown. Thus, until a specific ligand is discovered, these GPCRs are known as orphan receptors. According to the Human Genome Organization (HUGO) Gene Nomenclature Committee, these G protein–coupled orphan receptors should be named alphanumerically GPR followed by a number until their ligand is known. Once a specific ligand is identified, a more specific name is given the receptor.

The ligands for GPR40 were unknown when the receptor was first discovered. The HUGO Gene Nomenclature Committee changed the name of the receptor to free fatty acid receptor 1 (FFAR1) when the ligands were identified as medium- and long-chain fatty acids. With rare exceptions that are clearly identified, this chapter follows HUGO Gene Nomenclature Committee recommendations (see www.gene.ucl.ac.uk/nomenclature/index.html for more information on receptor nomenclature).

FFAR1 is one of several GRCRs for lipid mediators. Lipid mediators are intercellular lipid messengers that include sphingosine 1-phosphate, sphingosylphosphorylcholine, dioleoyl phosphatidic acid, lysophosphatidic acid, eicosatetraenoic acid, bile acids, and free fatty acids.[63] FFAR1 is activated by medium- and long-chain fatty acids, whereas FFAR2 (formerly known as GPR43) and FFAR3 (formerly known as GPR 41) are activated by shorter-chain fatty acids.[63] There is now evidence that FFAR1 activation by medium- and long-chain fatty acids has endocrine implications. FFAR1 is expressed in human pancreatic β-islet cells.[298] FFAR1 is involved in cholecystokinin secretion from I cells in response to fatty acids.[299] It has also been implicated in fatty acid stimulated GLP-1 and GIP secretion from L and K cells.[300] GPR120 is expressed in enteroendocrine cells and has a physiologic role in GLP-1 secretion.[301] FFAR2 and FFAR3 are expressed in adipose tissue and FFAR3 has been implicated in leptin production.[301]

Fatty acid–induced stimulation of FFAR1 in β-islet cells leads to activation of the $G\alpha_q$-phospholipase C second-messenger pathway, which in turn leads to release of calcium from the endoplasmic reticulum that augments insulin-mediated increases in intracellular calcium concentrations due to glucose-induced activation of voltage-gated calcium channels.[302-305] Because an increased intracellular calcium concentration induces insulin release, FFAR1- mediated augmentation of glucose-mediated increases in the intracellular calcium concentration leads to amplification of glucose-stimulated insulin release. [302-305]

An identified variant in FFAR1 (Gly180Ser), found in a Sicilian population, resulted in obesity, impaired glucose, and lipid stimulated insulin secretion.[306] Two other variants, Arg211His and Asp175Asn, are not associated

with alterations in insulin release.[306,307] TAK-875, an FFAR1 agonist, was shown to reduce hemoglobin A1c in patients with type 2 diabetes in a phase 2 clinical trial.[308] Wild-type mice placed on an 8-week high-fat diet develop glucose intolerance, insulin resistance, hypertriglyceridemia, and hepatic steatosis—whereas FFAR1 knockout mice on the same diet do not develop these conditions.[309] The clinical relevance for patients is not yet clear. However, an Arg211His polymorphism in the FFAR1 gene may explain some of the variation in insulin secretory capacity found in Japanese men: Arg/Arg homozygotes had lower serum insulin levels, homeostasis model of insulin resistance, and homeostasis model of beta-cell function than His/His homozygotes.[310]

KISS1 Receptor/GPR54

Homozygous inactivating mutations in the gene encoding the KISS1 receptor (GPR54) were initially described in French and Saudi Arabian patients with IHH; in both cases the affected subjects came from consanguineous families.[311-314] The Saudi patients carried a Leu148Ser mutation, whereas the French patients carried a 155bp deletion. Leu148 is highly conserved among class A GPCRs.[315] The mutation does not affect expression, ligand binding, or association with G_s but impairs ligand-induced catalytic activation of G_s.[315] At the same time, an African American patient with IHH was described who was compound heterozygous for inactivating GPR54 mutations.[313] Since publication of these initial reports, additional patients have been described. A boy with a Jamaican father and a Turkish-Cypriot mother, and with cryptorchidism and micropenis at birth and undetectable LH and FSH levels at 2 months of age, was found to have compound heterozygous GPR54 mutations.[316] Another missense mutation (Leu102Pro) that exhibits complete inactivation of GPR54 signaling has been identified.[317] Surprisingly, patients with this mutation exhibited spontaneous pulsatile LH and FSH secretion with normal frequency and a blunted amplitude and family members had partial pubertal development.[317]

Unlike patients with Kallmann syndrome, patients with GPR54 mutations have an intact sense of smell. Furthermore, in contrast to patients with IHH due to GnRH mutations, patients with GPR54 mutations increase serum gonadotropin levels in response to exogenous GnRH. Thus, biallelic loss of function mutations in GPR54 are a rare cause of normosmic IHH.[314,316]

Ligands for GPR54 derive from a single precursor protein, kisspeptin-1.[318,319] The longest derivative protein that acts as a ligand for GPR54 is metastin, so called because it is a metastasis suppressor gene in melanoma cells.[318] Metastin consists of kisspeptin-1 69-121.[318,319] However, shorter C-terminal peptides derived from kisspeptin-1 bind and activate GPR54.[318] Administration of metastin to adult male volunteers increases LH, FSH, and testosterone levels.[320] Studies in animal models suggests that Kiss1 expressing neurons in the hypothalamus modulate GnRH expressing neurons to initiate puberty and modulate sex steroid feedback on GnRH release.[314,321,]

An activating mutation in GPR54 was identified in a patient with central precocious puberty.[322] The adopted girl was found to have an Arg386Pro mutation, which led to prolonged activation of signaling in response to Kisspeptin.

Orexin Receptors

Orexins act on orexin receptors, located predominantly in the hypothalamus, to control food intake and play a role in the regulation of sleep/wakefulness.[323-325] There are two types of orexin receptors: the orexin-1 and the orexin-2 receptors.[325] There are also two types of orexins, orexin A and orexin B, formed from the precursor peptide preproorexin.[325] Orexins are also known as hypocretins, and orexin A is synonymous with hypocretin-1 and orexin B with hypocretin-2.[324,325] Orexin A acts on orexin-1 and orexin-2 receptors, whereas orexin B only acts on orexin-2 receptors.[323,326,327]

Like most class A GPCRs, orexin receptors couple with $G_{q/11}$ and G_i/G_o to activate phospholipase C and inhibit adenylyl cyclase, respectively.[325,328-330] Surprisingly, evidence suggests that orexin receptors also couple with G_s—which increases adenylyl cyclase activity.[330] Orexins increase food intake and duration of wakefulness.[323,324,331] Orexin A and activation of the orexin-1 receptor have greater orexigenic effects than orexin B and activation of the orexin-2 receptor.[332] The orexin-2 receptor mediates the arousal effect of orexins.[332] Most patients with narcolepsy with cataplexy have diminished levels of orexin A concentrations in cerebral spinal fluid and lack orexin-containing neurons.[324,333-336] This is thought to be due to postnatal cell death of orexin neurons in the hypothalamus.[337] HLA DQB1*0602 is associated with narcolepsy with cataplexy and an autoimmune process has been suggested but no autoantibodies have been identified. Thus far, no mutations in orexin receptors have been found in humans. A mutation in the orexin-2 receptor causes narcolepsy in dogs.[338] There is one described mutation (leu16ARG) in the HCRT gene in a child with early onset narcolepsy with cataplexy.[339] This mutation was shown to impair processing and trafficking of the mutant orexin leading to undetectable orexin A concentrations in the CSF.

Ghrelin Receptors

Another class A receptor that transduces hormone action is the ghrelin receptor. The receptor is also known as the growth hormone secretagogue receptor type 1a because the receptor is also activated by a family of synthetic growth hormone secretagogues.[105,332] Ghrelin is a product of posttranslational modification of the ghrelin gene product proghrelin.[340] Ghrelin is mainly produced in the stomach.[341,342] Activation of ghrelin receptors located in the hypothalamus and pituitary somatotrophs enhances growth hormone secretion.[343] The ghrelin receptor also has an orexigenic role.

Plasma ghrelin levels are elevated just prior to eating and decrease rapidly after eating.[344,345] In addition, intravenous administration of ghrelin to humans increases appetite and food intake.[346] Plasma ghrelin levels are

elevated in individuals with Prader Willi syndrome.[347] Thus, hyperphagia in patients with Prader Willi syndrome may be due at least in part to overactivation of ghrelin receptors by ghrelin. Screening of 184 extremely obese children and adolescents for mutations of the ghrelin receptor gene failed to identify a single mutation likely to cause obesity.[348]

Short individuals in two unrelated Moroccan kindreds were found to have a C to A transversion at position 611 in the first exon of the ghrelin receptor gene.[349] This transversion results in replacement of the apolar and neutral amino acid alanine at position 204 of the receptor by the polar and charged amino acid glutamate. This mutation interferes with normal constitutive activity of the receptor and decreases cell membrane expression of the receptor. Receptor activation by ghrelin, however, is preserved. Two thirds of individuals in the kindreds heterozygous for the mutation had height greater than or equal to 2 standard deviations below the mean. One heterozygous individual's height was 3.7 standard deviations below the mean. Prior to onset of growth hormone therapy, the only individual in the kindreds homozygous for the mutation had a height 3.7 standard deviations below the mean. Surprisingly, the patient homozygous for the mutation became overweight during puberty. The weight of the patients heterozygous for the mutation varied from underweight to overweight. Another patient who presented with severe short stature (−3 SD), vomiting, ketosis, hypoglycemia, and low BMI was identified to be a compound heterozygote for a W2X and an R237W mutation in the ghrelin receptor.[350] His IGF1 was low at 44 ng/mL and he failed GH stimulation testing and had a normal IGF1 generation test, and he had improvement in his height velocity and hypoglycemia in response to growth hormone treatment. Four more ghrelin receptor mutations were identified in a Japanese cohort with short stature (△Q36, P108L, C173R, and D246A).[351] △Q36 showed a minor reduction in activity. C173R led to intracellular retention. D246 caused impaired signaling and P108L led to reduced binding affinity to ghrelin. Two other mutations were identified in a cohort of patients with constitutional delay of growth and puberty in Brazil (Ser84Ile and Val182Ala).[352] Both resulted in decreased basal activity. The patients were short at presentation (−2.4 and −2.3 SD) but reached a normal adult height without treatment.

Another product of posttranslational modification of proghrelin, obestatin, appears to play a role in controlling appetite and weight.[340] Activation of the obestatin receptor, previously known as GPR39, in rats results in decreased food intake and weight.

Melanin-Concentrating Hormone Receptors

Formerly known as SLC-1 or GPR24, the type 1 melanin-concentrating hormone (MCH) receptor (MCHR1)—and the more recently discovered type 2 MCH receptor (MCHR2), formerly known as SLT or GPR145—may play a role in regulating feeding and energy metabolism in humans.[353-356] When activated, MCHR1 couples with $G_{q/11}$ and $G_{i/o}$ to increase phospholipase C activity and inhibit adenylyl cyclase activity, respectively.[353,354,357] MCHR2 couples with $G_{q/11}$, and MCH binding leads to increased phospholipase C activity.[355,356]

Studies in rodents reveal that MCH is an orexigenic hormone, and treatment of rodents with MCHR1 antagonists decreases food intake, weight, and body fat.[353,358,359,360,361] MCHR2 is not expressed in rodents.[360] Two loss-of-function mutations were identified in MCHR1 in humans (R210H and P377S).[362] Cells transfected with either mutant receptor failed to respond to MCH despite normal cell surface expression of the receptor, suggesting a receptor activation defect. These mutations were identified in two markedly underweight individuals and were not found in an obese cohort.[362,363] Analysis of the MCHR1 gene in more than 4000 obese German, Danish, French, and American children and adolescents revealed several single-nucleotide polymorphisms and gene variations in the German children and adolescents that may be associated with obesity.[364] Another study of 106 American subjects with early-onset obesity failed to definitively identify MCHR1 and MCHR2 mutations as a cause of obesity.[365]

CLASS B RECEPTORS THAT TRANSDUCE HORMONE ACTION

Growth Hormone–Releasing Hormone Receptor

The growth hormone–releasing (GHRH) receptor gene is located at 7p14.[366] GHRH receptors interact with G_s to stimulate adenylyl cyclase, resulting in increased intracellular cAMP levels that lead to somatotroph proliferation and growth hormone secretion.[367] Thus, it is not surprising that activating mutations in $G\alpha_s$ leading to constitutive activation of adenylyl cyclase have been found in some growth hormone–secreting pituitary adenomas in humans.[368]

Many mutations in the GHRH receptor that cause isolated growth hormone deficiency have been identified. These include 6 splice site mutations, 2 microdeletions, 2 nonsense mutations, 1 frameshift mutation, 10 missense mutations, and 1 mutation in the promoter.[369-372] The first naturally occurring mutation in the GHRH receptor (D60G) was found in the *little* mouse, which has a dwarf phenotype.[373,374] This mutation in a conserved amino acid in the extracellular domain impairs the ability to bind GHRH.[375] The first human mutation in the GHRH receptor (E72X) was identified in a consanguineous Indian family.[367] The same mutation was found in three apparently unrelated consanguineous kindreds from India, Pakistan, and Sri Lanka.[366,376,377] A different mutation (5′ splice site mutation in intron 1) was identified in a large Brazilian kindred of more than 100 individuals.[367] Both mutations result in the production of markedly truncated proteins with no receptor activity.[367,377] A frameshift mutation was identified in a patient with severe short stature and was the first documented case of early onset anterior pituitary hypoplasia.[371] In another family, two siblings with isolated growth hormone deficiency were found to be compound heterozygous for inactivating GHRH receptor gene mutations.[378]

Three more novel mutations were identified in families with severe short stature in the United Kingdom (W273S, R94L, and R162W).[369] The only mutation found in the promoter region of GHRH affects one of the Pit-1 binding sites.[379]

Studies of subjects in these large kindreds who are homozygous or compound heterozygous for inactivating GHRH receptor gene mutations have shown that affected children experience severe postnatal growth failure with proportionate short stature.[366,367,377,378] Males have high-pitched voices and moderately delayed puberty.[366,367,377] Unlike infants with complete growth hormone deficiency, they do not have frontal bossing, microphallus, or hypoglycemia.[366,377,378] Bone age is delayed with respect to chronologic age but advanced with respect to height age.[377] Some patients have been found to have pituitary hypoplasia.[366,377] Growth velocity increased with exogenous growth hormone therapy.[366,367,377,378] Remarkably, growth hormone treatment of two siblings from Turkey with the E72X mutation allowed them to reach a normal adult height despite pretreatment heights of −6.7 and –8.6 SD and initiation of growth hormone around age 14.[380]

Further studies in the Brazilian cohort revealed that homozygotes had increased abdominal obesity, a higher low-density lipoprotein (LDL), and total cholesterol but normal carotid wall thickness and no evidence of premature atherosclerosis.[381] Treatment of these patients with GH for 6 months improved body composition, reduced LDL and total cholesterol, and increased high-density lipoprotein (HDL). Surprisingly this was associated with increased carotid intima-media thickness and atherosclerotic plaques.[382] Reevaluation 5 years after the discontinuation of growth hormone showed a return to baseline for these measures.[383] Heterozygotes for the null mutation had normal adult heights and IGF1 SD scores but exhibited reduced body weight, body mass index (BMI), lean mass, fat mass, and increased insulin sensitivity.[384]

SNPs in the GHRH receptor have been shown to contribute to height-SDS variation,[385,386] but mutations remain a rare cause of isolated growth hormone deficiency.[387]

Gastric Inhibitory Polypeptide Receptors

The gastric inhibitory peptide receptor (GIPR) gene is located on the long arm of chromosome 19.[388] Two functional isoforms exist in humans due to alternate splicing.[389] GIPR activation induces $G\alpha_s$ activation of adenylyl cyclase.[389-391] Gastric inhibitory polypeptide (GIP) is also known as glucose-dependent insulinotropic polypeptide and is released by K cells in the small intestine in response to food. GIP has numerous physiologic actions, including stimulation of glucagon, somatostatin, and insulin release by pancreatic islet cells.[392,393] Human mutations in the GIP receptor have not been identified to date. One study identified a SNP in the GIPR that is associated with insulin resistance in obese German children.[394] Another study identified an SNP in the GIPR that is associated with reduced fasting and induced C-peptide levels.[395]

The GIPR is implicated in food-dependent Cushing syndrome. Circulating cortisol levels in patients with food-dependent or GIP-dependent Cushing syndrome rise abnormally in response to food intake.[94,95] GIP does not normally induce cortisol release from adrenocortical cells.[396,397] These patients may have adrenal adenomas or nodular bilateral adrenal hyperplasia that overexpresses GIPRs that abnormally stimulate cortisol secretion when activated.[396,398] Thus, in these patients postprandial GIP release leads to activation of these abnormally expressed and functioning adrenal GIPRs, resulting in excessive adrenal cortisol secretion.[396,397]

Parathyroid Hormone and Parathyroid Hormone–Related Peptide Receptors

Two types of parathyroid hormone (PTH) receptors have been identified. The type 1 PTH receptor (PTHR1) is activated by PTH and parathyroid hormone–related peptide (PTHrP) and mediates PTH effects in bone and kidney.[399] In spite of 51% homology to the PTHR1, the type 2 PTH receptor (PTHR2) is activated by PTH but not PTHrP.[399-402] The PTHR2 is particularly abundant in the brain and pancreas but is also expressed in the growth plate; its natural ligand is TIP39.[403-405] The function of the PTHR2 is largely unknown.[399,400]

The PTHR1 has a large aminoterminal extracellular domain containing six conserved cysteine residues.[399] Ligand binding induces the PTHR1 to interact with G_s and G_q proteins, leading to activation of the adenylyl cyclase/protein kinase A and phospholipase C/protein kinase C second-messenger pathways, respectively.[406-411] Interestingly, mutations in i2 interfere with coupling of the PTHR1 to G_q without interfering with coupling to G_s—whereas mutations in i3 disrupt coupling of the receptor to both G proteins.[412,413] Binding of PTH to the PTHR1 leads to internalization of a portion of plasma membrane containing a ternary complex of activated receptor-Gsα-adenylyl cyclase that exhibits sustained production of cyclic AMP.[414] By contrast, PTHrP binding to the PTHR1 receptor leads to formation of a complex that remains on the cell surface and generates cyclic AMP for only a short period of time.[414]

Biallelic loss-of-function mutations in the PTHR1 gene cause Blomstrand chondrodysplasia.[399] This lethal disorder is characterized by accelerated chondrocyte differentiation, resulting in short-limbed dwarfism, mandibular hypoplasia, lack of breast and nipple development, and severely impacted teeth.[399,415] One patient with this rare condition was found to be homozygous for a point mutation that resulted in a Pro132Leu substitution in the N-terminal domain that interferes with ligand binding.[416,417] Another patient was found to be homozygous for a frameshift mutation that results in a truncated receptor lacking TM5-7, and contiguous intracellular and extracellular domains.[418]

A third patient was found to have a maternally inherited mutation that altered splicing of maternal mRNA, resulting in a PTHR1 with a deletion of residues 373 through 383 in TM5 (which also interferes with ligand binding).[419] In spite of heterozygosity for the mutation, the patient was unable to produce normal PTHR1s because for unknown reasons the paternal allele was not expressed.[419] Heterozygosity for somatic or germline Arg150Cys missense mutations in the PTHR1 was

identified in two out of six patients with enchondromatosis,[420] a condition that is usually sporadic and which is attributed to a postzygotic somatic cell mutation. Enchondromas are benign cartilage tumors that develop in the metaphyses and may become incorporated into the diaphyses of long tubular bones, in close proximity to growth plate cartilage; there is an increased risk of malignant transformation to osteosarcoma.[16] Patients with multiple enchondromatosis (OMIM ID: 166000) have Ollier disease (World Health Organization terminology), a disorder characterized by the presence multiple enchondromas with an asymmetric distribution of lesions that vary in size, number, and location. When multiple enchondromatosis occurs with soft tissue hemangiomas, the disorder is known as Maffucci syndrome. In vitro studies showed that the Arg150Cys mutation was mildly activating but led to stimulation of phospholipase C rather than adenylyl cyclase.[420] A transgenic knockin mouse expressing the mutant PTHR1 under the control of the collagen type 2 promoter showed development of tumors that are similar to those observed in human enchondromatosis.[420] The clinical significance of these observations are uncertain, as most cases of enchondromatosis are caused by mutations in the IDH1 and IDH2 genes.[421,422]

Some cases of Jansen metaphyseal chondrodysplasia have been found to be caused by constitutively activating mutations of the PTHR1 gene.[423-425] This autosomal-dominant disorder is characterized by short-limbed dwarfism due to impaired terminal chondrocyte differentiation and delayed mineralization accompanied by hypercalcemia.[423,424] Interestingly, constitutive activation appears to result predominantly in excessive $G\alpha_s$ activity because adenylyl cyclase activity is increased and PLC activity is unchanged in COS-7 cells expressing mutated receptors.[423-425]

Other Class B Receptors That Transduce Hormone Action

Other class B receptors that transduce hormone action include glucagon-like peptide-1, glucagon, calcitonin, and corticotrophin-releasing factor receptors.[426] Class B receptors usually couple with heterotrimeric G_s proteins, leading to the activation of adenylyl cyclase—which in turn leads to elevated intracellular cAMP levels.[426,427] (See Chapter 19 for a discussion of the role of GLP1 in promoting insulin secretion and the use of GLP1 analogues or inhibitors of GLP1 breakdown in therapy.)

CLASS C RECEPTORS THAT TRANSDUCE HORMONE ACTION

Calcium-Sensing Receptors

The calcium-sensing receptor (CaSR) is located on the long arm of chromosome 3 (3q21.1).[428] The CaSR has a large aminoterminal domain that contains nine potential glycosylation sites.[429] Binding of ionized calcium to the CaSR leads to activation of phospholipase C via activation of $G_{q/11}$ proteins.[429,430]

The CaSR is an integral component of a feedback system that utilizes PTH and renal tubular calcium reabsorption to keep the serum concentrations of ionized calcium within a narrow physiologic range.[431] Increased extracellular ionized calcium concentrations activate CaSRs in parathyroid chief and renal tubular epithelial cells, leading to decreased PTH release and renal tubular calcium reabsorption.[429,432] When ionized calcium concentrations fall, CaSR activation decreases—leading to increased PTH release and enhanced renal tubular calcium reabsorption.[429,432]

The CaSR also binds magnesium, and thus PTH secretion can be inhibited by elevated serum concentrations of magnesium with consequent hypocalcemia.[433] The CaSR may participate in magnesium homeostasis by altering reabsorption of magnesium in the thick ascending limb of Henle in the kidneys.[434,435] It is probable that increased peritubular levels of magnesium activate renal CaSRs, leading to inhibition of reabsorption of magnesium from the thick ascending limb of Henle—which in turn leads to increased renal excretion of magnesium.[434,435]

Familial (benign) hypocalciuric hypercalcemia (FBH or FHH) and neonatal severe hyperparathyroidism (NSHPT) are caused by loss-of-function mutations of the CaSR gene.[436,437] Most of these mutations are located in the N-terminal extracellular domain.[438,439] With few exceptions, individuals heterozygous for loss-of-function mutations have FBH, a benign condition characterized by very low urinary calcium excretion, mild hypercalcemia, normal or slightly elevated serum PTH levels, and few if any symptoms of hypercalcemia or hyperparathyroidism. By contrast, individuals homozygous for such mutations will develop NSHPT, a life-threatening condition characterized by severe hypercalcemia, markedly elevated serum PTH levels and skeletal defects.[440,441] Therefore, children of consanguineous FBH parents are at risk for NSHPT.[438,442,443] Occasionally, infants with NSHPT are heterozygous for CaSR gene mutations.[444] In most cases FHH is transmitted in an autosomal dominant manner, but autosomal recessive inheritance has been described in one kindred in which the CaSR mutation was only weakly inactivating.[445]

Decreased CaSR function impedes calcium ion suppression of PTH release and renal tubular calcium reabsorption.[437] Thus, FBH is characterized by mild hypercalcemia that is accompanied by inappropriately normal or elevated serum PTH levels and by relatively low urinary calcium excretion.[440,446,447] Individuals with FBH may also have hypermagnesemia as a result of decreased peritubular inhibition of magnesium reabsorption from the thick ascending loops of the kidneys by the CaSR.[435] There are three type of FBH: FBH type 1 (FBH1), FBH type 2 (FBH2), and FBH type 3 (FBH3).[448]

FBH1 is due to heterozygous loss-of-function mutations of the CaSR gene on 3q21.1.[448] Two other chromosomal loci have been identified in patients with FBH who do not have CaSR gene mutations. FBH2 has been mapped to 19p13.3 and is biochemically and clinically similar to FBH1.[448,449] FBH3, which is also known as the Oklahoma variant (FBH_{OK}), has been mapped to 19q13.[448,450] Adults with FBH3 have hypophosphatemia, elevated serum PTH levels, and osteomalacia in addition to the clinical and biochemical findings found in individuals with FBH1 and FBH2.[448,451] NSHPT is characterized

by severe hypercalcemia accompanied by elevated circulating PTH levels, undermineralization of bone, rib cage deformity, and multiple long-bone and rib fractures.[440]

Activating mutations of the CaSR gene cause autosomal-dominant hypocalcemic hypercalciuria (ADHH), also known as autosomal dominant hypocalcemia, as increased CaSR function leads to increased calcium ion suppression of PTH release and suppression of renal tubular calcium reabsorption.[430,448,452-456] ADHH is characterized by hypocalcemia and hypomagnesemia accompanied by inappropriately normal or increased urinary calcium excretion and inappropriately normal or low serum PTH levels.[430,454-456] Patients with ADHH may be asymptomatic or may present with tetany, muscle cramps, or seizures during infancy or childhood.[454-457] Similar to inactivating mutations, most activating mutations are located in the N-terminal extracellular domain.[452,454,455,457] Treatment of patients with ADHH with activated forms of vitamin D (e.g., calcitriol) is complicated as normalization of serum calcium levels is associated with worsening of hypercalciuria, hence further increasing the risk of nephrolithiasis, in nephrocalcinosis, and in renal impairment.[454,455]

Some patients with activating mutations of the CaSR gene will develop Bartter syndrome type V, which similar to other types of Bartter syndrome, is characterized by hypokalemic metabolic alkalosis and by hyperaldosteronism due to elevated renin levels.[458,459] Patients with Bartter syndrome type V, unlike patients with other types of Bartter syndrome, may also have symptomatic hypocalcemia and are at risk for developing nephrocalcinosis due to hypercalciuria.[458,459] Evidence from in vitro functional expression studies suggests that patients with mild or moderate heterozygous gain-of-function mutations of the CaSR develop ADHH, whereas those with severe heterozygous gain-of-function mutations of the CaSR will also develop Bartter syndrome type V.[458-460]

Some single–amino-acid polymorphisms of the CaSR gene appear to predict whole-blood ionized and serum total calcium levels and may increase the risk for bone and mineral metabolism disorders in individuals with other genetic and environmental risk factors for these disorders.[461-463] Individuals heterozygous or homozygous for a Gln1011Glu CaSR gene polymorphism tend to have higher calcium levels than individuals with the polymorphism.[463,464] The 15.4% of 387 healthy young Canadian women with at least one CaSR gene allele with an Ala986Ser polymorphism were found to have higher total calcium levels than the remainder of the women without the polymorphism.[462]

Another study of 377 unrelated healthy Italian adult males and females found that 24% of study subjects were heterozygous or homozygous for the Ala986Ser polymorphism and confirmed the finding that individuals without the polymorphism have lower whole-blood ionized calcium levels than individuals with the polymorphism.[463] The Ala986Ser polymorphism has also been associated with Paget disease and primary hyperparathyroidism.[464-466] Individuals with a less common Arg990Gly polymorphism tend to have lower whole-blood ionized calcium levels than individuals without the polymorphism.[463] The Arg990Gly polymorphism has been found to be associated with hypercalciuria and nephrolithiasis.[466,467]

Auto-antibodies against the CaSR that interfere with binding of calcium to the receptor may cause autoimmune hypocalciuric hypercalcemia.[468] These patients have primary hyperparathyroidism with the clinical and biochemical features of patients with FBH.[468] Conversely, auto-antibodies that activate the CaSR are a cause of autoimmune-acquired hypoparathyroidism.[469] Both conditions may occur in association with other autoimmune conditions (such as autoimmune thyroiditis), with celiac disease in patients with autoimmune hypocalciuric hypercalcemia, and with autoimmune thyroiditis and autoimmune polyglandular syndrome types 1 and 2 in patients with autoimmune-acquired hypoparathyroidism.[468-470] Auto-antibodies that activate the CaSR were found in approximately one third of individuals with acquired hypoparathyroidism.[470]

G PROTEIN GENE DISORDERS

A growing number of human disorders are associated with somatic or germline mutations in genes that encode the subunits of G proteins and lead to either a gain of function or a loss of function in the signaling protein.[471] Here we will limit discussion to disorders associated with mutations of the *GNAS* gene that encodes $G\alpha_s$, as these mutations are the principal causes of endocrine disorders.

Inactivating Mutations of the *GNAS* Gene

Pseudohypoparathyroidism type 1a (PHP-1a; Albright hereditary osteodystrophy) and pseudopseudohypoparathyroidism (PPHP) are caused by heterozygous inactivating mutations of the GNAS gene that encodes $G\alpha_s$.[472-477] PHP-1a is characterized by end organ resistance to PTH with consequent hypocalcemia, hyperphosphatemia, and elevated circulating PTH levels. In addition, patients also manifest GHRH and TSH resistance as well as obesity and mildly impaired neurocognitive development. PHP Ia patients also show a constellation of developmental defects that have been termed Albright hereditary osteodystrophy (AHO) and which include heterotopic ossifications, short stature, mental retardation, brachydactyly of the hands and feet characterized by shortened fingers and short fourth and fifth metacarpals.[472-475] Patients with the associated condition PPHP have the same features of AHO as PHP-1a, but with the absence of hormone resistance to PTH.[472,473,476] The distinction between these two manifestations of the same gene defect is not stochastic but results from a complex mechanism of genomic imprinting that controls transcription of the GNAS gene. Hence, patients with a GNAS mutation on a maternal allele will develop a more severe form of Gsα deficiency with hormone resistance (i.e., PHP-1a), whereas patients with identical mutations on the paternal GNAS allele will have a milder condition with normal hormone responsiveness (i.e., PPHP).[472-476]

Although most cells express Gsα from both parental alleles, in some cells Gsα expression is suppressed from the paternal GNAS allele. Thus, patients with PHP Ia

develop hormone resistance that is limited to the thyroid, pituitary somatotrophs, and proximal renal tubule cells because in these cells $G\alpha_s$ is derived principally from the maternal GNAS allele.[478] Thus, patients with maternally inherited inactivating GNAS gene mutations express very little $G\alpha_s$ in these cells and develop hormone resistance.[478] Because Gsα is not expressed from the paternal allele in imprinted tissues, PPHP patients with paternally inherited inactivating GNAS gene mutations do not experience a deficit in Gsα protein in these cells, as they will have a normal amount of Gsα protein that is produced from the wild-type maternal GNAS allele.[478] Patients with either PHP Ia or PPHP express only 50% of the normal amount of Gsα protein in cells in which Gsα transcription is not controlled by the imprinting mechanism, which likely accounts for the similar features of AHO that occur in these two conditions. Subjects with paternally inherited GNAS mutations have variable features of AHO without hormonal resistance and have been described to have pseudopseudohypoparathyroidism (PPHP), progressive osseous heteroplasia (POH),[479] or osteoma cutis[480-482] based on the clinical phenotype. The basis for these distinctions is unknown.

Subjects with PHP type 1b (PHP Ib; MIM 603233) lack typical features of AHO but may have mild brachydactyly. PTH resistance is the principal manifestation of hormone resistance, but some patients have slightly elevated serum levels of TSH and normal serum concentrations of thyroid hormones as evidence of partial TSH resistance. It was initially thought that PHP-1b is caused by inactivating mutations in the PTHR1 gene.[483] However, no deleterious PTHR1 gene mutations have been found in patients with PHP-1b.[484,485]

Epigenetic defects in imprinting of GNAS are the cause of PHP-1b. There are three alternative first exons for GNAS (i.e., NESP55, XLαs, and exon A/B) that splice onto exons 2 through 13 and are associated with differentially methylated regions (DMRs). Most relevantly, the exon A/B DMR is methylated in the maternal germ cells and seems to be the principal control element for transcription from exon 1.[478,486] Loss of methylation in the DMR upstream of exon A/B of the maternal allele is a consistent finding in patients with PHP 1b, and this epigenetic defect accounts for decreased Gαs expression from the affected allele.[487-489] Most cases of autosomal dominant PHP 1b are caused by microdeletions on the maternal allele that include exons 3 to 5 or 4 to 6 of the gene encoding syntaxin-16 (STX16) (reviewed in the journal Current Molecular Medicine[487]). Three other maternally inherited microdeletions involving NESP55 and AS (delNESP55/ASdel3-4) or AS (delAS3-4) alone[490] have been identified, and these deletions produce a more global disruption of methylation that includes three GNAS DMRs (A/B, XL/AS, and NESP55). The genetic basis for most cases of sporadic PHP 1b remains unknown and does not appear to be associated with the GNAS locus.[491] These patients have global epigenetic defects in methylation that affect all three DMRs. In some cases, partial[491] or complete[492] paternal uniparental disomy (UPD) for chromosome 20 has been identified, in which two normal copies of GNAS are both derived from the father. Paternal UPD would predict a near complete deficiency of Gαs in imprinted cells and tissues in which Gαs is not transcribed from the paternal allele.

$G\alpha_s$ is not expressed in the renal proximal tubules of these patients due to lack of a GNAS allele with a maternal epigenotype. By contrast, both of the GNAS alleles with paternal epigenotypes are normally expressed in nonimprinted cells in which expression of Gsα from the paternal allele is not suppressed.[493]

Activating Mutations of the GNAS Gene

When a GPCR is activated by a ligand, the activated receptor interacts with the heterotrimeric G protein and allows release of bound GDP from the Gα subunit with replacement by GTP. The GTP-bound Gα dissociates from the Gβγ dimer, and both complexes are free to associate with downstream signal generating molecules.[6,7,9] The generation of second messengers is terminated by an intrinsic GTPase within the Gα subunit that acts as a timer; hydrolysis of GTP to GDP inactivates Gα and increases its affinity for Gβγ—leading to reassociation of an inactive heterotrimeric G protein that is ready for another cycle of receptor-induced activation.[6,7,9] For Gsα, the amino acids Arg^{200} and Gln^{226} are critical to GTPase activity.[493] GNAS mutations that result in substitutions of these amino acid residues lead to abrogation of GTPase activity and therefore prolong the active state of $G\alpha_s$ thereby leading to constitutive (i.e., receptor ligand-independent) stimulation of adenylyl cyclase.[493] Somatic mutations of these residues that disrupt GTPase activity are present in approximately 40% of growth-hormone–secreting and some ACTH-secreting and nonsecreting pituitary tumors; in some parathyroid, ovarian, testicular, thyroid, and adrenal tumors; and in some intramuscular myxomas.[92,494,495]

More widespread mosaic Arg^{200} $G\alpha_s$ mutations that decrease GTPase activity cause fibrous dysplasia or (when tissue distribution of the mutation is very widespread) McCune-Albright syndrome, which is characterized by the triad of café-au-lait spots, polyostotic fibrous dysplasia, and primary endocrine hyperfunction, particularly gonadotropin-independent precocious puberty.[496-500]

Patients with McCune-Albright syndrome may also have excessive growth hormone production, hyperthyroidism, and hypercortisolism, as well as nodularity of the pituitary, thyroid, and adrenal glands due to the growth-promoting effects of excessive cyclic AMP in these tissues.[497,501] Hypophosphatemia, which is not uncommon in patients with McCune-Albright syndrome, appears to be due to excessive production of the phosphatonin FGF-23 by fibrous dysplasia skeletal lesions.[502,503] Patients with McCune-Albright syndrome may also have nonendocrine problems such as hepatobiliary abnormalities, cardiomyopathy, optic neuropathy, and sudden death.[501,504,505]

CYTOKINE RECEPTORS

Cytokines are molecules produced by one cell that act on another cell.[506] Thus, the term *cytokine* can apply not only to molecules with immunologic functions but also to

hormones. Therefore, growth hormone, prolactin, and leptin are classified as type 1 cytokines.[507] These and other type 1 cytokines (including ILs 2–9, 11–13, and 15; erythropoietin; thrombopoietin; and granulocyte-colony–stimulating factor) are characterized by a four α-helical bundle structure and signaling via type 1 cytokine receptors.[507] Type 2 cytokines include the interferons and IL-10 and do not include hormones.[507]

Type 1 cytokines are divided into long-chain and short-chain cytokines.[507] Prolactin, leptin, and growth hormone belong to the long-chain subclass of type 1 cytokines because their helixes are 25 amino acids in length.[507] The short-chain type 1 cytokines, including IL-2 and stem cell factor, have helixes of approximately 15 amino acids in length.[507]

Structure and Function of Type 1 Cytokine Receptors

All type 1 cytokine receptors have four conserved cysteine residues, fibronectin type 2 modules, a Trp-Ser-X-Trp-Ser motif in the extracellular domain, and a proline-rich Box 1/Box 2 region in the cytoplasmic domain.[507,508] With the exception of stem cell factor, type 1 cytokine receptors do not contain catalytic domains such as kinases.[507]

Type 1 cytokine receptors for long-chain type 1 cytokines require homodimerization for activation.[507,509] First, the ligand binds a monomeric receptor.[507,509] Then, the ligand interacts with a second receptor to induce receptor dimerization and activation.[507,509] Activated receptors then stimulate members of the Janus family of tyrosine kinases (Jak kinases) to phosphorylate tyrosine residues on both the kinase itself and the cytoplasmic region of the receptors.[507,510] Signal transducers and activators of transcription (STATs) then dock on the phosphorylated cytoplasmic receptor domains or Jak kinases via an SH2 domain and are tyrosine phophorylated.[507] The phosphorylated STATs then dissociate from the receptors or Jak kinases, form homo- or heterodimers, and translocate to the nucleus.[507,511,512] In the nucleus, the STAT dimers bind and alter the activity of regulatory regions of target DNA.[507,511,513]

There are four Jak kinases.[511,514] Jak3 is only expressed in lymphohematopoietic cells, whereas Jak1, Jak2, and Tyk2 are expressed in every cell.[507,515,516] There are seven STATs (Stat1, Stat2, Stat3, Stat4, Stat5a, Stat5b, and Stat6), which have different SH2 domain sequences that confer different receptor specificities.[507,510-512]

Cytokine Receptors That Transduce Hormone Action

The actions of growth hormone, prolactin, and leptin are mediated via specific type 1 cytokine receptors.[507] Mutations of the growth hormone receptor (GHR) and the leptin receptor have been identified as the bases of specific endocrine disorders (Table 3-3).

Growth Hormone Receptors

The GHR gene is located on the short arm of chromosome 5 (5p13.1-p12), and 9 of the 13 exons of the gene encode the receptor.[517-520] A secretion signal sequence is encoded by exon 2, the N-terminal extracellular ligand binding domain is encoded by exons 3 through 7, the single transmembrane domain is encoded by exon 9, and the C-terminal cytoplasmic domain is encoded by exons 9 and 10.[517-520] Growth hormone binding protein (GHBP) is produced by proteolytic cleavage of the extracellular domain of the GHR from the rest of the receptor.[521] Approximately 50% of circulating growth hormone is bound to GHBP.[521]

Binding of growth hormone to its receptor induces receptor dimerization and association with JAK2, a member of the Janus kinase family, which results in self-phosphorylation of the JAK2 and a cascade of phosphorylation of cellular proteins, including Stat1, Stat3, and Stat5.[522-526] The most critical of these proteins is STAT5b, which couples GH binding to the activation of gene expression that leads to the intracellular effects of GH, including synthesis of IGF-I, insulin-like growth factor binding protein 3 (IGFBP3), and ALS. The phosphorylated STATs translocate to the nucleus, where they regulate growth hormone–responsive genes.[523-526] In particular, growth hormone indirectly controls growth by regulating production of insulin-like growth factor-1 (IGF-1)—which has direct effects on cell proliferation and hypertrophy.[527] Jak2 also activates the mitogen activated protein (MAP) kinase and insulin receptor substrate pathways.[528-530] However, the extent to which these pathways contribute to growth hormone action is as yet unknown.[520]

Patients are considered to have growth hormone insensitivity (GHI) if they do not exhibit appropriate growth and metabolic responses to physiologic levels of growth hormone.[521] The phenotype of GHI is variable and ranges from isolated moderate postnatal growth failure to severe postnatal growth failure accompanied by

TABLE 3-3 **Cytokine Receptors and Clinical Conditions Associated with Receptor Mutations**

Receptor	Germline Mutation	Endocrine Disorder
Growth hormone receptor	Some inactivating (heterozygous)	Partial growth hormone insensitivity with mild to moderate growth failure
	Inactivating (homozygous, compound heterozygous)	Growth hormone insensitivity/Laron syndrome with moderate to severe postnatal growth failure
Leptin receptor	Inactivating (homozygous)	Obesity and hypogonadotropic hypogonadism

the classic features of Laron syndrome in Ecuadorean patients with GHR deficiency.[521,531-535] Features of GHR deficiency include frontal temporal hairline recession, prominent forehead, decreased vertical dimension of face, hypoplastic nasal bridge, shallow orbits, blue sclera, small phallus prior to puberty, crowded permanent teeth, absent third molars, small hands and feet, hypoplastic fingernails, hypomuscularity, delayed age of onset for walking, high-pitched voice, increased total and low-density lipoprotein cholesterol, and fasting hypoglycemia.[521,535] All patients with GHI have normal or elevated circulating growth hormone levels, markedly decreased circulating IGF-1 levels, and a delayed bone age.[521]

Patients homozygous or compound heterozygous for deletion of exons 5 and 6—or homozygous or compound heterozygous for numerous nonsense, missense, frameshift, and splice-point mutations throughout the GHR gene—have been found to have GHI characterized by severe postnatal growth failure and usually low or absent circulating GHBP levels.[521,536-545] Patients homozygous or compound heterozygous for the Arg274Thr or the Gly223Gly splice mutations that result in a truncated receptor that cannot be anchored to the plasma membrane (or that result in the Asp152His missense mutation that interferes with GHR dimerization) have normal circulating GHBP levels.[521]

Patients heterozygous for mutations that alter the GHR have dimerization complexes that consist of two wild-type receptors, a wild-type receptor and a mutant receptor, and two mutant receptors. Thus, heterozygosity for loss-of-function GHR gene mutations may have a dominant negative effect because the wild-type receptor/mutant receptor dimers may not be able to function normally.[546] As expected from this phenomenon, some patients with moderate to severe growth failure have been found to be heterozygous for loss-of-function point or splice mutations of the GHR gene that alter the cytoplasmic or extracellular domains.[521,547-550] In some cases nonsense mediated mRNA decay can lead to degradation of the aberrant mRNA and prevent a potential dominant negative effect.[551]

Some patients with severe short stature and GHI do not have GHR mutations. Rather, they have defects in GHR-mediated intracellular signaling—including impaired STAT activation.[552] Some patients with GHI were found to have STAT5b mutations.[553-557] Unlike patients with GHR mutations, patients with STAT5b mutations also exhibited severe neurocognitive delay, chronic lung disease, and abnormal T-cell function.[554] Patients with GHI can now be successfully treated with recombinant IGF1,[558] but this does not result in a normal adult height, in contrast to GH deficiency in which GH therapy can achieve normal adult height.

LEPTIN RECEPTORS

The leptin receptor (LEPR, also known as Ob-R) gene is located at 1p31. There are five isoforms of LEPR due to alternative splicing of the LEPR gene transcript (Figure 3-5).[560] Only the Ob-Rb isoform contains both the Jak kinase binding and STAT motifs necessary to maximally transduce the effects of leptin.[560] The Ob-Ra, Ob-Rc, and Ob-Rd isoforms contain intact extracellular and transmembrane but are missing the STAT motif from their cytoplasmic domains.[560] The Ob-Re isoform is missing the transmembrane and cytoplasmic domains.[560] Thus, the Ob-Rb isoform is thought

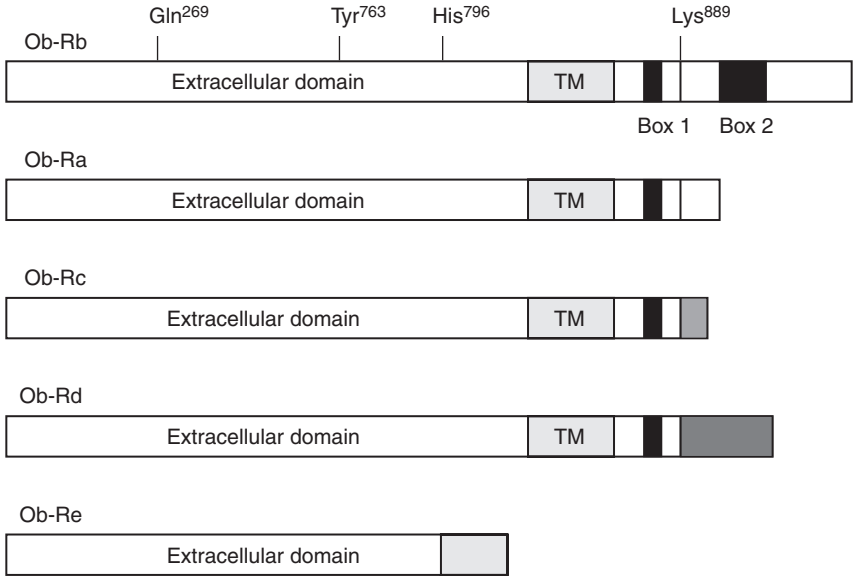


FIGURE 3-5 ■ Leptin receptor isoforms. There are five leptin receptor isoforms. Box 1 represents the Jak kinase binding motif, and box 2 represents the STAT motif. The Ob-Rb isoform is the only isoform that contains Jak kinase binding and STAT motifs, and it is thus thought to be the main isoform involved in mediating the effects of leptin. The Ob-Ra, Ob-Rc, and Ob-Rd isoforms are missing the STAT motif. The Ob-Re isoform is missing the transmembrane (TM) and cytoplasmic domains. (Reproduced with permission from Chen, D., & Garg, A. [1999]. Monogenic disorders of obesity and body fat distribution. *J Lipid Res, 40,* 1737.)

to be the main isoform involved in mediating the effects of leptin.[560]

Three sisters from a consanguineous kindred were found to be homozygous for a splice mutation in the LEPR gene that resulted in expression of an 831-amino- acid protein (Ob-Rhd) that lacks transmembrane and cytoplasmic domains.[561] They had been hyperphagic and morbidly obese since birth.[561] They were found to have elevated circulating leptin levels, decreased TSH and GH secretion, and failure of pubertal development due to hypogonadotropic hypogonadism.[561] Heterozygous carriers of the mutation are not morbidly obese and do not have delayed or absent puberty.[561]

Nonsense or missense LEPR mutations were identified in 3% of a selected cohort of 300 subjects with early onset obesity. Individuals with mutations had hyperphagia, severe obesity beginning in the first year of life with a mean BMISDS of +5.1, altered immune function, and delayed puberty due to hypogonadotropic hypogonadism. Importantly, circulating leptin levels were within the range predicted by the elevated fat mass, and clinical features were less severe than those of subjects with congenital leptin deficiency.[562] Heterozygous family members had an average BMISDS of +0.6 similar to that of family members with normal leptin receptors. Functional characterization of these missense mutations revealed defects causing intracellular retention, misfolding, or failure to signal to downstream pathways.[563] Additional novel mutations have been reported that cause similar phenotypes.[564,565]

RECEPTOR TYROSINE KINASES

The receptor tyrosine kinase (RTK) superfamily consists of 15 receptor tyrosine kinase families (Figure 3-6).[566] With one exception, these families consist of receptors with one membrane-spanning domain (see Figure 3-6).[566] The single-membrane–spanning receptors typically contain an N-terminal extracellular portion, a transmembrane helix, a juxtamembrane region, a tyrosine kinase (TK) domain, and a C-terminal region (see Figure 3-6).[566] These receptors require dimerization to be maximally activated.[566-568] Receptors belonging to insulin RTK family differ from other RTKs, as they contain two membrane-spanning polypeptide chains linked by disulfide bonds to two intervening extracellular peptide chains and thus do not dimerize (see Figure 3-6).[562]

Activation of RTKs leads to phosphorylation of tyrosine residues in the activation loop (A-loop) in the TK domain(s), resulting in activation of the TK(s).[562,569] Activation of the TK(s), in turn, induces the transfer of phosphate from adenosine triphosphate (ATP) to tyrosine residues in the cytosolic portion of the receptor and in cytosolic proteins that serve as docking sites for second messengers.[562]

There is a growing body of evidence suggesting that members of the receptor tyrosine kinase superfamily can directly and indirectly interact with heterotrimeric G proteins. The insulin, insulin-like growth factor-1, and insulin-like growth factor-2 receptors appear to directly interact with $G_{i/o}$ and $G_{q/11}$, $G_{i/o,}$ and G_i, respectively.[570] The fibroblast growth factor receptors appear to directly and indirectly interact with G_s.[570] Congenital alteration of function of receptors in the insulin and the fibroblast growth factor RTK families leads to endocrine disorders (Table 3-4).

Insulin Receptor Tyrosine Kinase Family

The insulin RTK family includes the insulin receptor (INSR) and the insulin-like growth factor-1 receptor (IGF1R).[570] These receptors are heterotetramers consisting of two α and β subunits in an αββα configuration (Figure 3-7).[571-573] The cysteine-rich extracellular α

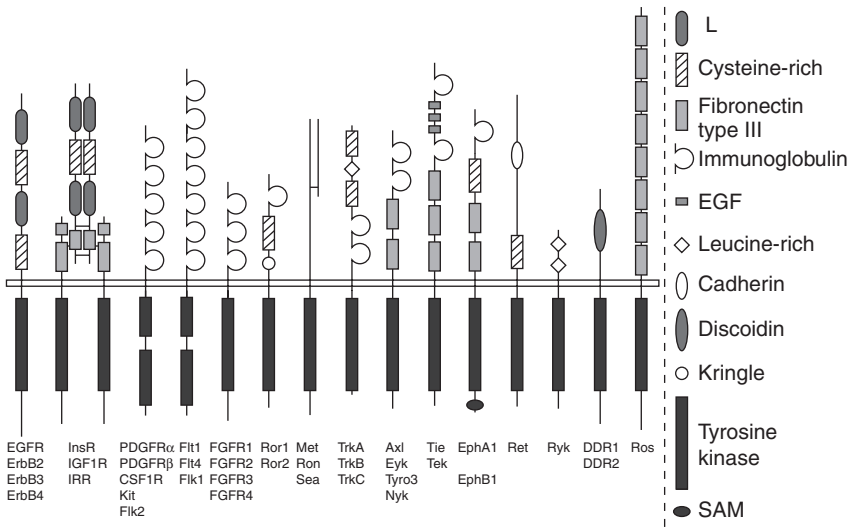


FIGURE 3-6 ■ The 15 RTK families. Each family has a characteristic extracellular portion and a cytoplasmic portion that contains a tyrosine kinase domain. (Reproduced with permission from Hubbard, S. R. [1999]. Structural analysis of receptor tyrosine kinases. *Prog Biophys Mol Biol, 71*, 344.)

TABLE 3-4 **Receptor Tyrosine Kinases and Clinical Conditions Associated with Receptor Mutations**

Receptor	Germ-Line Mutation	Endocrine Disorder
Insulin receptor	Inactivating (heterozygous)	Some cases of type A syndrome
	Inactivating (homozygous, compound heterozygous)	Rabson Mendenhall, Donohue (leprechaunism), and some cases of type A syndromes
IGF-1 receptor	Gene deletion (heterozygous)	Pre- and postnatal growth failure
FGFR1	Inactivating mutation (heterozygous)	Kallmann syndrome, missing teeth, cleft palate
FGFR3	Activating mutations (heterozygous)	Achondroplasia, severe achondroplasia with developmental delay and acanthosis nigricans, thanatophoric dysplasia types I and II, and platyspondylic lethal skeletal dysplasias (San Diego types)

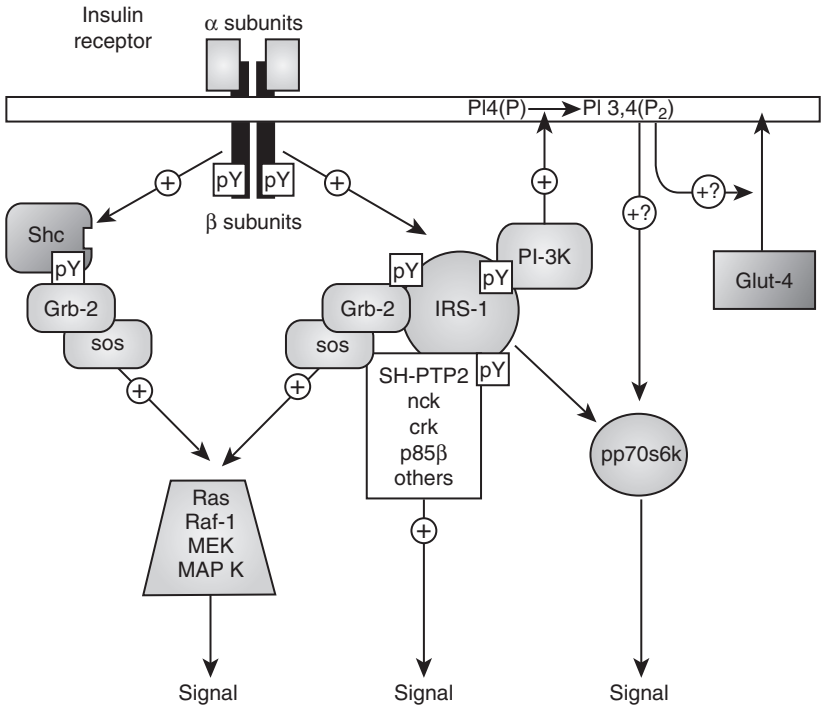


FIGURE 3-7 ■ Insulin receptor signaling. IRS proteins, SH2-domain–containing proteins (including Grb-2 and Shc), and other proteins (including SOS) interact to activate the Ras/Raf-1/MAP K cascade, the PI-3K/PKB cascades, and other enzymes—including SH-PTP2 (SHP2) and p70(s6k). (Adapted with permission from White, M. F. [1997]. The insulin signaling system and the IRS proteins. *Diabetologia, 40*, S10.)

subunits are linked by disulfide bonds, and each α subunit is linked to a plasma membrane-spanning and cytosolic β subunit by disulfide bonds.[573,574] Each β subunit contains a TK domain and a C-terminal region that contain tyrosine residues.[562]

Both insulin and insulin-like growth factor-1 (IGF-1) can bind INSRs and IGF1Rs. However, insulin has greater affinity for the INSR and IGF-1 has greater affinity for the IGF1R. Ligand binding alters the conformation of the receptor, resulting in *trans*-autophosphorylation of the C-terminal tyrosine residues on one β subunit by the TK on the other β subunit.[575,576] The phosphorylated tyrosine residues create motifs that can be bound by Src homology 2 (SH2)-domain-containing proteins, including Shc, Grb-2, SHP2, nck, phosphatidylinositol-3-kinase (PI3K), and Crk.[577-581] The receptor TK also phosphorylates tyrosine residues in insulin receptor substrate proteins (IRS), including IRS-1 and IRS-2, that bind INSRs and IGF1Rs.[581-584] When phosphorylated, these tyrosine residues create motifs that are bound by SH2-domain-containing proteins.[577,578,580,581,584] Thus, insulin receptor substrates can serve as docking proteins—allowing SH2-domain-containing proteins to indirectly interact with INSRs and IGF1Rs when stearic constraints do not permit direct interactions between the proteins and the receptors.[577,578,581] Ultimately, IRSs, SH2-domain-containing proteins, and other proteins (including mSOS) interact to activate the Ras/Raf/MAPKK/MAPK and PI3K/protein kinase B (PKB) cascades (see Figure 3-7).[580,581,584]

Activation of the Ras/Raf/ MAPKK/MAPK cascade increases mitogenesis and proliferation, and activation of the PI3K/PKB cascade increases glucose uptake and

glycogen synthesis.[573,585-590] Evidence suggests that IGF1 has a greater effect on cell growth than on glucose metabolism because activation of the IGF1R stimulates the Ras/MAPK cascade more than INSR activation.[573,589] Conversely, it appears that insulin has a greater effect on glucose metabolism because INSR activation stimulates the PI3K/PKB cascade more than IGF1R activation.[573,590]

THE INSULIN RECEPTOR

The INSR gene is located on 19p and contains 22 exons.[591] αβ half-receptor precursors are derived from proteolysis of a single proreceptor comprised of α and β subunits in tandem and disulfide linkage of these subunits.[581,591,592] These αβ half-receptor precursors then join to form a single αββα heterotetrameric insulin receptor.[592] Interestingly, αβ half-receptor precursors encoded by one allele may combine with αβ half-receptor precursors encoded by the other allele to form a single insulin receptor.[593] This phenomenon explains how heterozygous mutations resulting in impaired β subunit tyrosine kinase activity can have a dominant negative effect because activation of the INSR requires trans-autophosphorylation of one β subunit by the other β subunit.[593]

Mutations in the insulin receptor lead to Donohue syndrome (leprechaunism), Rabson-Mendenhall syndrome, or "type A insulin resistance syndrome." Patients with leprechaunism or Donohue syndrome are severely insulin resistant.[602,603] They present during infancy with severe intrauterine and postnatal growth retardation, lipoatrophy, and acanthosis nigricans.[600,602] They also have dysmorphic features that include globular eyes, micrognathia, and large ears.[600,602] Affected male infants commonly have penile enlargement, whereas affected female infants often have clitoromegaly and hirsutism.[600,602] In spite of hyperinsulinemia associated with glucose intolerance or diabetes mellitus, the major glucose metabolism problem for these patients is fasting hypoglycemia.[600,602] Many patients with this condition do not survive past the first year of life.[600,602] Unlike patients with Rabson-Mendenhall syndrome, patients with leprechaunism do not present with diabetic ketoacidosis. [601]

Patients with Rabson-Mendenhall syndrome present during childhood with severe insulin resistance.[599-601] Although patients with this disorder may present initially with fasting hypoglycemia, eventually they develop severe diabetic ketoacidosis that is refractory to insulin therapy.[601] Patients with this condition also have acanthosis nigricans, accelerated linear growth, dystrophic nails, premature and dysplastic dentition, coarse facial features, and pineal hyperplasia. [597,599-601]

Patients with "type A insulin resistance syndrome" have acanthosis nigricans and severe inherited insulin resistance in the absence of INSR autoantibodies.[594-596] Patients with this syndrome tend to be lean and develop glucose intolerance.[596,597] Females with this syndrome also exhibit signs of ovarian hyperandrogenism, including hirsutism, severe acne, clitoromegaly, oligomenorrhea, and infertility. [594-596]

Patients with "type B insulin resistance syndrome" are distinguished from patients with type A insulin resistance syndrome by the presence of anti-INSR antibodies in the plasma that block insulin binding.[594-596,598] Patients with "type B insulin resistance syndrome" present during adulthood with acanthosis nigricans, ovarian hyperandrogenism, and severe insulin resistance in association with signs of autoimmune disease—including alopecia areata, vitiligo, primary biliary cirrhosis, arthritis, and nephritis.[594-596,598] Surprisingly, these patients may present with fasting hypoglycemia that may or may not be accompanied by postprandial hyperglycemia.[594-596,598] Hodgkin disease and ataxia-telangiectasia are also associated with this syndrome.[596] The term *HAIR-AN* (hyperandrogenism, insulin resistance, and acanthosis nigricans) has also been used to describe women with features of types A and B insulin resistance syndromes in association with obesity.[596] However, this term is imprecise because many women who have been labeled as having HAIR-AN may actually have type A or B insulin resistance syndrome or severe polycystic ovary syndrome. [596]

Mutations in the INSR have been found in all patients with leprechaunism and Rabson-Mendenhall syndrome and in 10% to 15% of patients with type A insulin resistance syndrome.[596,603,604] These mutations are divided into five classes.[596,597,605] Class I mutations are frameshift or nonsense mutations that prematurely terminate translation and thus interfere with INSR synthesis. Class II mutations interfere with post-translational processing and intracellular trafficking of the INSR. Class III mutations decrease insulin binding to the INSR. Class IV mutations are point mutations usually located on the intracellular region of the β subunit that decrease INSR TK activity. Class V mutations increase INSR degradation by increasing insulin-induced endocytosis and degradation of the receptors.

Patients with Rabson-Mendenhall syndrome and leprechaunism are homozygous or compound heterozygous for these mutations.[596,606-616] Some patients with type A syndrome have been found to be heterozygous for dominant negative β-subunit mutations that reduce TK activity by 75%.[605,610,617-621] Other patients with type A syndrome have been found to be homozygous or compound heterozygous for α-subunit mutations that interfere with receptor trafficking to the plasma membrane, β-subunit mutations that interfere with TK activity, or mutations that interfere with proreceptor cleavage into α and β subunits. Still other patients have been found to have decreased INSR mRNA levels that may be due to a loss-of-function mutation in the INSR gene promoter.[597,622-626] Interestingly, one patient with leprechaunism with parents with type A syndrome has been described.[610] The proband was found to be homozygous for an INSR mutation that decreases TK activity, and the parents were found to be heterozygous for the mutation.[610]

THE INSULIN-LIKE GROWTH FACTOR-1 RECEPTOR

The growth-promoting effects of IGF-1 are mediated by IGF1Rs. IGF1R αβ subunits are encoded by a single

gene.[592] Like the insulin receptor, an αβ half-receptor precursor is produced that then joins with a half-receptor precursor that may be encoded from the other allele to form a complete heterotetrameric IGF1R.[592] The IGF1R has 100-fold less affinity for insulin than for IGF1.[627]

Patients who are heterozygous for a ring chromosome 15, resulting in deletion of the IGF1R gene, present with intrauterine growth retardation and postnatal growth failure accompanied by delayed bone age, mental retardation, cardiac abnormalities, cryptorchidism, and dysmorphic features that include microcephaly, triangular face, frontal bossing, hypertelorism, and brachydactyly.[628,629] Similarly, IUGR and postnatal growth failure are commonly found in patients heterozygous for deletion of distal 15q that results in deletion of the IGF1R gene. Patients with deletion of distal 15q often have microcephaly, triangular facies, hypertelorism, high-arched palate, micrognathia, cystic kidneys, and lung hypoplasia or dysplasia.[627,630-632] However, the ring chromosome and deleted area of distal 15q may also be missing other genes—and it is unknown to what extent absence of the IGF1R gene contributes to the phenotype found in these patients.[627,629]

It has been suggested African Efe Pygmies are short due to resistance to IGF1.[627] T-cell lines established from Efe Pygmies have decreased IGF1R gene expression, cell surface expression, receptor autophosphorylation, and intracellular signaling when compared with T-cell lines established from American controls.[627] However, no IGF1R gene mutation has been identified in Efe Pygmies that can account for these findings. [627]

No human homozygous IGF1R mutations have been identified and total loss of IGF1R function may be lethal as demonstrated in the mouse model.[633-635] Heterozygous and compound heterozygous mutations are associated with severe intrauterine growth retardation, microcephaly, and short stature with compound heterozygous mutations having a more severe phenotype.[633,636-638]

In addition to defective INSR function, some patients with leprechaunism and Rabson-Mendenhall syndrome are resistant to the glucose lowering or growth promotion of IGF1 and have abnormal IGF1R function—resulting in decreased ligand binding or altered intracellular signaling.[627,639-643] No deleterious IGF1R gene mutation has been identified in patients with these syndromes, and many patients with leprechaunism and Rabson-Mendenhall syndrome have normally functioning IGF1Rs and no evidence of IGF1 resistance.[627,644]

THE FIBROBLAST GROWTH FACTOR RECEPTOR FAMILY

There are four members of the fibroblast growth factor receptor (FGFR) tyrosine kinase family.[562] These are FGFR1, FGFR2, FGFR3, and FGFR4. These receptors consist of a single polypeptide chain that contains an N-terminal extracellular region, a transmembrane region, and a cytosolic region (see Figure 3-6).[562] The extracellular region contains three immunoglobulin-like domains: IgI, IgII, and IgIII (see Figure 3-6).[645] The cytosolic region contains a TK domain split into two segments (TK1 and TK2) by an intervening amino acid segment.[645]

Fibroblast growth factors, or FGFs, are a family of growth factors involved in angiogenesis, wound healing, and embryonic development. The FGFs are heparin-binding proteins and interactions with cell surface-associated heparan sulfate proteoglycans have been shown to be essential for FGF signal transduction. FGFs are key players in the processes of proliferation and differentiation of wide variety of cells and tissues. In humans, 22 members of the FGF family have been identified, all of which are structurally related signaling molecules.[645,646] As monomers, FGFs can only bind a single FGFR—forming an inactive 1:1 complex.[567] FGFR activation by dimerization occurs when two or more FGF molecules in 1:1 complexes are linked by heparan sulfate proteoglycans.[567]

Activation of FGFRs increases receptor TK activity.[645] Increased TK activity leads to autophosphorylation of a tyrosine residue in the C-terminal region, resulting in a binding site for the SH2 domain of phospholipase Cγ (PLCγ).[647,648] Once PLCγ is bound to this site, it is phosphorylated and activated.[647,648] In chondrocytes, activation of FGFR3 also induces activation of STAT1.[649]

Fibroblast Growth Factor Receptor 1

Inactivating mutations of the FGFR1 gene are a cause of autosomal-dominant Kallmann syndrome (KS).[650] Individuals with KS have anosmia and isolated hypogonadotropic hypogonadism.[651,652] The FGFR1, which is located on 8p12, plays a role in olfactory and GnRH neuronal migration from the nasal placode to the olfactory bulb and in the subsequent migration of the GnRH neurons to the hypothalamus.[650] Prior to identification of these FGFR1 gene mutations, X-linked KS was found to be caused by inactivating KAL1 gene mutations.[651,653,654] The KAL1 gene is located on the X chromosome and encodes anosmin-1.[651,653,654] Anosmin-1 is a ligand for the FGFR1 receptor.[655] Like FGFR1s, anosmin-1 plays a role in olfactory and GnRH neuronal migration to the nasal placode, and in the subsequent migration of GnRH neurons to the hypothalamus.[651,655,656]

There is a high penetrance for anosmia and signs of hypogonadotropic hypogonadism (including lack of puberty, microphallus, and cryptorchidism) in the 10% of KS patients with X-linked KS due to KAL1 gene mutations.[651] Female carriers of KAL1 gene mutations do not have anosmia or isolated hypogonadotropic hypogonadism.[651] In contrast to patients with KS due to KAL1 gene mutations, the approximately 10% of KS patients with FGFR1 gene mutations (even within the same kindred) exhibit variable phenotypes ranging from anosmia and complete hypogonadotropic hypogonadism (characterized by cryptorchidism and microphallus in males and absent pubertal development in both genders) to anosmia or delayed puberty.[650,657,658]

It has also been noted that in most kindreds with FGFR1 gene mutations females present with more mild KS phenotypes than males.[650,658] Female carriers may even be asymptomatic.[650,658] Because the KAL1 gene is located on the X chromosome, females may produce

more anosmin-1 than males.[650] Thus, a possible explanation for milder KS phenotypes in females with FGFR1 gene mutations may be that the increased anosmin-1 levels in females may lead to increased anosmin-1–induced activation of the mutant FGFR1s that may partially compensate for the mutation.[650] Interestingly, missing teeth and cleft palate are not an uncommon finding in individuals with KS due to FGFR1 gene mutations, whereas unilateral renal agenesis and bilateral synkinesia are associated with KS due to KAL1 gene mutations.[657]

Fibroblast Growth Factor Receptors 2-4

Gain-of-function mutations in the other three genes encoding FGFRs 2-4 cause many conditions—including Pfeiffer syndrome (activating mutations of FGFR1 and FGFR2), Crouzon syndrome (gain-of-function FGFR2 mutations), Crouzon syndrome with acanthosis nigricans (an FGFR3 mutation), Apert syndrome (FGFR2 mutations), and craniosynostosis (gain-of-function FGFR3 mutations).[645] Several autosomal-dominant short-limb dwarfism syndromes—including achondroplasia, severe achondroplasia with developmental delay and acanthosis nigricans (SADDAN), hypochondroplasia, and three types of platyspondylic lethal skeletal dysplasias (PLSD) [thanatophoric dysplasia I (TDI), thanatophoric dysplasia II (TDII), and San Diego types (PLSD-SD)]—are often caused by heterozygous constitutively activating FGFR3 gene mutations.[659-662]

Individuals with achondroplasia have activating mutations in the transmembrane domain of FGFR3, with the Gly380Asn found in > 95% of achondroplastic patients.[660,663-665] From 40% to 70% of individuals with hypochondroplasia have an activating Asn540Lys mutation in the TK1 domain.[660,666-669] All individuals with TDII have an activating Lys650Glu mutation in the activating loop of the TK2 domain, and > 90% of individuals with TDI and PLSD-SD have FGFR3 mutations.[660,662] Patients with SADDAN have an activating mutation in the same codon as patients with TDII.[670] Instead of the Lys650Glu mutation associated with TDII, patients with SADDAN have a Lys650Met mutation.[670] However, unlike patients with TDII patients with SADDAN do not have craniosynostosis and a cloverleaf skull—and often survive past childhood.[670]

The FGFR3 gene is primarily expressed in endochondral growth plates of long bones, brain, and skin pre- and postnatally.[671,672] Constitutional activation of FGFR3s in chondrocytes leads to growth arrest and apoptosis.[649,673,674] In addition, constitutive activation of FGFR3s is also postulated to alter neuronal migration because patients with SADDAN, TDI, and TDII have neurologic abnormalities that may include developmental delay, paucity of white matter, polymicrogyria, dysplastic temporal cortex, dysplasia of nuclei, and neuronal heterotopia.[670,675-677] Furthermore, constitutive activation of FGFRs in skin fibroblasts and keratinocytes is thought to cause the acanthosis nigricans seen in patients with SADDAN and Crouzon syndrome with acanthosis nigricans.[678] However, it is not yet known why some activating FGFR3 mutations affect the skeletal system, central nervous system, and the skin, whereas other activating FGFR3 mutations only affect the skeletal system.[670]

Loss of function mutations in FGFR3 also have been associated with human disease. An uncommon syndrome characterized by camptodactyly, tall stature, scoliosis, and hearing loss (CATSHL syndrome) has been associated with a heterozygous missense mutation that is predicted to cause a p.R621H substitution in the tyrosine kinase domain and partial loss of FGFR3 function.[679] These findings indicate that abnormal FGFR3 signaling can cause human anomalies by promoting as well as inhibiting endochondral bone growth.[680]

NUCLEAR RECEPTORS

Using a phylogenetic tree based on the evolution of two highly conserved nuclear receptor domains (the DNA-binding C domain and the ligand-binding E domain), Laudet divided nuclear receptors into six related subfamilies. Subfamily 0 contains receptors, such as the embryonic gonad (EGON) and DAX1 receptors, that do not have a conserved C or the E domain (Figure 3-8).[681,682] Subfamily 1 includes the peroxisome proliferator-activated retinoic acid, thyroid hormone, and vitamin D_3 receptors. Subfamily 2 includes the hepatocyte nuclear factor-4α (HNF-4α) and retinoid X receptors (RXRs). Subfamily 3 contains the steroid receptors. Evidence suggests that subfamily 3 (which includes the glucocorticoid, androgen, progesterone, and mineralocorticoid receptors) rapidly evolved from a common steroid receptor gene about 500 million years ago.[683]

Subfamilies 4 to 6 contain several nuclear receptors, namely NR4A1-3, NR5A1-2, NR6A1.[684-686] The nomenclature is as follows: nuclear receptor subfamily 4, group A, members 1 for NR4A1. NR5A1 is also known as steroidogenic factor-1 or SF-1.[685]

General Structure of the Nuclear Receptors

Nuclear receptors are made up of four domains: A/B, C, D, and E (Figure 3-9).[681] Supporting the notion that nuclear receptor subfamilies are derived from a common ancestral orphan receptor, the C and E domains are highly conserved among the subfamilies.[681] Mutation of several nuclear receptors are associated with endocrine disorders (Table 3-5).

The A/B domain is located at the N-terminal and contains the activation function 1 (AF-1)/τ 1 domain.[687] The AF-1/τ 1 domain regulates gene transcription by interacting with proteins (such as the Ada and TFIID complexes) that induce transcription.[688,689] This transactivation function of the AF-1/τ 1 domain is not dependent on binding of the nuclear hormone receptor to its ligand and is not specific in its choice of DNA target sequences.[681,690-692] Thus, specificity of action of the nuclear hormone receptor is determined by the function of other nuclear hormone receptor domains.

The C domain has characteristics that help to confer specificity of action on each nuclear hormone receptor.

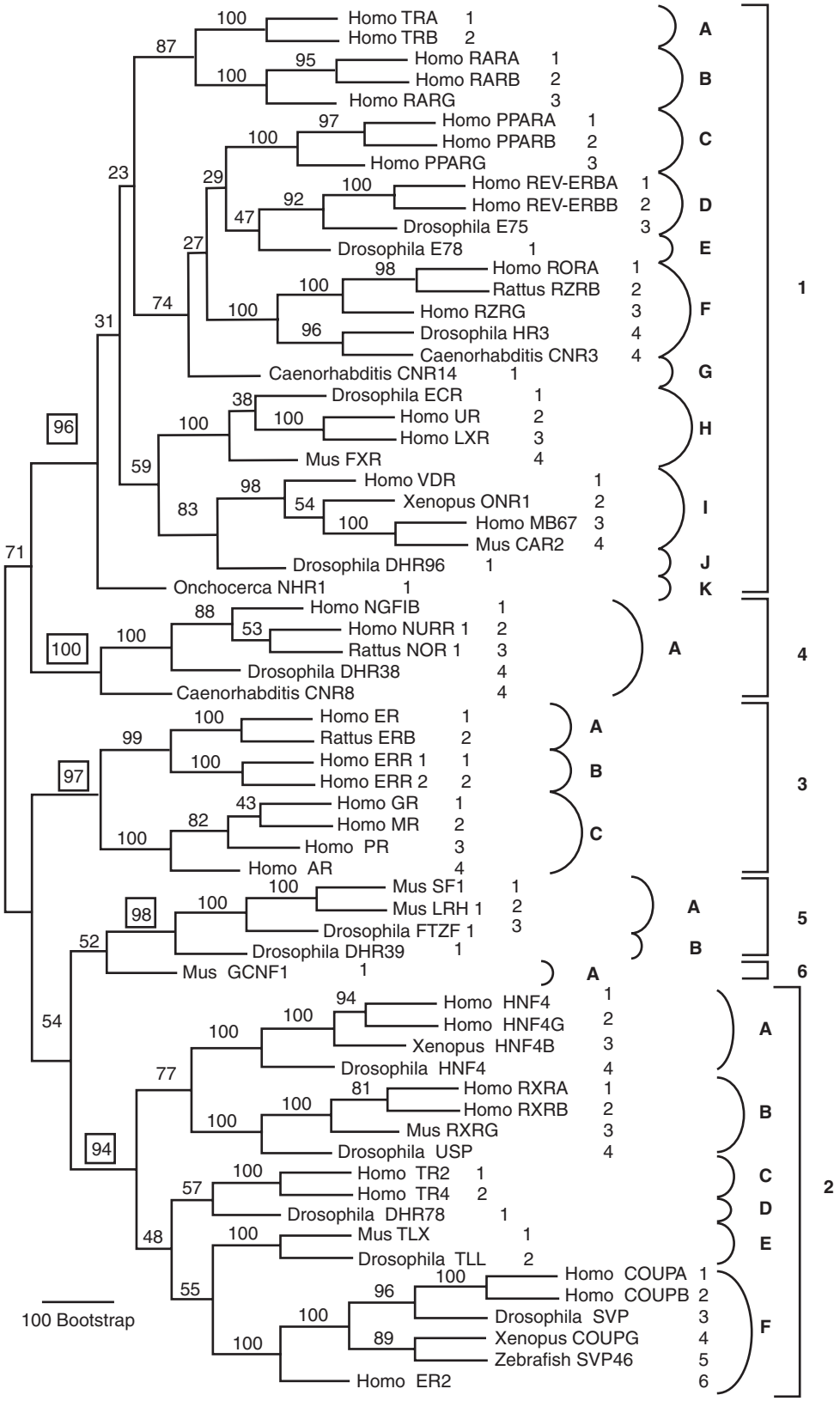


FIGURE 3-8 ■ Phylogenetic tree of nuclear receptors based on the evolution of the highly conserved C and E domains. Numbers at the right side of the figure represent subfamilies, and capital letters represent groups of more closely related receptors. The small numbers to the right of the receptor names are used in combination with the subfamily letters and group letters in a proposed nuclear receptor nomenclature. This nomenclature proposes that nuclear receptors should be named NR, followed by subfamily number, group letter, and individual receptor number. Thus, the mineralocorticoid receptor is named NR3C2 and the PPARγ receptor is named NR1C3 according to this nomenclature. Numbers to the left of the receptor names represent bootstrap values. Values that define subfamilies with more than one member are boxed. (Reproduced with permission from the Nuclear Receptors Nomenclature Committee [1999]. A unified nomenclature system for the nuclear receptor subfamily. *Cell*, *97*, 161.)

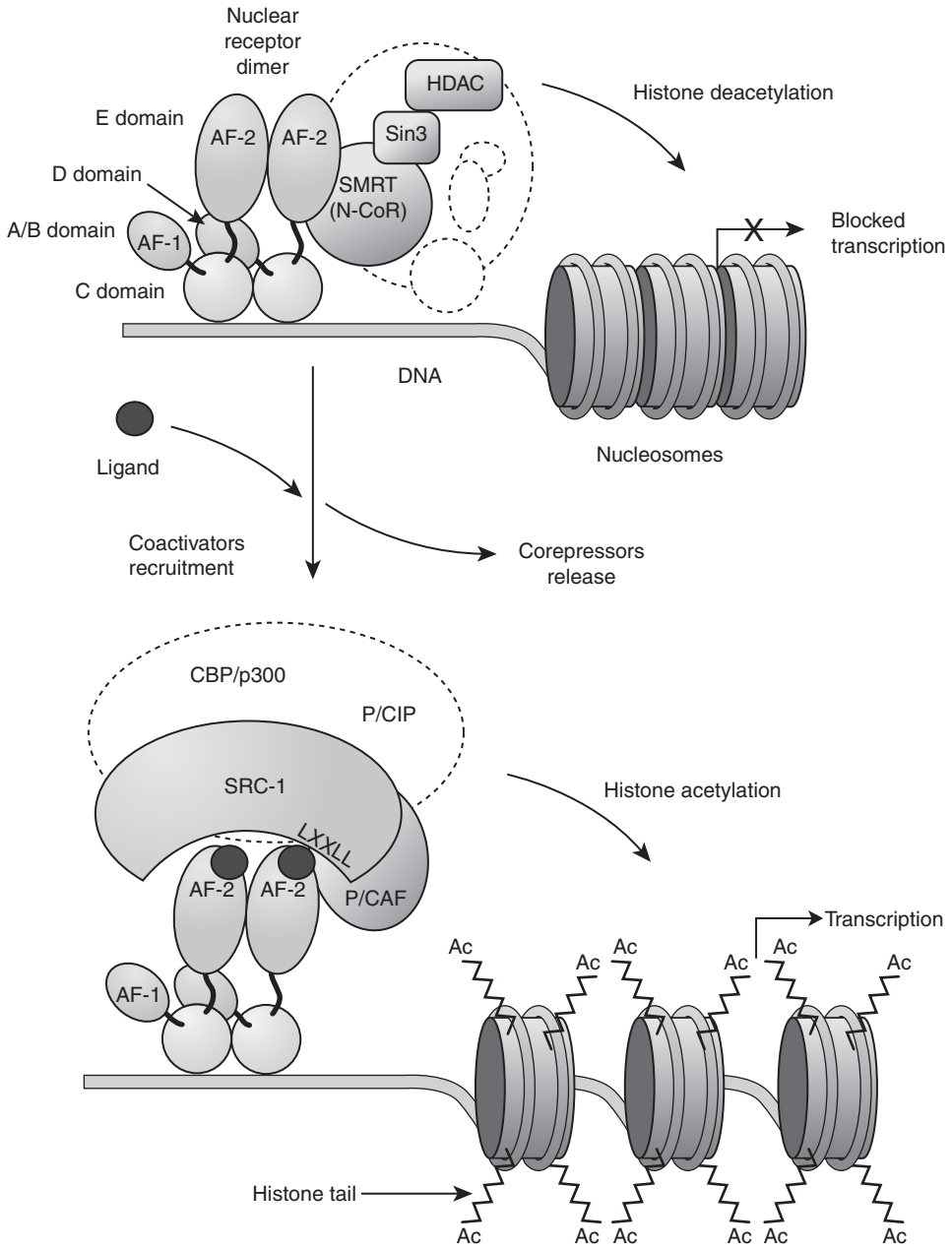


FIGURE 3-9 ■ Ligand-induced activation of transcription by nuclear receptors. Often, corepressors, including SMRT and nuclear receptor copressor (N-CoR), bind a nuclear receptor that is not bound by its ligand. These corepressors then associate with Sin3, which in turn associates with a histone deacetylase (HDAC). Then, HDAC represses transcription by deacetylating histone tails—resulting in compaction of the nucleosomes into structures that are inaccessible to transcription factors. Ligand binding induces structural changes in the E domain that result in release of the corepressor/Sin3/HDAC complexes from the receptor and binding of coactivator complexes that may include steroid receptor coactivator 1 (SRC-1), p300/cAMP responsive element binding protein (CBP), p300/CBP-associated factor (P/CAF), or p300/CBP cointegrator-associated protein (pCIP) to the LXXLL motif of the AF2-AD. Then, the coactivator complexes induce transcription by acetylating (Ac) the histone tails—resulting in decompaction of the nucleosomes into structures that are accessible to transcription factors. Dashed lines are used to represent coactivator and corepressor complexes because their composition in vivo is yet unknown. (Adapted with permission from Robyr, D., Wolffe, A. P., & Wahli, W. [2000]. Nuclear hormone receptor coregulators in action: diversity for shared tasks. *Mol Endocrinol, 14*, 339. Copyright 2000, The Endocrine Society.)

This domain consists of two zinc-finger motifs responsible for the DNA-binding activity of the receptor and the selection of dimerization partners.[693,694] Each zinc-finger module consists of a zinc ion surrounded by the sulfurs of four cysteine residues, resulting in a tertiary structure containing helices.[693,694] The P-box lies near the cysteines of the first zinc finger and contains the three to four amino acids responsible for specificity of binding to response elements.[694,695] The D-box consists of a loop of five amino acids attached to the first two cysteines of the second zinc finger that provides the interface for nuclear receptor dimerization.[694]

TABLE 3-5 **Nuclear Receptors and Clinical Conditions Associated with Receptor Mutations**

Receptor	Germ-Line Mutation	Endocrine Disorder
Thyroid hormone receptor β (TRβ)	Inactivating mutations (heterozygous and homozygous)	Generalized resistance to thyroid hormones
Vitamin D_3 receptor	Inactivating mutations (homozygous)	Vitamin D_3 resistance
PPARγ2	Inactivating mutations (heterozygous)	Obesity or early-onset type 2 diabetes mellitus
HNF-4	Inactivating mutations (heterozygous)	Maturity-onset diabetes of the young (MODY) type 1
Glucocorticoid receptor	Inactivating mutations (heterozygous)	Glucocorticoid resistance
Androgen receptor	Inactivating mutations (X-linked recessive)	Androgen insensitivity syndrome, Kennedy's disease
Estrogen receptor α (ERα)	Inactivating mutations (homozygous)	Tall stature and incomplete epiphyseal fusion
Mineralocorticoid receptor	Inactivating mutations (heterozygous, homozygous)	Pseudohypoaldosteronism type 1
	Activating mutations (homozygous)	Syndrome of apparent mineralocorticoid excess
DAX1	Inactivating mutations (X-linked recessive)	X-linked adrenal hypoplasia congenita

The D "hinge" domain contains nuclear localization signals and contributes to the function of the adjacent C and E domains.[681] Thus, the N-terminal portion of the domain contributes to DNA binding and heterodimerization and the C-terminal portion contributes to ligand binding.[695-698] The nuclear localization signal plays a particularly important role in the function of glucocorticoids and mineralocorticoid receptors because these receptors bind their ligand in the cytoplasm and must then localize to the nucleus to alter gene transcription.[687]

The E domain is known as the ligand-binding domain (LBD) or the hormone-binding domain. In addition to ligand binding, the E domain has effects on dimerization and transactivation.[681] The LBD consists of 11 to 12 α helices (named H1 through H12) and contains a ligand-binding pocket that is made up of portions of some of the different helices.[699-703] For example, the thyroid receptor (TR) LBD has a ligand-binding cavity that includes components from H2, H7, H8, H11, and H12.[703] The contribution of different parts of LBD to the ligand-binding pocket accounts for the finding that mutation of single–amino-acid molecules in different helices of the LBD can interfere with ligand binding.[687]

Unlike the AF-1/τ 1 transcriptional activating factor, the E domain activation factor 2 (AF2-AD) requires ligand binding to function (see Figure 3-9).[700-707] Often, when the receptor is not bound by its ligand, corepressor complexes simultaneously bind the LBD and transcriptional machinery consisting of protein complexes that place transcription factors on nucleosome binding sites (see Figure 3-9).[700-707] The corepressor complexes then suppress gene transcription by using histone deacetylases to compact the nucleosomes into inaccessible structures (see Figure 3-9).[708-711] Ligand binding induces structural rearrangements in the E domain that lead to release of these corepressor complexes from the transcriptional machinery and the LBD, and exposure of the transcriptional machinery and the LXXLL motif of the AF2-AD to coactivator complexes (see Figure 3-9).[700-707] These coactivator complexes have histone acetyltransferase activity that acts to relax nucleosome structures, enabling transcription factors to access nucleosome binding sites (see Figure 3-9).[712]

Most nuclear receptors are capable of binding their hormone response element and repress transcription when they are not bound by their ligand.[713] However, in the absence of ligand, steroid receptors are bound to a complex of heat-shock proteins instead of their response element and do not appear to repress transcription.[714]

Agonists and antagonists have different effects on the interaction between the ligand binding pocket and AF2-AD. For example, when 17β-estradiol binds to the estrogen receptor the position of the AF2-AD containing H12 is altered so that coactivators can access the LBD-binding coactivator binding site.[699] However, when the estrogen antagonist raloxifene binds at the same site the coactivator binding site on H12 remains blocked by other portions of H12.[699]

Although some of the nuclear receptors are fully active when bound as monomers to DNA, the hormone receptors in the nuclear receptor superfamily are most active when bound as homodimers or heterodimers (see Figure 3-9).[681] RXRs, hepatocyte nuclear factor 4, and the steroid receptors can bind DNA as homodimers or heterodimers.[681,715,716] The α isoform of the estrogen receptor (ESR1) is particularly promiscuous, and is able to heterodimerize with HNF4-α and retinoic acid receptors, the β isoform of the estrogen receptor (ESR2), RXR and the thyroid receptors.[715,716] As a homodimer, RXR binds the dINSRect repeat 1 (DR1).[717] It can also join the thyroid, vitamin D_3, and peroxisome proliferator–activated receptors to form heterodimers.[681,718-720]

Interestingly, it has also been suggested that some steroid hormones also act on transmembrane receptors—and these interactions may be responsible for the acute cellular effects of steroids.[721] Progesterone has been shown to interact with the G protein–coupled uterine oxytocin, nicotinic acetylcholine, $GABA_A$, NMDA, and sperm cell membrane progesterone receptors.[722-727] Cell membrane estrogen and glucocorticoids receptors have also been identified. [728-731]

SUBFAMILY 1 NUCLEAR RECEPTORS: THYROID HORMONE, VITAMIN D_3, AND PEROXISOME PROLIFERATOR–ACTIVATED RECEPTORS

Thyroid Hormone Receptors

The two thyroid hormone receptor (THR) isoforms—thyroid hormone receptor α (THRA) and thyroid hormone receptor β (THRB)—are encoded by different c-erbA genes on chromosomes 17 and 3, respectively.[698,732] Alternate splicing leads to expression of TRα1, TRβ1, and TRβ2 with different tissue distributions. TRα1 is the predominant subtype in cardiac and skeletal muscle, bone, and the central nervous system. TRβ1 is the predominant subtype in kidney and liver, whereas TRβ2 is expressed in hypothalamus and pituitary as well as retina and cochlea. THRs that are not occupied by the thyroid hormone triiodothyronine (T3) exist as homodimers or heterodimers with RXRs that are attached to DNA thyroid hormone response elements in association with corepressor proteins.[733] Thyroid hormone binding induces the release of the corepressors from the THR.[733] A coactivator, steroid receptor coactivator-1 (SRC-1), is then able to attach to the THR—enabling activation of transcription.[733]

Generalized resistance to thyroid hormones (GRTH) is due to mutations in the THRB gene.[732,734,735] Patients with this syndrome have impaired receptor response to triiodothyronine (T3).[732] They have elevated T3 and thyroxine (T4) levels with normal TSH levels.[732,734,735] The clinical manifestations are variable but may include goiter, attention deficit disorder, hearing defects, learning disabilities, poor weight gain, mental retardation, and delayed bone age.[732,734,735]

Mutations have been found in the D and E domains of THRBs of GRTH patients.[732,736,737] Thus, these mutations alter ligand binding or transactivation.[732,737,738] However, most mutant THRBs retain the ability to repress transactivation of target genes through interactions with corepressors.[737,738] Some of the GRTH mutant receptors continue to associate with corepressors and are unable to bind the coactivator SRC-1 even when bound by T3.[738,739] Thus, mutant THRBs have a dominant negative effect in the heterozygous state because they are able to interfere with the function of wild-type receptors by repressing transcription of target DNA.[732,737,738]

Patients who are homozygous for deleterious mutations of THRB demonstrate more severe clinical abnormalities than patients who are heterozygous for these mutations. One patient with a deletion of both THRB alleles presented with deaf mutism, dysmorphic features, and stippled epiphyses.[740] This condition is inherited in an autosomal recessive mode because the mutant allele is missing the THRB gene and is therefore unable to produce a THRB with corepressor function.[740] Another patient homozygous for a THRB mutant ("kindred S receptor") with an amino acid deletion in the ligand-binding domain presented with mental retardation, very delayed bone age, and very elevated T3 and T4 levels.[736,741] Heterozygous carriers of the kindred S receptor mutation have milder clinical manifestations of GRTH because this mutant THRB retains corepressor activity and thus has dominant negative effects.[736,741,742]

Mutations in THRA were not identified until recently.[743,744] The first described proband is heterozygous for a nonsense mutation (E403X) that inhibits the wild-type receptor in a dominant negative fashion.[743] The patient had growth retardation developmental delay and constipation in the setting of low T4 and free T4, low reverse T3, normal T3 and free T3, and normal TSH. After thyroxine treatment, normalizing T4 levels, T3 levels became elevated. The mutant receptor failed to bind radiolabeled T3 and failed to activate a thyroid hormone responsive reporter gene and inhibited the activity of the wild-type receptor. The second report described a 1 base insertion in a father and a daughter causing a frameshift and premature termination at codon 406.[744] Analyses of the mutant receptor in vitro after expression in cultured cells revealed that the mutant receptor fails to respond to stimulation by T3 and exerts a strong dominant negative effect on the wild-type receptor.

Vitamin D Receptor

Severe rickets, hypocalcemia, secondary hyperparathyroidism, and increased 1,25-dihydroxyvitamin D (calcitriol) levels occur in patients with the autosomal recessive syndrome of "vitamin D resistance."[745] These patients have defective vitamin D receptors (VDRs). Mutations causing this syndrome have been found in the zinc fingers of the DNA-binding domain (C domain), leading to decreased or abolished receptor binding to regulatory elements of target genes.[746] Causative mutations have also been found that lead to the production of receptors that have decreased or abolished ability to bind calcitriol and heterodimerize with retinoid X receptors (RXRs), which are required for the VDR to maximally transactivate target genes.[746,747]

Less severe mutations in the VDR are associated with decreased gastrointestinal calcium absorption and bone mineral density, even during childhood, and an increased risk for osteoporosis and fractures.[748-753] However, it has not been possible to replicate these findings in some ethnic groups.[754-758] Thus, other factors (such as estrogen receptor genotype, dietary calcium, and age) probably contribute to the effects of VDR polymorphisms on bone mineral metabolism.[751,753,759,760] Certain VDR polymorphisms are associated with decreased pre- and postnatal linear growth.[761,762]

Other important associations have been found with VDR polymorphisms. Homozygous polymorphisms have been reported to be associated with primary hyperparathyroidism.[763] In addition, the presence of certain VDR alleles is associated with increased risk for the development of early-onset periodontal disease.[764] Conversely, absence of such alleles has been associated with familial calcium nephrolithiasis.[765] Absence of certain alleles may be a risk factor for the development of sarcoidosis.[766] However, the presence of these alleles is associated with hypercalciuria and nephrolithiasis and increased risk in women for the development of metastatic breast cancer.[767,768] VDR polymorphisms have also been associated with increased susceptibility to psoriasis, tuberculosis, leprosy, and other infections.[769-773]

PPARγ

PPARγ has a role in regulating adipocyte differentiation and metabolism. This formerly orphan receptor was adopted by the ligand prostaglandin J2. Mutations in the gene encoding PPARγ cause familial partial lipodystrophy type 3 (FPLD3).[774]

Patients with FPLD3 exhibit fat loss in the arms and legs, severe insulin resistance, early-onset type 2 diabetes mellitus, and severe hypertriglyceridemia Some heterozygous mutations have a dominant negative effect on the wild-type receptor (V290M and P467L).[775,776] These mutations lead to amino acid substitutions that disturb the orientation of H12 in the E domain leading to decreased ligand-dependent transactivation by AF-2/AD and coactivator recruitment.[775] Other mutations are point mutations that cause FPLD3 simply due to haploinsufficiency without a dominant negative effect.[777-784] In addition a frameshift mutation that causes a similar phenotype was identified.[785] A mutation in PPARγ expanded the phenotype to include muscular, immune, and hematologic features.[786] This mutation is thought to induce a conformational change affecting transcriptional activation by the receptor.

A missense mutation leading to a Pro115Gln substitution near a site of serine phosphorylation at position 114 that suppresses transcriptional activation in PPARγ2 was found in some morbidly obese patients.[787] The Pro115Gln substitution interferes with phosphorylation of the serine at position 114, leading to increased transcriptional activation by PPARγ2—which in turn leads to increased adipocyte differentiation and triglyceride accumulation.[787]

SUBFAMILY 2 NUCLEAR RECEPTORS: HEPATOCYTE NUCLEAR FACTOR AND RETINOID X RECEPTORS

This subfamily includes the HNF receptors and the RXR. RXRs form heterodimers with other nuclear receptors (including the estrogen, vitamin D, and thyroid hormone receptors) and with PPARγ (RXRs are discussed elsewhere in this chapter).

HNF Receptors

Alteration of another orphan nuclear receptor, HNF-4α, also causes an endocrine disorder. Mutations of the HNF-4α gene on chromosome 20 that alter the ligand-binding domain (E domain) or the DNA-binding domain (C domain) have been found in patients with maturity-onset diabetes of the young type 1 (MODY1).[788-791] Patients with MODY usually develop diabetes mellitus by the end of the third decade of life. They have a defect in glucose-mediated stimulation of insulin secretion.[789,792] Several studies have now described a biphasic phenotype of neonatal hyperinsulinism and macrosomia with later onset of diabetes in patients with HNF-4α mutations.[793-797] In most cases, the hyperinsulinism is transient but can be persistent requiring diazoxide treatment.[795,796]

Carriers of a glycine-to-serine substitution in codon 115 in the DNA-binding domain (C domain) appear to be at increased risk for developing low-insulin diabetes mellitus.[798] Hepatocyte nuclear factors 3 (HNF-3α, -3β, and -3γ) are also regulators of the early-onset type 2 diabetes genes HNF-1 α, HNF-4 α, and IPF-1/PDX-1—which are associated with MODY types 3, 1, and 4, respectively.[799-802]

SUBFAMILY 3 NUCLEAR RECEPTORS: THE STEROID RECEPTORS AND GLUCOCORTICOID, ANDROGEN, ESTROGEN, AND MINERALOCORTICOID RECEPTORS

Glucocorticoid Receptors

Mutations in glucocorticoid receptors cause primary generalized glucocorticoid resistance (PGGR) or primary generalized glucocorticoid hypersensitivity (PGGH).[803] PGGR is clinically characterized by the presence of elevated plasma cortisol and ACTH levels, accompanied by the effects of hyperaldosteronism and hyperandrogenism in the absence of striae and central fat deposition.[698,803,804,805] ACTH excess leads to adrenocortical hyperplasia increased cortisol secretion and increased production of adrenal steroids with androgenic (androstenedione, DHEA, DHEA-S) and mineralocorticoid activity (cortisol, deoxycorticosterone and corticosterone). The clinical spectrum is broad, ranging from asymptomatic to severe hyperandrogenism (characterized by severe acne, hirsutism, irregular menses, and infertility), mineralocorticoid excess (characterized by hypokalemic alkalosis and hypertension), and fatigue.[804-806,] Girls may present with ambiguous genitalia and both girls and boys may present with isosexual precocity.[807] Clinical manifestations of glucocorticoid deficiency are infrequent and largely limited to fatigue.[806,808,809] However, there were reports of childhood hypoglycemia.[810,811]

Both homozygous[809,810,812,813] and heterozygous[813-817] mutations have been described. Heterozygous mutations that cause PGGR generally do so by exerting a dominant negative effect on the wild-type receptor.[803]

ACTH-secreting pituitary macroadenomas can also be caused by a frameshift mutation in the GR gene that interferes with signal transduction.[818] Patients with this mutation manifest the symptoms of glucocorticoid resistance. The tumor develops as a result of impaired negative feedback regulation by glucocorticoids of the hypothalamic-pituitary axis.

PGGH was described in a patient who presented with obesity, hypertension, and hyperlipidemia and was found to have a mutation in the GR (D401H) that demonstrated increased transactivation glucocorticoid responsive genes.[819]

Androgen Receptors

The human androgen receptor (AR) gene is located on the X chromosome. Known disorders characterized by AR dysfunction due to AR gene mutations are only expressed in patients with a 46 XY karyotype.[820] Thus, these disorders are either transmitted by X-linked recessive inheritance or are due to sporadic mutations.

More than 1000 mutations have been described in the AR gene with more than 500 mutations causing androgen insensitivity syndrome (AIS; testicular feminization).[821] The phenotype of this syndrome can vary in severity and has been divided into mild, partial and complete forms (MAIS, PAIS, and CAIS).[822] CAIS is characterized by intraabdominal testes, absence of mullerian structures, absence of androgen-induced body hair such as pubic and axillary hair, and a female appearance.[820,823,824] PAIS refers to individuals with ambiguous external genitalia with an enlarged clitoris or microphallus and patients with Reifenstein syndrome.[823] Reifenstein syndrome is characterized by severe hypospadias with scrotal development and severe gynecomastia.[823] MAIS refers to patients with AR mutations who are otherwise normal phenotypic males who present with adolescent gynecomastia or later infertility.

The phenotypic heterogeneity of AIS is due to the wide variety of locations for the mutations causing AIS. Functional consequences of each mutation causing AIS relate to the function of the domain in which the mutation is located. However, the degree of impairment of mutated receptor function in in vitro studies does not always correlate with the phenotypic severity of the syndrome.[825]

Mutations in exons that code for the AR hormone-binding domain decrease hormone-binding affinity.[826] However, these mutations do not abolish the hormone-binding capability of the receptor.[826] Thus, patients with these mutations usually present with PAIS or occasionally with CAIS.[826-829] Patients with mutations in the hormone-binding domain do not appear to respond to treatment with high doses of testosterone.[826] Mutations in the DNA-binding domain lead to failure of target gene regulation. Thus, patients with these mutations usually manifest the CAIS.[830,831]

CAIS is also caused by a point mutation that results in a premature termination condition, leading to transcription beginning downstream of the termination signal and the AF1/tau1 domain.[832] Numerous other mutations have been described that cause truncation or deletion of the AR and complete AIS.[833-836] Two patients with ambiguous genitalia and partial virilization were found to be mosaic for mutant ARs.[837,838]

Some patients with Reifenstein syndrome have been found to have a mutation in the DNA-binding domain that abolishes receptor dimerization.[839] Other patients have been found to have a mutation in a different area of the DNA-binding domain that does not affect receptor dimerization, or to have mutations in the hormone-binding domain in the E domain.[840-843] The Ala596Thr mutation in the D-box area of the DNA-binding domain has been associated with an increased risk of breast cancer.[844]

AIS is also a feature of Kennedy disease, which is an X-linked recessive condition causing spinal and muscular atrophy.[827] This condition is caused by extension of a poly-CAG segment in the AR gene exon that codes for the N-terminus of the AR, leading to an increased number of glutamine residues in the A/B domain.[845,846] ARs with a polyQ region increased to 48 glutamine residues accumulate abnormally in transfected cells due to misfolding and aberrant proteolytic processing.[847] Because polyQ extension does not completely abolish of transactivation, patients with Kennedy disease exhibit a mild partial AIS phenotype consisting of normal virilization accompanied by testicular atrophy, gynecomastia, and infertility.

Gain-of-function mutations in the AR have been described in prostate, breast, testicular, liver, and laryngeal cancers.[821]

Estrogen Receptors

Two major full-length estrogen receptor isoforms have been identified in mammals. Estrogen receptor α (ESR1) was discovered first and mediates most of the known actions of estrogens.[698] ESR1 is expressed primarily in the uterus, ovaries, testes, epididymis, adrenal cortices, and kidneys.[848] Estrogen receptor β (ESR2) was discovered in 1996.[849] ESR1 and ESR2 share 95% and 50% homology in the DBD and LBD, respectively.[850] There is little homology in the N-terminal between the two isoforms.[850] ESR2 is expressed primarily in the uterus, ovaries, testes, prostate, bladder, lung, and brain.[848] Although ESR2 has a high affinity for estrogens, it has less transactivating ability than ESR1 and has not yet been found to be involved in any pathologic condition.[698,851,852]

There is evidence supporting the existence of other functional estrogen receptors.[853] Some of these putative estrogen receptors localize to the cell membrane instead of or in addition to the nucleus. A 46-kDa amino terminal truncated product of ESR1, named ER46, localizes to the cell membrane and mediates estrogen actions that are initiated at the cell membrane.[854] Another of these putative estrogen receptors has been named ER-X and is postulated to be a G protein–coupled receptor that localizes to the cell membrane.[855] Another putative receptor is the aptly named heterodimeric putative estrogen receptor (pER), which has been found on the cell and nuclear membranes.[856] The pER acts as a serine phosphatase.[856] Five other estrogen-binding proteins have also been identified, and at least three of them localize to the cell membrane.[857-860]

It had been thought that androgens are the main hormones responsible for closing the epiphyses during puberty. In 1994, however, it was shown from extensive studies of a 28-year-old man with incomplete epiphyseal closure and continued linear growth (in spite of otherwise normal pubertal development) that the estrogen receptor mediates epiphyseal closure.[861] He was found to have a homozygous C-to-T point mutation of codon 157 of the second exon in the ESR1 gene, introducing a premature termination signal. Expression of this gene leads to the production of a nonfunctional ESR1 lacking both the DNA- and hormone-binding domains. He was also found to have increased estradiol levels, impaired glucose tolerance with hyperinsulinemia, and decreased bone density.

Further strengthening the association between ESR1 and epiphyseal closure is the observation that women with ESR1-positive breast cancer and a mutation in the B domain (B′ allele) of ESR1 have an increased incidence of spontaneous abortion and tall stature.[862] These

associations were not found in female carriers of the allele without breast cancer or with ESR1-negative breast cancer. Thus, a second (as yet undiscovered) mutation is likely to play a role in the development of tall stature and spontaneous abortions in female carriers with ESR1-positive breast cancer.

Mineralocorticoid Receptors

Mineralocorticoid resistance is also known as pseudohypoaldosteronism (PHA). Both sporadic and familial cases with either autosomal-dominant or autosomal-recessive cases have been reported.[863-867] Clinical presentation of patients with PHA ranges from asymptomatic salt wasting; to growth failure; to chronic failure to thrive, lethargy, and emesis; to life-threatening dehydration accompanied by severe salt wasting.[864-866,868-870] Patients with the severe forms of PHA typically present within a year of birth and may even present in utero with polyhydramnios due to polyuria.[871] Biochemically, the condition is characterized by urinary salt wasting, hyponatremia, elevated plasma potassium, aldosterone, and renin activity and urinary aldosterone metabolism that are unresponsive to the mineralocorticoid treatment.[872-874]

Two forms of PHA are recognized (types I and II). Autosomal recessive (generalized) PHAI results from mutations in the epithelial sodium channel (ENaC), whereas autosomal dominant (renal) PHAI is due to mutations in the mineralocorticoid receptor (MR). PHAII is the result of mutations in a family of serine-threonine kinases known as WNK1 and WNK4, which are downstream of aldosterone action.[875] PHAI is characterized by renal tubular mineralocorticoid resistance, whereas PHA II results from mineralocorticoid resistance in the kidney, intestine, salivary or sweat gland and is also known as Gordon syndrome.[876] Patients with these conditions present with hyperkalemia that only responds to treatment with nonchloride ions, such as bicarbonate or sulfate, which increase delivery of sodium to the distal tubule.[877] In general, patients with autosomal dominant PHA1 have a mild salt wasting syndrome and improve with age, whereas patients with autosomal recessive PHA1 have severe salt wasting, hyperkalemia, as well as increased sweat and salivary sodium and frequent respiratory tract infections that fails to improve with age.[878]

More than 50 different mutations of the MR causing autosomal dominant PHAI have been described (all heterozygous).[878-889] There is clear heterogeneity of clinical manifestations within families. Haploinsufficiency due to mRNA or protein degradation is clearly sufficient to cause autosomal dominant PHAI.[884,887,890] Yet some mutations have been shown to exert dominant negative effects on the wild-type receptor.[887]

Mutations causing constitutive activation of the MR have been described and cause severe early-onset hypertension.[890] An interesting syndrome involving overactivation of the MRs is the syndrome of apparent mineralocorticoid excess. Patients with this autosomal-recessive condition can exhibit pre- and postnatal growth failure, hypervolemic hypertension, medullary nephrocalcinosis, and hypokalemic metabolic alkalosis accompanied by hyporeninemic hypoaldosteronism.[892-895] Patients with this syndrome may also be asymptomatic and exhibit only biochemical abnormalities.[896] Patients with this syndrome also have increased serum and urinary cortisol-to-cortisone ratios.[895] This syndrome is caused by mutations in the 11 β-hydroxysteroid dehydrogenase type 2 (11 β-HSD2) genes that reduce the activity of the enzyme.[894,895,897-899] 11 β-HSD2 converts the active glucocorticoid cortisol to inactive cortisone. Thus, decreased activity of 11 β-HSD2 increases cortisol levels in MR-containing tissues, leading to increased binding and activation of MRs by cortisol.[894,895]

A transient form of mineralocorticoid resistance, probably due to abnormal maturation of aldosterone receptor function, also exists.[891] This variant of PHA is known as the syndrome of early-childhood hyperkalemia. Children with this disorder present with failure to thrive or linear growth failure accompanied by hyperkalemia and metabolic acidosis. This condition resolves spontaneously by the second half of the first decade of life.

SUBFAMILY 0 NUCLEAR RECEPTORS: DAX1

Subfamily 0 nuclear receptors include DAX1.[682] DAX1 plays a role in the regulation of steroid, mullerian-inhibiting substance, and gonadotropin production.

DAX1

The dosage-sensitive sex-reversal adrenal hypoplasia congenital critical region on the X chromosome gene 1 (DAX1) is an orphan nuclear receptor because its ligand has not yet been identified.[900] It has homologies in the E domain to other orphan receptors, including RXRs.[901] However, DAX1 has an unusual DNA-binding C domain that contains a tract of amino acid repeats instead of zinc finger motifs.[901] DAX1 inhibits steroidogenic factor 1 (SF-1)-mediated transcription. SF-1 is another orphan nuclear receptor that regulates transcription of adrenal and gonadal steroid hydroxylases, mullerian-inhibiting substance, and gonadotropin genes.[902,903]

DAX1 gene mutations have been identified that cause X-linked adrenal hypoplasia congenita.[901] Patients with this condition have congenital adrenal insufficiency and are therefore deficient in glucocorticoid, mineralocorticoid, and androgen production.[901,904] Gonadotropin deficiency and azoospermia also occur in these patients.[904,905] Female carriers may have delayed puberty.[905] All mutations that have been found to cause X-linked congenital adrenal hypoplasia are either located in or prevent transcription of the area of the E domain that inhibits SF-1- mediated transcription.[900,906-908] Thus, DAX1 mutations may cause X-linked congenital adrenal hypoplasia by altering SF-1 regulation of gonado- and adrenogenesis.[906] DAX1 deletion may also occur in the setting of the contiguous gene deletion syndrome, resulting in complex glycerol kinase deficiency (cGKD) if individuals have deletions extending from the GK gene into the Duchene muscular dystrophy (DMD) gene or involving a significant extension telomeric from DAX1.[909]

SUMMARY

Understanding of receptors that transduce or influence hormone action has increased dramatically. As molecular biology techniques improve, it is expected that knowledge of receptor action will continue to increase at a rapid pace. It is likely that subtle defects in receptor function (such as regulatory or promoter region mutations that increase or decrease receptor gene expression, or mutations in second messenger proteins) will be found that cause endocrine disorders. It is also likely that new receptors will be discovered that transduce or influence hormone action and that endocrine roles will be found for receptors that were not previously thought to mediate or alter hormone action.

REFERENCES

1. Bockaert, J., & Pin, J. P. (1999). Molecular tinkering of G protein-coupled receptors: an evolutionary success. *EMBO J*, *18*, 1723–1729.
2. Vassart, G., & Costagliola, S. (2011). G protein-coupled receptors: mutations and endocrine diseases. *Nat Rev Endocrinol*, 7, 362–372.
3. Horn, F., Weare, J., Beukers, M. W., et al. (1998). GPCRDB: an information system for G protein-coupled receptors. *Nucleic Acids Res*, *26*, 275–279.
4. Vassart, G., Pardo, L., & Costagliola, S. (2004). A molecular dissection of the glycoprotein hormone receptors. *Trends Biochem Sci*, *29*, 119–126.
5. Baldwin, J. M. (1993). The probable arrangement of the helices in G protein-coupled receptors. *EMBO J*, *12*, 1693–1703.
6. Spengler, D., Waeber, C., Pantaloni, C., et al. (1993). Differential signal transduction by five splice variants of the PACAP receptor. *Nature*, *365*, 170–175.
7. Wess, J. (1997). G-protein-coupled receptors: molecular mechanisms involved in receptor activation and selectivity of G-protein recognition. *Faseb J*, *11*, 346–354.
8. Smrcka, A. V. (2008). G protein betagamma subunits: central mediators of G protein-coupled receptor signaling. *Cell Mol Life Sci*, *65*, 2191–214.
9. Daaka, Y., Luttrell, L. M., Ahn, S., et al. (1998). Essential role for G protein-coupled receptor endocytosis in the activation of mitogen-activated protein kinase. *J Biol Chem*, *273*, 685–688.
10. Eason, M. G., & Liggett, S. B. (1995). Identification of a Gs coupling domain in the amino terminus of the third intracellular loop of the alpha 2A-adrenergic receptor: evidence for distinct structural determinants that confer Gs versus Gi coupling. *J Biol Chem*, *270*, 24753–24760.
11. Fields, T. A., & Casey, P. J. (1997). Signaling functions and biochemical properties of pertussis toxin-resistant G-proteins. *Biochem J*, *321*, 561–571.
12. Limbird, L. E. (1988). Receptors linked to inhibition of adenylate cyclase: additional signaling mechanisms. *FASEB J*, *2*, 2686–2695.
13. Rivkees, S. A., Barbhaiya, H, & IJzerman, A. P. (1999). Identification of the adenine binding site of the human A1 adenosine receptor. *J Biol Chem*, *274*, 3617–3621.
14. Reppert, S. M., Weaver, D. R., Stehle, J. H., & Rivkees, S. A. (1991). Molecular cloning and characterization of a rat A1-adenosine receptor that is widely expressed in brain and spinal cord. *Mol Endocrinol*, *5*, 1037–1048.
15. Levitzki, A., Marbach, I., & Bar-Sinai, A. (1993). The signal transduction between beta-receptors and adenylyl cyclase. *Life Sci*, *52*, 2093–2100.
16. Thompson, M. D., Burnham, W. M., & Cole, D. E. (2005). The G protein-coupled receptors: pharmacogenetics and disease. *Crit Rev Clin Lab Sci*, *42*, 311–392.
17. Lu, D., Yan, H., Othman, T., et al. (2004). Cytoskeletal protein 4.1G binds to the third intracellular loop of the A1 adenosine receptor and inhibits receptor action. *Biochem J*, *377*, 51–59.
18. Lu, D., Yan, H., Othman, T., & Rivkees, S. A. (2004). Cytoskeletal protein 4.1G is a binding partner of the metabotropic glutamate receptor subtype 1 alpha. *J Neurosci Res*, *78*, 49–55.
19. Csaba, Z., & Dournaud, P. (2001). Cellular biology of somatostatin receptors. *Neuropeptides*, *35*, 1–23.
20. Rocheville, M., Lange, D. C., Kumar, U., et al. (2000). Subtypes of the somatostatin receptor assemble as functional homo- and heterodimers. *J Biol Chem*, *275*, 7862–7869.
21. Rocheville, M., Lange. D. C., Kumar. U., et al. (2000). Receptors for dopamine and somatostatin: formation of hetero-oligomers with enhanced functional activity. *Science*, *288*, 154–157.
22. Pfeiffer, M., Koch, T., Schroder, H., et al. (2001). Homo- and heterodimerization of somatostatin receptor subtypes: inactivation of sst(3) receptor function by heterodimerization with sst(2A). *J Biol Chem*, *276*, 14027–14036.
23. Born, W., Fischer, J. A., & Muff, R. (2002). Receptors for calcitonin gene-related peptide, adrenomedullin, and amylin: the contributions of novel receptor-activity-modifying proteins. *Receptors Channels*, *8*, 201–209.
24. Born, W., Muff, R., & Fischer, J. A. (2002). Functional interaction of G protein-coupled receptors of the adrenomedullin peptide family with accessory receptor-activity-modifying proteins (RAMP). *Microsc Res Tech*, *57*, 14–22.
25. Lerner, U. H. (2006). Deletions of genes encoding calcitonin/alpha-CGRP, amylin and calcitonin receptor have given new and unexpected insights into the function of calcitonin receptors and calcitonin receptor-like receptors in bone. *J Musculoskelet Neuronal Interact*, *6*, 87–95.
26. Christopoulos, A., Christopoulos, G., Morfis, M., et al. (2003). Novel receptor partners and function of receptor activity-modifying proteins. *J Biol Chem*, *278*, 3293–3297.
27. Angers, S., Salahpour, A., & Bouvier, M. (2002). Dimerization: an emerging concept for G protein-coupled receptor ontogeny and function. *Annu Rev Pharmacol Toxicol*, *42*, 409–435.
28. McLatchie, L. M., Fraser, N. J., Main, M. J., et al. (1998). RAMPs regulate the transport and ligand specificity of the calcitonin-receptor-like receptor. *Nature*, *393*, 333–339.
29. Metherell, L. A., Chapple, J. P., Cooray, S., et al. (2005). Mutations in MRAP, encoding a new interacting partner of the ACTH receptor, cause familial glucocorticoid deficiency type 2. *Nat Genet*, *37*, 166–170.
30. Webb, T. R., Chan, L., Cooray, S. N., et al. (2009). Distinct melanocortin 2 receptor accessory protein domains are required for melanocortin 2 receptor interaction and promotion of receptor trafficking. *Endocrinology*, *150*, 720–726.
31. Cooray, S. N., Chung, T. T., Mazhar, K., et al. (2011). Bioluminescence resonance energy transfer reveals the adrenocorticotropin (ACTH)-induced conformational change of the activated ACTH receptor complex in living cells. *Endocrinology*, *152*, 495–502.
32. Clark, A. J., & Weber, A. (1998). Adrenocorticotropin insensitivity syndromes. *Endocr Rev*, *19*, 828–843.
33. Biebermann, H., Krude, H., Elsner, A., et al. (2003). Autosomal-dominant mode of inheritance of a melanocortin-4 receptor mutation in a patient with severe early-onset obesity is due to a dominant-negative effect caused by receptor dimerization. *Diabetes*, *52*, 2984–2988.
34. Tarnow, P., Rediger, A., Brumm, H., et al. (2008). A heterozygous mutation in the third transmembrane domain causes a dominant-negative effect on signalling capability of the MC4R. *Obes Facts*, *1*, 155–162.
35. Morello, J. P., Salahpour, A., Petaja-Repo, U. E., et al. (2001). Association of calnexin with wild type and mutant AVPR2 that causes nephrogenic diabetes insipidus. *Biochemistry*, *40*, 6766–6775.
36. Zhu, X., & Wess, J. (1998). Truncated V2 vasopressin receptors as negative regulators of wild-type V2 receptor function. *Biochemistry*, *37*, 15773–15784.
37. Oksche, A., & Rosenthal, W. (1998). The molecular basis of nephrogenic diabetes insipidus. *J Mol Med* (Berl), *76*, 326–337.
38. Calebiro, D., de Filippis, T., Lucchi, S., et al. (2005). Intracellular entrapment of wild-type TSH receptor by oligomerization with mutants linked to dominant TSH resistance. *Hum Mol Genet*, *14*, 2991–3002.
39. Ulloa-Aguirre, A., Janovick, J. A., Leanos-Miranda, A., & Conn, P. M. (2004). Misrouted cell surface GnRH receptors as a disease aetiology for congenital isolated hypogonadotrophic hypogonadism. *Hum Reprod Update*, *10*, 177–192.

40. Leanos-Miranda, A., Ulloa-Aguirre, A., Ji, T. H., et al. (2003). Dominant-negative action of disease-causing gonadotropin-releasing hormone receptor (GnRHR) mutants: a trait that potentially coevolved with decreased plasma membrane expression of GnRHR in humans. *J Clin Endocrinol Metab*, *88*, 3360–3367.
41. Brothers, S. P., Cornea, A., Janovick, J. A., & Conn, P. M. (2004). Human loss-of-function gonadotropin-releasing hormone receptor mutants retain wild-type receptors in the endoplasmic reticulum: molecular basis of the dominant-negative effect. *Mol Endocrinol*, *18*, 1787–1797.
42. Seifert, R., & Wenzel-Seifert, K. (2002). Constitutive activity of G-protein-coupled receptors: cause of disease and common property of wild-type receptors. *Naunyn-Schmiedebergs Arch Pharmacol*, *366*, 381–416.
43. Milligan, G. (2003). Constitutive activity and inverse agonists of G protein-coupled receptors: a current perspective. *Mol Pharmacol*, *64*, 1271–1276.
44. Tiberi, M., & Caron, M. G. (1994). High agonist-independent activity is a distinguishing feature of the dopamine D1B receptor subtype. *J Biol Chem*, *269*, 27925–27931.
45. Milligan, G., Bond, R. A., & Lee, M. (1995). Inverse agonism: pharmacological curiosity or potential therapeutic strategy? *Trends Pharmacol Sci*, *16*, 10–13.
46. Ferguson, S. S. (2001). Evolving concepts in G protein-coupled receptor endocytosis: the role in receptor desensitization and signaling. *Pharmacol Rev*, *53*, 1–24.
47. Mason, D., Hassan, A., Chacko, S., & Thompson, P. (2002). Acute and chronic regulation of pituitary receptors for vasopressin and corticotropin releasing hormone. *Arch Physiol Biochem*, *110*, 74–89.
48. Lohse, M. J., Benovic, J. L., Codina, J., et al. (1990). Beta-arrestin: a protein that regulates beta-adrenergic receptor function. *Science*, *248*, 154–1550.
49. Mukherjee, S., Palczewski, K., Gurevich, V., et al. (1999). A direct role for arrestins in desensitization of the luteinizing hormone/choriogonadotropin receptor in porcine ovarian follicular membranes. *Proc Natl Acad Sci USA*, *96*, 493–498.
50. Bouvier, M., Hausdorff, W. P., De Blasi, A., et al. (1988). Removal of phosphorylation sites from the beta 2-adrenergic receptor delays onset of agonist-promoted desensitization. *Nature*, *333*, 370–373.
51. Hunzicker-Dunn, M., Gurevich, V. V., Casanova, J. E., & Mukherjee, S. (2002). ARF6: a newly appreciated player in G protein-coupled receptor desensitization. *FEBS Lett*, *521*, 3–8.
52. Troispoux, C., Guillou, F., Elalouf, J. M., et al. (1999). Involvement of G protein-coupled receptor kinases and arrestins in desensitization to follicle-stimulating hormone action. *Mol Endocrinol*, *13*, 1599–1614.
53. Lazari, M. F., Liu, X., Nakamura, K., et al. (1999). Role of G protein-coupled receptor kinases on the agonist-induced phosphorylation and internalization of the follitropin receptor. *Mol Endocrinol*, *13*, 866–878.
54. Nakamura, K., Lazari, M. F., Li, S., et al. (1999). Role of the rate of internalization of the agonist-receptor complex on the agonist-induced down-regulation of the lutropin/choriogonadotropin receptor. *Mol Endocrinol*, *13*, 1295–1304.
55. Nakamura, K., Liu, X., Ascoli, M. (2000). Seven non-contiguous intracellular residues of the lutropin/choriogonadotropin receptor dictate the rate of agonist-induced internalization and its sensitivity to non-visual arrestins. *J Biol Chem*, *275*, 241–247.
56. Frenzel, R., Voigt, C., Paschke, R. (2006). The human thyrotropin receptor is predominantly internalized by beta-arrestin 2. *Endocrinology*, *147*, 3114–3122.
57. Oakley, R. H., Laporte, S. A., Holt, J. A., et al. (2000). Differential affinities of visual arrestin, beta arrestin1, and beta arrestin2 for G protein-coupled receptors delineate two major classes of receptors. *J Biol Chem*, *275*, 17201–17210.
58. Lohse, M. J. (1993). Molecular mechanisms of membrane receptor desensitization. *Biochim Biophys*, *1179*, 171–188.
59. Morris, A. J., & Malbon, C. C. (1999). Physiological regulation of G protein-linked signaling. *Physiol Rev*, *79*, 1373–1430.
60. Scearce-Levie, K., Lieberman, M. D., Elliott, H. H., & Conklin, B. R. (2005). Engineered G protein coupled receptors reveal independent regulation of internalization, desensitization and acute signaling. *BMC Biol*, *3*, 3.
61. Barak, L. S., Oakley, R. H., Laporte, S. A., & Caron, M. G. (2001). Constitutive arrestin-mediated desensitization of a human vasopressin receptor mutant associated with nephrogenic diabetes insipidus. *Proc Natl Acad Sci USA*, *98*, 93–98.
62. Tao, Y. X. (2006). Inactivating mutations of G protein-coupled receptors and diseases: structure-function insights and therapeutic implications. *Pharmacol Ther*, *111*, 949–973.
63. Im, D. S. (2004). Discovery of new G protein-coupled receptors for lipid mediators. *J Lipid Res*, *45*, 410–418.
64. Krasnoperov, V. G., Bittner, M. A., Beavis, R., et al. (1997). Alpha-latrotoxin stimulates exocytosis by the interaction with a neuronal G-protein-coupled receptor. *Neuron*, *18*, 925–937.
65. O'Hara, P. J., Sheppard, P. O., Thogersen, H., et al. (1993). The ligand-binding domain in metabotropic glutamate receptors is related to bacterial periplasmic binding proteins. *Neuron*, *11*, 41–52.
66. Silve, C., Petrel, C., Leroy, C., et al. (2005). Delineating a Ca2+ binding pocket within the Venus flytrap module of the human calcium-sensing receptor. *J Biol Chem*, *280*, 37917–37923.
67. Magenis, R. E., Smith, L., Nadeau, J. H., et al. (1994). Mapping of the ACTH, MSH, and neural (MC3 and MC4) melanocortin receptors in the mouse and human. *Mamm Genome*, *5*, 503–508.
68. Mesiano, S., & Jaffe, R. B. (1997). Developmental and functional biology of the primate fetal adrenal cortex. *Endocr Rev*, *18*, 378–403.
69. Miller, W. L. (1988). Molecular biology of steroid hormone synthesis. *Endocr Rev*, *9*, 295–318.
70. Tiosano, D., Pannain, S., Vassart, G., et al. (1999). The hypothyroidism in an inbred kindred with congenital thyroid hormone and glucocorticoid deficiency is due to a mutation producing a truncated thyrotropin receptor. *Thyroid*, *9*, 887–894.
71. Clark, A. J., & Weber, A. (1998). Adrenocorticotropin insensitivity syndromes. *Endocr Rev*, *19*, 828–843.
72. Clark, A. J., McLoughlin, L., & Grossman, A. (1993). Familial glucocorticoid deficiency associated with point mutation in the adrenocorticotropin receptor. *Lancet*, *341*, 461–462.
73. Tsigos, C., Arai, K., Hung, W., & Chrousos, G. P. (1993). Hereditary isolated glucocorticoid deficiency is associated with abnormalities of the adrenocorticotropin receptor gene. *J Clinical Invest*, *92*, 2458–2461.
74. Weber, A., Toppari, J., Harvey, R. D., et al. (1995). Adrenocorticotropin receptor gene mutations in familial glucocorticoid deficiency: relationships with clinical features in four families. *J Clin Endocrinol Metab*, *80*, 65–71.
75. Tsigos, C., Arai, K., Latronico, A. C., et al. (1995). A novel mutation of the adrenocorticotropin receptor (ACTH-R) gene in a family with the syndrome of isolated glucocorticoid deficiency, but no ACTH-R abnormalities in two families with the triple A syndrome. *J Clin Endocrinol Metab*, *80*, 2186–2189.
76. Naville, D., Barjhoux, L., Jaillard, C., et al. (1996). Demonstration by transfection studies that mutations in the adrenocorticotropin receptor gene are one cause of the hereditary syndrome of glucocorticoid deficiency. *J Clin Endocrinol Metab*, *81*, 1442–1448.
77. Slavotinek, A. M., Hurst, J. A., Dunger, D., & Wilkie, A. O. (1998). ACTH receptor mutation in a girl with familial glucocorticoid deficiency. *Clin Genet*, *53*, 57–62.
78. Chung, T. T., Chan, L. F., Metherell, L. A., & Clark, A. J. (2010). Phenotypic characteristics of familial glucocorticoid deficiency (FGD) type 1 and 2. *Clin Endocrinol* (Oxf), *72*, 589–594.
79. Tullio-Pelet, A., Salomon, R., Hadj-Rabia, S., et al. (2000). Mutant WD-repeat protein in triple-A syndrome. *Nat Genet*, *26*, 332–335.
80. Cronshaw, J. M., & Matunis, M. J. (2003). The nuclear pore complex protein ALADIN is mislocalized in triple A syndrome. *Proc Natl Acad Sci USA*, *100*, 5823–5827.
81. Allgrove, J., Clayden, G. S., Grant, D. B., & Macaulay, J. C. (1978). Familial glucocorticoid deficiency with achalasia of the cardia and deficient tear production. *Lancet*, *1*, 1284–1286.
82. Shepard, T. H., Landing, B. H., Mason, & D. G. (1959). Familial Addison's disease. *Am J Dis Child*, *97*, 154–162.
83. Migeon, C. J., Kenny, E. M., Kowarski, A., et al. (1968). The syndrome of congenital adrenocortical unresponsiveness to ACTH: report of six cases. *Pediatr Res*, *2*, 501–513.
84. Monteleone, J. A., & Monteleone, P. L. (1970). Hereditary adrenocortical unresponsiveness to ACTH—another case. *Pediatrics*, *46*, 321–322.

85. Spark, R. F., & Etzkorn, J. R. (1977). Absent aldosterone response to ACTH in familial glucocorticoid deficiency. *N Engl J Med*, *297*, 917–920.
86. Davidai, G., Kahana, L., & Hochberg, Z. (1984). Glomerulosa failure in congenital adrenocortical unresponsiveness to ACTH. *Clin Endocrinol*, *20*, 515–520.
87. Kelch, R. P., Kaplan, S. L., Biglieri, E. G., et al. (1972). Hereditary adrenocortical unresponsiveness to adrenocorticotropic hormone. *J Pediatr*, *81*, 726–736.
88. Turan, S., Hughes, C., Atay, Z., et al. (2012). An atypical case of familial glucocorticoid deficiency without pigmentation caused by coexistent homozygous mutations in MC2R (T152K) and MC1R (R160W). *J Clin Endocrinol Metab*, *97*, E771–E774.
89. Lacy, D. E., Nathavitharana, K. A., & Tarlow, M. J. (1993). Neonatal hepatitis and congenital insensitivity to adrenocorticotropin (ACTH). *J Pediatr Gastroenterol Nutr*, *17*, 438–440.
90. Clark, A. J., Cammas, F. M., Watt, A., et al. (1997). Familial glucocorticoid deficiency: one syndrome, but more than one gene. *J Mol Med* (Berlin, Germany), *75*, 394–399.
91. Petrykowski, W., Burmeister, P., & Bohm, N. (1975). Familiar glucocorticoid insufficiency. *Klin Padiatr*, *187*, 198–215.
92. Lyons, J., Landis, C. A., Harsh, G., et al. (1990). Two G protein oncogenes in human endocrine tumors. *Science*, *249*, 655–659.
93. Reincke, M., Mora, P., Beuschlein, F., et al. (1997). Deletion of the adrenocorticotropin receptor gene in human adrenocortical tumors: implications for tumorigenesis. *J Clin Endocrinol Metab*, *82*, 3054–3058.
94. Lacroix, A., Bolte, E., Tremblay, J., et al. (1992). Gastric inhibitory polypeptide-dependent cortisol hypersecretion: a new cause of Cushing's syndrome. *N Engl J Med*, *327*, 974–980.
95. Reznik, Y., Allali-Zerah, V., Chayvialle, J. A., et al. (1992). Food-dependent Cushing's syndrome mediated by aberrant adrenal sensitivity to gastric inhibitory polypeptide. *N Engl J Med*, *327*, 981–986.
96. Lacroix, A., N'Diaye, N., Mircescu, H., et al. (1998). Abnormal expression and function of hormone receptors in adrenal Cushing's syndrome. *Endocr Res*, *24*, 835–843.
97. Arnaldi, G., Gasc, J. M., de Keyzer, Y., et al. (1998). Variable expression of the V1 vasopressin receptor modulates the phenotypic response of steroid-secreting adrenocortical tumors. *J Clin Endocrinol Metab*, *83*, 2029–2035.
98. Daidoh, H., Morita, H., Hanafusa, J., et al. (1998). In vivo and in vitro effects of AVP and V1a receptor antagonist on Cushing's syndrome due to ACTH-independent bilateral macronodular adrenocortical hyperplasia. *Clin Endocrinol*, *49*, 403–409.
99. Bourdeau, I., & Stratakis, C. A. (2002). Cyclic AMP-dependent signaling aberrations in macronodular adrenal disease. *Ann NY Acad Sci*, *968*, 240–255.
100. Tao, Y. X. (2010). Mutations in the melanocortin-3 receptor (MC3R) gene: impact on human obesity or adiposity. *Curr Opin Investig Drugs*, *11*, 1092–1096.
101. Yang, F., & Tao, Y. X. (2012). Functional characterization of nine novel naturally occurring human melanocortin-3 receptor mutations. *Biochim Biophys Acta*, *1822*, 1752–1761.
102. Butler, A. A., Kesterson, R. A., Khong, K., et al. (2000). A unique metabolic syndrome causes obesity in the melanocortin-3 receptor-deficient mouse. *Endocrinology*, *141*, 3518–3521.
103. Chen, A. S., Marsh, D. J., Trumbauer, M. E., et al. (2000). Inactivation of the mouse melanocortin-3 receptor results in increased fat mass and reduced lean body mass. *Nat Genet*, *26*, 97–102.
104. Feng, N., Young, S. F., Aguilera, G., et al. (2005). Co-occurrence of two partially inactivating polymorphisms of MC3R is associated with pediatric-onset obesity. *Diabetes*, *54*, 2663–2667.
105. Smit, M. J., Vischer, H. F., Bakker, R. A., et al. (2007). Pharmacogenomic and structural analysis of constitutive G protein-coupled receptor activity. *Annu Rev Pharmacol Toxicol*, *47*, 53–87.
106. Siegrist, W., Drozdz, R., Cotti, R., et al. (1997). Interactions of alpha-melanotropin and agouti on B16 melanoma cells: evidence for inverse agonism of agouti. *J Recept Signal Transduct Res*, *17*, 75–98.
107. Vergoni, A. V., Bertolini, A., Wikberg, J. E., & Schioth, H. B. (1999). Selective melanocortin MC4 receptor blockage reduces immobilization stress-induced anorexia in rats. *Eur J Pharmacol*, *369*, 11–15.
108. Vergoni, A. V., Bertolini, A., Guidetti, G., et al. (2000). Chronic melanocortin 4 receptor blockage causes obesity without influencing sexual behavior in male rats. *J Endocrinol*, *166*, 419–426.
109. Yeo, G. S., Farooqi, I. S., Aminian, S., et al. (1998). A frameshift mutation in MC4R associated with dominantly inherited human obesity. *Nat Genet*, *20*, 111–112.
110. Farooqi, I. S., Yeo, G. S., Keogh, J. M., et al. (2000). Dominant and recessive inheritance of morbid obesity associated with melanocortin 4 receptor deficiency. *J Clini Invest*, *106*, 271–279.
111. Farooqi, I. S., Keogh, J. M., Yeo, G. S., et al. (2003). Clinical spectrum of obesity and mutations in the melanocortin 4 receptor gene. *N Engl J Med*, *348*, 1085–1095.
112. Vink, T., Hinney, A., van Elburg, A. A., et al. (2001). Association between an agouti-related protein gene polymorphism and anorexia nervosa. *Mol Psychiatry*, *6*, 325–328.
113. Adan, R. A., & Vink, T. (2001). Drug target discovery by pharmacogenetics: mutations in the melanocortin system and eating disorders. *Eur Neuropsychopharmacol*, *11*, 483–490.
114. Chagnon, Y. C., Chen, W. J., Perusse, L., et al. (1997). Linkage and association studies between the melanocortin receptors 4 and 5 genes and obesity- related phenotypes in the Quebec Family Study. *Mol Med*, *3*, 663–673.
115. Farooqi, I. S., & O'Rahilly, S. (2005). Monogenic obesity in humans. *Annu Rev Med*, *56*, 443–458.
116. Farooqi, I. S., Keogh, J. M., Yeo, G. S., et al. (2003). Clinical spectrum of obesity and mutations in the melanocortin 4 receptor gene. *N Engl J Med*, *348*, 1085–1095.
117. Jacobson, P., Ukkola, O., Rankinen, T., et al. (2002). Melanocortin 4 receptor sequence variations are seldom a cause of human obesity: the Swedish Obese Subjects, the HERITAGE Family Study, and a Memphis cohort. *J Clin Endocrinol Metab*, *87*, 4442–4446.
118. Miraglia Del Giudice, E., Cirillo, G., Nigro, V., et al. (2002). Low frequency of melanocortin-4 receptor (MC4R) mutations in a Mediterranean population with early-onset obesity. *Int J Obes Relat Metab Disord*, *26*, 647–651.
119. Vaisse, C., Clement, K., Durand, E., et al. (2000). Melanocortin-4 receptor mutations are a frequent and heterogeneous cause of morbid obesity. *J Clin Invest*, *106*, 253–262.
120. Wakamatsu, K., Graham, A., Cook, D., & Thody, A. J. (1997). Characterisation of ACTH peptides in human skin and their activation of the melanocortin-1 receptor. *Pigment Cell Res*, *10*, 288–297.
121. Graham, A., Wakamatsu, K., Hunt, G., et al. (1997). Agouti protein inhibits the production of eumelanin and phaeomelanin in the presence and absence of alpha-melanocyte stimulating hormone. *Pigment Cell Res*, *10*, 298–303.
122. Mendiratta, M. S., Yang, Y., Balazs, A. E., et al. (2011). Early onset obesity and adrenal insufficiency associated with a homozygous POMC mutation. *Int J Pediatr Endocrinol*, *2011*, 5.
123. Rees, J. L. (2003). Genetics of hair and skin color. *Annu Rev Genet*, *37*, 67–90.
124. Challis, B. G., Pritchard, L. E., Creemers, J. W., et al. (2002). A missense mutation disrupting a dibasic prohormone processing site in pro-opiomelanocortin (POMC) increases susceptibility to early-onset obesity through a novel molecular mechanism. *Hum Mol Genet*, *11*, 1997–2004.
125. Farooqi, I. S., Drop, S., Clements, A., et al. (2006). Heterozygosity for a POMC-null mutation and increased obesity risk in humans. *Diabetes*, *55*, 2549–2553.
126. Lee, Y. S., Challis, B. G., Thompson, D. A., et al. (2006). A POMC variant implicates beta-melanocyte-stimulating hormone in the control of human energy balance. *Cell Metab*, *3*, 135–140.
127. Farooqi, I. S., Volders, K., Stanhope, R., et al. (2007). Hyperphagia and early-onset obesity due to a novel homozygous missense mutation in prohormone convertase 1/3. *J Clin Endocrinol Metab*, *92*, 3369–3373.
128. Jackson, R. S., Creemers, J. W., Ohagi, S. et al. (1997). Obesity and impaired prohormone processing associated with mutations in the human prohormone convertase 1 gene. *Nat Genet*, *16*, 303–306.
129. Creemers, J. W., Choquet, H., Stijnen, P., et al. (2012). Heterozygous mutations causing partial prohormone convertase 1 deficiency contribute to human obesity. *Diabetes*, *61*, 383–3890.
130. Drucker, D. J. (2007). The role of gut hormones in glucose homeostasis. *J Clin Invest*, *117*, 24–32.

131. Pasel, K., Schulz, A., Timmermann, K., et al. (2000). Functional characterization of the molecular defects causing nephrogenic diabetes insipidus in eight families. *J Clin Endocrinol Metab*, *85*, 1703–1710.
132. Bichet, D. G., Hendy, G. N., Lonergan, M., et al. (1992). X-linked nephrogenic diabetes insipidus: from the ship Hopewell to RFLP studies. *Am J Hum Genet*, *51*, 1089–1102.
133. Rosenthal, W., Seibold, A., Antaramian, A., et al. (1992). Molecular identification of the gene responsible for congenital nephrogenic diabetes insipidus. *Nature*, *359*, 233–235.
134. Lolait, S. J., O'Carroll, A. M., McBride, O. W., et al. (1992). Cloning and characterization of a vasopressin V2 receptor and possible link to nephrogenic diabetes insipidus. *Nature*, *357*, 336–339.
135. Knoers, N., van den Ouweland, A., Dreesen, J., et al. (1993). Nephrogenic diabetes insipidus: identification of the genetic defect. *Pediatr Nephrol*, *7*, 685–688.
136. Anesi, L., de Gemmis, P., Galla, D., & Hladnik, U. (2012). Two new large deletions of the AVPR2 gene causing nephrogenic diabetes insipidus and a review of previously published deletions. *Nephrol Dial Transplant*, *27*, 3705–312.
137. Wesche, D., Deen, P. M., & Knoers, N. V. (2012). Congenital nephrogenic diabetes insipidus: the current state of affairs. *Pediatr Nephrol*, 2012 Dec;27(12):2183–204.
138. Deen, P. M., Verdijk, M. A., Knoers, N. V., et al. (1994). Requirement of human renal water channel aquaporin-2 for vasopressin-dependent concentration of urine. *Science*, *264*, 92–95.
139. van Lieburg, A. F., Verdijk, M. A., Knoers, V. V., et al. (1994). Patients with autosomal nephrogenic diabetes insipidus homozygous for mutations in the aquaporin 2 water-channel gene. *Am J Hum Genet*, *55*, 648–652.
140. Ala, Y., Morin, D., Mouillac, B., et al. (1998). Functional studies of twelve mutant V2 vasopressin receptors related to nephrogenic diabetes insipidus: molecular basis of a mild clinical phenotype. *J Am Soc Nephrol*, *9*, 1861–1872.
141. Feldman, B. J., Rosenthal, S. M., Vargas, G. A., et al. (2005). Nephrogenic syndrome of inappropriate antidiuresis. *N Engl J Med*, *352*, 1884–1890.
142. Cheung, C. C., Cadnapaphornchai, M. A., Ranadive, S. A., et al. (2012). Persistent elevation of urine aquaporin-2 during water loading in a child with nephrogenic syndrome of inappropriate antidiuresis (NSIAD) caused by a R137L mutation in the V2 vasopressin receptor. *Int J Pediatr Endocrinol*, *2012*, 3.
143. Ben-Menahem, D., Hyde, R., Pixley, M., et al. (1999). Synthesis of multi-subunit domain gonadotropin complexes: a model for alpha/beta heterodimer formation. *Biochemistry*, *38*, 15070–15077.
144. Lustbader, J. W., Lobel, L., Wu, H., & Elliott, M. M. (1998). Structural and molecular studies of human chorionic gonadotropin and its receptor. *Recent Prog Horm Res*, *53*, 395–424.
145. Themmen, A. P., Martens, J. W., & Brunner, H. G. (1998). Activating and inactivating mutations in LH receptors. *Mol Cell Endocrinol*, *145*, 137-142.
146. Tsai-Morris, C. H., Buczko, E., Wang, W., et al. (1991). Structural organization of the rat luteinizing hormone (LH) receptor gene. *J Biol Chem*, *266*, 11355–11359.
147. Minegishi, T., Nakamura, K., Takakura, Y., et al. (1990). Cloning and sequencing of human LH/hCG receptor cDNA. *Biochem Biophys Res Commun*, *172*, 1049–1054.
148. Schwartz, M., Imperato-McGinley, J., Peterson, R. E., et al. (1981). Male pseudohermaphroditism secondary to an abnormality in Leydig cell differentiation. *J Clin Endocrinol Metab*, *53*, 123–127.
149. Toledo, S. P., Arnhold, I. J., Luthold, W, et al. (1985). Leydig cell hypoplasia determining familial hypergonadotropic hypogonadism. *Prog Clin Biol Res*, *200*, 311–314.
150. el-Awady, M. K., Temtamy, S. A., et al. (1987). Familial Leydig cell hypoplasia as a cause of male pseudohermaphroditism. *Hum Hered*, *37*, 36–40.
151. Martinez-Mora, J., Saez, J. M., Toran, N., et al. (1991). Male pseudohermaphroditism due to Leydig cell agenesia and absence of testicular LH receptors. *Clin Endocrinol*, *34*, 485–491.
152. Toledo, S. P. (1992). Leydig cell hypoplasia leading to two different phenotypes: male pseudohermaphroditism and primary hypogonadism. *Clin Endocrinol*, *36*, 521–522.
153. Kremer, H., Kraaij, R., Toledo, S. P., et al. (1995). Male pseudohermaphroditism due to a homozygous missense mutation of the luteinizing hormone receptor gene. *Nat Genet*, *9*, 160–164.
154. Latronico, A. C., Anasti, J., Arnhold, I. J., et al. (1996). Brief report: testicular and ovarian resistance to luteinizing hormone caused by inactivating mutations of the luteinizing hormone-receptor gene. *N Engl J Med*, *334*, 507–512.
155. Chan, W. Y. (1998). Molecular genetic, biochemical, and clinical implications of gonadotropin receptor mutations. *Mol Genet Metab*, *63*, 75–84.
156. Latronico, A. C., Chai, Y., Arnhold, I. J., et al. (1998). A homozygous microdeletion in helix 7 of the luteinizing hormone receptor associated with familial testicular and ovarian resistance is due to both decreased cell surface expression and impaired effector activation by the cell surface receptor. *Mol Endocrinol*, *12*, 442–450.
157. Martens, J. W., Verhoef-Post, M., Abelin, N., et al. (1998). A homozygous mutation in the luteinizing hormone receptor causes partial Leydig cell hypoplasia: correlation between receptor activity and phe type. *Mol Endocrinol*, *12*, 775–784.
158. Laue, L. L., Wu, S. M., Kudo, M., et al. (1996). Compound heterozygous mutations of the luteinizing hormone receptor gene in Leydig cell hypoplasia. *Mol Endocrinol*, *10*, 987–997.
159. Misrahi, M., Meduri, G., Pissard, S., et al. (1997). Comparison of immunocytochemical and molecular features with the phenotype in a case of incomplete male pseudohermaphroditism associated with a mutation of the luteinizing hormone receptor. *J Clin Endocrinol Metab*, *82*, 2159–2165.
160. Gromoll, J., Eiholzer, U., Nieschlag, E., & Simoni, M. (2000). Male hypogonadism caused by homozygous deletion of exon 10 of the luteinizing hormone (LH) receptor: differential action of human chorionic gonadotropin and LH. *J Clin Endocrinol Metab*, *85*, 2281–2286.
161. Arnhold, I. J., Lofrano-Porto, A., & Latronico, A. C. (2009). Inactivating mutations of luteinizing hormone beta-subunit or luteinizing hormone receptor cause oligo-amenorrhea and infertility in women. *Horm Res*, *71*, 75–82.
162. Schedewie, H. K., Reiter, E. O., Beitins, I. Z., et al. (1981). Testicular leydig cell hyperplasia as a cause of familial sexual precocity. *J Clin Endocrinol Metab*, *52*, 271–278.
163. Rosenthal, S. M., Grumbach, M. M., & Kaplan, S. L. (1983). Gonadotropin-independent familial sexual precocity with premature Leydig and germinal cell maturation (familial testotoxicosis): effects of a potent luteinizing hormone-releasing factor agonist and medroxyprogesterone acetate therapy in four cases. *J Clin Endocrinol Metab*, *57*, 571–579.
164. Gondos, B., Egli, C. A., Rosenthal, S. M., & Grumbach, M. M. (1985). Testicular changes in gonadotropin-independent familial male sexual precocity: familial testotoxicosis. *Arch Pathol Lab Med*, *109*, 990–995.
165. Egli, C. A., Rosenthal, S. M., Grumbach, M. M., et al. (1985). Pituitary gonadotropin-independent male-limited autosomal dominant sexual precocity in nine generations: familial testotoxicosis. *J Pediatr*, *106*, 33–40.
166. Kremer, H., Mariman, E., Otten, B. J., et al. (1993). Cosegregation of missense mutations of the luteinizing hormone receptor gene with familial male-limited precocious puberty. *Hum Mol Genet*, *2*, 1779–1783.
167. Rosenthal, I. M., Refetoff, S., Rich, B., et al. (1996). Response to challenge with gonadotropin- releasing hormone agonist in a mother and her two sons with a constitutively activating mutation of the luteinizing hormone receptor: a clinical research center study. J Clin Endocrinol Metab, 81, 3802–3806.
168. Oerter, K. E., Uriarte, M. M., Rose, S. R., et al. (1990). Gonadotropin secretory dynamics during puberty in normal girls and boys. *J Clin Endocrinol Metab*, *71*, 1251–1258.
169. Latronico, A. C., Abell, A. N., Arnhold, I. J., et al. (1998). A unique constitutively activating mutation in third transmembrane helix of luteinizing hormone receptor causes sporadic male gonadotropin-independent precocious puberty. *J Clin Endocrinol Metab*, *83*, 2435–2440.
170. Nagasaki, K., Katsumata, N., Ogawa, Y., et al. (2011). Novel C617Y mutation in the 7th transmembrane segment of luteinizing hormone/choriogonadotropin receptor in a Japanese boy with peripheral precocious puberty. *Endocr J*, *57*, 1055–1060.

171. Goji, K., Teraoka, Y., Hosokawa, Y., et al. (2009). Gonadotropin-independent precocious puberty associated with a somatic activating mutation of the LH receptor gene: detection of a mutation present in only a small fraction of cells from testicular tissue using wild-type blocking polymerase chain reaction and laser-capture microdissection. *Endocrine*, *35*, 397–401.
172. Sangkhathat, S., Kanngurn, S., Jaruratanasirikul, S., et al. (2010). Peripheral precocious puberty in a male caused by Leydig cell adenoma harboring a somatic mutation of the LHR gene: report of a case. *J Med Assoc Thai*, *93*, 1093–1097.
173. Rosenthal, I. M., Refetoff, S., Rich, B., et al. (1996). Response to challenge with gonadotropin-releasing hormone agonist in a mother and her two sons with a constitutively activating mutation of the luteinizing hormone receptor: a clinical research center study. *J Clin Endocrinol Metab*, *81*, 3802–3806.
174. Toledo, S. P., Brunner, H. G., Kraaij, R., et al. (1996). An inactivating mutation of the luteinizing hormone receptor causes amenorrhea in a 46,XX female. J Clin Endocrinol Metab, *81*, 3850–3854.
175. Tapanainen, J. S., Vaskivuo, T., Aittomaki, K., & Huhtaniemi, I. T. (1998). Inactivating FSH receptor mutations and gonadal dysfunction. *Mol Cell Endocrinol*, *145*, 129–135.
176. Minegishi, T., Nakamura, K., Takakura, Y., et al. (1991). Cloning and sequencing of human FSH receptor cDNA. *Biochem Biophys Res Commun*, *175*, 1125–1130.
177. Heckert, L. L., Daley, I. J., & Griswold, M. D. (1992). Structural organization of the follicle-stimulating hormone receptor gene. *Mol Endocrinol*, *6*, 70–80.
178. Jones, G. S., Acosta, A. A., Garcia, J. E., et al. (1985). The effect of follicle-stimulating hormone without additional luteinizing hormone on follicular stimulation and oocyte development in normal ovulatory women. *Fertil Steril*, *43*, 696–702.
179. Durham, C. R., Zhu, H., Masters, B. S., et al. (1985). Regulation of aromatase activity of rat granulosa cells: induction of synthesis of NADPH-cytochrome P-450 reductase by FSH and dibutyryl cyclic AMP. *Mol Cell Endocrinol*, *40*, 211–219.
180. Knobil, E. (1980). The neuroendocrine control of the menstrual cycle. *Recent Prog Horm Res*, *36*, 53–88.
181. Aittomaki, K., Herva, R., Stenman, U. H., et al. (1996). Clinical features of primary ovarian failure caused by a point mutation in the follicle-stimulating hormone receptor gene. *J Clin Endocrinol Metab*, *81*, 3722–3726.
182. Aittomaki, K., Lucena, J. L., Pakarinen, P., et al. (1995). Mutation in the follicle-stimulating hormone receptor gene causes hereditary hypergonadotropic ovarian failure. *Cell*, *82*, 959–968.
183. Tapanainen, J. S., Aittomaki, K., Min, J., et al. (1997). Men homozygous for an inactivating mutation of the follicle-stimulating hormone (FSH) receptor gene present variable suppression of spermatogenesis and fertility. *Nat Genet*, *15*, 205–206.
184. Jiang, M., Aittomaki, K., Nilsson, C., et al. (1998). The frequency of an inactivating point mutation (566C → T) of the human follicle-stimulating hormone receptor gene in four populations using allele-specific hybridization and time-resolved fluorometry. *J Clin Endocrinol Metab*, *83*, 4338–4343.
185. Tapanainen, J. S., Vaskivuo, T., Aittomaki, K., & Huhtaniemi, I. T. (1998). Inactivating FSH receptor mutations and gonadal dysfunction. *Mol Cell Endocrinol*, *145*, 129–135.
186. Touraine, P., Beau, I., Gougeon, A., et al. (1999). New natural inactivating mutations of the follicle-stimulating hormone receptor: correlations between receptor function and phenotype. *Mol Endocrinol*, *13*, 1844–1854.
187. Gromoll, J., Simoni, M., & Nieschlag, E. (1996). An activating mutation of the follicle-stimulating hormone receptor autonomously sustains spermatogenesis in a hypophysectomized man. *J Clin Endocrinol Metab*, *81*, 1367–1370.
188. Doherty, E., Pakarinen, P., Tiitinen, A., et al. (2002). A Novel mutation in the FSH receptor inhibiting signal transduction and causing primary ovarian failure. *J Clin Endocrinol Metab*, *87*, 1151–1155.
189. Kotlar, T. J., Young, R. H., Albanese, C., et al. (1997). A mutation in the follicle-stimulating hormone receptor occurs frequently in human ovarian sex cord tumors. *J Clin Endocrinol Metab*, *82*, 1020–1026.
190. Allen, L. A., Achermann, J. C., Pakarinen, P., et al. (2003). A novel loss of function mutation in exon 10 of the FSH receptor gene causing hypergonadotrophic hypogonadism: clinical and molecular characteristics. *Hum Reprod*, *18*, 251–256.
191. Meduri, G., Touraine, P., Beau, I., et al. (2003). Delayed puberty and primary amenorrhea associated with a novel mutation of the human follicle-stimulating hormone receptor: clinical, histological, and molecular studies. *J Clin Endocrinol Metab*, *88*, 3491–3498.
192. Beau, I., Touraine, P., Meduri, G., et al. (1998). A novel phenotype related to partial loss of function mutations of the follicle stimulating hormone receptor. *J Clini Invest*, *102*, 1352–1359.
193. Touraine, P., Beau, I., Gougeon, A., et al. (1999). New natural inactivating mutations of the follicle-stimulating hormone receptor: correlations between receptor function and phenotype. *Mol Endocrinol*, *13*, 1844–1854.
194. Gromoll, J., Simoni, M., Nieschlag, E. (1996). An activating mutation of the follicle-stimulating hormone receptor autonomously sustains spermatogenesis in a hypophysectomized man. *J Clin Endocrinol Metab*, *81*, 1367–1370.
195. Laue, L., Chan, W. Y., Hsueh, A. J., et al. (1995). Genetic heterogeneity of constitutively activating mutations of the human luteinizing hormone receptor in familial male-limited precocious puberty. *Proc Natl Acad Sci USA*, *92*, 1906–1910.
196. Tonacchera, M., Van Sande, J., Parma, J., et al. (1996). TSH receptor and disease. *Clin Endocrinol*, *44*, 621–633.
197. Smits, G., Olatunbosun, O., Delbaere, A., et al. (2003). Ovarian hyperstimulation syndrome due to a mutation in the follicle-stimulating hormone receptor. *N Engl J Med*, *349*, 760–766.
198. Vasseur, C., Rodien, P., Beau, I., et al. (2003). A chorionic gonadotropin-sensitive mutation in the follicle-stimulating hormone receptor as a cause of familial gestational spontaneous ovarian hyperstimulation syndrome. *N Engl J Med*, *349*, 753–759.
199. Montanelli, L., Delbaere, A., Di Carlo, C., et al. (2004). A mutation in the follicle-stimulating hormone receptor as a cause of familial spontaneous ovarian hyperstimulation syndrome. *J Clin Endocrinol Metab*, *89*, 1255–1258.
200. De Leener, A., Montanelli, L., Van Durme, J., et al. (2006). Presence and absence of follicle-stimulating hormone receptor mutations provide some insights into spontaneous ovarian hyperstimulation syndrome physiopathology. *J Clin Endocrinol Metab*, *91*, 555–562.
201. Libert, F., Lefort, A., Gerard, C., et al. (1989). Cloning, sequencing and expression of the human thyrotropin (TSH) receptor: evidence for binding of autoantibodies. *Biochem Biophys Res Commun*, *165*, 1250–1255.
202. Nagayama, Y., Kaufman, K. D., Seto, P., & Rapoport, B. (1989). Molecular cloning, sequence and functional expression of the cDNA for the human thyrotropin receptor. *Biochem Biophys Res Commun*, *165*, 1184–1190.
203. Misrahi, M., Loosfelt, H., Atger, M., et al. (1990). Cloning, sequencing and expression of human TSH receptor. *Biochem Biophys Res Commun*, *166*, 394–403.
204. Gross, B., Misrahi, M., Sar, S., & Milgrom, E. (1991). Composite structure of the human thyrotropin receptor gene. *Biochem Biophys Res Commun*, *177*, 679–687.
205. Dumont, J. E., Lamy, F., Roger, P., & Maenhaut, C. (1992). Physiological and pathological regulation of thyroid cell proliferation and differentiation by thyrotropin and other factors. *Physiol Rev*, *72*, 667–697.
206. Allgeier, A., Offermanns, S., Van Sande, J., et al. (1994). The human thyrotropin receptor activates G-proteins Gs and Gq/11. *J Biol Chem*, *269*, 13733–13735.
207. Russo, D., Chazenbalk, G. D., Nagayama, Y., et al. (1991). A new structural model for the thyrotropin (TSH) receptor, as determined by covalent cross-linking of TSH to the recombinant receptor in intact cells: evidence for a single polypeptide chain. *Mol Endocrinol*, *5*, 1607–1612.
208. Rapoport, B., Chazenbalk, G. D., Jaume, J. C., & McLachlan, S. M. (1998). The thyrotropin (TSH) receptor: interaction with TSH and autoantibodies. *Endocr Rev*, *19*, 673–716.
209. Chazenbalk, G. D., Tanaka, K., McLachlan, S. M., & Rapoport, B. (1999). On the functional importance of thyrotropin receptor intramolecular cleavage. *Endocrinology*, *140*, 4516-4520.
210. Tanaka, K., Chazenbalk, G. D., McLachlan, S. M., & Rapoport, B. (1999). Thyrotropin receptor cleavage at site 1 involves two discontinuous segments at each end of the unique 50-amino acid insertion. *J Biol Chem*, *274*, 2093–2096.

211. Kajita, Y., Rickards, C. R., Buckland, P. R., et al. (1985). Analysis of thyrotropin receptors by photoaffinity labelling: orientation of receptor subunits in the cell membrane. *Biochem J*, *227*, 413–420.
212. Loosfelt, H., Pichon, C., Jolivet, A., et al. (1992). Two-subunit structure of the human thyrotropin receptor. *Proc Natl Acad Sci USA*, *89*, 3765–3769.
213. Misrahi, M., Ghinea, N., Sar, S., et al. (1994). Processing of the precursors of the human thyroid-stimulating hormone receptor in various eukaryotic cells (human thyrocytes, transfected L cells and baculovirus-infected insect cells). *Eur J Biochem/FEBS*, *222*, 711–719.
214. Tanaka, K., Chazenbalk, G. D., McLachlan, S. M., & Rapoport, B. (1999). Subunit structure of thyrotropin receptors expressed on the cell surface. *J Biol Chem*, *274*, 33979–33984.
215. Nagayama, Y., Wadsworth, H. L., Chazenbalk, G. D., et al. (1991). Thyrotropin-luteinizing hormone/chorionic gonadotropin receptor extracellular domain chimeras as probes for thyrotropin receptor function. *Proc Natl Acad Sci USA*, *88*, 902–905.
216. Nagayama, Y., & Rapoport, B. (1992). Role of the carboxyl-terminal half of the extracellular domain of the human thyrotropin receptor in signal transduction. *Endocrinology*, *131*, 548–552.
217. Kopp, P., Muirhead, S., Jourdain, N., et al. (1997). Congenital hyperthyroidism caused by a solitary toxic adenoma harboring a novel somatic mutation (serine281 → isoleucine) in the extracellular domain of the thyrotropin receptor. *J Clin Invest*, *100*, 1634–1639.
218. Duprez, L., Parma, J., Costagliola, S., et al. (1997). Constitutive activation of the TSH receptor by spontaneous mutations affecting the N-terminal extracellular domain. *FEBS Letters*, *409*, 469–474.
219. Gruters, A., Schoneberg, T., Biebermann, H., et al. (1998). Severe congenital hyperthyroidism caused by a germ-line neo mutation in the extracellular portion of the thyrotropin receptor. *J Clin Endocrinol Metab*, *83*, 1431–1436.
220. Parma, J., Duprez, L., Van Sande, J., et al. (1993). Somatic mutations in the thyrotropin receptor gene cause hyperfunctioning thyroid adenomas. *Nature*, *365*, 649–651.
221. Porcellini, A., Ciullo, I., Laviola, L., et al. (1994). Novel mutations of thyrotropin receptor gene in thyroid hyperfunctioning adenomas: rapid identification by fine needle aspiration biopsy. *J Clin Endocrinol Metab*, *79*, 657–661.
222. Paschke, R., Tonacchera, M., Van Sande, J., et al. (1994). Identification and functional characterization of two new somatic mutations causing constitutive activation of the thyrotropin receptor in hyperfunctioning autonomous adenomas of the thyroid. *J Clin Endocrinol Metab*, *79*, 1785–1789.
223. Russo, D., Arturi, F., Wicker, R., et al. (1995). Genetic alterations in thyroid hyperfunctioning adenomas. *J Clin Endocrinol Metab*, *80*, 1347–1351.
224. Russo, D., Arturi, F., Suarez, H. G., et al. (1996). Thyrotropin receptor gene alterations in thyroid hyperfunctioning adenomas. *J Clin Endocrinol Metab*, *81*, 1548–1551.
225. Parma, J., Duprez, L., Van Sande, J., et al. (1997). Diversity and prevalence of somatic mutations in the thyrotropin receptor and Gs alpha genes as a cause of toxic thyroid adenomas . *J Clin Endocrinol Metab*, *82*, 2695–2701.
226. Fuhrer, D., Holzapfel, H. P., Wonerow, P., et al. (1997). Somatic mutations in the thyrotropin receptor gene and not in the Gs alpha protein gene in 31 toxic thyroid nodules. *J Clin Endocrinol Metab*, *82*, 3885–3891.
227. Krohn, K., Fuhrer, D., Holzapfel, H. P., & Paschke, R. (1998). Clonal origin of toxic thyroid nodules with constitutively activating thyrotropin receptor mutations. *J Clin Endocrinol Metab*, *83*, 130–134.
228. Parma, J., Van Sande, J., Swillens, S., et al. (1995). Somatic mutations causing constitutive activity of the thyrotropin receptor are the major cause of hyperfunctioning thyroid adenomas: identification of additional mutations activating both the cyclic adenosine 3',5'-monophosphate and inositol phosphate-Ca2+ cascades. *Mol Endocrinol*, *9*, 725–733.
239. Paschke, R., & Ludgate, M. (1997). The thyrotropin receptor in thyroid diseases. *N Engl J Med*, *337*, 1675–1681.
230. Takeshita, A., Nagayama, Y., Yokoyama, N., et al. (1995). Rarity of oncogenic mutations in the thyrotropin receptor of autonomously functioning thyroid nodules in Japan. *J Clin Endocrinol Metab*, *80*, 2607–2611.
231. Nogueira, C. R., Kopp, P., Arseven, O. K., et al. (1999). Thyrotropin receptor mutations in hyperfunctioning thyroid adenomas from Brazil. *Thyroid*, *9*, 1063–1068.
232. Holzapfel, H. P., Fuhrer, D., Wonerow, P., et al. (1997). Identification of constitutively activating somatic thyrotropin receptor mutations in a subset of toxic multinodular goiters. *J Clin Endocrinol Metab*, *82*, 4229–4233.
233. Russo, D., Arturi, F., Schlumberger, M., et al. (1995). Activating mutations of the TSH receptor in differentiated thyroid carcinomas. *Oncogene*, *11*, 1907–1911.
234. Spambalg, D., Sharifi, N., Elisei, R., et al. (1996). Structural studies of the thyrotropin receptor and Gs alpha in human thyroid cancers: low prevalence of mutations predicts infrequent involvement in malignant transformation. *J Clin Endocrinol Metab*, *81*, 3898–3901.
235. Russo, D., Tumino, S., Arturi, F., et al. (1997). Detection of an activating mutation of the thyrotropin receptor in a case of an autonomously hyperfunctioning thyroid insular carcinoma. *J Clin Endocrinol Metab*, 82, 735–738.
236. O'Sullivan, C., Barton, C. M., Staddon, S. L., et al. (1991). Activating point mutations of the gsp oncogene in human thyroid adenomas. *Mol Carcinog*, *4*, 345–349.
237. Suarez, H. G., du Villard, J. A., Caillou, B., et al. (1991). gsp mutations in human thyroid tumours. *Oncogene*, *6*, 677–679.
238. Schaarschmidt, J., Paschke, S., Ozerden, M., et al. (2012). Late manifestation of subclinical hyperthyroidism after goitrogenesis in an index patient with a N670S TSH receptor germline mutation masquerading as TSH receptor antibody negative Graves' disease. *Horm Metab Res*, *44*, 962–965.
239. Duprez, L., Parma, J., Van Sande, J., et al. (1994). Germline mutations in the thyrotropin receptor gene cause non-autoimmune autosomal dominant hyperthyroidism. *Nat Genet*, 7, 396–401.
240. Fuhrer, D., Wonerow, P., Willgerodt, H., & Paschke, R. (1997). Identification of a new thyrotropin receptor germline mutation (Leu629Phe) in a family with neonatal onset of autosomal dominant nonautoimmune hyperthyroidism. *J Clin Endocrinol Metab*, *82*, 4234–4238.
241. Tonacchera, M., Van Sande, J., Cetani, F., et al. (1996). Functional characteristics of three new germline mutations of the thyrotropin receptor gene causing autosomal dominant toxic thyroid hyperplasia. *J Clin Endocrinol Metab*, *81*, 547–554.
242. Schwab, K. O., Sohlemann, P., Gerlich, M., et al. (1996). Mutations of the TSH receptor as cause of congenital hyperthyroidism. *Exp Clin Endocrinol Diabetes*, *104*, 124–128.
243. Kopp, P., van Sande, J., Parma, J., et al. (1995). Brief report: congenital hyperthyroidism caused by a mutation in the thyrotropin-receptor gene. *N Engl J Med*, *332*, 150–154.
244. de Roux, N., Polak, M., Couet, J., et al. (1996). A neomutation of the thyroid-stimulating hormone receptor in a severe neonatal hyperthyroidism. *J Clin Endocrinol Metab*, *81*, 2023–2026.
245. Holzapfel, H. P., Wonerow, P., von Petrykowski, W., et al. (1997). Sporadic congenital hyperthyroidism due to a spontaneous germline mutation in the thyrotropin receptor gene. *J Clin Endocrinol Metab*, *82*, 3879–3884.
246. Schwab, K. O., Gerlich, M., Broecker, M., et al. (1997). Constitutively active germline mutation of the thyrotropin receptor gene as a cause of congenital hyperthyroidism. *J Pediatr*, *131*, 899–904.
247. Esapa, C. T., Duprez, L., Ludgate, M., et al. (1999). A novel thyrotropin receptor mutation in an infant with severe thyrotoxicosis. *Thyroid*, *9*, 1005–1010.
248. Biebermann, H., Winkler, F., Handke, D., et al. (2011). Molecular description of non-autoimmune hyperthyroidism at a neonate caused by a new thyrotropin receptor germline mutation. *Thyroid Res*, *4*(Suppl. 1), S8.
249. Costagliola, S., Sunthorntepvarakul, T., Migeotte, I., et al. (1999). Structure-function relationships of two loss-of-function mutations of the thyrotropin receptor gene. *Thyroid*, *9*, 995–1000.
250. de Roux, N., Misrahi, M., Brauner, R., et al. (1996). Four families with loss of function mutations of the thyrotropin receptor. *J Clin Endocrinol Metab*, *81*, 4229–4235.
251. Biebermann, H., Winkler, F., Handke, D., et al. (2011). New pathogenic thyrotropin receptor mutations decipher differentiated activity switching at a conserved helix 6 motif of family A GPCR. *J Clin Endocrinol Metab*, *97*, E228–E232.

252. Tonacchera, M., Agretti, P., Pinchera, A., et al. (2000). Congenital hypothyroidism with impaired thyroid response to thyrotropin (TSH) and absent circulating thyroglobulin: evidence for a new inactivating mutation of the TSH receptor gene. *J Clin Endocrinol Metab*, *85*, 1001–1008.
253. Sunthorntepvarakul T., Gottschalk, M. E., Hayashi, Y., & Refetoff, S. (1995). Brief report: resistance to thyrotropin caused by mutations in the thyrotropin-receptor gene. *N Engl J Med*, *332*, 155–160.
254. Clifton-Bligh, R. J., Gregory, J. W., Ludgate, M., et al. (1997). Two novel mutations in the thyrotropin (TSH) receptor gene in a child with resistance to TSH. *J Clin Endocrinol Metab*, *82*, 1094–1100.
255. Biebermann, H., Schoneberg, T., Krude, H., et al. (1997). Mutations of the human thyrotropin receptor gene causing thyroid hypoplasia and persistent congenital hypothyroidism. *J Clin Endocrinol Metab*, *82*, 3471–3480.
256. Abramowicz, M. J., Duprez, L., Parma, J., et al. (1997). Familial congenital hypothyroidism due to inactivating mutation of the thyrotropin receptor causing profound hypoplasia of the thyroid gland. *J Clini Invest*, *99*, 3018–3024.
257. Gagne, N., Parma, J., Deal, C., et al. (1998). Apparent congenital athyreosis contrasting with normal plasma thyroglobulin levels and associated with inactivating mutations in the thyrotropin receptor gene: are athyreosis and ectopic thyroid distinct entities? *J Clin Endocrinol Metab*, *83*, 1771–1775.
258. Alves, E. A., Cruz, C. M., Pimentel, C. P., et al. (2010). High frequency of D727E polymorphisms in exon 10 of the TSHR gene in Brazilian patients with congenital hypothyroidism. *J Pediatr Endocrinol Metab*, *23*, 1321–1328.
259. Cangul, H., Aycan, Z., Saglam. H., et al. (2012). TSHR is the main causative locus in autosomal recessively inherited thyroid dysgenesis. *J Pediatr Endocrinol Metab*, *25*, 419–426.
260. Chang, W. C., Liao, C. Y., Chen, W. C., et al. (2012). R450H TSH receptor mutation in congenital hypothyroidism in Taiwanese children. *Clin Chim Acta*, *413*, 1004–1007.
261. Narumi, S., Muroya, K., Abe, Y., et al. (2009). TSHR mutations as a cause of congenital hypothyroidism in Japan: a population-based genetic epidemiology study. *J Clin Endocrinol Metab*, *94*, 1317–1223.
262. Saito, T., Endo, T., Kawaguchi, A., et al. (1997). Increased expression of the Na+/I- symporter in cultured human thyroid cells exposed to thyrotropin and in Graves' thyroid tissue. *J Clin Endocrinol Metab*, *82*, 3331–3336.
263. Narumi, S., Nagasaki, K., Ishii, T., et al. (2011). Nonclassic TSH resistance: TSHR mutation carriers with discrepantly high thyroidal iodine uptake. *J Clin Endocrinol Metab*, *96*, E1340–E1345.
264. Mimouni, M., Mimouni-Bloch, A., Schachter, J., & Shohat, M. (1996). Familial hypothyroidism with autosomal dominant inheritance. *Arch Dis Child*, *75*, 245–246.
265. Xie, J., Pannain, S., Pohlenz, J., et al. (1997). Resistance to thyrotropin (TSH) in three families is not associated with mutations in the TSH receptor or TSH. *J Clin Endocrinol Metab*, *82*, 3933–3940.
266. Grossmann, M., Weintraub, B. D., & Szkudlinski, M. W. (1997). Novel insights into the molecular mechanisms of human thyrotropin action: structural, physiological, and therapeutic implications for the glycoprotein hormone family. *Endocr Rev*, *18*, 476–501.
267. Harada, A., Hershman, J. M., Reed, A. W., et al. (1979). Comparison of thyroid stimulators and thyroid hormone concentrations in the sera of pregnant women. *J Clin Endocrinol Metab*, *48*, 793-797.
268. Hershman, J. M. (1999). Human chorionic gonadotropin and the thyroid: hyperemesis gravidarum and trophoblastic tumors. *Thyroid*, *9*, 653–657.
269. Karp, P. J., Hershman, J. M., Richmond, S., et al. (1973). Thyrotoxicosis from molar thyrotropin. *Arch Intern Med*, *132*, 432–436.
270. Kenimer, J. G., Hershman, J. M., & Higgins, H. P. (1975). The thyrotropin in hydatidiform moles is human chorionic gonadotropin. *J Clin Endocrinol Metab*, *40*, 482–491.
271. Higgins, H. P., Hershman, J. M., Kenimer, J. G., et al. (1975). The thyrotoxicosis of hydatidiform mole. *Ann Intern Med*, *83*, 307–311.
272. Nagataki, S., Mizuno, M., Sakamoto, S., et al. (1977). Thyroid function in molar pregnancy. *J Clin Endocrinol Metab*, *44*, 254–263.
273. Anderson, N. R., Lokich, J. J., McDermott, W. V., Jr., et al. (1979). Gestational choriocarcinoma and thyrotoxicosis. *Cancer*, *44*, 304–306.
274. Walkington, L., Webster, J., Hancock, B. W., et al. (2011). Hyperthyroidism and human chorionic gonadotrophin production in gestational trophoblastic disease. *Br J Cancer*, *104*, 1665–1669.
275. Lockwood, C. M., Grenache, D. G., & Gronowski, A. M. (2009). Serum human chorionic gonadotropin concentrations greater than 400,000 IU/L are invariably associated with suppressed serum thyrotropin concentrations. *Thyroid*, *19*, 863–868.
276. Rodien, P., Bremont, C., Sanson, M. L., et al. (1998). Familial gestational hyperthyroidism caused by a mutant thyrotropin receptor hypersensitive to human chorionic gonadotropin. *N Engl J Med*, *339*, 1823–1826.
277. Smits, G., Olatunbosun, O., Delbaere, A., et al. (2003). Ovarian hyperstimulation syndrome due to a mutation in the follicle-stimulating hormone receptor. *N Engl J Med*, *349*, 760–766.
278. Montanelli, L., Delbaere, A., Di Carlo, C., et al. (2004). A mutation in the follicle-stimulating hormone receptor as a cause of familial spontaneous ovarian hyperstimulation syndrome. *J Clin Endocrinol Metab*, *89*, 1255–1258.
279. De Leener, A., Montanelli, L., Van Durme, J., et al. (2006). Presence and absence of follicle-stimulating hormone receptor mutations provide some insights into spontaneous ovarian hyperstimulation syndrome physiopathology. *J Clin Endocrinol Metab*, *91*, 555–562.
280. Fan, N. C., Jeung, E. B., Peng, C., et al. (1994). The human gonadotropin-releasing hormone (GnRH) receptor gene: cloning, genomic organization and chromosomal assignment. *Mol Cell Endocrinol*, *103*, R1–R6.
281. Kottler, M. L., Lorenzo, F., Bergametti, F., et al. (1995). Subregional mapping of the human gonadotropin-releasing hormone receptor (GnRH-R) gene to 4q between the markers D4S392 and D4S409. *Hum Genet*, *96*, 477–480.
282. Kakar, S. S., Musgrove, L. C., Devor, D. C., et al. (1992). Cloning, sequencing, and expression of human gonadotropin releasing hormone (GnRH) receptor. *Biochem Biophys Res Commun*, *189*, 289–295.
283. Chi, L., Zhou, W., Prikhozhan, A., et al. (1993). Cloning and characterization of the human GnRH receptor. *Mol Cell Endocrinol*, *91*, R1–R6.
284. Stojilkovic, S. S., Reinhart, J., & Catt, K. J. (1994). Gonadotropin-releasing hormone receptors: structure and signal transduction pathways. *Endocr Rev*, *15*, 462–499.
285. Hokin, L. E. (1985). Receptors and phosphoinositide-generated second messengers. *Annu Rev Biochem*, *54*, 205–235.
286. Berridge, M. J. (1987). Inositol trisphosphate and diacylglycerol: two interacting second messengers. *Annu Rev Biochem*, *56*, 159–193.
287. de Roux, N., Young, J., Misrahi, M., et al. (1997). A family with hypogonadotropic hypogonadism and mutations in the gonadotropin-releasing hormone receptor. *N Engl J Med*, *337*, 1597–1602.
288. Seminara, S. B., Hayes, F. J., & Crowley, W. F., Jr. (1998). Gonadotropin-releasing hormone deficiency in the human (idiopathic hypogonadotropic hypogonadism and Kallmann's syndrome): pathophysiological and genetic considerations. *Endocr Rev*, *19*, 521–539.
289. Pralong, F. P., Gomez, F., Castillo, E., et al. (1999). Complete hypogonadotropic hypogonadism associated with a novel inactivating mutation of the gonadotropin-releasing hormone receptor. *J Clin Endocrinol Metab*, *84*, 3811–3816.
290. Brothers, S. P., Cornea, A., Janovick, J. A., & Conn, P. M. (2004). Human loss-of-function gonadotropin-releasing hormone receptor mutants retain wild-type receptors in the endoplasmic reticulum: molecular basis of the dominant-negative effect. *Mol Endocrinol*, *18*, 1787–1797.
291. de Roux, N., Young, J., Brailly-Tabard, S,, et al. (1999). The same molecular defects of the gonadotropin-releasing hormone receptor determine a variable degree of hypogonadism in affected kindred. *J Clin Endocrinol Metab*, *84*, 567–572.
292. Seminara, S. B., Beranova, M., Oliveira, L. M., et al. (2000). Successful use of pulsatile gonadotropin-releasing hormone (GnRH) for ovulation induction and pregnancy in a patient with GnRH receptor mutations. *J Clin Endocrinol Metab*, *85*, 556–562.
293. Chanson, P., De Roux, N., Young, J., et al. (1998). Absence of activating mutations in the GnRH receptor gene in human pituitary gonadotroph adenomas. *Eur J Endocrinol*, *139*, 157–160.

294. Gershengorn, M. C. (1989). Mechanism of signal transduction by TRH. *Ann NY Acad Sci*, *553*, 191–196.
295. Collu, R., Tang, J., Castagne, J., et al. (1997). A novel mechanism for isolated central hypothyroidism: inactivating mutations in the thyrotropin-releasing hormone receptor gene. *J Clin Endocrinol Metab*, *82*, 1561–1565.
296. Bonomi, M., Busnelli, M., Beck-Peccoz, P., et al. (2009). A family with complete resistance to thyrotropin-releasing hormone. *N Engl J Med*, *360*, 731–734.
297. Rabeler, R., Mittag, J., Geffers, L., et al. (2004). Generation of thyrotropin-releasing hormone receptor 1-deficient mice as an animal model of central hypothyroidism. *Mol Endocrinol*, *18*, 1450–1460.
298. Tomita, T., Masuzaki, H., Iwakura, H., et al. (2006). Expression of the gene for a membrane-bound fatty acid receptor in the pancreas and islet cell tumours in humans: evidence for GPR40 expression in pancreatic beta cells and implications for insulin secretion. *Diabetologia*, *49*, 962–968.
299. Liou, A. P., Lu, X., Sei, Y. et al. (2010). The G-protein-coupled receptor GPR40 directly mediates long-chain fatty acid-induced secretion of cholecystokinin. *Gastroenterology*, *140*, 903–912.
300. Edfalk, S., Steneberg, P., & Edlund, H. (2008). Gpr40 is expressed in enteroendocrine cells and mediates free fatty acid stimulation of incretin secretion. *Diabetes*, *57*, 2280–2287.
301. Ichimura, A., Hirasawa, A., Hara, T., & Tsujimoto, G, (2009). Free fatty acid receptors act as nutrient sensors to regulate energy homeostasis. *Prostaglandins Other Lipid Mediat*, *89*, 82–88.
302. Itoh, Y., Kawamata, Y., Harada, M., et al. (2003). Free fatty acids regulate insulin secretion from pancreatic beta cells through GPR40. *Nature*, *422*, 173–176.
303. Fujiwara, K., Maekawa, F., & Yada, T. (2005). Oleic acid interacts with GPR40 to induce Ca2+ signaling in rat islet beta-cells: mediation by PLC and L-type Ca2+ channel and link to insulin release. *Am J Physiol*, *289*, E670–E677.
304. Shapiro, H., Shachar, S., Sekler, I., et al. (2005). Role of GPR40 in fatty acid action on the beta cell line INS-1E. *Biochem Biophys Res Commun*, *335*, 97–104.
305. Itoh, Y., & Hinuma, S. (2005). GPR40, a free fatty acid receptor on pancreatic beta cells, regulates insulin secretion. *Hepatol Res*, *33*, 171–173.
306. Vettor, R., Granzotto, M., De Stefani, D., et al. (2008). Loss-of-function mutation of the GPR40 gene associates with abnormal stimulated insulin secretion by acting on intracellular calcium mobilization. *J Clin Endocrinol Metab*, *93*, 3541–3550.
307. Hamid, Y. H., Vissing, H., Holst, B., et al. (2005). Studies of relationships between variation of the human G protein-coupled receptor 40 gene and type 2 diabetes and insulin release. *Diabet Med*, *22*, 74–80.
308. Burant, C. F., Viswanathan, P., Marcinak, J., et al. (2012). TAK-875 versus placebo or glimepiride in type 2 diabetes mellitus: a phase 2, randomised, double-blind, placebo-controlled trial. *Lancet*, *379*, 1403–1411.
309. Steneberg, P., Rubins, N., Bartoov-Shifman, R., et al. (2005). The FFA receptor GPR40 links hyperinsulinemia, hepatic steatosis, and impaired glucose homeostasis in mouse. *Cell Metab*, *1*, 245–258.
310. Ogawa, T., Hirose, H., Miyashita, K., et al. (2005). GPR40 gene Arg211His polymorphism may contribute to the variation of insulin secretory capacity in Japanese men. *Metabolism*, *54*, 296–299.
311. Bo-Abbas, Y., Acierno, J. S., Jr., Shagoury, J. K., et al. (2003). Autosomal recessive idiopathic hypogonadotropic hypogonadism: genetic analysis excludes mutations in the gonadotropin-releasing hormone (GnRH) and GnRH receptor genes. *J Clin Endocrinol Metab*, *88*, 2730–2737.
312. de Roux, N., Genin, E., Carel, J. C., et al. (2003). Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54. *Proc Natl Acad Sci USA*, *100*, 10972–10976.
313. Seminara, S. B., Messager, S., Chatzidaki, E. E., et al. (2003). The GPR54 gene as a regulator of puberty. *N Engl J Med*, *349*, 1614–1627.
314. Seminara, S. B. (2005). Metastin and its G protein-coupled receptor, GPR54: critical pathway modulating GnRH secretion. *Front Neuroendocrinol*, *26*, 131–138.
315. Wacker, J. L., Feller, D. B., Tang, X. B., et al. (2008). Disease-causing mutation in GPR54 reveals the importance of the second intracellular loop for class A G-protein-coupled receptor function. *J Biol Chem*, *283*, 31068–31078.
316. Semple, R. K., Achermann, J. C., Ellery, J., et al. (2005). Two novel missense mutations in g protein-coupled receptor 54 in a patient with hypogonadotropic hypogonadism. *J Clin Endocrinol Metab*, *90*, 1849–1855.
317. Tenenbaum-Rakover, Y., Commenges-Ducos, M., Iovane, A., et al. (2007). Neuroendocrine phenotype analysis in five patients with isolated hypogonadotropic hypogonadism due to a L102P inactivating mutation of GPR54. *J Clin Endocrinol Metab*, *92*, 1137–1144.
318. Kotani, M., Detheux, M., Vandenbogaerde, A., et al. (2001). The metastasis suppressor gene KiSS-1 encodes kisspeptins, the natural ligands of the orphan G protein-coupled receptor GPR54. *J Biol Chem*, *276*, 34631–34636.
319. Ohtaki, T., Shintani, Y., Honda, S., et al. (2001). Metastasis suppressor gene KiSS-1 encodes peptide ligand of a G-protein-coupled receptor. *Nature*, *411*, 613–617.
320. Dhillo, W. S., Chaudhri, O. B., Patterson, M., et al. (2005). Kisspeptin-54 stimulates the hypothalamic-pituitary gonadal axis in human males. *J Clin Endocrinol Metab*, *90*, 6609–6615.
321. Dungan Lemko, H. M., & Elias, C. F. (2012). Kiss of the mutant mouse: how genetically altered mice advanced our understanding of kisspeptin's role in reproductive physiology. *Endocrinology*, *153*, 5119–5129.
322. Teles, M. G., Bianco, S. D., Brito, V. N., et al. (2008). A GPR54-activating mutation in a patient with central precocious puberty. *N Engl J Med*, *358*, 709–715.
323. Sakurai, T., Amemiya, A., Ishii, M., et al. (1998). Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell*, *92*, 573–585.
324. Sutcliffe, J. G., & de Lecea, L. (2000). The hypocretins: excitatory neuromodulatory peptides for multiple homeostatic systems, including sleep and feeding. *J Neurosci Res*, *62*, 161–168.
325. Spinazzi, R., Andreis, P. G., Rossi, G. P., & Nussdorfer, G. G. (2006). Orexins in the regulation of the hypothalamic-pituitary-adrenal axis. *Pharmacol Rev*, *58*, 46–57.
326. de Lecea, L., Kilduff, T. S., Peyron, C., et al. (1998). The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. *Proc Natl Acad Sci USA*, *95*, 322–327.
327. Ammoun, S., Holmqvist, T., Shariatmadari, R., et al. (2003). Distinct recognition of OX1 and OX2 receptors by orexin peptides. *J Pharmacol Exp Ther*, *305*, 507–514.
328. Smart, D., Jerman, J. C., Brough, S. J., et al. (1999). Characterization of recombinant human orexin receptor pharmacology in a Chinese hamster ovary cell-line using FLIPR. *Br J Pharmacol*, *128*, 1–3.
329. Kane, J. K., Tanaka, H., Parker, S. L., et al. (2000). Sensitivity of orexin-A binding to phospholipase C inhibitors, neuropeptide Y, and secretin. *Biochem Biophys Res Commun*, *272*, 959–965.
330. Holmqvist, T., Johansson, L., Ostman, M., et al. (2005). OX1 orexin receptors couple to adenylyl cyclase regulation via multiple mechanisms. *J Biol Chem*, *280*, 6570–6579.
331. Sakurai, T. (1999). Orexins and orexin receptors: implication in feeding behavior. *Regulatory Peptides*, *85*, 25–30.
332. Xu, Y. L., Jackson, V. R., Civelli, O. (2004). Orphan G protein-coupled receptors and obesity. *Eur J Pharmacol*, *500*, 243–253.
333. Nishino, S., Ripley, B., Overeem, S., et al. (2000). Hypocretin (orexin) deficiency in human narcolepsy. *Lancet*, *355*, 39–40.
334. Peyron, C., Faraco, J., Rogers, W., et al. (2000). A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. *Nat Med*, *6*, 991–997.
335. Nishino, S., Ripley, B., Overeem, S., et al. (2001). Low cerebrospinal fluid hypocretin (Orexin) and altered energy homeostasis in human narcolepsy. *Ann Neurol*, *50*, 381–388.
336. Sutcliffe, J. G., & de Lecea, L. (2002). The hypocretins: setting the arousal threshold. *Nat Rev*, *3*, 339–349.
337. Nishino, S., Okuro, M., Kotorii, N., et al. (2010). Hypocretin/orexin and narcolepsy: new basic and clinical insights. *Acta Physiol* (Oxf), *198*, 209–222.

338. Lin, L., Faraco, J., Li, R., et al. (1999). The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. *Cell*, *98*, 365–376.
339. Peyron, C., Faraco, J., Rogers, W., et al. (2000). A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. *Nat Med*, *6*, 991–997.
340. Zhang, J. V., Ren, P. G., Avsian-Kretchmer, O., et al. (2005). Obestatin, a peptide encoded by the ghrelin gene, opposes ghrelin's effects on food intake. *Science*, *310*, 996–999.
341. Kojima, M., Hosoda, H., Date, Y., et al. (1999). Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature*, *402*, 656–660.
342. Date, Y., Kojima, M., Hosoda, H., et al. (2000). Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. *Endocrinology*, *141*, 4255–4261.
343. Davenport, A. P., Bonner, T. I., Foord, S. M., et al. (2005). International Union of Pharmacology. LVI: ghrelin receptor nomenclature, distribution, and function. *Pharmacol Rev*, *57*, 541–546.
344. Cummings, D. E., Purnell, J. Q., Frayo, R. S., et al. (2001). A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes*, *50*, 1714–1719.
345. Tschop, M., Wawarta, R., Riepl, R. L., et al. (2001). Post-prandial decrease of circulating human ghrelin levels. *J Endocrinol Invest*, *24*, RC19–RC21.
346. Wren, A. M., Seal, L. J., Cohen, M. A., et al. (2001). Ghrelin enhances appetite and increases food intake in humans. *J Clin Endocrinol Metab*, *86*, 5992.
347. Cummings, D. E., Clement, K., Purnell, J. Q., et al. (2002). Elevated plasma ghrelin levels in Prader Willi syndrome. *Nat Med*, *8*, 643–644.
348. Wang, H. J., Geller, F., Dempfle, A., et al. (2004). Ghrelin receptor gene: identification of several sequence variants in extremely obese children and adolescents, healthy normal-weight and underweight students, and children with short normal stature. *J Clin Endocrinol Metab*, *89*, 157–162.
349. Pantel, J., Legendre, M., Cabrol, S., et al. (2006). Loss of constitutive activity of the growth hormone secretagogue receptor in familial short stature. *J Clini Invest*, *116*, 760–768.
350. Pantel, J., Legendre, M., Nivot, S., et al. (2009). Recessive isolated growth hormone deficiency and mutations in the ghrelin receptor. *J Clin Endocrinol Metab*, *94*, 4334–4341.
351. Inoue, H., Kangawa, N., Kinouchi, A., et al. (2012). Identification and functional analysis of novel human growth hormone secretagogue receptor (GHSR) gene mutations in Japanese subjects with short stature. *J Clin Endocrinol Metab*, *96*, E373–E378.
352. Pugliese-Pires, P. N., Fortin, J. P., Arthur, T., et al. (2011). Novel inactivating mutations in the GH secretagogue receptor gene in patients with constitutional delay of growth and puberty. *Eur J Endocrinol*, *165*, 233–241.
353. Chambers, J., Ames, R. S., Bergsma, D., et al. (1999). Melanin-concentrating hormone is the cognate ligand for the orphan G-protein-coupled receptor SLC-1. *Nature*, *400*, 261–265.
354. Saito, Y., Nothacker, H. P., Wang, Z., et al. (1999). Molecular characterization of the melanin-concentrating-hormone receptor. *Nature*, *400*, 265–269.
355. Hill, J., Duckworth, M., Murdock, P., et al. (2001). Molecular cloning and functional characterization of MCH2, a novel human MCH receptor. *J Biol Chem*, *276*, 20125–20129.
356. Wang, S., Behan, J., O'Neill, K., et al. (2001). Identification and pharmacological characterization of a novel human melanin-concentrating hormone receptor, mch-r2. *J Biol Chem*, *276*, 34664–34670.
357. Hawes, B. E., Kil, E., Green, B., et al. (2000). The melanin-concentrating hormone receptor couples to multiple G proteins to activate diverse intracellular signaling pathways. *Endocrinology*, *141*, 4524–4532.
358. Borowsky, B., Durkin, M. M., Ogozalek, K., et al. (2002). Antidepressant, anxiolytic and anorectic effects of a melanin-concentrating hormone-1 receptor antagonist. *Nat Med*, *8*, 825–830.
359. Shearman, L. P., Camacho, R. E., Sloan Stribling, D., et al. (2003). Chronic MCH-1 receptor modulation alters appetite, body weight and adiposity in rats. *Eur J Pharmacol*, *475*, 37-47.
360. Chung, S., Parks, G. S., Lee, C., & Civelli, O. (2010). Recent updates on the melanin-concentrating hormone (MCH) and its receptor system: lessons from MCH1R antagonists. *J Mol Neurosci*, *43*, 115–121.
361. Gehlert, D. R., Rasmussen, K., Shaw, J., et al. (2009). Preclinical evaluation of melanin-concentrating hormone receptor 1 antagonism for the treatment of obesity and depression. *J Pharmacol Exp Ther*, *329*, 429–438.
362. Goldstein, C., Schroeder, J. C., Fortin, J. P., et al. (2010). Two naturally occurring mutations in the type 1 melanin-concentrating hormone receptor abolish agonist-induced signaling. *J Pharmacol Exp Ther*, *335*, 799–806.
363. Wermter, A. K., Reichwald, K., Buch, T., et al. (2005). Mutation analysis of the MCHR1 gene in human obesity. *Eur J Endocrinol*, *152*, 851–862.
364. Wermter, A. K., Reichwald, K., Buch, T., et al. (2005). Mutation analysis of the MCHR1 gene in human obesity. *Eur J Endocrinol*, *152*, 851-862.
365. Gibson, W. T., Pissios, P., Trombly, D. J., et al. (2004). Melanin-concentrating hormone receptor mutations and human obesity: functional analysis. *Obes Res*, *12*, 743–749.
366. Maheshwari, H. G., Silverman, B. L., Dupuis, J., & Baumann, G. (1998). Phenotype and genetic analysis of a syndrome caused by an inactivating mutation in the growth hormone-releasing hormone receptor: dwarfism of Sindh. *J Clin Endocrinol Metab*, *83*, 4065–4074.
367. Salvatori, R., Hayashida, C. Y., Aguiar-Oliveira, M. H., et al. (1999). Familial dwarfism due to a novel mutation of the growth hormone-releasing hormone receptor gene. *J Clin Endocrinol Metab*, *84*, 917–923.
368. Vallar, L., Spada, A., & Giannattasio, G. (1987). Altered Gs and adenylate cyclase activity in human GH-secreting pituitary adenomas. *Nature*, *330*, 566–568.
369. Alatzoglou, K. S., Turton, J. P., Kelberman, D., et al. (2009). Expanding the spectrum of mutations in GH1 and GHRHR: genetic screening in a large cohort of patients with congenital isolated growth hormone deficiency. *J Clin Endocrinol Metab*, *94*, 3191–3199.
370. Martari, M., & Salvatori, R. (2009). Diseases associated with growth hormone-releasing hormone receptor (GHRHR) mutations. *Prog Mol Biol Transl Sci*, *88*, 57–84.
371. Shohreh, R., Sherafat-Kazemzadeh, R., Jee, Y. H., et al. (2012). A novel frame shift mutation in the GHRH receptor gene in familial isolated GH deficiency: early occurrence of anterior pituitary hypoplasia. *J Clin Endocrinol Metab*, *96*, 2982–2986.
372. Wang, Q., Diao, Y., Xu, Z., et al. (2009). Identification of a novel splicing mutation in the growth hormone (GH)-releasing hormone receptor gene in a Chinese family with pituitary dwarfism. *Mol Cell Endocrinol*, *313*, 50–56.
373. Godfrey, P., Rahal, J. O., Beamer, W. G., et al. (1993). GHRH receptor of little mice contains a missense mutation in the extracellular domain that disrupts receptor function. *Nat Genet*, *4*, 227–232.
374. Lin, S. C., Lin, C. R., Gukovsky, I., et al. (1993). Molecular basis of the little mouse phenotype and implications for cell type-specific growth. *Nature*, *364*, 208–213.
375. Gaylinn, B. D., Dealmeida, V. I., Lyons, C. E., Jr., et al. (1999). The mutant growth hormone-releasing hormone (GHRH) receptor of the little mouse does not bind GHRH. *Endocrinology*, *140*, 5066–50674.
376. Wajnrajch, M. P., Gertner, J. M., Harbison, M. D., et al. (1996). Nonsense mutation in the human growth hormone-releasing hormone receptor causes growth failure analogous to the little (lit) mouse. *Nat Genet*, *12*, 88–90.
377. Netchine, I., Talon, P., Dastot, F., et al. (1998). Extensive phenotypic analysis of a family with growth hormone (GH) deficiency caused by a mutation in the GH-releasing hormone receptor gene. *J Clin Endocrinol Metab*, *83*, 432–436.
378. Salvatori, R., Fan, X., Phillips, J. A., III, et al. (2001). Isolated growth hormone (GH) deficiency due to compound heterozygosity for two new mutations in the GH-releasing hormone receptor gene. *Clin Endocrinol*, *54*, 681–687.
379. Salvatori, R., Fan, X., Mullis, P. E., et al. (2002). Decreased expression of the GHRH receptor gene due to a mutation in a Pit-1 binding site. *Mol Endocrinol*, *16*, 450–458.

380. Siklar, Z., Berberoglu, M., Legendre, M., et al. (2011). Two siblings with isolated GH deficiency due to loss-of-function mutation in the GHRHR gene: successful treatment with growth hormone despite late admission and severe growth retardation . *J Clin Res Pediatr Endocrinol*, *2*, 164–167.
381. Menezes Oliveira, J. L., Marques-Santos, C, Barreto-Filho, J. A., et al. (2006). Lack of evidence of premature atherosclerosis in untreated severe isolated growth hormone (GH) deficiency due to a GH-releasing hormone receptor mutation. *J Clin Endocrinol Metab*, *91*, 2093–2099.
382. Oliveira, J. L., Aguiar-Oliveira, M. H., D'Oliveira, A., Jr., et al. (2007). Congenital growth hormone (GH) deficiency and atherosclerosis: effects of GH replacement in GH-naive adults. *J Clin Endocrinol Metab*, *92*, 4664–4670.
383. Araujo, V. P., Aguiar-Oliveira, M. H., Oliveira, J. L., et al. (2012). Arrest of atherosclerosis progression after interruption of GH replacement in adults with congenital isolated GH deficiency. *Eur J Endocrinol*, *166*, 977–982.
384. Pereira, R. M., Aguiar-Oliveira, M. H., Sagazio, A., et al. (2007). Heterozygosity for a mutation in the growth hormone-releasing hormone receptor gene does not influence adult stature, but affects body composition . J Clin Endocrinol Metab, *92*, 2353–2357.
385. Camats, N., Fernandez-Cancio, M., Carrascosa, A., et al. (2012). Contribution of human growth hormone-releasing hormone receptor (GHRHR) gene sequence variation to isolated severe growth hormone deficiency (ISGHD) and normal adult height. *Clin Endocrinol* (Oxf), *77*, 564–574.
386. Johansson, A., Jonasson, I., & Gyllensten, U. (2009). Extended haplotypes in the growth hormone releasing hormone receptor gene (GHRHR) are associated with normal variation in height. *PLoS One*, *4*, e4464.
387. Franca, M. M., Jorge, A. A., Alatzoglou, K. S., et al. (2011). Absence of GH-releasing hormone (GHRH) mutations in selected patients with isolated GH deficiency. *J Clin Endocrinol Metab*, *96*, E1457–E1460.
388. Yamada, Y., Hayami, T., Nakamura, K., et al. (1995). Human gastric inhibitory polypeptide receptor: cloning of the gene (GIPR) and cDNA. *Genomics*, *29*, 773–776.
389. Gremlich. S., Porret, A., Hani, E. H., et al. (1995). Cloning, functional expression, and chromosomal localization of the human pancreatic islet glucose-dependent insulinotropic polypeptide receptor. *Diabetes*, *44*, 1202–1208.
390. Wheeler, M. B., Gelling, R. W., McIntosh, C. H., et al. (1995). Functional expression of the rat pancreatic islet glucose-dependent insulinotropic polypeptide receptor: ligand binding and intracellular signaling properties. *Endocrinology*, *136*, 4629–4639.
391. Tseng, C. C., & Zhang, X. Y. (1998). Role of regulator of G protein signaling in desensitization of the glucose-dependent insulinotropic peptide receptor. *Endocrinology*, *139*, 4470–4475.
392. Beck, B. (1989). Gastric inhibitory polypeptide: a gut hormone with anabolic functions. J Mol Endocrinol, *2*, 169–174.
393. Fehmann, H. C., Goke, R., & Goke, B. (1995). Cell and molecular biology of the incretin hormones glucagon-like peptide-I and glucose-dependent insulin releasing polypeptide. *Endocr Rev*, *16*, 390–410.
394. Sauber, J., Grothe, J., Behm, M., et al. (2010). Association of variants in gastric inhibitory polypeptide receptor gene with impaired glucose homeostasis in obese children and adolescents from Berlin. *Eur J Endocrinol*, *163*, 259–264.
395. Almind, K., Ambye, L., Urhammer, S. A., et al. (1998). Discovery of amino acid variants in the human glucose-dependent insulinotropic polypeptide (GIP) receptor: the impact on the pancreatic beta cell responses and functional expression studies in Chinese hamster fibroblast cells. *Diabetologia*, *41*, 1194–1198.
396. N'Diaye, N., Tremblay, J., Hamet, P., et al. (1998). Adrenocortical overexpression of gastric inhibitory polypeptide receptor underlies food-dependent Cushing's syndrome. *J Clin Endocrinol Metab*, *83*, 2781–2785.
397. Lebrethon, M. C., Avallet, O., Reznik, Y., et al. (1998). Food-dependent Cushing's syndrome: characterization and functional role of gastric inhibitory polypeptide receptor in the adrenals of three patients. *J Clin Endocrinol Metab*, *83*, 4514–4519.
398. de Herder, W. W., Hofland, L. J., Usdin, T. B., et al. (1996). Food-dependent Cushing's syndrome resulting from abundant expression of gastric inhibitory polypeptide receptors in adrenal adenoma cells. *J Clin Endocrinol Metab*, *81*, 3168–3172.
399. Mannstadt, M., Juppner, H., & Gardella, T. J. (1999). Receptors for PTH and PTHrP: their biological importance and functional properties. *Am J Physiol*, *277*, F665–F675.
400. Usdin, T. B., Gruber, C., & Bonner, T. I. (1995). Identification and functional expression of a receptor selectively recognizing parathyroid hormone, the PTH2 receptor. *J Biol Chem*, *270*, 15455–15458.
401. Behar, V., Pines, M., Nakamoto, C., et al. (1996). The human PTH2 receptor: binding and signal transduction properties of the stably expressed recombinant receptor. *Endocrinology*, *137*, 2748–2757.
402. Turner, P. R., Mefford, S., Bambino, T., & Nissenson, R. A. (1998). Transmembrane residues together with the amino terminus limit the response of the parathyroid hormone (PTH) 2 receptor to PTH-related peptide. *J Biol Chem*, *273*, 3830–3837.
403. Bhattacharya, P., Yan, Y. L., Postlethwait, J., & Rubin, D. A. (2011). Evolution of the vertebrate pth2 (tip39) gene family and the regulation of PTH type 2 receptor (pth2r) and its endogenous ligand pth2 by hedgehog signaling in zebrafish development. *J Endocrinol*, *211*, 187–200.
404. Dobolyi, A., Palkovits, M., & Usdin, T. B. (2010). The TIP39-PTH2 receptor system: unique peptidergic cell groups in the brainstem and their interactions with central regulatory mechanisms. *Prog Neurobiol*, *90*, 29–59.
405. Usdin, T. B. (2000). The PTH2 receptor and TIP39: a new peptide-receptor system. *Trends Pharmacol Sci*, *21*, 128–130.
406. Abou-Samra, A. B., Jueppner, H., Westerberg, D., et al. (1989). Parathyroid hormone causes translocation of protein kinase-C from cytosol to membranes in rat osteosarcoma cells. *Endocrinology*, *124*, 1107–1113.
407. Tamura, T., Sakamoto, H., & Filburn, C. R. (1989). Parathyroid hormone 1–34, but not 3–34 or 7–34, transiently translocates protein kinase C in cultured renal (OK) cells. *Biochem Biophys Res Commun*, *159*, 1352–1358.
408. Dunlay, R., & Hruska, K. (1990). PTH receptor coupling to phospholipase C is an alternate pathway of signal transduction in bone and kidney. *Am J Physiol*, *258*, F223–F231.
409. Partridge, N. C., Bloch, S. R., & Pearman, A. T. (1994). Signal transduction pathways mediating parathyroid hormone regulation of osteoblastic gene expression. *J Cell Biochem*, *55*, 321–327.
410. Offermanns, S., Iida-Klein, A., Segre, G. V., & Simon, M. I. (1996). G alpha q family members couple parathyroid hormone (PTH)/PTH-related peptide and calcitonin receptors to phospholipase C in COS-7 cells. *Mol Endocrinol*, *10*, 566–574.
411. Schwindinger, W. F., Fredericks, J., Watkins, L., et al. (1998). Coupling of the PTH/PTHrP receptor to multiple G-proteins: direct demonstration of receptor activation of Gs, Gq/11, and Gi(1) by [alpha- 32P]GTP-gamma-azidoanilide photoaffinity labeling. *Endocrine*, *8*, 201–209.
412. Iida-Klein, A., Guo, J., Takemura, M., et al. (1997). Mutations in the second cytoplasmic loop of the rat parathyroid hormone (PTH)/PTH-related protein receptor result in selective loss of PTH-stimulated phospholipase C activity. *J Biol Chem*, *272*, 6882–6889.
413. Huang, Z., Chen, Y., Pratt, S., et al. (1996). The N-terminal region of the third intracellular loop of the parathyroid hormone (PTH)/PTH-related peptide receptor is critical for coupling to cAMP and inositol phosphate/Ca2+ signal transduction pathways. *J Biol Chem*, *271*, 33382–33389.
414. Ferrandon, S., Feinstein, T. N., Castro, M., et al. (2009). Sustained cyclic AMP production by parathyroid hormone receptor endocytosis. *Nat Chem Biol*, *5*, 734–742.
415. Wysolmerski, J. J., Cormier, S., Philbrick, W. M., et al. (2001). Absence of functional type 1 parathyroid hormone (PTH)/PTH-related protein receptors in humans is associated with abnormal breast development and tooth impaction. *J Clin Endocrinol Metab*, *86*, 1788–1794.
416. Karaplis, A. C., He, B., Nguyen, M. T., et al. (1998). Inactivating mutation in the human parathyroid hormone receptor type 1 gene in Blomstrand chondrodysplasia. *Endocrinology*, *139*, 5255–5258.

417. Zhang, P., Jobert, A. S., Couvineau, A., & Silve, C. (1998). A homozygous inactivating mutation in the parathyroid hormone/parathyroid hormone-related peptide receptor causing Blomstrand chondrodysplasia. *J Clin Endocrinol Metab*, *83*, 3365–3368.
418. Karperien, M., van der Harten, H. J., van Schooten, R., et al. (1999). A frame-shift mutation in the type I parathyroid hormone (PTH)/PTH-related peptide receptor causing Blomstrand lethal osteochondrodysplasia. *J Clin Endocrinol Metab*, *84*, 3713–3720.
419. Jobert, A. S., Zhang, P., Couvineau, A., et al. (1998). Absence of functional receptors for parathyroid hormone and parathyroid hormone-related peptide in Blomstrand chondrodysplasia. *J Clini Invest*, *102*, 34–40.
420. Hopyan, S., Gokgoz, N., Poon, R., et al. (2002). A mutant PTH/PTHrP type I receptor in enchondromatosis. *Nat Genet*, *30*, 306–310.
421. Alderton, G. K. (2011). Genetics: IDH mosaicism in enchondromatosis syndromes. *Nat Rev Cancer*, *12*, 6.
422. Pansuriya, T. C., van Eijk, R., d'Adamo, P., et al. (2011). Somatic mosaic IDH1 and IDH2 mutations are associated with enchondroma and spindle cell hemangioma in Ollier disease and Maffucci syndrome. *Nat Genet*, *43*, 1256–1261.
423. Schipani, E., Kruse, K., & Juppner, H. (1995). A constitutively active mutant PTH-PTHrP receptor in Jansen-type metaphyseal chondrodysplasia. *Science*, *268*, 98–100.
424. Schipani, E., Langman, C. B., Parfitt, A. M., et al. (1996). Constitutively activated receptors for parathyroid hormone and parathyroid hormone-related peptide in Jansen's metaphyseal chondrodysplasia. *N Engl J Med*, *335*, 708–714.
425. Schipani, E., Langman, C., Hunzelman, J., et al. (1999). A novel parathyroid hormone (PTH)/PTH-related peptide receptor mutation in Jansen's metaphyseal chondrodysplasia. *J Clin Endocrinol Metab*, *84*, 3052–3057.
426. Holz, G. G., & Chepurny, O. G. (2003). Glucagon-like peptide-1 synthetic analogs: new therapeutic agents for use in the treatment of diabetes mellitus. *Curr Med Chem*, *10*, 2471–2483.
427. Holz, G. G., Leech, C. A., & Habener, J. F. (2000). Insulinotropic toxins as molecular probes for analysis of glucagon-like peptide-1 receptor-mediated signal transduction in pancreatic beta-cells. *Biochimie*, *82*, 915–926.
428. Janicic, N, Soliman, E., Pausova, Z., et al. (1995). Mapping of the calcium-sensing receptor gene (CASR) to human chromosome 3q13.3–21 by fluorescence in situ hybridization, and localization to rat chromosome 11 and mouse chromosome 16. *Mamm Genome*, *6*, 798–801.
429. Brown, E. M., Gamba, G., Riccardi, D., et al. (1993). Cloning and characterization of an extracellular Ca(2+)-sensing receptor from bovine parathyroid. *Nature*, *366*, 575–580.
430. Mancilla, E. E., De Luca, F., & Baron, J. (1998). Activating mutations of the Ca2+-sensing receptor. *Mol Geneti Metab*, *64*, 198–204.
431. Brown, E. M., Pollak, M., Chou, Y. H., et al. (1995). The cloning of extracellular Ca(2+)-sensing receptors from parathyroid and kidney: molecular mechanisms of extracellular Ca(2+)-sensing. *J Nutr*, *125*, 1965S–1970S.
432. Brown, E. M., & Hebert, S. C. (1995). A cloned Ca(2+)-sensing receptor: a mediator of direct effects of extracellular Ca2+ on renal function? *J Am Soc Nephrol*, *6*, 1530–1540.
433. Cholst, I. N., Steinberg, S. F., Tropper, P. J., et al. (1984). The influence of hypermagnesemia on serum calcium and parathyroid hormone levels in human subjects. *N Engl J Med*, *310*, 1221–1225.
434. Hebert, S. C., Brown, E. M., & Harris, H. W. (1997). Role of the Ca(2+)-sensing receptor in divalent mineral ion homeostasis. *J Exp Biol*, *200*, 295–302.
435. Chattopadhyay, N. (2000). Biochemistry, physiology and pathophysiology of the extracellular calcium-sensing receptor. *Int J Biochem Cell Biol*, *32*, 789–804.
436. Thakker, R. V. (1998). Disorders of the calcium-sensing receptor. *Biochim Biophys Acta*, *1448*, 166–170.
437. Pearce, S., & Steinmann, B. (1999). Casting new light on the clinical spectrum of neonatal severe hyperparathyroidism. *Clin Endocrinol*, *50*, 691–693.
438. Chou, Y. H., Pollak, M. R., Brandi, M. L., et al. (1995). Mutations in the human Ca(2+)-sensing-receptor gene that cause familial hypocalciuric hypercalcemia. *Am J Hum Genet*, *56*, 1075–1079.
439. Health, H., III, Odelberg, S., Jackson, C. E., et al. (1996). Clustered inactivating mutations and benign polymorphisms of the calcium receptor gene in familial benign hypocalciuric hypercalcemia suggest receptor functional domains. *J Clin Endocrinol Metab*, *81*, 1312–1317.
440. Marx, S. J., Attie, M. F., Spiegel, A. M., et al. (1982). An association between neonatal severe primary hyperparathyroidism and familial hypocalciuric hypercalcemia in three kindreds. *N Engl J Med*, *306*, 257–264.
441. Marx, S. J., Fraser, D., Rapoport, A. (1985). Familial hypocalciuric hypercalcemia: mild expression of the gene in heterozygotes and severe expression in homozygotes. *Am J Med*, *78*, 15–22.
442. Pollak, M. R., Brown, E. M., Chou, Y. H., et al. (1993). Mutations in the human Ca(2+)-sensing receptor gene cause familial hypocalciuric hypercalcemia and neonatal severe hyperparathyroidism. *Cell*, *75*, 1297–1303.
443. Janicic, N., Pausova, Z., Cole, D. E., Hendy, G. N., (1995). Insertion of an Alu sequence in the Ca(2+)-sensing receptor gene in familial hypocalciuric hypercalcemia and neonatal severe hyperparathyroidism. *Am J Hum Genet*, *56*, 880–886.
444. Pearce, S. H., Trump, D., Wooding, C., et al. (1995). Calcium-sensing receptor mutations in familial benign hypercalcemia and neonatal hyperparathyroidism. *J Clini Invest*, *96*, 2683–2692.
445. Lietman, S. A., Tenenbaum-Rakover, Y., Jap, T. S., et al. (2009). A novel loss-of-function mutation, Gln459Arg, of the calcium-sensing receptor gene associated with apparent autosomal recessive inheritance of familial hypocalciuric hypercalcemia. *J Clin Endocrinol Metab*, *94*, 4372–4379.
446. Marx, S. J., Attie, M. F., Levine, M. A., et al. (1981). The hypocalciuric or benign variant of familial hypercalcemia: clinical and biochemical features in fifteen kindreds. *Medicine* (Baltimore), *60*, 397–412.
447. Marx, S. J., Spiegel, A. M., Levine, M. A., et al. (1982). Familial hypocalciuric hypercalcemia: the relation to primary parathyroid hyperplasia. *N Engl J Med*, *307*, 416–426.
448. Thakker, R. V. (2004). Diseases associated with the extracellular calcium-sensing receptor. *Cell Calcium*, *35*, 275–282.
449. Heath, H., III, Jackson, C. E., Otterud, B., & Leppert, M. F. (1993). Genetic linkage analysis in familial benign (hypocalciuric) hypercalcemia: evidence for locus heterogeneity. *Am J Hum Genet*, *53*, 193–200.
450. Lloyd, S. E., Pannett, A. A., Dixon, P. H., et al. (1999). Localization of familial benign hypercalcemia, Oklahoma variant (FBHOk), to chromosome 19q13. *Am J Hum Genet*, *64*, 189–195.
451. McMurtry, C. T., Schranck, F. W., Walkenhorst, D. A., et al. (1992). Significant developmental elevation in serum parathyroid hormone levels in a large kindred with familial benign (hypocalciuric) hypercalcemia. *Am J Med*, *93*, 247–258.
452. Pollak, M. R., Brown, E. M., Estep, H. L., et al. (1994). Autosomal dominant hypocalcaemia caused by a Ca(2+)-sensing receptor gene mutation. Nat Genet 8, 303-307.
453. Finegold D. N., Armitage M. M., Galiani M, et al. (1994). Preliminary localization of a gene for autosomal dominant hypoparathyroidism to chromosome 3q13. *Pediatr Res*, *36*, 414–417.
454. Baron, J., Winer, K. K., Yanovski, J. A., et al. (1996). Mutations in the Ca(2+)-sensing receptor gene cause autosomal dominant and sporadic hypoparathyroidism. *Hum Mol Genet*, *5*, 601–606.
455. Pearce, S. H., Williamson, C., Kifor, O., et al. (1996). A familial syndrome of hypocalcemia with hypercalciuria due to mutations in the calcium-sensing receptor. *N Engl J Med*, *335*, 1115–1122.
456. De Luca, F., Ray, K., Mancilla, E. E., et al. (1997). Sporadic hypoparathyroidism caused by de novo gain-of-function mutations of the Ca(2+)-sensing receptor. *J Clin Endocrinol Metab*, *82*, 2710–2715.
457. Pearce, S. (1999). Extracellular "calcistat" in health and disease. *Lancet*, *353*, 83–84.
458. Vargas-Poussou, R., Huang, C., Hulin, P., et al. (2002). Functional characterization of a calcium-sensing receptor mutation in severe autosomal dominant hypocalcemia with a Bartter-like syndrome. *J Am Soc Nephrol*, *13*, 2259–2266.
459. Watanabe, S., Fukumoto, S., Chang, H., et al. (2002). Association between activating mutations of calcium-sensing receptor and Bartter's syndrome. *Lancet*, *360*, 692–694.
460. Hebert, S. C. (2003). Bartter syndrome. *Curr Opin Nephrol Hypertens*, *12*, 527–532.

461. Cole, D. E., Peltekova, V. D., Rubin, L. A., et al. (1999). A986S polymorphism of the calcium-sensing receptor and circulating calcium concentrations. *Lancet*, *353*, 112–115.
462. Cole, D. E., Vieth, R., Trang, H. M., et al. (2001). Association between total serum calcium and the A986S polymorphism of the calcium-sensing receptor gene. *Mol Genet Metab*, *72*, 168–174.
463. Scillitani, A., Guarnieri, V., De Geronimo, S., et al. (2004). Blood ionized calcium is associated with clustered polymorphisms in the carboxyl-terminal tail of the calcium-sensing receptor. *J Clin Endocrinol Metab*, *89*, 5634–5638.
464. Miedlich, S., Lamesch, P., Mueller, A., & Paschke, R. (2001). Frequency of the calcium-sensing receptor variant A986S in patients with primary hyperparathyroidism. *Eur J Endocrinol*, *145*, 421–427.
465. Donath, J., Speer, G., Poor, G., et al. (2004). Vitamin D receptor, oestrogen receptor-alpha and calcium-sensing receptor genotypes, bone mineral density and biochemical markers in Paget's disease of bone. *Rheumatology* (Oxford, England), *43*, 692–695.
466. Scillitani, A., Guarnieri, V., Battista, C., et al. (2007). Primary hyperparathyroidism and the presence of kidney stones are associated with different haplotypes of the calcium-sensing receptor. *J Clin Endocrinol Metab*, *92*, 277–283.
467. Vezzoli, G., Tanini, A., Ferrucci, L., et al. (2002). Influence of calcium-sensing receptor gene on urinary calcium excretion in stone-forming patients. *J Am Soc Nephrol*, *13*, 2517–2523.
468. Kifor, O., Moore, F. D., Jr., Delaney, M., et al. (2003). A syndrome of hypocalciuric hypercalcemia caused by autoantibodies directed at the calcium-sensing receptor. *J Clin Endocrinol Metab*, *88*, 60–72.
469. Li, Y., Song, Y. H., Rais, N., et al. (1996). Autoantibodies to the extracellular domain of the calcium sensing receptor in patients with acquired hypoparathyroidism. *J Clin Invest*, *97*, 910–914.
470. Mayer, A., Ploix, C., Orgiazzi, J., et al. (2004). Calcium-sensing receptor autoantibodies are relevant markers of acquired hypoparathyroidism. *J Clin Endocrinol Metab*, *89*, 4484–4488.
471. Milligan, G., & Kostenis, E. (2006). Heterotrimeric G-proteins: a short history. *Br J Pharmacol*, *147*(Suppl. 1), S46–S55.
472. Hayward, B. E., Kamiya, M., Strain, L., et al. (1998). The human GNAS1 gene is imprinted and encodes distinct paternally and biallelically expressed G proteins. *Proc Natl Acad Sci USA*, *95*, 10038–10043.
473. Hayward, B. E., Moran, V., Strain, L., & Bonthron, D. T. (1998). Bidirectional imprinting of a single gene: GNAS1 encodes maternally, paternally, and biallelically derived proteins. *Proc Natl Acad Sci USA*, *95*, 15475–15480.
474. Juppner, H., Schipani, E., Bastepe, M., et al. (1998). The gene responsible for pseudohypoparathyroidism type Ib is paternally imprinted and maps in four unrelated kindreds to chromosome 20q13.3. *Proc Natl Acad Sci USA*, *95*, 11798–11803.
475. Nakamoto, J. M., Sandstrom, A. T., Brickman, A. S., et al. (1998). Pseudohypoparathyroidism type Ia from maternal but not paternal transmission of a Gs alpha gene mutation. *Am J Med Genet*, *77*, 261–267.
476. Wilson, L. C., Oude Luttikhuis, M. E., Clayton, P. T., et al. (1994). Parental origin of Gs alpha gene mutations in Albright's hereditary osteodystrophy. *J Med Genet*, *31*, 835–839.
477. Levine, M. A. (2012). An update on the clinical and molecular characteristics of pseudohypoparathyroidism. *Curr Opin Endocrinol Diabetes Obes*, *19*, 443–451.
478. Weinstein, L. S., Yu, S., Warner, D. R., & Liu, J. (2001). Endocrine manifestations of stimulatory G protein alpha-subunit mutations and the role of genomic imprinting. *Endocr Rev*, *22*, 675–705.
479. Lebrun, M., Richard, N., Abeguile, G., et al. (2010). Progressive osseous heteroplasia: a model for the imprinting effects of GNAS inactivating mutations in humans. *J Clin Endocrinol Metab*, *95*, 3028–3038.
480. Lau, K., Willig, R. P., Hiort, O., & Hoeger, P. H. (2012). Linear skin atrophy preceding calcinosis cutis in pseudo-pseudohypoparathyroidism. *Clin Exp Dermatol*, *37*, 646–648.
481. Martin, J., Tucker, M., & Browning, J. C. (2012). Infantile osteoma cutis as a presentation of a GNAS mutation. *Pediatr Dermatol*, *29*, 483–484.
482. Ward, S., Sugo, E., Verge, C. F., & Wargon, O. (2011). Three cases of osteoma cutis occurring in infancy: a brief overview of osteoma cutis and its association with pseudo-pseudohypoparathyroidism. *Australas J Dermatol*, *52*, 127–131.
483. Silve, C., Santora, A., Breslau, N., et al. (1986). Selective resistance to parathyroid hormone in cultured skin fibroblasts from patients with pseudohypoparathyroidism type Ib. *J Clin Endocrinol Metab*, *62*, 640–644.
484. Fukumoto, S., Suzawa, M., Kikuchi, T., et al. (1998). Cloning and characterization of kidney-specific promoter of human PTH/PTHrP receptor gene: absence of mutation in patients with pseudohypoparathyroidism type Ib. *Mol Cell Endocrinol*, *141*, 41–47.
485. Bettoun, J. D., Minagawa, M., Kwan, M. Y., et al. (1997). Cloning and characterization of the promoter regions of the human parathyroid hormone (PTH)/PTH-related peptide receptor gene: analysis of deoxyribonucleic acid from normal subjects and patients with pseudohypoparathyroidism type 1b. *J Clin Endocrinol Metab*, *82*, 1031–1040.
486. Liu, J., Litman, D., Rosenberg, M. J., et al. (2000). A GNAS1 imprinting defect in pseudohypoparathyroidism type IB. *J Clini Invest*, *106*, 1167–1174.
487. Izzi, B., Van Geet, C., & Freson, K. (2012). Recent advances in GNAS epigenetic research of pseudohypoparathyroidism. *Curr Mol Med*, *12*, 566–573.
488. Mantovani, G. (2011). Clinical review: pseudohypoparathyroidism: diagnosis and treatment. *J Clin Endocrinol Metab*, *96*, 3020–3030.
489. Mantovani, G., Elli, F. M., & Spada, A. (2012). GNAS epigenetic defects and pseudohypoparathyroidism: time for a new classification? *Horm Metab Res*, *44*, 716–723.
490. Chillambhi, S., Turan, S., Hwang, D. Y., et al. (2010). Deletion of the noncoding GNAS antisense transcript causes pseudohypoparathyroidism type Ib and biparental defects of GNAS methylation in cis. *J Clin Endocrinol Metab*, *95*, 3993–4002.
491. Fernandez-Rebollo, E., Perez, D. N., Lecumberri, B., et al. (2011). Exclusion of the GNAS locus in PHP-Ib patients with broad GNAS methylation changes: evidence for an autosomal recessive form of PHP-Ib? *J Bone Miner Res*, *26*, 1854–1863.
492. Bastepe, M., Altug-Teber, O., Agarwal, C., et al. (2011). Paternal uniparental isodisomy of the entire chromosome 20 as a molecular cause of pseudohypoparathyroidism type Ib (PHP-Ib). *Bone*, *48*, 659–662.
493. Spiegel, A. M., & Weinstein, L. S. (2004). Inherited diseases involving g proteins and g protein-coupled receptors. *Ann Rev Med*, *55*, 27–39.
494. Williamson, E. A., Ince, P. G., Harrison, D., et al. (1995). G-protein mutations in human pituitary adrenocorticotrophic hormone-secreting adenomas. *Eur J Clin Invest*, *25*, 128–131.
495. Okamoto, S., Hisaoka, M., Ushijima, M., et al. (2000). Activating Gs(alpha) mutation in intramuscular myxomas with and without fibrous dysplasia of bone. *Virchows Arch*, *437*, 133–137.
496. Shenker, A., Weinstein, L. S., Sweet, D. E., & Spiegel, A. M. (1994). An activating Gs alpha mutation is present in fibrous dysplasia of bone in the McCune-Albright syndrome. *J Clin Endocrinol Metab*, *79*, 750–755.
497. Weinstein, L. S., Shenker, A., Gejman, P. V., et al. (1991). Activating mutations of the stimulatory G protein in the McCune-Albright syndrome. *N Engl J Med*, *325*, 1688–1695.
498. Collins, M. T., Singer, F. R., & Eugster, E. (2012). McCune-Albright syndrome and the extraskeletal manifestations of fibrous dysplasia. *Orphanet J Rare Dis*, 7(Suppl. 1), S4.
499. Dumitrescu, C. E., & Collins, M. T. (2008). McCune-Albright syndrome. *Orphanet J Rare Dis*, *3*, 12.
500. Lietman, S. A., Schwindinger, W. F., & Levine, M. A. (2007). Genetic and molecular aspects of McCune-Albright syndrome. *Pediatr Endocrinol Rev*, 2007 Aug; 4 Suppl 4:380–5.
501. Shenker, A., Weinstein, L. S., Moran, A., et al. (1993). Severe endocrine and nonendocrine manifestations of the McCune-Albright syndrome associated with activating mutations of stimulatory G protein GS. *J Pediatr*, *123*, 509–518.
502. Riminucci, M., Collins, M. T., Fedarko, N. S., et al. (2003). FGF-23 in fibrous dysplasia of bone and its relationship to renal phosphate wasting. *J Clin Invest*, *112*, 683–692.

503. Yamamoto, T., Imanishi, Y., Kinoshita, E., et al. (2005). The role of fibroblast growth factor 23 for hypophosphatemia and abnormal regulation of vitamin D metabolism in patients with McCune-Albright syndrome. *J Bone Miner Metab*, *23*, 231–237.
504. Boyce, A. M., Glover, M., Kelly, M. H., et al. (2013). Optic neuropathy in McCune-Albright syndrome: effects of early diagnosis and treatment of growth hormone excess. *J Clin Endocrinol Metab*, *98*, E126–E34.
505. Lee, J. S., FitzGibbon, E., Butman, J. A., et al. (2002). Normal vision despite narrowing of the optic canal in fibrous dysplasia. *N Engl J Med*, *347*, 1670–1676.
506. Cohen, S., Bigazzi, P. E., & Yoshida, T. (1974). Commentary. Similarities of T cell function in cell-mediated immunity and antibody production. *Cell Immunol*, *12*, 150–159.
507. Leonard, W. J., & Lin, J.-X. (2000). Cytokine receptor signaling pathways. *J Allergy Clin Immunol*, *105*, 877–888.
508. Davies, D. R., & Wlodawer, A. (1995). Cytokines and their receptor complexes. *Faseb J*, *9*, 50–56.
509. de Vos, A. M., Ultsch, M., & Kossiakoff, A. A. (1992). Human growth hormone and extracellular domain of its receptor: crystal structure of the complex. *Science*, *255*, 306–312.
510. Greenlund, A. C., Morales, M. O., Viviano, B. L., et al. (1995). Stat recruitment by tyrosine-phosphorylated cytokine receptors: an ordered reversible affinity-driven process. *Immunity*, *2*, 677–687.
511. Darnell, J. E., Jr., Kerr, I. M., & Stark, G. R. (1994). Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. *Science*, *264*, 1415–1421.
512. Horvath, C. M., & Darnell, J. E. (1997). The state of the STATs: recent developments in the study of signal transduction to the nucleus. *Curr Opin Cell Biol*, *9*, 233–239.
513. Darnell, J. E., Jr. (1997). STATs and gene regulation. *Science*, *277*, 1630–1635.
514. Leonard, W. J., & O'Shea, J. J. (1998). Jaks and STATs: biological implications. *Annu Rev Immunol*, *16*, 293–322.
515. Witthuhn, B. A., Silvennoinen, O., Miura, O., et al. (1994). Involvement of the Jak-3 Janus kinase in signalling by interleukins 2 and 4 in lymphoid and myeloid cells. *Nature*, *370*, 153–157.
516. Johnston, J. A., Kawamura, M., Kirken, R. A., et al. (1994). Phosphorylation and activation of the Jak-3 Janus kinase in response to interleukin-2. *Nature*, *370*, 151–153.
517. Leung, D. W., Spencer, S. A., Cachianes, G., et al. (1987). Growth hormone receptor and serum binding protein: purification, cloning and expression. *Nature*, *330*, 537–543.
518. Kelly, P. A., Ali, S., Rozakis, M., et al. (1993). The growth hormone/prolactin receptor family. *Recent Prog Horm Res*, *48*, 123–164.
519. Rosenfeld, R. G., Rosenbloom, A. L., & Guevara-Aguirre, J. (1994). Growth hormone (GH) insensitivity due to primary GH receptor deficiency. *Endocr Rev*, *15*, 369–390.
520. Campbell, G. S. (1997). Growth-hormone signal transduction. *J Pediatr*, *131*, S42–S44.
521. Rosenbloom, A. L. (1999). Growth hormone insensitivity: physiologic and genetic basis, phenotype, and treatment. *J Pediatr*, *135*, 280–289.
522. Argetsinger, L. S., Campbell, G. S., Yang, X., et al. (1993). Identification of JAK2 as a growth hormone receptor-associated tyrosine kinase. *Cell*, *74*, 237–244.
523. Meyer, D. J., Campbell, G. S., Cochran, B. H., et al. (1994). Growth hormone induces a DNA binding factor related to the interferon-stimulated 91-kDa transcription factor. *J Biol Chem*, *269*, 4701–4704.
524. Wood, T. J., Sliva, D., Lobie, P. E., et al. (1995). Mediation of growth hormone-dependent transcriptional activation by mammary gland factor/Stat 5. *J Biol Chem*, *270*, 9448–9453.
525. Smit, L. S., Meyer, D. J., Billestrup, N., et al. (1996). The role of the growth hormone (GH) receptor and JAK1 and JAK2 kinases in the activation of Stats 1, 3, and 5 by GH. *Mol Endocrinol*, *10*, 519–533.
526. Hansen, L. H., Wang, X., Kopchick, J. J., et al. (1996). Identification of tyrosine residues in the intracellular domain of the growth hormone receptor required for transcriptional signaling and Stat5 activation. *J Biol Chem*, *271*, 12669–12673.
527. Rajaram, S., Baylink, D. J., & Mohan, S. (1997). Insulin-like growth factor-binding proteins in serum and other biological fluids: regulation and functions. *Endocr Rev*, *18*, 801–831.
528. VanderKuur, J., Allevato, G., Billestrup, N., et al. (1995). Growth hormone-promoted tyrosyl phosphorylation of SHC proteins and SHC association with Grb2. *J Biol Chem*, *270*, 7587–7593.
529. Argetsinger, L. S., Hsu, G. W., Myers, M. G., Jr., et al. (1995). Growth hormone, interferon-gamma, and leukemia inhibitory factor promoted tyrosyl phosphorylation of insulin receptor substrate-1. *J Biol Chem*, *270*, 14685–14692.
530. Argetsinger, L. S., Norstedt, G., Billestrup, N., et al. (1996). Growth hormone, interferon-gamma, and leukemia inhibitory factor utilize insulin receptor substrate-2 in intracellular signaling. *J Biol Chem*, *271*, 29415–29421.
531. Savage, M. O., Hwa, V., David, A., et al. (2011). Genetic defects in the growth hormone-IGF-I axis causing growth hormone insensitivity and impaired linear growth. *Front Endocrinol* (Lausanne), *2*, 95.
532. Laron, Z., Pertzelan, A., & Mannheimer, S. (1966). Genetic pituitary dwarfism with high serum concentration of growth hormone: a new inborn error of metabolism? *Isr J Med Sci*, *2*, 152–155.
533. Laron, Z., Pertzelan, A., & Karp, M. (1968). Pituitary dwarfism with high serum levels of growth hormone. *Isr J Med Sci*, *4*, 883–894.
534. Laron, Z. (1974). The syndrome of familial dwarfism and high plasma immunoreactive human growth hormone. *Birth Defects Orig Artic Ser*, *10*, 231–238.
535. Rosenbloom, A. L., Guevara-Aguirre, J., Rosenfeld, R. G., & Francke, U. (1999). Growth hormone receptor deficiency in Ecuador. *J Clin Endocrinol Metab*, *84*, 4436–4443.
536. Wojcik, J., Berg, M. A., Esposito, N., et al. (1998). Four contiguous amino acid substitutions, identified in patients with Laron syndrome, differently affect the binding affinity and intracellular trafficking of the growth hormone receptor. *J Clin Endocrinol Metab*, *83*, 4481–4489.
537. Walker, J. L., Crock, P. A., Behncken, S. N., et al. (1998). A novel mutation affecting the interdomain link region of the growth hormone receptor in a Vietnamese girl, and response to long-term treatment with recombinant human insulin-like growth factor-I and luteinizing hormone-releasing hormone analogue. *J Clin Endocrinol Metab*, *83*, 2554–2561.
538. Aisenberg, J., Auyeung, V., Pedro, H. F., et al. (2010). Atypical GH insensitivity syndrome and severe insulin-like growth factor-I deficiency resulting from compound heterozygous mutations of the GH receptor, including a novel frameshift mutation affecting the intracellular domain. *Horm Res Paediatr*, *74*, 406–411.
539. Akinci, A., Rosenfeld, R. G., & Hwa, V. (2013). A novel exonic GHR splicing mutation (c.784G > C) in a patient with classical growth hormone insensitivity syndrome. *Horm Res Paediatr*, *79*, 32–38.
540. Arman, A., Ozon, A., Isguven, P. S., et al. (2008). Novel splice site mutation in the growth hormone receptor gene in Turkish patients with Laron-type dwarfism. *J Pediatr Endocrinol Metab*, *21*, 47–58.
541. David, A., Miraki-Moud, F., Shaw, N. J., et al. (2010). Identification and characterisation of a novel GHR defect disrupting the polypyrimidine tract and resulting in GH insensitivity. *Eur J Endocrinol*, *162*, 37–42.
542. David, A., Hwa, V., Metherell, L. A., et al. (2011). Evidence for a continuum of genetic, phenotypic, and biochemical abnormalities in children with growth hormone insensitivity. *Endocr Rev*, *32*, 472–497.
543. Fang, P., Riedl, S., Amselem, S., et al. (2007). Primary growth hormone (GH) insensitivity and insulin-like growth factor deficiency caused by novel compound heterozygous mutations of the GH receptor gene: genetic and functional studies of simple and compound heterozygous states. *J Clin Endocrinol Metab*, *92*, 2223–2231.
544. Kang, J. H., Kim, O. S., Kim, J. H., et al. (2012). A novel mutation of exon 7 in growth hormone receptor mRNA in a patient with growth hormone insensitivity syndrome and neurofibromatosis type I. *Int J Mol Med*, *30*, 713–717.
545. Woods, K. (2007). Genetic defects of the growth-hormone-IGF axis associated with growth hormone insensitivity. *Endocr Dev*, *11*, 6–15.

546. Derr, M. A., Aisenberg, J., Fang, P., et al. (2011). The growth hormone receptor (GHR) c.899dupC mutation functions as a dominant negative: insights into the pathophysiology of intracellular GHR defects. *J Clin Endocrinol Metab*, *96*, E1896–E904.
547. Goddard, A. D., Covello, R., Luoh, S. M., et al. (1995). Mutations of the growth hormone receptor in children with idiopathic short stature. The Growth Hormone Insensitivity Study Group. *N Engl J Med*, *333*, 1093–1098.
548. Goddard, A. D., Dowd, P., Chernausek, S., et al. (1997). Partial growth-hormone insensitivity: the role of growth-hormone receptor mutations in idiopathic short stature. *J Pediatr*, *131*, S51–S55.
549. Ayling, R. M., Ross, R., Towner, P., et al. (1997). A dominant-negative mutation of the growth hormone receptor causes familial short stature. *Nat Genet*, *16*, 13–14.
550. Iida, K., Takahashi, Y., Kaji, H., et al. (1998). Growth hormone (GH) insensitivity syndrome with high serum GH-binding protein levels caused by a heterozygous splice site mutation of the GH receptor gene producing a lack of intracellular domain. *J Clin Endocrinol Metab*, *83*, 531–537.
551. Gorbenko del Blanco, D., de Graaff, L. C., Visser, T. J., & Hokken-Koelega, A. C. (2012). Growth hormone insensitivity syndrome caused by a heterozygous GHR mutation: phenotypic variability owing to moderation by nonsense-mediated decay. *Clin Endocrinol* (Oxf), *76*, 706–712.
552. Freeth, J. S., Silva, C. M., Whatmore, A. J., & Clayton, P. E. (1998). Activation of the signal transducers and activators of transcription signaling pathway by growth hormone (GH) in skin fibroblasts from normal and GH binding protein-positive Laron syndrome children. *Endocrinology*, *139*, 20–28.
553. Hwa, V., Little, B., Adiyaman, P., et al. (2005). Severe growth hormone insensitivity resulting from total absence of signal transducer and activator of transcription 5b. *J Clin Endocrinol Metab*, *90*, 4260–4266.
554. Hwa, V., Nadeau, K., Wit, J. M., & Rosenfeld, R. G. (2011). STAT5b deficiency: lessons from STAT5b gene mutations. Best Pract Res Clin Endocrinol Metab, *25*, 61–75.
555. Kofoed, E. M., Hwa, V., Little, B., et al. (2003). Growth hormone insensitivity associated with a STAT5b mutation. *N Engl J Med*, *349*, 1139–1147.
556. Walenkamp, M. J., Vidarsdottir, S., Pereira, A. M., et al. (2007). Growth hormone secretion and immunological function of a male patient with a homozygous STAT5b mutation. *Eur J Endocrinol*, *156*, 155–165.
557. Wit, J. M., van Duyvenvoorde, H. A., Scheltinga, S. A., et al. (2012). Genetic analysis of short children with apparent growth hormone insensitivity. *Horm Res Paediatr*, *77*, 320–333.
558. Laron, Z. (2008). Insulin-like growth factor-I treatment of children with Laron syndrome (primary growth hormone insensitivity). *Pediatr Endocrinol Rev*, *5*, 766–771.
559. Chernausek, S. D., Backeljauw, P. F., Frane, J., et al. (2007). Long-term treatment with recombinant insulin-like growth factor (IGF)-I in children with severe IGF-I deficiency due to growth hormone insensitivity. *J Clin Endocrinol Metab*, *92*, 902–910.
560. Chen, D., & Garg, A. (1999). Monogenic disorders of obesity and body fat distribution. *J Lipid Res*, *40*, 1735–1746.
561. Clement, K., Vaisse, C., Lahlou, N., et al. (1998). A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature*, *392*, 398–401.
562. Farooqi, I. S., Wangensteen, T., Collins, S., et al. (2007). Clinical and molecular genetic spectrum of congenital deficiency of the leptin receptor. *N Engl J Med*, *356*, 237–247.
563. Kimber, W., Peelman, F., Prieur, X., et al. (2008). Functional characterization of naturally occurring pathogenic mutations in the human leptin receptor. *Endocrinology*, *149*, 6043–6052.
564. Andiran, N., Celik, N., & Andiran, F. (2012). Homozygosity for two missense mutations in the leptin receptor gene (P316:W646C) in a Turkmenian girl with severe early-onset obesity. *J Pediatr Endocrinol Metab*, *24*, 1043–1045.
565. Mazen, I., El-Gammal, M., Abdel-Hamid, M., et al. (2011). Homozygosity for a novel missense mutation in the leptin receptor gene (P316T) in two Egyptian cousins with severe early onset obesity. *Mol Genet Metab*, *102*, 461–464.
566. Hubbard, S. R. (1999). Structural analysis of receptor tyrosine kinases. *Prog Biophys Mol Biol*, *71*, 343–358.
567. Spivak-Kroizman, T., Lemmon, M. A., Dikic, I., et al. (1994). Heparin-induced oligomerization of FGF molecules is responsible for FGF receptor dimerization, activation, and cell proliferation. *Cell*, *79*, 1015–1024.
568. Heldin, C. H. (1995). Dimerization of cell surface receptors in signal transduction. *Cell*, *80*, 213–223.
569. Pawson, T. (1995). Protein modules and signalling networks. *Nature*, *373*, 573–580.
570. Patel, T. B. (2004). Single transmembrane spanning heterotrimeric g protein-coupled receptors and their signaling cascades. *Pharmacol Rev*, *56*, 371–385.
571. Ebina, Y., Ellis, L., Jarnagin, K., et al. (1985). The human insulin receptor cDNA: the structural basis for hormone-activated transmembrane signalling. *Cell*, *40*, 747–758.
572. Ullrich, A., Gray, A., Tam, A. W., et al. (1986). Insulin-like growth factor I receptor primary structure: comparison with insulin receptor suggests structural determinants that define functional specificity. *EMBO J*, *5*, 2503–2512.
573. Urso, B., Cope, D. L., Kalloo-Hosein, H. E., et al. (1999). Differences in signaling properties of the cytoplasmic domains of the insulin receptor and insulin-like growth factor receptor in 3T3-L1 adipocytes. *J Biol Chem*, *274*, 30864–30873.
574. Garrett, T. P., McKern, N. M., Lou, M., et al. (1998). Crystal structure of the first three domains of the type-1 insulin-like growth factor receptor. *Nature*, *394*, 395–399.
575. Treadway, J. L., Morrison, B. D., Soos, M. A., et al. (1991). Transdominant inhibition of tyrosine kinase activity in mutant insulin/insulin-like growth factor I hybrid receptors. *Proc Natl Acad Sci U S A*, *88*, 214–218.
576. Frattali, A. L., Treadway, J. L., & Pessin, J. E. (1992). Transmembrane signaling by the human insulin receptor kinase: relationship between intramolecular beta subunit trans- and cis-autophosphorylation and substrate kinase activation. *J Biol Chem*, *267*, 19521–19528.
577. Lee, C. H., Li, W., Nishimura, R., et al. (1993). Nck associates with the SH2 domain-docking protein IRS-1 in insulin-stimulated cells. *Proc Natl Acad Sci USA*, *90*, 11713–11717.
578. Skolnik, E. Y., Batzer, A., Li, N., et al. (1993). The function of GRB2 in linking the insulin receptor to Ras signaling pathways. *Science*, *260*, 1953–1955.
579. Myers, M. G., Jr., Grammer, T. C., Wang, L. M., et al. (1994). Insulin receptor substrate-1 mediates phosphatidylinositol 3'-kinase and p70S6k signaling during insulin, insulin-like growth factor-1, and interleukin-4 stimulation. *J Biol Chem*, *269*, 28783–28789.
580. Myers, M. G., Jr., Wang, L. M., Sun, X. J., et al. (1994). Role of IRS-1-GRB-2 complexes in insulin signaling. *Mol Cell Biol*, *14*, 3577–3587.
581. White, M. F. (1997). The insulin signalling system and the IRS proteins. *Diabetologia*, *40*(Suppl. 2), S2–S17.
582. Gustafson, T. A., He, W., Craparo, A., et al. (1995). Phosphotyrosine-dependent interaction of SHC and insulin receptor substrate 1 with the NPEY motif of the insulin receptor via a novel non-SH2 domain. *Mol Cell Biol*, *15*, 2500–2508.
583. Craparo, A., O'Neill, T. J., & Gustafson, T. A. (1995). Non-SH2 domains within insulin receptor substrate-1 and SHC mediate their phosphotyrosine-dependent interaction with the NPEY motif of the insulin-like growth factor I receptor. *J Biol Chem*, *270*, 15639–15643.
584. White, M. F., & Yenush, L. (1998). The IRS-signaling system: a network of docking proteins that mediate insulin and cytokine action. *Curr Top Microbiol Immunol*, *228*, 179–208.
585. Denton, R. M., & Tavare, J. M. (1995). Does mitogen-activated-protein kinase have a role in insulin action? The cases for and against. *Eur J Biochem/FEBS*, *227*, 597–611.
586. Hara, K., Yonezawa, K., Sakaue, H., et al. (1994). 1-Phosphatidylinositol 3-kinase activity is required for insulin-stimulated glucose transport but not for RAS activation in CHO cells. *Proc Natl Acad Sci USA*, *91*, 7415–7419.
587. Clarke, J. F., Young, P. W., Yonezawa, K., et al. (1994). Inhibition of the translocation of GLUT1 and GLUT4 in 3T3-L1 cells by the phosphatidylinositol 3-kinase inhibitor, wortmannin. *Biochem J*, *300*, 631–635.

588. Okada, T., Kawano, Y., Sakakibara, T., et al. (1994). Essential role of phosphatidylinositol 3-kinase in insulin-induced glucose transport and antilipolysis in rat adipocytes. Studies with a selective inhibitor wortmannin. *J Biol Chem*, *269*, 3568–3573.
589. Lammers, R., Gray, A., Schlessinger, J., Ullrich, A. (1989). Differential signalling potential of insulin- and IGF-1-receptor cytoplasmic domains. *EMBO J*, *8*, 1369–1375.
590. Kalloo-Hosein, H. E., Whitehead, J. P., Soos, M., et al. (1997). Differential signaling to glycogen synthesis by the intracellular domain of the insulin versus the insulin-like growth factor-1 receptor: evidence from studies of TrkC-chimeras. *J Biol Chem*, *272*, 24325–24332.
591. Seino, S., Seino, M., & Bell, G. I. (1990). Human insulin-receptor gene. *Diabetes*, *39*, 129–133.
592. Frattali, A. L., Treadway, J. L., & Pessin, J. E. (1992). Insulin/IGF-1 hybrid receptors: implications for the dominant-negative phenotype in syndromes of insulin resistance. *J Cell Biochem*, *48*, 43–50.
593. Treadway, J. L., Frattali, A. L., & Pessin, J. E. (1992). Intramolecular subunit interactions between insulin and insulin-like growth factor 1 alpha beta half-receptors induced by ligand and Mn/MgATP binding. *Biochemistry*, *31*, 11801–11805.
594. Kahn, C. R., Flier, J. S., Bar, R. S., et al. (1976). The syndromes of insulin resistance and acanthosis nigricans: insulin-receptor disorders in man. *N Engl J Med*, *294*, 739–745.
595. Moller, D. E., & Flier, J. S. (1991). Insulin resistance: mechanisms, syndromes, and implications. *N Engl J Med*, *325*, 938–948.
596. Tritos, N. A., & Mantzoros, C. S. (1998). Clinical review 97: syndromes of severe insulin resistance. *J Clin Endocrinol Metab*, *83*, 3025–3030.
597. Hunter, S. J., & Garvey, W. T. (1998). Insulin action and insulin resistance: diseases involving defects in insulin receptors, signal transduction, and the glucose transport effector system. *Am J Med*, *105*, 331345.
598. Taylor, S. I., Grunberger, G., Marcus-Samuels, B., et al. (1982). Hypoglycemia associated with antibodies to the insulin receptor. *N Engl J Med*, *307*, 1422–1426.
599. Mendenhall, E. (1950). Tumor of the pineal gland with high insulin resistance. *J Indiana State Med Assoc*, *43*, 32–36.
600. Mantzoros, C. S., & Flier, J. S. (1995). Insulin resistance: the clinical spectrum. *Adv Endocrinol Metab*, *6*, 193–232.
601. Longo, N., Wang, Y., & Pasquali, M. (1999). Progressive decline in insulin levels in Rabson-Mendenhall syndrome. *J Clin Endocrinol Metab*, *84*, 2623–2629.
602. Donohue, W., & Uchida, I. (1954). Leprechaunism: a euphemism for a rare familial disorder. *J Pediatr*, *45*, 505–519.
603. Whitehead, J. P., Soos, M. A., Jackson, R, et al. (1998). Multiple molecular mechanisms of insulin receptor dysfunction in a patient with Donohue syndrome. *Diabetes*, *47*, 1362–1364.
604. Whitehead, J. P., Humphreys, P., Krook, A., et al. (1998). Molecular scanning of the insulin receptor substrate 1 gene in subjects with severe insulin resistance: detection and functional analysis of a naturally occurring mutation in a YMXM motif. *Diabetes*, *47*, 837–839.
605. Taylor, S., Wertheimer, E., Hone, J., et al. (1994). Mutations in the insulin receptor gene in patients with genetic syndromes of extreme insulin resistance. In B. Draznin, D. LeRoith (Eds.), *Molecular biology of diabetes* (pp. 1–23). Philadelphia: Humana Press.
606. Kadowaki, T., Bevins, C. L., Cama, A., et al. (1988). Two mutant alleles of the insulin receptor gene in a patient with extreme insulin resistance. *Science*, *240*, 787–790.
607. Krook, A., Brueton, L., & O'Rahilly, S. (1993). Homozygous nonsense mutation in the insulin receptor gene in infant with leprechaunism. *Lancet*, *342*, 277–278.
608. Wertheimer, E., Lu, S. P., Backeljauw, P. F., et al. (1993). Homozygous deletion of the human insulin receptor gene results in leprechaunism. *Nat Genet*, *5*, 71–73.
609. Psiachou, H., Mitton, S., Alaghband-Zadeh, J., et al. (1993). Leprechaunism and homozygous nonsense mutation in the insulin receptor gene. *Lancet*, *342*, 924.
610. Takahashi, Y., Kadowaki, H., Momomura, K., et al. (1997). A homozygous kinase-defective mutation in the insulin receptor gene in a patient with leprechaunism. *Diabetologia*, *40*, 412–420.
611. Kawashima, Y., Nishimura, R., Utsunomiya, A., et al. (2013). Leprechaunism (Donohue syndrome): a case bearing novel compound heterozygous mutations in the insulin receptor gene. *Endocr J*, *60*, 107–112.
612. Kim, D., Cho, S. Y., Yeau, S. H., et al. (2012). Two novel insulin receptor gene mutations in a patient with Rabson-Mendenhall syndrome: the first Korean case confirmed by biochemical, and molecular evidence. *J Korean Med Sci*, *27*, 565–568.
613. Nobile, S., Semple, R. K., & Carnielli, V. P. (2012). A novel mutation of the insulin receptor gene in a preterm infant with Donohue syndrome and heart failure. *J Pediatr Endocrinol Metab*, *25*, 363–366.
614. Thiel, C. T., Knebel, B., Knerr, I., et al. (2008). Two novel mutations in the insulin binding subunit of the insulin receptor gene without insulin binding impairment in a patient with Rabson-Mendenhall syndrome. *Mol Genet Metab*, *94*, 356–362.
615. Tuthill, A., Semple, R. K., Day, R., et al. (2007). Functional characterization of a novel insulin receptor mutation contributing to Rabson-Mendenhall syndrome. *Clin Endocrinol* (Oxf), *66*, 21–26.
616. Unal, S., Aycan, Z., Halsall, D. J., et al. (2009). Donohue syndrome in a neonate with homozygous deletion of exon 3 of the insulin receptor gene. *J Pediatr Endocrinol Metab*, *22*, 669–674.
617. Moller, D. E., & Flier, J. S. (1988). Detection of an alteration in the insulin-receptor gene in a patient with insulin resistance, acanthosis nigricans, and the polycystic ovary syndrome (type A insulin resistance). *N Engl J Med*, *319*, 1526–1529.
618. Odawara, M., Kadowaki, T., Yamamoto, R., et al. (1989). Human diabetes associated with a mutation in the tyrosine kinase domain of the insulin receptor. *Science*, *245*, 66–68.
619. Taira, M., Hashimoto, N., Shimada, F., et al. (1989). Human diabetes associated with a deletion of the tyrosine kinase domain of the insulin receptor. *Science*, *245*, 63–66.
620. Cama, A., de la Luz Sierra, M., Ottini, L., et al. (1991). A mutation in the tyrosine kinase domain of the insulin receptor associated with insulin resistance in an obese woman. *J Clin Endocrinol Metab*, *73*, 894–901.
621. Moller, D. E., Cohen, O., Yamaguchi, Y., et al. (1994). Prevalence of mutations in the insulin receptor gene in subjects with features of the type A syndrome of insulin resistance. *Diabetes*, *43*, 247–255.
622. Yoshimasa, Y., Seino, S., Whittaker, J., et al. (1988). Insulin-resistant diabetes due to a point mutation that prevents insulin proreceptor processing. *Science*, *240*, 784–787.
623. Accili, D., Frapier, C., Mosthaf, L., et al. (1989). A mutation in the insulin receptor gene that impairs transport of the receptor to the plasma membrane and causes insulin-resistant diabetes. *EMBO J*, *8*, 2509–2517.
624. Kadowaki, T., Kadowaki, H., Rechler, M. M., et al. (1990). Five mutant alleles of the insulin receptor gene in patients with genetic forms of insulin resistance. *J Clin Invest*, *86*, 254–264.
625. Kusari, J., Takata, Y., Hatada, E., et al. (1991). Insulin resistance and diabetes due to different mutations in the tyrosine kinase domain of both insulin receptor gene alleles. *J Biol Chem*, *266*, 5260–5267.
626. O'Rahilly, S., & Moller, D. E. (1992). Mutant insulin receptors in syndromes of insulin resistance. *Clinical Endocrinology*, *36*, 121–132.
627. Jain, S., Golde, D. W., Bailey, R., & Geffner, M. E. (1998). Insulin-like growth factor-I resistance. *Endocr Rev*, *19*, 625–646.
628. Butler, M. G., Fogo, A. B., Fuchs, D. A., et al. (1988). Two patients with ring chromosome 15 syndrome. *Am J Med Genet*, *29*, 149-154.
629. de Lacerda, L., Carvalho, J. A., Stannard, B., et al. (1999). In vitro and in vivo responses to short-term recombinant human insulin-like growth factor-1 (IGF-I) in a severely growth-retarded girl with ring chromosome 15 and deletion of a single allele for the type 1 IGF receptor gene. *Clin Endocrinol*, *51*, 541–550.
630. Pasquali, F., Zuffardi, O., Severi, F., et al. (1973). Tandem translocation 15-13. *Ann Genet*, *16*, 47–50.
631. Kristoffersson, U., Heim, S., Mandahl, N., et al. (1987). Monosomy and trisomy of 15q24— —qter in a family with a translocation t(6;15)(p25;q24). *Clin Genet*, *32*, 169–171.
632. Roback, E. W., Barakat, A. J., Dev, V. G., et al. (1991). An infant with deletion of the distal long arm of chromosome 15 (q26.1— —qter) and loss of insulin-like growth factor 1 receptor gene. *Am J Med Genet*, *38*, 74–79.

633. Abuzzahab, M. J., Schneider, A., Goddard, A., et al. (2003). IGF-I receptor mutations resulting in intrauterine and postnatal growth retardation. *N Engl J Med*, *349*, 2211–2222.
634. Baker, J., Liu, J. P., Robertson, E. J., & Efstratiadis, A. (1993). Role of insulin-like growth factors in embryonic and postnatal growth. *Cell*, *75*, 73–82.
635. Liu, J. P., Baker, J., Perkins, A. S., et al. (1993). Mice carrying null mutations of the genes encoding insulin-like growth factor I (Igf-1) and type 1 IGF receptor (Igf1r). *Cell*, *75*, 59–72.
636. Fang, P., Cho, Y. H., Derr, M. A., et al. (2012). Severe short stature caused by novel compound heterozygous mutations of the insulin-like growth factor 1 receptor (IGF1R). *J Clin Endocrinol Metab*, *97*, E243–E247.
637. Labarta, J. I., Barrio, E., Audi, L., et al. (2013). Familial short stature and intrauterine growth retardation associated with a novel mutation in the IGF-I receptor (IGF1R) gene. *Clin Endocrinol* (Oxf), *78*, 255–262.
638. Walenkamp, M. J., van der Kamp, H. J., Pereira, A. M., et al. (2006). A variable degree of intrauterine and postnatal growth retardation in a family with a missense mutation in the insulin-like growth factor I receptor. *J Clin Endocrinol Metab*, *91*, 3062–3070.
639. Rechler, M. M. (1982). Leprechaunism and related syndromes with primary insulin resistance: heterogeneity of molecular defects. *Prog Clin Biol Res*, *97*, 245–281.
640. Kaplowitz, P. B., & D'Ercole, A. J. (1982). Fibroblasts from a patient with leprechaunism are resistant to insulin, epidermal growth factor, and somatomedin C. *J Clin Endocrinol Metab*, *55*, 741-748.
641. Backeljauw, P. F., Alves, C., Eidson, M., et al. (1994). Effect of intravenous insulin-like growth factor I in two patients with leprechaunism. *Pediatr Res*, *36*, 749–754.
642. Longo, N., Singh, R., Griffin, L. D., et al. (1994). Impaired growth in Rabson-Mendenhall syndrome: lack of effect of growth hormone and insulin-like growth factor-I. *J Clin Endocrinol Metab*, *79*, 799–805.
643. Desbois-Mouthon, C., Danan, C., Amselem, S., et al. (1996). Severe resistance to insulin and insulin-like growth factor-I in cells from a patient with leprechaunism as a result of two mutations in the tyrosine kinase domain of the insulin receptor. *Metabolism*, *45*, 1493–1500.
644. Kuzuya, H., Matsuura, N., Sakamoto, M., et al. (1993). Trial of insulinlike growth factor I therapy for patients with extreme insulin resistance syndromes. *Diabetes*, *42*, 696–705.
645. Burke, D., Wilkes, D., Blundell, T. L., & Malcolm, S. (1998). Fibroblast growth factor receptors: lessons from the genes. *Trends Biochem Sci*, *23*, 59–62.
646. Brooks, A. N., Kilgour, E., & Smith, P. D. (2012). Molecular pathways: fibroblast growth factor signaling: a new therapeutic opportunity in cancer. *Clin Cancer Res*, *18*, 1855–1862.
647. Mohammadi, M., Dionne, C. A., Li, W., et al. (1992). Point mutation in FGF receptor eliminates phosphatidylinositol hydrolysis without affecting mitogenesis. *Nature*, *358*, 681–684.
648. Peters, K. G., Marie, J., Wilson, E., et al. (1992). Point mutation of an FGF receptor abolishes phosphatidylinositol turnover and Ca2+ flux but not mitogenesis. *Nature*, *358*, 678–681.
649. Su, W. C., Kitagawa, M., Xue N, et al. (1997). Activation of Stat1 by mutant fibroblast growth-factor receptor in thanatophoric dysplasia type II dwarfism. *Nature*, *386*, 288–292.
650. Dode, C., Levilliers, J., Dupont, J. M., et al. (2003). Loss-of-function mutations in FGFR1 cause autosomal dominant Kallmann syndrome. *Nat Genet*, *33*, 463–465.
651. Oliveira, L. M., Seminara, S. B., Beranova, M., et al. (2001). The importance of autosomal genes in Kallmann syndrome: genotype-phenotype correlations and neuroendocrine characteristics. *J Clin Endocrinol Metab*, *86*, 1532–1538.
652. Pitteloud, N., Hayes, F. J., Boepple, P. A., et al. (2002). The role of prior pubertal development, biochemical markers of testicular maturation, and genetics in elucidating the phenotypic heterogeneity of idiopathic hypogonadotropic hypogonadism. *J Clin Endocrinol Metab*, *87*, 152–160.
653. Franco, B., Guioli, S., Pragliola, A., et al. (1991). A gene deleted in Kallmann's syndrome shares homology with neural cell adhesion and axonal path-finding molecules. *Nature*, *353*, 529–536.
654. Legouis, R., Hardelin, J. P., Levilliers, J., et al. (1991). The candidate gene for the X-linked Kallmann syndrome encodes a protein related to adhesion molecules. *Cell*, *67*, 423–435.
655. Gonzalez-Martinez, D., Kim, S. H., Hu, Y., et al. (2004). Anosmin-1 modulates fibroblast growth factor receptor 1 signaling in human gonadotropin-releasing hormone olfactory neuroblasts through a heparan sulfate-dependent mechanism. *J Neurosci*, *24*, 10384–10392.
656. Bick, D. P., Schorderet, D. F., Price, P. A., et al. (1992). Prenatal diagnosis and investigation of a fetus with chondrodysplasia punctata, ichthyosis, and Kallmann syndrome due to an Xp deletion. *Prenatal Diagnosis*, *12*, 19–29.
657. Albuisson, J., Pecheux, C., Carel, J. C., et al. (2005). Kallmann syndrome: 14 novel mutations in KAL1 and FGFR1 (KAL2). *Hum Mutat*, *25*, 98–99.
658. Pitteloud, N., Meysing, A., Quinton, R., et al. (2006). Mutations in fibroblast growth factor receptor 1 cause Kallmann syndrome with a wide spectrum of reproductive phenotypes. *Mol Cell Endocrinol*, *254/255*, 60–69.
659. Bonaventure, J., Rousseau, F., Legeai-Mallet, L., et al. (1996). Common mutations in the gene encoding fibroblast growth factor receptor 3 account for achondroplasia, hypochondroplasia and thanatophoric dysplasia. *Acta Paediatr Suppl*, *417*, 33–38.
660. Bonaventure, J., Rousseau, F., Legeai-Mallet, L., et al. (1996). Common mutations in the fibroblast growth factor receptor 3 (FGFR 3) gene account for achondroplasia, hypochondroplasia, and thanatophoric dwarfism. *Am J Med Genet*, *63*, 148–154.
661. Brodie, S. G., Kitoh, H., Lipson, M., et al. (1998). Thanatophoric dysplasia type I with syndactyly. *Am J Med Genet*, *80*, 260–262.
662. Brodie, S. G., Kitoh, H., Lachman, R. S., et al. (1999). Platyspondylic lethal skeletal dysplasia, San Diego type, is caused by FGFR3 mutations. *Am J Med Genet*, *84*, 476–480.
663. Shiang, R., Thompson, L. M., Zhu, Y. Z., et al. (1994). Mutations in the transmembrane domain of FGFR3 cause the most common genetic form of dwarfism, achondroplasia. *Cell*, *78*, 335–342.
664. Bellus, G. A., Hefferon, T. W., Ortiz de Luna, R. I., et al. (1995). Achondroplasia is defined by recurrent G380R mutations of FGFR3. *Am J Hum Genet*, *56*, 368–373.
665. Ezquieta Zubicaray, B., Iguacel, A. O., Varela Junquera, J. M., et al. (1999). [Gly380Arg and Asn540Lys mutations of fibroblast growth factor receptor 3 in achondroplasia and hypochondroplasia in the Spanish population]. *Med Clin* (Barc), *112*, 290–293.
666. Bellus, G. A., McIntosh, I., Smith, E. A., et al. (1995). A recurrent mutation in the tyrosine kinase domain of fibroblast growth factor receptor 3 causes hypochondroplasia. *Nat Genet*, *10*, 357–359.
667. Prinos, P., Costa, T., Sommer, A., et al. (1995). A common FGFR3 gene mutation in hypochondroplasia. *Hum Mol Genet*, *4*, 2097–2101.
668. Ramaswami, U., Rumsby, G., Hindmarsh, P. C., & Brook, C. G. (1998). Genotype and phenotype in hypochondroplasia. *J Pediatr*, *133*, 99–102.
669. Fofanova, O. V., Takamura, N., Kinoshita, E., et al. (1998). A missense mutation of C1659 in the fibroblast growth factor receptor 3 gene in Russian patients with hypochondroplasia. *Endocr J*, *45*, 791–795.
670. Bellus, G. A., Bamshad, M. J., Przylepa, K. A., et al. (1999). Severe achondroplasia with developmental delay and acanthosis nigricans (SADDAN): phenotypic analysis of a new skeletal dysplasia caused by a Lys650Met mutation in fibroblast growth factor receptor 3. *Am J Med Genet*, *85*, 53–65.
671. Peters, K., Ornitz, D., Werner, S., Williams, L. (1993). Unique expression pattern of the FGF receptor 3 gene during mouse organogenesis. *Dev Biol*, *155*, 423–430.
672. Werner, S., Weinberg, W., Liao, X., et al. (1993). Targeted expression of a dominant-negative FGF receptor mutant in the epidermis of transgenic mice reveals a role of FGF in keratinocyte organization and differentiation. *EMBO J*, *12*, 2635–2643.
673. Naski, M. C., Colvin, J. S., Coffin, J. D., & Ornitz, D. M. (1998). Repression of hedgehog signaling and BMP4 expression in growth plate cartilage by fibroblast growth factor receptor 3. *Development*, *125*, 4977–4988.
674. Legeai-Mallet, L., Benoist-Lasselin, C., Delezoide, A. L., et al. (1998). Fibroblast growth factor receptor 3 mutations promote apoptosis but do not alter chondrocyte proliferation in thanatophoric dysplasia. *J Biol Chem*, *273*, 13007–13014.

675. Wongmongkolrit, T., Bush, M., & Roessmann, U. (1983). Neuropathological findings in thanatophoric dysplasia. *Arch Pathol Lab Med*, *107*, 132–135.
676. Ho, K. L., Chang, C. H., Yang, S. S., & Chason, J. L. (1984). Neuropathologic findings in thanatophoric dysplasia. *Acta Neuropathol*, *63*, 218–228.
677. Shigematsu, H., Takashima, S., Otani, K., & Ieshima, A. (1985). Neuropathological and Golgi study on a case of thanatophotoric dysplasia. *Brain Dev*, *7*, 628–632.
678. Cruz, P. D., Jr., & Hud, J. A., Jr. (1992). Excess insulin binding to insulin-like growth factor receptors: proposed mechanism for acanthosis nigricans. *J Invest Dermatol*, *98*, 82S–85S.
679. Toydemir, R. M., Brassington, A. E., Bayrak-Toydemir, P., et al. (2006). A novel mutation in FGFR3 causes camptodactyly, tall stature, and hearing loss (CATSHL) syndrome. *Am J Hum Genet*, *79*, 935–941.
680. Foldynova-Trantirkova, S., Wilcox, W. R., & Krejci, P. (2012). Sixteen years and counting: the current understanding of fibroblast growth factor receptor 3 (FGFR3) signaling in skeletal dysplasias. *Hum Mutat*, *33*, 29–41.
681. Laudet, V. (1997). Evolution of the nuclear receptor superfamily: early diversification from an ancestral orphan receptor. *J Mol Endocrinol*. *19*, 207–226.
682. Laudet, V., Auwerx, J., Gustafsson, J.-A., & Wahli, W., for the Nuclear Receptors Nomenclature Committee (1999). A unified nomenclature system for the nuclear receptor superfamily. *Cell*, *97*, 161–163.
683. Baker, M. E. (1997). Steroid receptor phylogeny and vertebrate origins. *Mol Cell Endocrinol*, *135*, 101–107.
684. Mohan HM, Aherne CM, Rogers AC, et al. (2012). Molecular pathways: the role of NR4A orphan nuclear receptors in cancer. Clin Cancer Res. 12, 3223-3228.
685. Miyamoto K, Yazawa T, Mizutani T, et al. (2011). Stem cell differentiation into steroidogenic cell lineages by NR5A family. Mol Cell Endocrinol. 1-2, 123-126.
686. Greschik H, Schüle R. (1998). Germ cell nuclear factor: an orphan receptor with unexpected properties. J Mol Med. 76. 800-810.
687. Kumar, R., & Thompson, E. B. (1999). The structure of the nuclear hormone receptors. *Steroids*, *64*, 310–319.
688. Ford, J., McEwan, I. J., Wright, A. P., & Gustafsson, J. A. (1997). Involvement of the transcription factor IID protein complex in gene activation by the N-terminal transactivation domain of the glucocorticoid receptor in vitro. *Mol Endocrinol*, *11*, 1467–1475.
689. Henriksson, A., Almlof, T., Ford, J., et al. (1997). Role of the Ada adaptor complex in gene activation by the glucocorticoid receptor. *Mol Cell Biol*, *17*, 3065–3073.
690. Hollenberg, S. M., & Evans, R. M. (1988). Multiple and cooperative trans-activation domains of the human glucocorticoid receptor. *Cell*, *55*, 899–906.
691. Bocquel, M. T., Kumar, V., Stricker, C., et al. (1989). The contribution of the N- and C-terminal regions of steroid receptors to activation of transcription is both receptor and cell-specific. *Nucleic Acids Res*, *17*, 2581–2595.
692. Tasset, D., Tora, L., Fromental, C., et al. (1990). Distinct classes of transcriptional activating domains function by different mechanisms. *Cell*, *62*, 1177–1187.
693. Freedman, L. P., Luisi, B. F., Korszun, Z. R., et al. (1988). The function and structure of the metal coordination sites within the glucocorticoid receptor DNA binding domain. *Nature*, *334*, 543–546.
694. Luisi, B. F., Xu, W. X., Otwinowski, Z., et al. (1991). Crystallographic analysis of the interaction of the glucocorticoid receptor with DNA. *Nature*, *352*, 497–505.
695. Lee, M. S., Kliewer, S. A., Provencal, J., et al. (1993). Structure of the retinoid X receptor alpha DNA binding domain: a helix required for homodimeric DNA binding. *Science*, *260*, 1117–1121.
696. Wilson, T. E., Paulsen, R. E., Padgett, K. A., & Milbrandt, J. (1992). Participation of non-zinc finger residues in DNA binding by two nuclear orphan receptors. *Science*, *256*, 107-110.
697. Laudet, V., & Adelmant, G. (1995). Nuclear receptors: lonesome orphans. *Curr Biol*, *5*, 124-127.
698. Tenbaum, S, & Baniahmad, A. (1997). Nuclear receptors: structure, function and involvement in disease. *Int J Biochem Cell Biol*, *29*, 1325–1341.
699. Brzozowski, A. M., Pike, A. C., Dauter, Z., et al. (1997). Molecular basis of agonism and antagonism in the oestrogen receptor. *Nature*, *389*, 753–758.
700. Bourguet, W., Ruff, M., Chambon, P., et al. (1995). Crystal structure of the ligand-binding domain of the human nuclear receptor RXR-alpha. *Nature*, *375*, 377-382.
701. Renaud, J. P., Rochel, N., Ruff, M., et al. (1995). Crystal structure of the RAR-gamma ligand-binding domain bound to all-trans retinoic acid. *Nature*, *378*, 681–689.
702. Wagner, R. L., Apriletti, J. W., McGrath, M. E., et al. (1995). A structural role for hormone in the thyroid hormone receptor. *Nature*, *378*, 690–697.
703. Danielian, P. S., White, R., Lees, J. A., & Parker, M. G. (1992). Identification of a conserved region required for hormone dependent transcriptional activation by steroid hormone receptors. *EMBO J*, *11*, 1025–1033.
704. Barettino, D., Vivanco Ruiz, M. M., & Stunnenberg, H. G. (1994). Characterization of the ligand-dependent transactivation domain of thyroid hormone receptor. *EMBO J*, *13*, 3039–3049.
705. Durand, B., Saunders, M., Gaudon, C., et al. (1994). Activation function 2 (AF-2) of retinoic acid receptor and 9-cis retinoic acid receptor: presence of a conserved autonomous constitutive activating domain and influence of the nature of the response element on AF-2 activity. *EMBO J*, *13*, 5370–5382.
706. Schwabe, J. W. (1996). Transcriptional control: how nuclear receptors get turned on. *Curr Biol*, *6*, 372–374.
707. Wurtz, J. M., Bourguet, W., Renaud, J. P., et al. (1996). A canonical structure for the ligand-binding domain of nuclear receptors. *Nat Struct Biol*, *3*, 87–94.
708. Chen, H., Lin, R. J., Schiltz, R. L., et al. (1997). Nuclear receptor coactivator ACTR is a novel histone ace-tyltransferase and forms a multimeric activation complex with P/CAF and CBP/p300. *Cell*, *90*, 569–580.
709. Nagy, L., Kao, H. Y., Chakravarti, D., et al. (1997). Nuclear receptor repression mediated by a complex containing SMRT, mSin3A, and histone deacetylase. *Cell*, *89*, 373–380.
710. Alland, L., Muhle, R., Hou, H., Jr., et al. (1997). Role for N-CoR and histone deacetylase in Sin3- mediated transcriptional repression. *Nature*, *387*, 49–55.
711. Heinzel, T., Lavinsky, R. M., Mullen, T. M., et al. (1997). A complex containing N-CoR, mSin3 and histone deacetylase mediates transcriptional repression. *Nature*, *387*, 43–48.
712. Grunstein, M. (1997). Histone acetylation in chromatin structure and transcription. *Nature*, *389*, 349–352.
713. Jenster, G. (1998). Coactivators and corepressors as mediators of nuclear receptor function: an update. *Mol Cell Endocrinol*, *143*, 1–7.
714. Smith, D. F., & Toft, D. O. (1993). Steroid receptors and their associated proteins. *Mol Endocrinol*, *7*, 4–11.
715. Lee, S. K., Choi, H. S., Song, M. R., et al. (1998). Estrogen receptor, a common interaction partner for a subset of nuclear receptors. *Mol Endocrinol*, *12*, 1184–1192.
716. Barrett, T. J., & Spelsberg, T. C. (1998). Steroid receptors at the nexus of transcriptional regulation. *J Cell Biochem Suppl*, *31*, 185–193.
717. Zhang, X. K., Lehmann, J., Hoffmann, B., et al. (1992). Homodimer formation of retinoid X receptor induced by 9-cis retinoic acid. *Nature*, *358*, 587–591.
718. Hermann, T., Hoffmann, B., Zhang, X. K., et al. (1992). Heterodimeric receptor complexes determine 3,5,3'-triiodothyronine and retinoid signaling specificities. *Mol Endocrinol*, *6*, 1153–1162.
719. Zhang, X. K., Hoffmann, B., Tran, P. B., et al. (1992). Retinoid X receptor is an auxiliary protein for thyroid hormone and retinoic acid receptors. *Nature*, *355*, 441–446.
720. Chen, Z. P., Shemshedini, L., Durand, B., et al. (1994). Pure and functionally homogeneous recombinant retinoid X receptor. *J Biol Chem*, *269*, 25770–25776.
721. Watson, C. S., & Gametchu, B. (1999). Membrane-initiated steroid actions and the proteins that mediate them. *Proc Soc Exp Biol Med*, *220*, 9–19.
722. Chen, H. C., & Farese, R. V. (1999). Steroid hormones: interactions with membrane-bound receptors. *Curr Biol*, *9*, R478–R481.
723. Baldi, E., Luconi, M., Bonaccorsi, L., & Forti, G. (1998). Nongenomic effects of progesterone on spermatozoa: mechanisms of signal transduction and clinical implications. *Front Biosci*, *3*, D1051–D1059.

724. Bergeron, R., de Montigny, C., & Debonnel, G. (1996). Potentiation of neuronal NMDA response induced by dehydroepiandrosterone and its suppression by progesterone: effects mediated via sigma receptors. *J Neurosci*, *16*, 1193–1202.
725. Ke, L., & Lukas, R. J. (1996). Effects of steroid exposure on ligand binding and functional activities of diverse nicotinic acetylcholine receptor subtypes. *J Neurochem*, *67*, 1100–1112.
726. Maitra, R., & Reynolds, J. N. (1999). Subunit dependent modulation of GABAA receptor function by neuroactive steroids. *Brain Research*, *819*, 75–82.
727. Grazzini, E., Guillon, G., Mouillac, B., & Zingg, H. H. (1998). Inhibition of oxytocin receptor function by direct binding of progesterone. *Nature*, *392*, 509–512.
728. Pietras, R. J., & Szego, C. M. (1977). Specific binding sites for oestrogen at the outer surfaces of isolated endometrial cells. *Nature*, *265*, 69–72.
729. Berthois, Y., Pourreau-Schneider, N., Gandilhon, P., et al. (1986). Estradiol membrane binding sites on human breast cancer cell lines: use of a fluorescent estradiol conjugate to demonstrate plasma membrane binding systems. *J Steroid Biochem*, *25*, 963–972.
730. Gametchu, B., Watson, C. S., & Pasko, D. (1991). Size and steroid-binding characterization of membrane-associated glucocorticoid receptor in S-49 lymphoma cells. *Steroids*, *56*, 402–410.
731. Nenci, I., Marchetti, E., Marzola, A., & Fabris, G. (1981). Affinity cytochemistry visualizes specific estrogen binding sites on the plasma membrane of breast cancer cells . J Steroid Biochem, *14*, 1139–1146.
732. Refetoff, S. (1994). Resistance to thyroid hormone: an historical overview. *Thyroid*, *4*, 345–349.
733. Apriletti, J. W., Ribeiro, R. C., Wagner, R. L., et al. (1998). Molecular and structural biology of thyroid hormone receptors. *Clin Exp Pharmacol Physiol Suppl*, *25*, S2–S11.
734. Usala, S. J., Bale, A. E., Gesundheit, N., et al. (1988). Tight linkage between the syndrome of generalized thyroid hormone resistance and the human c-erbA beta gene. *Mol Endocrinol*, *2*, 1217–1220.
735. Refetoff, S., Weiss, R. E., & Usala, S. J. (1993). The syndromes of resistance to thyroid hormone. *Endocr Rev*, *14*, 348–399.
736. Usala, S. J., Menke, J. B., Watson, T. L., et al. (1991). A homozygous deletion in the c-erbA beta thyroid hormone receptor gene in a patient with generalized thyroid hormone resistance: isolation and characterization of the mutant receptor. *Mol Endocrinol*, *5*, 327–335.
737. Piedrafita, F. J., Ortiz, M. A., & Pfahl, M. (1995). Thyroid hormone receptor-beta mutants associated with generalized resistance to thyroid hormone show defects in their ligand-sensitive repression function. *Mol Endocrinol*, *9*, 1533–1548.
738. Yoh, S. M., Chatterjee, V. K., & Privalsky, M. L. (1997). Thyroid hormone resistance syndrome manifests as an aberrant interaction between mutant T3 receptors and transcriptional corepressors. *Mol Endocrinol*, *11*, 470–480.
739. Liu, Y., Takeshita, A., Misiti, S., et al. (1998). Lack of coactivator interaction can be a mechanism for dominant negative activity by mutant thyroid hormone receptors. *Endocrinology*, *139*, 4197–4204.
740. Takeda, K., Sakurai, A., DeGroot, L. J., & Refetoff, S. (1992). Recessive inheritance of thyroid hormone resistance caused by complete deletion of the protein-coding region of the thyroid hormone receptor-beta gene. *J Clin Endocrinol Metab*, *74*, 49–55.
741. Ono, S., Schwartz, I. D., Mueller, O. T., et al. (1991). Homozygosity for a dominant negative thyroid hormone receptor gene responsible for generalized resistance to thyroid hormone. *J Clin Endocrinol Metab*, *73*, 990–994.
742. Sakurai, A., Miyamoto, T., Refetoff, S., & DeGroot, L. J. (1990). Dominant negative transcriptional regulation by a mutant thyroid hormone receptor-beta in a family with generalized resistance to thyroid hormone. *Mol Endocrinol*, *4*, 1988–1994.
743. Bochukova, E., Schoenmakers, N., Agostini, M., et al. (2011). A mutation in the thyroid hormone receptor alpha gene. *N Engl J Med*, *366*, 243–249.
744. van Mullem, A., van Heerebeek, R., Chrysis, D., et al. (2012). Clinical phenotype and mutant TRalpha1. *N Engl J Med*, *366*, 1451–1453.
745. Hughes, M. R., Malloy, P. J., Kieback, D. G., et al. (1988). Point mutations in the human vitamin D receptor gene associated with hypocalcemic rickets. *Science*, *242*, 1702–1705.
746. Whitfield, G. K., Selznick, S. H., Haussler, C. A., et al. (1996). Vitamin D receptors from patients with resistance to 1,25-dihydroxyvitamin D_3: point mutations confer reduced transactivation in response to ligand and impaired interaction with the retinoid X receptor heterodimeric partner. *Mol Endocrinol*, *10*, 1617–1631.
747. Kristjansson, K., Rut, A. R., Hewison, M., et al. (1993). Two mutations in the hormone binding domain of the vitamin D receptor cause tissue resistance to 1,25 dihydroxyvitamin D_3. *J Clin Invest*, *92*, 12–16.
748. Ames, S. K., Ellis, K. J., Gunn, S. K., et al. (1999). Vitamin D receptor gene Fok1 polymorphism predicts calcium absorption and bone mineral density in children. *J Bone Miner Res*, *14*, 740–746.
749. Gennari, L., Becherini, L., Masi, L., et al. (1997). Vitamin D receptor genotypes and intestinal calcium absorption in postmenopausal women. *Calcif Tissue Int*, *61*, 460–463.
750. Ferrari, S., Rizzoli, R., Chevalley, T., et al. (1995). Vitamin-D-receptor-gene polymorphisms and change in lumbar-spine bone mineral density. *Lancet*, *345*, 423–424.
751. Feskanich, D., Hunter, D. J., Willett, W. C., et al. (1998). Vitamin D receptor genotype and the risk of bone fractures in women. *Epidemiology*, *9*, 535–539.
752. Gennari, L., Becherini, L., Mansani, R., et al. (1999). FokI polymorphism at translation initiation site of the vitamin D receptor gene predicts bone mineral density and vertebral fractures in postmenopausal Italian women. *J Bone Miner Res*, *14*, 1379–1386.
753. Gong, G., Stern, H. S., Cheng, S. C., et al. (1999). The association of bone mineral density with vitamin D receptor gene polymorphisms. *Osteoporos Int*, *9*, 55–64.
754. Eccleshall, T. R., Garnero, P., Gross, C., et al. (1998). Lack of correlation between start codon polymorphism of the vitamin D receptor gene and bone mineral density in premenopausal French women: the OFELY study. *J Bone Miner Res*, *13*, 31–35.
755. Cheng, W. C., & Tsai, K. S. (1999). The vitamin D receptor start codon polymorphism (Fok1) and bone mineral density in premenopausal women in Taiwan. *Osteoporos Int*, *9*, 545-549.
756. Deng, H. W., Li, J., Li, J. L., et al. (1999). Association of VDR and estrogen receptor genotypes with bone mass in postmenopausal Caucasian women: different conclusions with different analyses and the implications. *Osteoporos Int*, *9*, 499–507.
757. Gross, C., Krishnan, A. V., Malloy, P. J., et al. (1998). The vitamin D receptor gene start codon polymorphism: a functional analysis of FokI variants. *J Bone Miner Res*, *13*, 1691–1699.
758. Hansen, T. S., Abrahamsen, B., Henriksen, F. L., et al. (1998). Vitamin D receptor alleles do not predict bone mineral density or bone loss in Danish perimenopausal women. *Bone*, *22*, 571–575.
759. Ferrari, S. L., Rizzoli, R., Slosman, D. O., & Bonjour, J. P. (1998). Do dietary calcium and age explain the controversy surrounding the relationship between bone mineral density and vitamin D receptor gene polymorphisms? *J Bone Miner Res*, *13*, 363–370.
760. Gennari, L., Becherini, L., Masi, L., et al. (1998). Vitamin D and estrogen receptor allelic variants in Italian postmenopausal women: evidence of multiple gene contribution to bone mineral density. *J Clin Endocrinol Metab*, *83*, 939–944.
761. Suarez, F., Rossignol, C., & Garabedian, M. (1998). Interactive effect of estradiol and vitamin D receptor gene polymorphisms as a possible determinant of growth in male and female infants. *J Clin Endocrinol Metab*, *83*, 3563–3568.
762. Tao, C., Yu, T., Garnett, S., et al. (1998). Vitamin D receptor alleles predict growth and bone density in girls. *Arch Dis Child*, *79*, 488–493; discussion 493-484.
763. Carling, T., Ridefelt, P., Hellman, P., et al. (1998). Vitamin D receptor gene polymorphism and parathyroid calcium sensor protein (CAS/gp330) expression in primary hyperparathyroidism. *World J Surg*, *22*, 700–706; discussion 706-707.
764. Hennig, B. J., Parkhill, J. M., Chapple, I. L., et al. (1999). Association of a vitamin D receptor gene polymorphism with localized early-onset periodontal diseases. *J Periodontol*, *70*, 1032–1038.
765. Jackman, S. V., Kibel, A. S., Ovuworie, C. A., et al. (1999). Familial calcium stone disease: TaqI polymorphism and the vitamin D receptor. *J Endourol*, *13*, 313–316.

766. Niimi, T., Tomita, H., Sato, S., et al. (1999). Vitamin D receptor gene polymorphism in patients with sarcoidosis. *Am J Respir Crit Care Med*, *160*, 1107–1109.
767. Ruggiero, M., Pacini, S., Amato, M., et al. (1999). Association between vitamin D receptor gene polymorphism and nephrolithiasis. *Miner Electrolyte Metab*, *25*, 185–190.
768. Ruggiero, M., Pacini, S., Aterini, S., et al. (1998). Vitamin D receptor gene polymorphism is associated with metastatic breast cancer. *Oncol Res*, *10*, 43–46.
769. Park, B. S., Park, J. S., Lee, D. Y., et al. (1999). Vitamin D receptor polymorphism is associated with psoriasis. *J Invest Dermatol*, *112*, 113–116.
770. Roy, S., Frodsham, A., Saha, B., et al. (1999). Association of vitamin D receptor genotype with leprosy type. *J Infect Dis*, *179*, 187–191.
771. Hill, A. V. (1998). The immunogenetics of human infectious diseases. *Annu Rev Immunol*, *16*, 593–617.
772. Roth, D. E., Soto, G., Arenas, F., et al. (2004). Association between vitamin D receptor gene polymorphisms and response to treatment of pulmonary tuberculosis. *J Infect Dis*, *190*, 920–927.
773. Saito, M., Eiraku, N., Usuku, K., et al. (2005). ApaI polymorphism of vitamin D receptor gene is associated with susceptibility to HTLV-1-associated myelopathy/tropical spastic paraparesis in HTLV-1 infected individuals. *J Neurol Sci*, *232*, 29–35.
774. Fiorenza, C. G., Chou, S. H., & Mantzoros, C. S. (2010). Lipodystrophy: pathophysiology and advances in treatment. *Nat Rev Endocrinol*, *7*, 137–150.
775. Barroso, I., Gurnell, M., Crowley, V. E., et al. (1999). Dominant negative mutations in human PPARgamma associated with severe insulin resistance, diabetes mellitus and hypertension. *Nature*, *402*, 880–883.
776. Savage, D. B., Tan, G. D., Acerini, C. L., et al. (2003). Human metabolic syndrome resulting from dominant-negative mutations in the nuclear receptor peroxisome proliferator-activated receptor-gamma. *Diabetes*, *52*, 910–917.
777. Agarwal, A. K., & Garg, A. (2002). A novel heterozygous mutation in peroxisome proliferator-activated receptor-gamma gene in a patient with familial partial lipodystrophy. *J Clin Endocrinol Metab*, *87*, 408–411.
778. Francis, G. A., Li, G., Casey, R., et al. (2006). Peroxisomal proliferator activated receptor-gamma deficiency in a Canadian kindred with familial partial lipodystrophy type 3 (FPLD3). *BMC Med Genet*, *7*, 3.
779. Hegele, R. A., Cao, H., Frankowski, C., et al. (2002). PPARG F388L, a transactivation-deficient mutant, in familial partial lipodystrophy. *Diabetes*, *51*, 3586–3590.
780. Jeninga, E. H., van Beekum, O., van Dijk, A. D., et al. (2007). Impaired peroxisome proliferator-activated receptor gamma function through mutation of a conserved salt bridge (R425C) in familial partial lipodystrophy. *Mol Endocrinol*, *21*, 1049–1065.
781. Ludtke, A., Buettner, J., Schmidt, H. H., & Worman, H. J. (2007). New PPARG mutation leads to lipodystrophy and loss of protein function that is partially restored by a synthetic ligand. *J Med Genet*, *44*, e88.
782. Ludtke, A., Buettner, J., Wu, W., et al. (2007). Peroxisome proliferator-activated receptor-gamma C190S mutation causes partial lipodystrophy. *J Clin Endocrinol Metab*, *92*, 2248–2255.
783. Monajemi, H., Zhang, L., Li, G., et al. (2007). Familial partial lipodystrophy phenotype resulting from a single-base mutation in deoxyribonucleic acid-binding domain of peroxisome proliferator-activated receptor-gamma. *J Clin Endocrinol Metab*, *92*, 1606–1612.
784. Visser, M. E., Kropman, E., Kranendonk, M. E., et al. (2011). Characterisation of non-obese diabetic patients with marked insulin resistance identifies a novel familial partial lipodystrophy-associated PPARgamma mutation (Y151C). *Diabetologia*, *54*, 1639–1644.
785. Hegele, R. A., Ur, E., Ransom, T. P., & Cao, H. (2006). A frameshift mutation in peroxisome-proliferator-activated receptor-gamma in familial partial lipodystrophy subtype 3 (FPLD3; MIM 604367). *Clin Genet*, *70*, 360–362.
786. Campeau, P. M., Astapova, O., Martins, R., et al. (2012). Clinical and molecular characterization of a severe form of partial lipodystrophy expanding the phenotype of PPARgamma deficiency. *J Lipid Res*, *53*, 1968–1978.
787. Ristow, M., Muller-Wieland, D., Pfeiffer, A., et al. (1998). Obesity associated with a mutation in a genetic regulator of adipocyte differentiation. *N Engl J Med*, *3392*, 953–959.
788. Sladek, F. M., Dallas-Yang, Q., & Nepomuceno, L. (1998). MODY1 mutation Q268X in hepatocyte nuclear factor 4alpha allows for dimerization in solution but causes abnormal subcellular localization. *Diabetes*, *47*, 985–990.
789. Lindner, T., Gragnoli, C., Furuta, H., et al. (1997). Hepatic function in a family with a nonsense mutation (R154X) in the hepatocyte nuclear factor-4alpha/MODY1 gene. *J Clin Invest*, *100*, 1400–1405.
790. Furuta, H., Iwasaki, N., Oda, N., et al. (1997). Organization and partial sequence of the hepatocyte nuclear factor-4 alpha/MODY1 gene and identification of a missense mutation, R127W, in a Japanese family with MODY. *Diabetes*, *46*, 1652–1657.
791. Bulman, M. P., Dronsfield, M. J., Frayling, T., et al. (1997). A missense mutation in the hepatocyte nuclear factor 4 alpha gene in a UK pedigree with maturity-onset diabetes of the young. *Diabetologia*, *40*, 859–862.
792. Yamagata, K., Furuta, H., Oda, N., et al. (1996). Mutations in the hepatocyte nuclear factor-4alpha gene in maturity-onset diabetes of the young (MODY1). *Nature*, *384*, 458–460.
793. Conn, J. J., Simm, P. J., Oats, J. J., et al. (2009). Neonatal hyperinsulinaemic hypoglycaemia and monogenic diabetes due to a heterozygous mutation of the HNF4A gene. *Aust NZ J Obstet Gynaecol*, *49*, 328–330.
794. Fajans, S. S., & Bell, G. I. (2007). Macrosomia and neonatal hypoglycaemia in RW pedigree subjects with a mutation (Q268X) in the gene encoding hepatocyte nuclear factor 4alpha (HNF4A). *Diabetologia*, *50*, 2600–2601.
795. Flanagan, S. E., Kapoor, R. R., Mali, G., et al. (2010). Diazoxide-responsive hyperinsulinemic hypoglycemia caused by HNF4A gene mutations . Eur J Endocrinol, *162*, 987–992.
796. Kapoor, R. R., Locke, J., Colclough, K., et al. (2008). Persistent hyperinsulinemic hypoglycemia and maturity-onset diabetes of the young due to heterozygous HNF4A mutations. *Diabetes*, *57*, 1659–1663.
797. Pearson, E. R., Boj, S. F., Steele, A. M., et al. (2007). Macrosomia and hyperinsulinaemic hypoglycaemia in patients with heterozygous mutations in the HNF4A gene. *PLoS Med*, *4*, e118.
798. Malecki, M. T., Yang, Y., Antonellis, A., et al. (1999). Identification of new mutations in the hepatocyte nuclear factor 4alpha gene among families with early onset Type 2 diabetes mellitus. *Diabet Med*, *16*, 193–200.
799. Fajans, S. S., & Bell, G. I. (2006). Phenotypic heterogeneity between different mutations of MODY subtypes and within MODY pedigrees. *Diabetologia*, *49*, 1106–1108.
800. Fajans, S. S., Bell, G. I., & Polonsky, K. S. (2001). Molecular mechanisms and clinical pathophysiology of maturity-onset diabetes of the young. *N Engl J Med*, *345*, 971–980.
801. Malecki, M. T., & Klupa, T. (2005). Type 2 diabetes mellitus: from genes to disease. *Pharmacol Rep*, *57*, 20–32.
802. Shih, D. Q., & Stoffel, M. (2002). Molecular etiologies of MODY and other early-onset forms of diabetes. *Curr Diab Rep*, *2*, 125–134.
803. Charmandari, E. (2011). Primary generalized glucocorticoid resistance and hypersensitivity. *Horm Res Paediatr*, *76*, 145–155.
804. Lamberts, S. W., Koper, J. W., Biemond, P., et al. (1992). Cortisol receptor resistance: the variability of its clinical presentation and response to treatment. *J Clin Endocrinol Metab*, *74*, 313–321.
805. Werner, S., Thoren, M., Gustafsson, J. A., & Bronnegard, M. (1992). Glucocorticoid receptor abnormalities in fibroblasts from patients with idiopathic resistance to dexamethasone diagnosed when evaluated for adrenocortical disorders. *J Clin Endocrinol Metab*, *75*, 1005–1009.
806. Charmandari, E., Kino, T., Ichijo, T., & Chrousos, G. P. (2008). Generalized glucocorticoid resistance: clinical aspects, molecular mechanisms, and implications of a rare genetic disorder. *J Clin Endocrinol Metab*, *93*, 1563–1572.
807. Malchoff, C. D., Javier, E. C., Malchoff, D. M., et al. (1990). Primary cortisol resistance presenting as isosexual precocity. *J Clin Endocrinol Metab*, *70*, 503–507.
808. Chrousos, G. P., Detera-Wadleigh, S. D., & Karl, M. (1993). Syndromes of glucocorticoid resistance. *Ann Intern Med*, *119*, 1113–1124.

809. Chrousos, G. P., Vingerhoeds, A., Brandon D, et al. (1982). Primary cortisol resistance in man: a glucocorticoid receptor-mediated disease. *J Clin Invest*, *69*, 1261–1269.
810. McMahon, S. K., Pretorius, C. J., Ungerer, J. P., et al. (2010). Neonatal complete generalized glucocorticoid resistance and growth hormone deficiency caused by a novel homozygous mutation in Helix 12 of the ligand binding domain of the glucocorticoid receptor gene (NR3C1). *J Clin Endocrinol Metab*, *95*, 297–302.
811. Nader, N., Bachrach, B. E., Hurt, D. E., et al. (2010). A novel point mutation in helix 10 of the human glucocorticoid receptor causes generalized glucocorticoid resistance by disrupting the structure of the ligand-binding domain. *J Clin Endocrinol Metab*, *95*, 2281–2285.
812. Malchoff, D. M., Brufsky, A., Reardon, G., et al. (1993). A mutation of the glucocorticoid receptor in primary cortisol resistance. *J Clin Invest*, *91*, 1918–1925.
813. Mendonca, B. B., Leite, M. V., de Castro, M., et al. (2002). Female pseudohermaphroditism caused by a novel homozygous missense mutation of the GR gene. *J Clin Endocrinol Metab*, *87*, 1805–1809.
814. Charmandari, E., Kino, T., Ichijo, T., et al. (2007). A novel point mutation in helix 11 of the ligand-binding domain of the human glucocorticoid receptor gene causing generalized glucocorticoid resistance. *J Clin Endocrinol Metab*, *92*, 3986–3990.
815. Charmandari, E., Kino, T., Souvatzoglou, E., et al. (2004). Natural glucocorticoid receptor mutants causing generalized glucocorticoid resistance: molecular genotype, genetic transmission, and clinical phenotype. *J Clin Endocrinol Metab*, *89*, 1939–1949.
816. Karl, M., Lamberts, S. W., Koper, J. W., et al. (1996). Cushing's disease preceded by generalized glucocorticoid resistance: clinical consequences of a novel, dominant-negative glucocorticoid receptor mutation. *Proc Assoc Am Physicians*, *108*, 296–307.
817. Kino, T., Stauber, R. H., Resau, J. H., et al. (2001). Pathologic human GR mutant has a transdominant negative effect on the wild-type GR by inhibiting its translocation into the nucleus: importance of the ligand-binding domain for intracellular GR trafficking. *J Clin Endocrinol Metab*, *86*, 5600–5608.
818. Karl, M., Lamberts, S. W., Koper, J. W., et al. (1996). Cushing's disease preceded by generalized glucocorticoid resistance: clinical consequences of a novel, dominant-negative glucocorticoid receptor mutation. *Proc Assoc Am Physicians*, *108*, 296–307.
819. Charmandari, E., Ichijo, T., Jubiz, W., et al. (2008). A novel point mutation in the amino terminal domain of the human glucocorticoid receptor (hGR) gene enhancing hGR-mediated gene expression. *J Clin Endocrinol Metab*, *93*, 4963–4968.
820. McPhaul, M. J., Marcelli, M., Tilley, W. D., et al. (1991). Androgen resistance caused by mutations in the androgen receptor gene . Faseb J, *5*, 2910–2915.
821. Gottlieb, B., Beitel, L. K., Nadarajah, A., et al. (2012). The androgen receptor gene mutations database: 2012 update. *Hum Mutat*, *33*, 887–894.
822. Hughes, I. A., Werner, R., Bunch, T., & Hiort, O. (2010). Androgen insensitivity syndrome. *Semin Reprod Med*, *30*, 432–442.
823. Jirasek, J. E. (1971). Androgen-insensitive male pseudohermaphroditism. *Birth Defects Orig Artic Ser*, 7, 179–184.
824. Lubahn, D. B., Brown, T. R., Simental, J. A., et al. (1989). Sequence of the intron/exon junctions of the coding region of the human androgen receptor gene and identification of a point mutation in a family with complete androgen insensitivity. *Proc Natl Acad Sci USA*, *86*, 9534–9538.
825. Bevan, C. L., Hughes, I. A., & Patterson, M. N. (1997). Wide variation in androgen receptor dysfunction in complete androgen insensitivity syndrome. *J Steroid Biochem Mol Biol*, *61*, 19–26.
826. Tincello, D. G., Saunders, P. T., Hodgins, M. B., et al. (1997). Correlation of clinical, endocrine and molecular abnormalities with in vivo responses to high-dose testosterone in patients with partial androgen insensitivity syndrome. *Clin Endocrinol*, *46*, 497–506.
827. MacLean, H. E., Warne, G. L., & Zajac, J. D. (1995). Defects of androgen receptor function: rom sex reversal to motor neurone disease. *Mol Cell Endocrinol*, *112*, 133–141.
828. Peterziel, H., Culig, Z., Stober, J., et al. (1995). Mutant androgen receptors in prostatic tumors distinguish between amino-acid-sequence requirements for transactivation and ligand binding. *Int J Cancer*, *63*, 544–550.
829. Brown, T. R., Lubahn, D. B., Wilson, E. M., et al. (1990). Functional characterization of naturally occurring mutant androgen receptors from subjects with complete androgen insensitivity. *Mol Endocrinol*, *4*, 1759–1772.
830. McPhaul, M. J., Marcelli, M., Zoppi, S., et al. (1993). Genetic basis of endocrine disease. 4. The spectrum of mutations in the androgen receptor gene that causes androgen resistance. *J Clin Endocrinol Metab*, *76*, 17–23.
831. Marcelli, M., Zoppi, S., Grino, P. B., et al. (1991). A mutation in the DNA-binding domain of the androgen receptor gene causes complete testicular feminization in a patient with receptor-positive androgen resistance. *J Clini Invest*, *87*, 1123–1126.
832. Zoppi, S., Wilson, C. M., Harbison, M. D., et al. (1993). Complete testicular feminization caused by an amino-terminal truncation of the androgen receptor with downstream initiation. *J Clini Invest*, *91*, 1105–1112.
833. Marcelli, M., Tilley, W. D., Wilson, C. M., et al. (1990). Definition of the human androgen receptor gene structure permits the identification of mutations that cause androgen resistance: premature termination of the receptor protein at amino acid residue 588 causes complete androgen resistance. *Mol Endocrinol*, *4*, 1105–1116.
834. Trifiro, M., Gottlieb, B., Pinsky, L., et al. (1991). The 56/58 kDa androgen-binding protein in male genital skin fibroblasts with a deleted androgen receptor gene. *Mol Cell Endocrinol*, *75*, 37–47.
835. Quigley, C. A., Friedman, K. J., Johnson, A., et al. (1992). Complete deletion of the androgen receptor gene: definition of the null phenotype of the androgen insensitivity syndrome and determination of carrier status. *J Clin Endocrinol Metab*, *74*, 927–933.
836. Ris-Stalpers, C., Kuiper, G. G., Faber, P. W., et al. (1990). Aberrant splicing of androgen receptor mRNA results in synthesis of a nonfunctional receptor protein in a patient with androgen insensitivity. *Proc Natl Acad Sci USA*, *87*, 7866–7870.
837. Holterhus, P. M., Wiebel, J., Sinnecker, G. H., et al. (1999). Clinical and molecular spectrum of somatic mosaicism in androgen insensitivity syndrome. *Pediatr Res*, *46*, 684–690.
838. Holterhus, P. M., Bruggenwirth, H. T., Hiort, O., et al. (1997). Mosaicism due to a somatic mutation of the androgen receptor gene determines phenotype in androgen insensitivity syndrome. *J Clin Endocrinol Metab*, *82*, 3584–3589.
839. Gast, A., Neuschmid-Kaspar, F., Klocker, H., & Cato, A. C. (1995). A single amino acid exchange abolishes dimerization of the androgen receptor and causes Reifenstein syndrome. *Mol Cell Endocrinol*, *111*, 93–98.
840. Kaspar, F., Klocker, H., Denninger, A., & Cato, A. C. (1993). A mutant androgen receptor from patients with Reifenstein syndrome: identification of the function of a conserved alanine residue in the D box of steroid receptors. *Mol Cell Biol*, *13*, 7850–7858.
841. Klocker, H., Kaspar, F., Eberle, J., et al. (1992). Point mutation in the DNA binding domain of the androgen receptor in two families with Reifenstein syndrome. *Am J Hum Genet*, *50*, 1318–1327.
842. Nakao, R., Yanase, T., Sakai, Y., et al. (1993). A single amino acid substitution (gly743 → val) in the steroid-binding domain of the human androgen receptor leads to Reifenstein syndrome. *J Clin Endocrinol Metab*, *77*, 103–107.
843. McPhaul, M. J., Marcelli, M., Zoppi, S., et al. (1992). Mutations in the ligand-binding domain of the androgen receptor gene cluster in two regions of the gene. *J Clin Invest*, *90*, 2097–2101.
844. Wooster, R., Mangion, J., Eeles, R., et al. (1992). A germline mutation in the androgen receptor gene in two brothers with breast cancer and Reifenstein syndrome. *Nat Genet*, *2*, 132–134.
845. MacLean, H. E., Warne, G. L., & Zajac, J. D. (1996). Spinal and bulbar muscular atrophy: androgen receptor dysfunction caused by a trinucleotide repeat expansion. *J Neurol Sci*, *135*, 149–157.
846. Chamberlain, N. L., Driver, E. D., & Miesfeld, R. L. (1994). The length and location of CAG trinucleotide repeats in the androgen receptor N-terminal domain affect transactivation function. *Nucleic Acids Res*, *22*, 3181–3186.

847. Stenoien, D. L., Cummings, C. J., Adams, H. P., et al. (1999). Polyglutamine-expanded androgen receptors form aggregates that sequester heat shock proteins, proteasome components and SRC-1, and are suppressed by the HDJ-2 chaperone. *Hum Mol Genet*, *8*, 731–741.
848. Kuiper, G. G., Carlsson, B., Grandien, K., et al. (1997). Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. *Endocrinology*, *138*, 863–870.
849. Mosselman, S., Polman, J., & Dijkema, R. (1996). ER beta: identification and characterization of a novel human estrogen receptor. *FEBS Lett*, *392*, 49–53.
850. Kuiper, G. G., Enmark, E., Pelto-Huikko, M., et al. (1996). Cloning of a novel receptor expressed in rat prostate and ovary. *Proc Natl Acad Sci USA*, *93*, 5925–5930.
851. McInerney, E. M., Tsai, M. J., O'Malley, B. W., & Katzenellenbogen, B. S. (1996). Analysis of estrogen receptor transcriptional enhancement by a nuclear hormone receptor coactivator. *Proc Natl Acad Sci USA*, *93*, 10069–10073.
852. Pettersson, K., Grandien, K., Kuiper, G. G., & Gustafsson, J. A. (1997). Mouse estrogen receptor beta forms estrogen response element-binding heterodimers with estrogen receptor alpha. *Mol Endocrinol*, *11*, 1486–1496.
853. Toran-Allerand, C. D. (2004). Minireview: a plethora of estrogen receptors in the brain: where will it end? *Endocrinology*, *145*, 1069–1074.
854. Li, L., Haynes, M. P., & Bender, J. R. (2003). Plasma membrane localization and function of the estrogen receptor alpha variant (ER46) in human endothelial cells. *Proc Natl Acad Sci USA*, *100*, 4807–4812.
855. Toran-Allerand, C. D., Guan, X., MacLusky, N. J., et al. (2002). ER-X: a novel, plasma membrane-associated, putative estrogen receptor that is regulated during development and after ischemic brain injury. *J Neurosci*, *22*, 8391–8401.
856. Rao, B. R. (1998). Isolation and characterization of an estrogen binding protein which may integrate the plethora of estrogenic actions in non-reproductive organs. *J Steroid Biochem Mol Biol*, *65*, 3–41.
857. Asaithambi, A., Mukherjee, S., & Thakur, M. K. (1997). Expression of 112-kDa estrogen receptor in mouse brain cortex and its autoregulation with age. *Biochem Biophys Res Commun*, *231*, 683–685.
858. Ramirez, V. D., Kipp, J. L., & Joe, I. (2001). Estradiol, in the CNS, targets several physiologically relevant membrane-associated proteins. *Brain Res Rev*, *37*, 141–152.
859. Joe, I., & Ramirez, V. D. (2001). Binding of estrogen and progesterone-BSA conjugates to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and the effects of the free steroids on GAPDH enzyme activity: physiological implications. *Steroids*, *66*, 529–538.
860. Zheng, J., & Ramirez, V. D. (1999). Purification and identification of an estrogen binding protein from rat brain: oligomycin sensitivity-conferring protein (OSCP), a subunit of mitochondrial F0F1-ATP synthase/ATPase. *J Steroid Biochem Mol Biol*, *68*, 65–75.
861. Smith, E. P., Boyd, J., Frank, G. R., et al. (1994). Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. *N Engl J Med*, *331*, 1056–1061.
862. Lehrer, S., Rabin, J., Stone, J., & Berkowitz, G. S. (1994). Association of an estrogen receptor variant with increased height in women. *Horm Metab Res*, *26*, 486–488.
863. Kuhnle, U., Nielsen, M. D., Tietze, H. U., et al. (1990). Pseudohypoaldosteronism in eight families: different forms of inheritance are evidence for various genetic defects. *J Clin Endocrinol Metab*, *70*, 638–641.
864. Chitayat, D., Spirer, Z., Ayalon, D., & Golander, A. (1985). Pseudohypoaldosteronism in a female infant and her family: diversity of clinical expression and mode of inheritance. *Acta Paediatr Scand*, *74*, 619–622.
865. Hanukoglu, A., Fried, D., & Gotlieb, A. (1978). Inheritance of pseudohypoaldosteronism. Lancet, 1, 1359.
866. Limal, J. M., Rappaport, R., Dechaux, M., & Morin, C. (1978). Familial dominant pseudohypoaldosteronism. *Lancet*, *1*, 51.
867. Bonnici, F. (1977). Autosomal recessive transmission of familial pseudohypoaldosteronism. *Arch Fr Pediatr*, *34*, 915–916.
868. Rosler, A. (1984). The natural history of salt-wasting disorders of adrenal and renal origin. *J Clin Endocrinol Metab*, *59*, 689–700.
869. Shigetomi, S., Ojima, M., Ueno, S., et al. (1986). Two adult familial cases of selective hypoaldosteronism due to insufficiency of conversion of corticosterone to aldosterone. *Endocrinol Jpn*, *33*, 787–794.
870. Keszler, M., & Sivasubramanian, K. N. (1983). Pseudohypoaldosteronism. *Am J Dis Child*, *137*, 738–740.
871. Abramson, O., Zmora, E., Mazor, M., & Shinwell, E. S. (1992). Pseudohypoaldosteronism in a preterm infant: intrauterine presentation as hydramnios. *J Pediatr*, *120*, 129–132.
872. Kuhnle, U., Keller, U., Armanini, D., et al. (1994). Immunofluorescence of mineralocorticoid receptors in peripheral lymphocytes: presence of receptor-like activity in patients with the autosomal dominant form of pseudohypoaldosteronism, and its absence in the recessive form. *J Steroid Biochem Mol Biol*, *51*, 267–273.
873. Arai, K., & Chrousos, G. P. (1995). Syndromes of glucocorticoid and mineralocorticoid resistance. *Steroids*, *60*, 173–179.
874. Oberfield, S. E., Levine, L. S., Carey, R. M., et al. (1979). Pseudohypoaldosteronism: multiple target organ unresponsiveness to mineralocorticoid hormones. *J Clin Endocrinol Metab*, *48*, 228–234.
875. Furgeson, S. B., & Linas, S. (2010). Mechanisms of type I and type II pseudohypoaldosteronism. *J Am Soc Nephrol*, *21*, 1842–1845.
876. Hanukoglu, A. (1991). Type I pseudohypoaldosteronism includes two clinically and genetically distinct entities with either renal or multiple target organ defects. *J Clin Endocrinol Metab*, *73*, 936–944.
877. Schambelan, M., Sebastian, A., & Rector, F. C., Jr. (1981). Mineralocorticoid-resistant renal hyperkalemia without salt wasting (type II pseudohypoaldosteronism): role of increased renal chloride reabsorption. *Kidney Int*, *19*, 716–727.
878. Zennaro, M. C., Hubert, E. L., & Fernandes-Rosa, F. L. (2011). Aldosterone resistance: structural and functional considerations and new perspectives. *Mol Cell Endocrinol*, *350*, 206–215.
879. Fernandes-Rosa, F. L., de Castro, M., Latronico, A. C., et al. (2006). Recurrence of the R947X mutation in unrelated families with autosomal dominant pseudohypoaldosteronism type 1: evidence for a mutational hot spot in the mineralocorticoid receptor gene. *J Clin Endocrinol Metab*, *91*, 3671–3675.
880. Geller, D. S., Rodriguez-Soriano, J., Vallo Boado, A., et al. (1998). Mutations in the mineralocorticoid receptor gene cause autosomal dominant pseudohypoaldosteronism type I. *Nat Genet*, *19*, 279–281.
881. Geller, D. S., Zhang, J., Zennaro, M. C., et al. (2006). Autosomal dominant pseudohypoaldosteronism type 1: mechanisms, evidence for neonatal lethality, and phenotypic expression in adults. *J Am Soc Nephrol*, *17*, 1429–1436.
882. Nystrom, A. M., Bondeson, M. L., Skanke, N., et al. (2004). A novel nonsense mutation of the mineralocorticoid receptor gene in a Swedish family with pseudohypoaldosteronism type I (PHA1). *J Clin Endocrinol Metab*, *89*, 227–231.
883. Riepe, F. G. (2009). Clinical and molecular features of type 1 pseudohypoaldosteronism. *Horm Res*, *72*, 1–9.
884. Riepe, F. G., Finkeldei, J., de Sanctis, L., et al. (2006). Elucidating the underlying molecular pathogenesis of NR3C2 mutants causing autosomal dominant pseudohypoaldosteronism type 1. *J Clin Endocrinol Metab*, *91*, 4552–4561.
885. Riepe, F. G., Krone, N., Morlot, M., et al. (2003). Identification of a novel mutation in the human mineralocorticoid receptor gene in a German family with autosomal-dominant pseudohypoaldosteronism type 1: further evidence for marked interindividual clinical heterogeneity. *J Clin Endocrinol Metab*, *88*, 1683–1686.
886. Riepe, F. G., Krone, N., Morlot, M., et al. (2004). Autosomal-dominant pseudohypoaldosteronism type 1 in a Turkish family is associated with a novel nonsense mutation in the human mineralocorticoid receptor gene. *J Clin Endocrinol Metab*, *89*, 2150–2152.
887. Sartorato, P., Lapeyraque, A. L., Armanini, D., et al. (2003). Different inactivating mutations of the mineralocorticoid receptor in fourteen families affected by type I pseudohypoaldosteronism. *J Clin Endocrinol Metab*, *88*, 2508–2517.
888. Tajima, T., Kitagawa, H., Yokoya, S., et al. (2000). A novel missense mutation of mineralocorticoid receptor gene in one Japanese family with a renal form of pseudohypoaldosteronism type 1. *J Clin Endocrinol Metab*, *85*, 4690–4694.

889. Viemann, M., Peter, M., Lopez-Siguero, J. P., et al. (2001). Evidence for genetic heterogeneity of pseudohypoaldosteronism type 1: identification of a novel mutation in the human mineralocorticoid receptor in one sporadic case and no mutations in two autosomal dominant kindreds. *J Clin Endocrinol Metab*, *86*, 2056–2059.
890. Geller, D. S., Farhi, A., Pinkerton, N., et al. (2000). Activating mineralocorticoid receptor mutation in hypertension exacerbated by pregnancy. *Science*, *289*, 119–123.
891. McSherry, E. (1981). Renal tubular acidosis in childhood. *Kidney Int*, *20*, 799–809.
892. New, M. I., Stoner, E., & DiMartino-Nardi, J. (1986). Apparent mineralocorticoid excess causing hypertension and hypokalemia in children. *Clin Exp Hypertens* [A], *8*, 751–772.
893. Muller-Berghaus, J., Homoki, J., Michalk, D. V., & Querfeld, U. (1996). Diagnosis and treatment of a child with the syndrome of apparent mineralocorticoid excess type 1. *Acta Paediatr*, *85*, 111–113.
894. Dave-Sharma, S., Wilson, R. C., Harbison, M. D., et al. (1998). Examination of genotype and phenotype relationships in 14 patients with apparent mineralocorticoid excess. *J Clin Endocrinol Metab*, *83*, 2244–2254.
895. Morineau, G., Marc, J. M., Boudi, A., et al. (1999). Genetic, biochemical, and clinical studies of patients with A328V or R213C mutations in 11betaHSD2 causing apparent mineralocorticoid excess. *Hypertension*, *34*, 435–441.
896. Ugrasbul, F., Wiens, T., Rubinstein, P., et al. (1999). Prevalence of mild apparent mineralocorticoid excess in Mennonites. *J Clin Endocrinol Metab*, *84*, 4735–4738.
897. Monder, C., Shackleton, C. H., Bradlow, H. L., et al. (1986). The syndrome of apparent mineralocorticoid excess: its association with 11 beta-dehydrogenase and 5 beta-reductase deficiency and some consequences for corticosteroid metabolism. *J Clin Endocrinol Metab*, *63*, 550–557.
898. Mune, T., Rogerson, F. M., Nikkila, H., et al. (1995). Human hypertension caused by mutations in the kidney isozyme of 11 beta-hydroxysteroid dehydrogenase. *Nat Genet*, *10*, 394–399.
899. Wilson, R. C., Harbison, M. D., Krozowski, Z. S., et al. (1995). Several homozygous mutations in the gene for 11 beta-hydroxysteroid dehydrogenase type 2 in patients with apparent mineralocorticoid excess. *J Clin Endocrinol Metab*, *80*, 3145–3150.
900. Bassett, J. H., O'Halloran, D. J., Williams, G. R., et al. (1999). Novel DAX1 mutations in X-linked adrenal hypoplasia congenita and hypogonadotrophic hypogonadism. *Clin Endocrinol*, *50*, 69–75.
901. Zanaria, E., Muscatelli, F., Bardoni, B., et al. (1994). An unusual member of the nuclear hormone receptor superfamily responsible for X-linked adrenal hypoplasia congenita. *Nature*, *372*, 635–641.
902. Lynch, J. P., Lala, D. S., Peluso, J. J., et al. (1993). Steroidogenic factor 1, an orphan nuclear receptor, regulates the expression of the rat aromatase gene in gonadal tissues. *Mol Endocrinol*, *7*, 776–786.
903. Ito, M., Yu, R. N., & Jameson, J. L. (1998). Steroidogenic factor-1 contains a carboxy-terminal transcriptional activation domain that interacts with steroid receptor coactivator-1. *Mol Endocrinol*, *12*, 290–301.
904. Muscatelli, F., Strom, T. M., Walker, A. P., et al. (1994). Mutations in the DAX-1 gene give rise to both X-linked adrenal hypoplasia congenita and hypogonadotropic hypogonadism. *Nature*, *372*, 672–676.
905. Seminara, S. B., Achermann, J. C., Genel, M., et al. (1999). X-linked adrenal hypoplasia congenita: a mutation in DAX1 expands the phenotypic spectrum in males and females. *J Clin Endocrinol Metab*, *84*, 4501–4509.
906. Ito, M., Yu, R., & Jameson, J. L. (1997). DAX-1 inhibits SF-1-mediated transactivation via a carboxy-terminal domain that is deleted in adrenal hypoplasia congenita. *Mol Cell Biol*, *17*, 1476–1483.
907. Zhang, Y. H., Guo, W., Wagner, R. L., et al. (1998). DAX1 mutations map to putative structural domains in a deduced three-dimensional model. *Am J Hum Genet*, *62*, 855–864.
908. Hamaguchi, K., Arikawa, M., Yasunaga, S., et al. (1998). Novel mutation of the DAX1 gene in a patient with X-linked adrenal hypoplasia congenita and hypogonadotropic hypogonadism. *Am J Med Genet*, *76*, 62-66.
909. Zhang, Y. H., Huang, B. L., Niakan, K. K., et al. (2004). IL-1RAPL1 is associated with mental retardation in patients with complex glycerol kinase deficiency who have deletions extending telomeric of DAX1. *Hum Mutat*, *24*, 273.

QUESTIONS

1. A 15-year-old male presents with hypertension. He has a family history of stroke in father and grandfather. Lab evaluation reveals a potassium level of 3.4mEq/L. plasma renin activity is suppressed and aldosterone level is high. Urine and serum metanephrines and catecholamines were normal. Renal ultrasound with Doppler was normal. Adrenal CT was normal.

Which of the following tests is most likely to make the diagnosis?
   a. Measuring the cortisol to cortisone ratio
   b. Normal saline and dexamethasone suppression of aldosterone
   c. Sequencing of the mineralocorticoid receptor
   d. Adrenal venous sampling
   e. MRI of the adrenal glands

Answer: b

2. A 16-year-old female presents with 1 year of oligomenorrhea and hirsutism. She had menarche at age 12 and had regular periods for 1.5 years followed by a steady decline in frequency of menses. Her last menstrual period was 1 year ago. On examination, her weight and height are at the 50th percentile and have not changed significantly over the past year. Her blood pressure is 156/102. She is Tanner 5 for breast and pubic hair development without clitoromegaly. She has facial acne, back acne and facial hair (sideburns, upper lip and chin). She has no striae or a buffalo hump. The LH to FSH ratio is not increased.

What is the most likely diagnosis?
   a. ACTH dependent Cushing syndrome
   b. Late onset Congenital adrenal hyperplasia
   c. Glucocorticoid resistance
   d. Polycystic Ovarian Syndrome
   e. Glucocorticoid-remediable aldosteronism

Answer: c

3. You are called to see an 18-day-old boy in the cardiac ICU. He was found unresponsive and in ventricular tachycardia. EMTs resuscitated him. His potassium was 9 and his sodium was 114. His newborn screen for congenital adrenal hyperplasia was normal. Serum 17-hydroxyprogesterone is pending. Adrenal ultrasound was performed and is normal. His ACTH on arrival was 10 times the upper limit of normal and his cortisol was 2.34. His triglycerides were elevated.

What is the most likely diagnosis?
   a. 21-hydroxylase deficiency
   b. Pseudohypoaldosteronism type I
   c. Adrenal hypoplasia congenita
   d. Addison's disease
   e. Adrenoleukodystrophy

Answer: c

4. You are called by the newborn nursery to see a baby with ambiguous genitalia. Pregnancy and delivery were unremarkable. Prenatal ultrasound demonstrated hypospadias vs. ambiguous genitalia. Amniocentesis revealed a karyotype of 46XY. Exam is remarkable for presence of testes in a bifid scrotum with incomplete fusion of the labioscrotal folds, distal shaft hypospadias and a stretched phallic length of 3.2cm. Electrolytes were normal. Anti-Mullerian Hormone was >180ng/ml on day of life 2. Abdominal ultrasound revealed no Mullerian structures. Labs obtained on week 3 of life reveal an LH of 4.7, testosterone of 898ng/dL, a dihydrotestosterone of 245ng/dL, 17-hydroxyprogesterone of 241ng/dL, 17-hydroxypregnenolone of 839ng/dL, androstenedione of 78ng/dL.

What is the most likely diagnosis?
   a. 3 beta HSD deficiency
   b. 5 alpha reductase deficiency
   c. SRY deletion
   d. Partial androgen insensitivity
   e. P450 oxidoreductase deficiency

Answer: d

5. A 3-year-old boy is found to have an elevated calcium after she was admitted to the hospital with a bronchiolitis. He is otherwise healthy. His mother and father have no significant past medical history. There is no family history of kidney stones. His lab evaluation reveals an ionized calcium of 6.0 mg/dL (4.5-5.6) and calcium of 12.5mg/dL. Magnesium was 2.6 mg/dL (1.8-2.4) and phosphorus was 3.5 mg/dL (3.5-6). Intact PTH done on the same sample was 28pg/ml(16-87). Fractional excretion of calcium was 0.92%. 25-hyroxyvitamin D and 1,25-hyroxyvitamin D levels are normal. Renal ultrasound was normal.

What is the most appropriate next step?
   a. Technetium sestamibi scan
   b. Measure serum calcium in the parents
   c. Skeletal survey
   d. PTHrp
   e. Parathyroid surgery

Answer: b

6. A 4-year-old boy is referred for short stature. His height SDS is -4. His weight is $<3^{rd}$ percentile. His birth weight was 3.5SD below the mean and his birth length was 5SD below the mean. He was proportional. IGF1, IGFBP3 and acid-labile subunit levels were elevated. Bone age was delayed. Growth hormone stimulation testing with clonidine and arginine peaked at 50ng/ml.

Which of the following will make the diagnosis?
   a. Overnight growth hormone sampling
   b. IGF1 generation test
   c. SNP array
   d. Trial of growth hormone
   e. Sequencing of the IGF-1R gene

Answer: e

7. A 4-year-old Caucasian boy is referred for obesity. His BMISDS is +5. His parents report an insatiable appetite since birth. He is on hydrocortisone for a diagnosis of adrenal insufficiency. On exam he has red hair.

What is the most appropriate test?
   a. Obtain an MRI of the hypothalamus and pituitary
   b. Sequence the MC4R gene
   c. Sequence the POMC gene
   d. Sequence the PC1/3 gene
   e. Indirect calorimetry

Answer: c

CHAPTER 4

Laboratory Methods in Pediatric Endocrinology

Donald Walt Chandler, PhD • Dennis J. Chia, MD • Jon Nakamoto, MD, PhD • Kelly Y. Chun, PhD • Samuel H. Pepkowitz, MD • Robert Rapaport, MD

CHAPTER OUTLINE

INTRODUCTION

Pediatric endocrinology evaluations are often accompanied by requests for laboratory tests. Each clinician must determine the correct lab test to be ordered, establish the circumstances for proper testing (e.g., fasting, time of day, stimulation protocol), and choose a specific laboratory method to be utilized. When the results return, the pediatric endocrinologist must interpret them correctly to arrive at the appropriate diagnosis and to determine management. If treatment is initiated, monitoring methods, which may include specific laboratory tests, must be instituted. The evaluation of laboratory assays is an essential component of pediatric endocrinology practice. One could suggest that of all specialties in medicine, pediatric endocrinology is the one most dependent on laboratory tests. It is thereby apparent that for optimal endocrine diagnosis and management, thorough understanding of laboratory methodology is crucial. Therefore, a section on laboratory techniques is an integral component of any instructional manual of pediatric endocrinology.

Hormones circulate at remarkably low levels, yet relatively small perturbations can distinguish health from disease states. As a frame of reference, the major extracellular cation, sodium, circulates at approximately 140 millimolar (10^{-3} mol/L), whereas normal glucose levels are in the 4 millimolar range. In contrast, relatively abundant hormones such as cortisol have concentrations in the low micromolar range (10^{-6} mol/L), and others such as growth hormone and thyroglobulin are only in the low picomolar range (10^{-12} mol/L) (Figure 4-1). Given this thousand- to billion-fold difference, it is not surprising that assays using chemical analysis are not applicable in the measurement of hormones. To reach an appropriate level of sensitivity and specificity at these concentrations of an analyte, two types of assays are generally employed: immunoassays and tandem mass spectrometry (MS/MS) coupled with chromatography.

Endocrine hormone assays have applied improved technology to evolve from colorimetric tests and radioimmunoassay (RIA) with monoclonal antibodies, sensitive detection techniques, extensive automation, and, increasingly, tandem mass spectrometry. With automation, the sudden availability of platforms with a menu of commonly ordered hormone tests has rapidly broadened access of endocrine testing so that currently more than 3000 laboratories offer a wide range of endocrine tests.[1] In most general laboratory settings, one of five common immunoassay analyzers is used for hormone detection (Table 4-1). These analyzers consolidate more than 20 to 30 hormone or other assays on a single platform that can operate in batch or random access mode with unattended capability, timely turnaround, and cost-effective throughput. Despite these practical advantages, there are some challenges to consider. In many laboratories utilizing such high-throughput instruments, appropriate in-depth knowledge of endocrinology and the technical aspects of the analytic systems is limited, such that troubleshooting is often outsourced to device manufacturers whose primary focus may not be on analytic accuracy. Furthermore, platforms of consolidated analyzers may not be appropriate for specimens from pediatric-age patients for several reasons: available normal

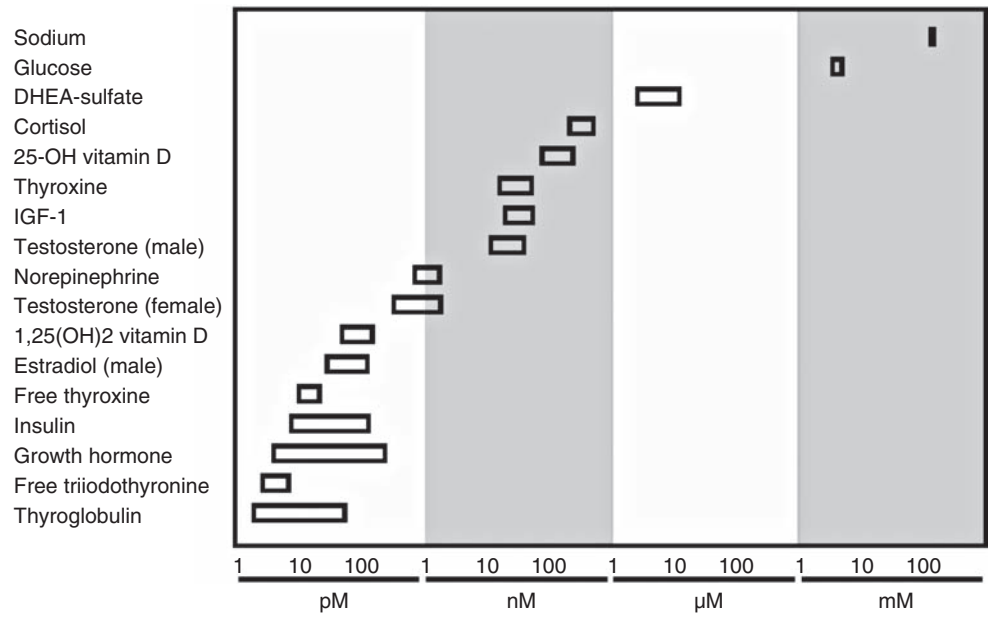


FIGURE 4-1 ■ Relative concentrations of selected hormones. Concentrations are rounded for illustration purposes.

TABLE 4-1 **Prevalent Commercial Immunoassay Analyzers**

Analyzer	Number of Units Worldwide	Methods Supported	Protein Hormones	Steroid Hormones	Thyroid Hormones
Abbott Architect	> 6500	Chemiluminescence	FSH, hCG (total B-hCG), LH, prolactin, SHBG, C-peptide, insulin, intact, TSH, total β-hCG LH, FSH, prolactin, iPTH thyroglobulin, many others	Vitamin D, DHEA-S, cortisol (serum and urine), estradiol, estriol, progesterone, testosterone and others	TT3, TT4, FT3, FT4, T3-uptake, and others
Beckman Access	> 2500	Chemiluminescence			
Roche Elecsys	> 6000	Electrochemiluminescence			
Siemens Dimension	> 1000	Chemiluminescence			
Ortho Vitros	> 3100	Chemiluminescence + enzyme immunoassays, nephelometry			

reference range data may be inadequate; the assay may perform well at a normal adult range but be unsatisfactory in the pediatric range; or the failure to remove cross-reacting contaminants by extraction and chromatography steps may lead to falsely elevated results.[2-4] Similar pitfalls may be encountered as mass spectrometry technology is expanded to more laboratories.

This chapter is intended as a reference work for pediatric endocrinologists in training or in practice to assist in the requisition and interpretation of laboratory tests to facilitate optimal clinical diagnosis and management. It will first cover the two main assay formats used to measure hormone levels, namely (1) antibody-based immunoassays and (2) mass spectrometry. Later sections discuss preanalytic variables and then validation techniques, both analytic and clinical. Dispersed throughout the chapter are short clinical vignettes that illustrate how a strong foundation in laboratory methods can impact clinical care. This chapter is not intended to provide a comprehensive review of all laboratory assays. Principles of molecular endocrinology testing are discussed in Chapter 2.

HORMONAL ASSAYS

Immunoassays

Immunoassay is an important methodology in the endocrine laboratory. Understanding the basic principles involved allows specialists to (1) identify when a particular immunoassay is or is not appropriate for a particular clinical scenario, (2) anticipate potential physiologic and technical issues affecting the interpretation of laboratory results, and (3) understand how to work with the clinical laboratory to investigate unanticipated or clinically discordant test results.

Competitive Immunoassay versus Immunometric (Sandwich) Assay

There are two main immunoassay formats relevant to endocrinology laboratory testing, and understanding some of the basic differences between these formats helps both interpretation of results and troubleshooting of unexpected situations.[5] The first class of assays is termed *competitive*, with the classic radioimmunoassay (RIA) as the archetypal example. A primary antibody against the substance ("analyte") of interest is added to the patient's sample, together with a radiolabeled version of the analyte ("tracer") that competes with the endogenous analyte for binding to the primary antibody (Figure 4-2, upper panel). After a sufficiently long incubation time, the primary antibody is precipitated using a second anti-IgG antibody, polyethylene glycol, or (most commonly nowadays) by using primary antibody attached to a solid support such as a bead, which allows a simple centrifugation step to collect the primary antibody. Any unbound tracer or analyte is washed away, and the amount of tracer in the precipitate is then quantified.

To convert this tracer signal into a concentration, a standard curve is prepared from samples where varying concentrations of a known amount of analyte have been added. For a competitive immunoassay, the amount of signal detected (amount of tracer bound to antibody) decreases as the analyte in the patient's sample increases. Importantly, *anything* that prevents the tracer from binding to the primary antibody would also decrease signal and increase apparent analyte concentration. For example, taking an immunoassay optimized for serum and using it for an extremely concentrated urine sample or in a body fluid where the protein concentration is extremely high can significantly alter the results. Because high salt or high protein concentration can inhibit tracer binding to antibody, tracer signal will be low and the apparent analyte concentration very high, even in the complete absence of any analyte.

The second class of immunoassays is termed *immunometric*, with the most important example being the noncompetitive, so-called sandwich assay. Typically, as shown in Figure 4-2 (lower panel), an antibody attached to a solid support (plate, wall of a tube, or a bead) is used to

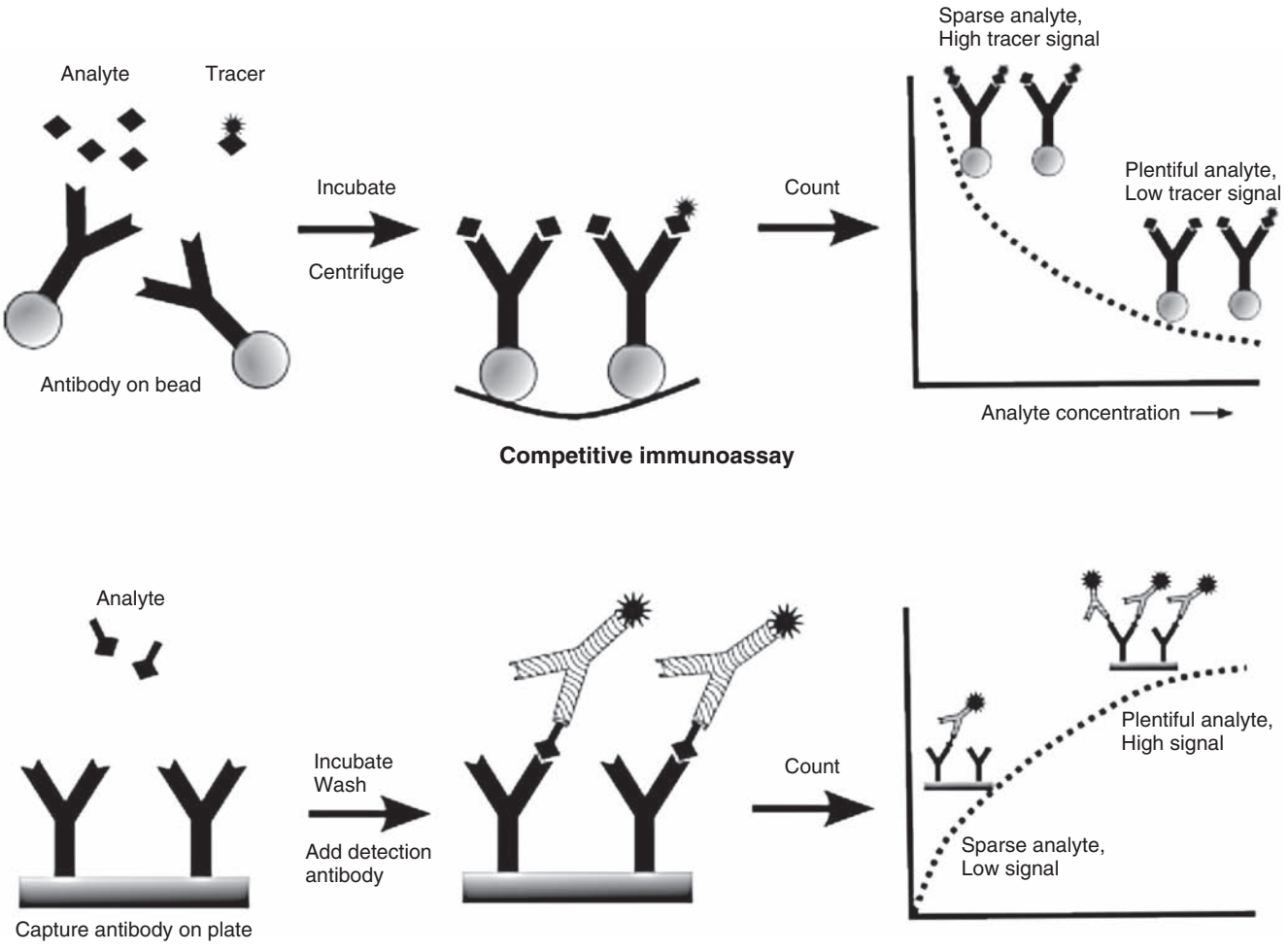


FIGURE 4-2 ■ Comparison of competitive versus immunometric immunoassays. In competitive immunoassay (top), the labeled tracer (signal conjugated analyte) and unlabeled (analyte) compete to bind specific antibody sites. In the two-site method (bottom), analyte of interest is sandwich between the capture and signal antibodies.

capture the analyte of interest, followed by the addition of a second, labeled antibody that binds to a different site on the analyte, creating an antibody-analyte-antibody sandwich. After unbound detection antibody is washed away, that which remains generates a radioactive (immunoradiometric [IRMA]), chemiluminescent (immunochemiluminometric [ICMA]), colorimetric (enzyme-linked immunosorbent assay [ELISA]), or fluorescent (immunofluorescence assay [IFMA]) signal, depending on the label chosen.

The immunometric assays offer certain inherent advantages over competitive immunoassays. First of all, the fact that two antibodies are involved, each binding to a separate epitope on the analyte, greatly increases analytic specificity, including the ability to select very specific isoforms of an analyte.[6] For example, one antibody can be directed at the N-terminus of a peptide, whereas the other antibody can be directed at the C-terminus, ensuring that only full-length peptide is detected. A second advantage is that immunometric assays tend to be more analytically sensitive than their competitive assay counterparts.[7] This arises from the fact that the sensitivity of a competitive immunoassay largely depends on the affinity of the antibody used in the assay—and developing very high affinity antibodies is not an easy or predictable task. In contrast, the sensitivity of an immunometric assay can be improved by use of a higher activity signal, which is more easily detected even when analyte concentrations are very low. As signal detection technology continues to improve, increasingly sensitive immunometric assays can be developed. A third advantage of immunometric assays arises from the ability to use monoclonal antibodies, which can be more easily produced in quantity and have more predictable characteristics than polyclonal antibodies.

Potential Confounders in Immunoassays

Clinical Scenario

A 16-year-old girl with schizophrenia has recently developed galactorrhea and secondary amenorrhea. Her prolactin is elevated at 43 μg/mL, which her attending psychiatrist states is a level commonly seen for young women treated with risperidone. A medical student on the service asks whether the level could also be consistent with a prolactinoma and a "hook effect" accounting for a falsely low level.

Sandwich immunometric assays are potentially vulnerable to what has been termed the "hook" or "prozone" effect that can lead to falsely normal or low values in the presence of large amounts of the analyte[8] (Figure 4-3). Generally, as analyte concentration increases, more antibody-analyte-antibody sandwiches are formed, leading to more signal detected. At extremely high analyte concentrations, however, it is possible for both capture and detection antibodies to be saturated with analyte prior to formation of a sandwich complex, leading to the detection antibody being washed away and the low signal being misinterpreted as a low concentration of analyte. This phenomenon is not seen with competitive immunoassays. To minimize the chances of a hook effect, most immunometric assays have now modified their methods as follows: analyte is allowed to bind to capture antibody, a thorough wash step removes excess unbound analyte, and only then is the detection antibody added. In the now extremely rare situation where a hook effect is still suspected, serial dilutions of the sample may be performed to see if the apparent concentration of the analyte actually rises with increasing sample dilution.

There is some inconsistency in the literature around the terms *heterophilic antibody* and *human antianimal antibody* (HAAA) or *human antimouse antibody* (HAMA).

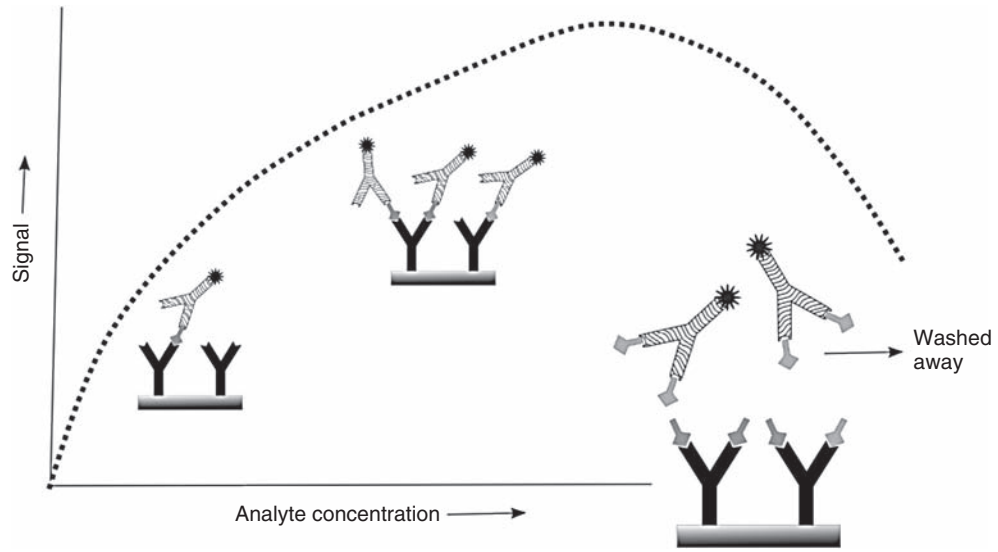


FIGURE 4-3 ■ Hook (prozone) effect leading to falsely low results. Extremely high analyte concentration leads to occupancy of all binding sites without creating the assay "sandwich" configuration. Signal antibody is washed away and the low signal is interpreted as a low concentration of analyte (dotted line). Dilution of sample may lead to increasing signal and an apparent increase in the measured analyte concentration.

Some authors use these terms interchangeably; others reserve the term *heterophilic* for low-affinity antibodies that spontaneously arise against multiple, poorly defined antigens and use HAMA or HAAA only for those patients who develop specific high-affinity antibodies due to treatment with a murine monoclonal antibody or due to recurrent exposure to a particular animal species (e.g., scratches endured by a rabbit handler). Regardless of the term used, any given patient may have antibodies present that bind to and interfere with animal (mouse monoclonal; rabbit, goat, or donkey polyclonal) antibodies used in a particular immunoassay,[9] even without previous exposure to that particular animal. These antibodies can bridge the capture and detection antibodies in an immunometric assay, as shown in Figure 4-4 (left panel), creating a sandwich and a high signal (false elevation) even in the absence of analyte. Alternatively, they could attach at the binding site of the capture antibody and prevent binding of the analyte (Figure 4-4, right panel), eliminating formation of the sandwich, and causing a falsely low value. Competitive immunoassays are less often affected, although they may show falsely high values if the interfering antibody outcompetes the assay antibody for tracer, or falsely low values if they bind preferentially to the analyte over the tracer.

When a heterophilic/HAAA/HAMA effect is suspected, the sample should be assayed on a different immunoassay platform, as the interference is often specific for one manufacturer's assay but not another's. Such a maneuver is often the quickest to accomplish, but the sending lab must ensure that the second lab is truly using a different platform. Another option is to use heterophilic antibody blocking agents, which are a mixture of animal immunoglobulin fragments or other proprietary agents that soak up the interfering antibodies. A disadvantage of this approach for pediatrics is that a significantly larger volume of sample is required to assay both with and without use of the blocking agent. A third, more labor-intensive option is to assay the sample on serial dilutions, as those showing antibody interference effects tend not to show linear responses on dilution.

Clinical Scenario

An adolescent with papillary thyroid cancer is treated by total thyroidectomy and radioactive iodine. Several months postoperatively, a thyroglobulin measured by ICMA (Tg-ICMA) is undetectable and thyroglobulin antibodies (TgAb) are negative, which is taken as proof that cancer has not recurred. However, great consternation occurs when a sample is sent for a different TgAb assay that is positive (the original TgAb assay remains negative), calling into question the undetectable Tg-ICMA. Thyroglobulin measured by a competitive assay (Tg-RIA) is consistently detectable at a low level and does not decrease despite other favorable prognostic indicators (decreasing titer of TgAb, no lymph node involvement) or additional radioactive iodine treatment.

Specific autoantibody interferences appear to be rare compared to the heterophilic/HAAA/HAMA interferences described in the previous section, with the notable exception of TgAb in patients with thyroid cancer. TgAb is present in about 20% to 25% of thyroid cancer patients and leads to false depression of thyroglobulin levels by immunometric assay (e.g., Tg-ICMA). In addition, different thyroglobulin antibody assays may report discordant (negative versus positive) results in some cases. Although a competitive assay such as Tg-RIA is less often affected by TgAb interference than is the Tg-ICMA, if the autoantibody is of sufficiently high affinity to outcompete the assay antibody, a Tg-RIA may show false elevation in the presence of such TgAb.[10] Overall, any Tg immunoassay result, whether performed by Tg-ICMA or Tg-RIA, must be viewed as potentially affected in a patient with TgAb present (and even possibly in patients whose TgAb is reportedly negative). Helpful proxies for Tg include following TgAb titer, which should fall over time in patients without cancer recurrence.[11] Imaging studies may also help assess the probability of recurrence. Although rare, autoantibodies may be of sufficiently high affinity to affect both competitive and immunometric assays alike. Consequently, they would be less likely to show differences when assayed on different platforms and are not affected by heterophilic antibody blocking agents. In these scenarios, diagnosis may require measurement of specific autoantibody

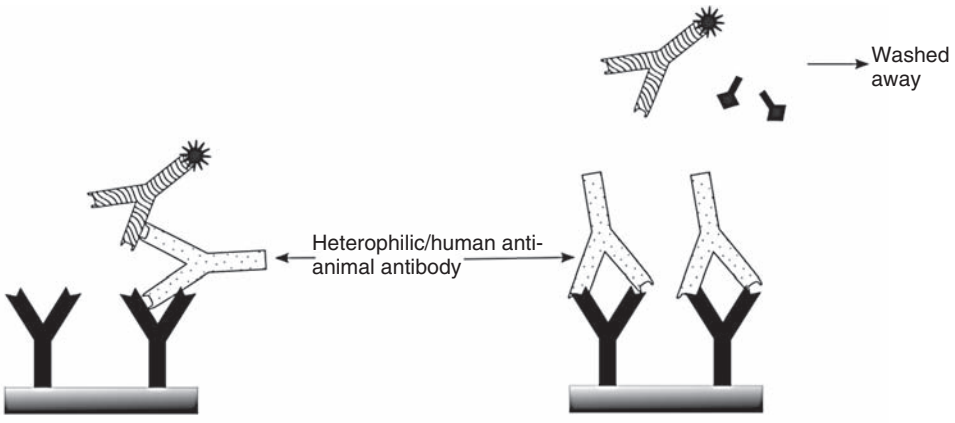


FIGURE 4-4 ■ Mechanism of immunometric assay interference by heterophilic or human antianimal antibodies. See text for details.

(e.g., a parathyroid hormone [PTH] antibody assay in someone with a discordantly high or low PTH level).

Limitations of Immunoassays

The ability of an antibody to bind a specific target with high affinity is remarkable, yet there are limitations. The small size and relatively poor immunogenicity of steroid molecules make it difficult to obtain an antibody that can clearly distinguish a specific steroid from other similar steroids. For example, an antibody raised against testosterone may show significant cross-reactivity with a conjugated form such as testosterone glucuronide, or with the structurally similar dihydrotestosterone molecule. A solvent extraction step to remove water-soluble conjugates such as glucuronides, coupled with a chromatographic step to separate out structurally similar molecules, will greatly improve the analytic specificity of the assay[12] (Figure 4-5A). The extraction step also separates testosterone from binding proteins that can interfere with accurate measurement.

Testosterone immunoassays that do not involve such extraction and chromatography steps, including the majority of assays found on most automated laboratory platforms, may perform adequately at the higher levels found in adult males (> 300 ng/dL) but will frequently measure inappropriately high values in the range most pertinent to women and prepubertal children (Figure 4-5B). Because in practice the lower values are more concordant with the clinical picture, endocrinologists caring for women and children have traditionally relied on immunoassays that involve extraction and chromatography steps, or assays involving chromatography and tandem mass spectrometry.

Free and Bioavailable Hormone Tests

Clinical Scenario

A pediatrician caring for a newborn male infant is informed of an abnormal newborn screening result with a low total T4 and normal TSH. He contacts his colleague in pediatric endocrinology about how to initiate a workup for hypopituitarism that may accompany central hypothyroidism. Instead, the pediatric endocrinologist recommends a repeat set of tests that includes a free T4 measurement.

The free hormone hypothesis states that free, or unbound, steroids or thyronines, vitamins, or peptides are

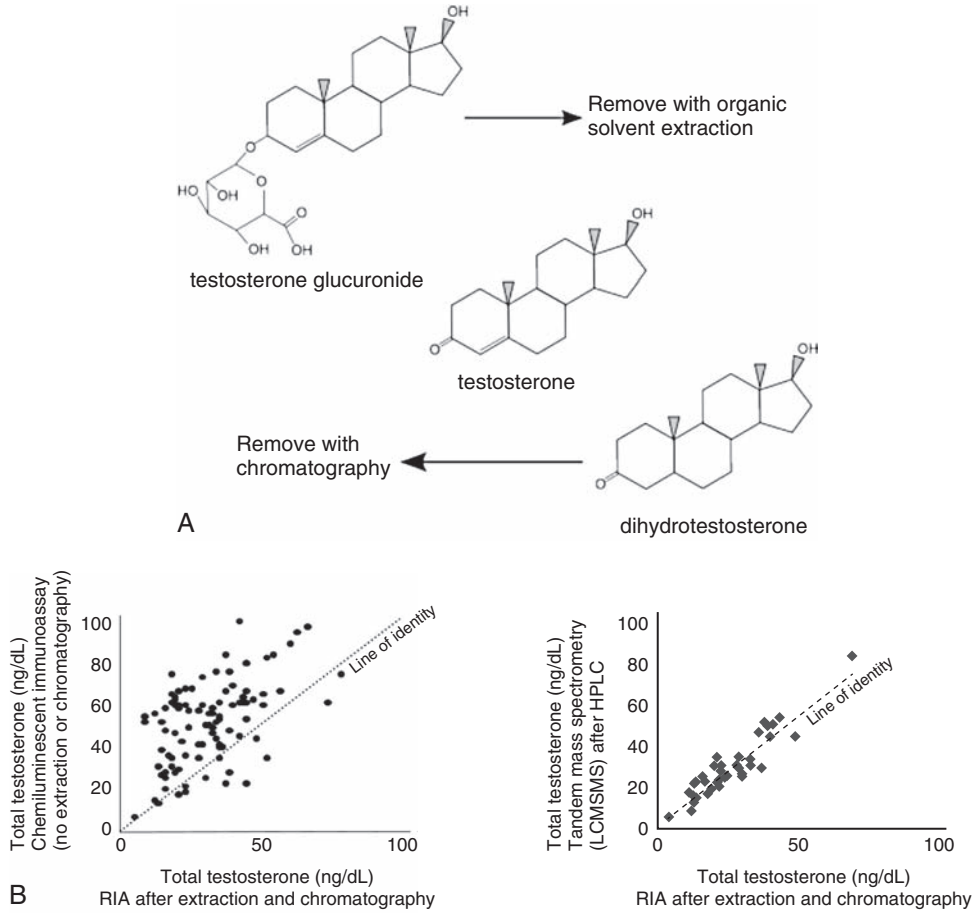
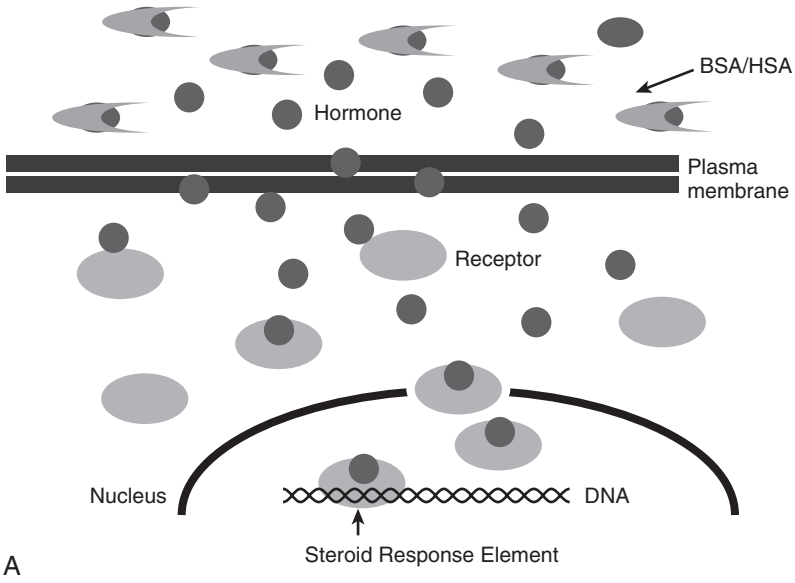


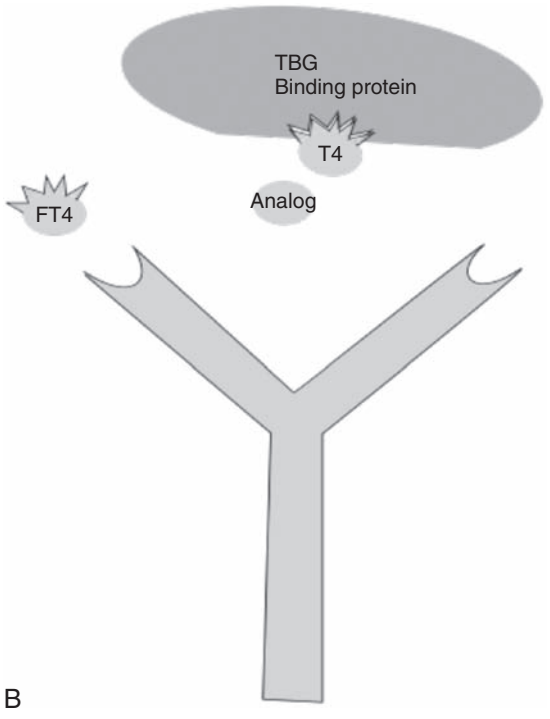
FIGURE 4-5 ■ **A,** Increasing specificity of analysis via extraction and chromatography. After organic solvent extraction, water-soluble conjugates remain in the aqueous phase while steroid molecules generally remain in the organic phase. Similar steroid molecules can be separated by an additional chromatography step. **B,** Correlation of testosterone assays (extraction/chromatography/RIA versus nonextraction immunoassay or tandem mass spectrometry). Left panel: Steroid immunoassays without additional preparation steps prior to assay tend to give higher values than immunoassays after extraction and chromatography, likely because of steroid conjugates and compounds of similar structure (occasional lower values may reflect issues with incompletely removed steroid binding proteins). Right panel: Measurement of steroids by HPLC-mass spectrometry (which also involves extraction and chromatography) produces testosterone results very similar to those obtained with immunoassay after extraction and chromatography.

available to enter cells or interact with cellular proteins by virtue of their small size and sometimes their hydrophobic nature.[13] Free hormone in this section means not liganded to a binding protein. As free and bound hormone pass through a tissue bed, the free hormone may be taken up by the tissues, additional free hormone may become available from the bound fraction, or the complexed hormone may be taken up by cells.[14] The systems may be further complicated by multiple binding proteins. Clearly, the activities of several steroids, thyronines, and even protein hormones are reduced by binding to specific high–affinity binding proteins and albumin. Conversely, the absence of binding proteins can reduce the total hormone level, but the free hormone level may be unaffected. For example, the majority of thyroid hormone in circulation is carried by thyroxine-binding globulin (TBG). TBG deficiency is characterized by low total thyroid hormone levels, normal free thyroid hormone levels, normal TSH (in equilibrium with the free T4), and clinical euthyroidism.

Testosterone binds to sex hormone binding globulin (SHBG) and serum albumin. Testosterone binds SHBG with much higher relative affinity, but SHBG is present in much lower concentrations than albumin. Free testosterone may be measured by equilibrium dialysis, which is accepted as the best indication of the physiologic availability of testosterone (Figure 4-6A). The free fraction may also be estimated mathematically from the testosterone, SHBG, and albumin concentration; however, not all assumptions may be valid in calculated free



A



B

FIGURE 4-6 ■ **A,** Free steroids may enter cells by passive diffusion through plasma membranes. Bound steroids may not diffuse through membranes. Once in the cell, steroids may interact with enzymes, or nuclear receptors. **B,** Free thyroxine (FT4) measurements, routinely made by analog immunoassay largely reflect total thyroxine. (Fritz KS, Wilcox RB, Nelson JC [2007]. A direct free thyroxine [T4] immunoassay with the characteristics of a total T4 immunoassay. Clin Chem 53:911-915.) Dialysis with HPLC and tandem mass spectrometry or dialysis and immunoassay methods provide theoretic accuracy. Dialysis methods should be used where binding proteins vary from the norm. T4 interacts with the antibody used in the FT4 test and competes with the analog; however, the analog does not bind thyroxine-binding globulin (TBG).

testosterone.[15, 16] Analog methods measure free testosterone directly using a competitive test in automated assay instruments (Figure 4-6B), but the analog methods may at best weakly reflect free hormone activity. Similarly, free T4 measured by analog methods can perform poorly in some settings.

Bioanalytic testosterone refers to the fractions of testosterone bound to serum albumin plus the free hormone; for this reason this fraction is also called free and weakly bound testosterone. The dissociation rate of albumin bound is very fast relative to that of the SHBG bound, and for that reason it is thought to be available to the tissues for actions. This idea, supported by empirical evidence, is in contrast to the free hormone hypothesis, as free and protein-associated hormone may be available to the tissues.[14] In practicality, measurement of free or bioavailable hormones provides the same information to the clinical encounter.

Mass Spectrometry

Mass spectrometry is a qualitative and quantitative analytic technique that separates molecular ions based on their mass and charge, or more exactly according to the mass/charge ratio (Figure 4-7). In clinical laboratories, mass spectrometry is invariably preceded by chromatographic assays that separate hormones. Separation techniques include high-pressure liquid chromatography (HPLC), column chromatography, gas chromatography, thin-layer chromatography, electrophoresis, and other variations limited only by the imaginations of the inventors. In all chromatography assays, the hormone is attracted to both a mobile phase and a stationary phase, and separation occurs by variations in attraction of hormone to the two phases. Table 4-2 highlights some of the chromatographic techniques used in endocrinology.

Clinical Scenario

A pediatrician is notified that a male infant she discharged on day-of-life 2 had a filter paper newborn screen performed by mass spectrometry, and the result of this test was highly suspicious for congenital adrenal hyperplasia. She reaches the family by phone, and the baby is evaluated in the office on day-of-life 7. Laboratory testing reveals sodium 134, potassium 6.1, and markedly elevated 17-OH progesterone concentration, confirming the diagnosis. The baby is admitted to the hospital, and the pediatric endocrinology team is consulted.

Mass spectrometry is increasingly employed in endocrine laboratories. In the late 1990s, incorporation of mass spectrometry technology led to a significant expansion of newborn screening programs, particularly for inborn errors of metabolism and extending to congenital adrenal hyperplasia, as illustrated in the preceding case scenario. Detection systems have become increasingly sensitive and sophisticated. New-generation liquid chromatography (LC)-MS/MS steroid assays have shown

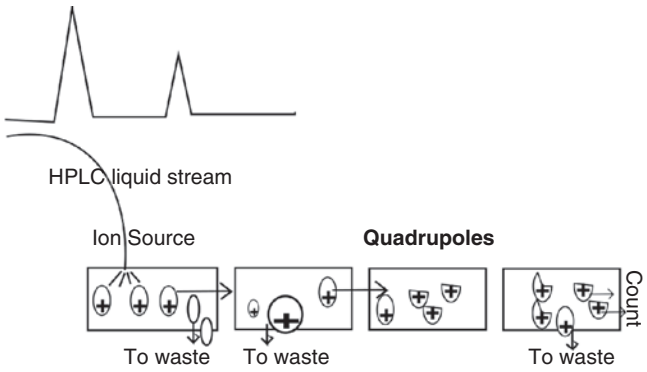


FIGURE 4-7 ■ (left to right) Box 1. The liquid stream from HPLC is vaporized and ions are formed. Un-ionized molecules are swept to waste. Box 2. Ions are selected by mass/charge whereas other mass/charge molecules are swept to waste. Box 3. Ions are fragmented. Box 4. Fragments of the selected mass/charge are counted, others are swept to waste.

TABLE 4-2 **Common Chromatographic Assay Types**

Acronym	Description	Typical Mobile Phase	Typical Stationary Phase
GC	Gas chromatography	Helium, nitrogen	Polymer coated inside a capillary column
HPLC	High pressure liquid chromatography	Water, acetonitrile, methanol	Polar or nonpolar additions to very tiny bead particles
Column chromatography	Various sorbents in a traditional column	Polar or nonpolar ion exchange or affinity	Buffers and solvents Salts or ligands that affect affinity
TLC	Thin layer chromatography	Methanol, acetone	Particles of silica coated on a glass plate
CE	Capillary electrophoresis	Electrical charge moves ions	Resin with fixed charge coated in a capillary

improved accuracy, precision, sensitivity, selectivity, and clinical utility over simple direct and complex reference immunoassays.[17, 18] Assay technology that employs mass spectrometry, especially for steroids and biogenic amines, is regarded as more accurate than immunoassay, such that it is now the standard assay for these molecules in reference laboratories. The performance characteristics of immunoassay and mass spectrometry are summarized in Table 4-3.

It is important to note that, as with immunoassay, assay design is crucial for accuracy and specificity with mass spectrometry. Detection systems such as flame ionization detection for gas chromatography (GC), visible or ultraviolet detection coupled with HPLC, and staining of thin layer chromatography (TLC) have a place in research but are generally not sufficiently specific, sensitive, and robust for clinical hormone measurement. Gas chromatography with mass spectrometry can have similarly useful specificity and sensitivity characteristics; however, generation of derivatives is required to help the molecules evaporate into the gas phase, adding complexity.

The mass/charge ratio is the fundamental feature that allows discrimination of compounds by mass spectrometry. Steroid hormones, normally neutral, for example, are charged in an interface and then accelerated as gas-phase ions into a mass spectrometer operated under vacuum. At the same time the solvent is dried and swept away along with other uncharged molecules. In tandem mass spectrometry (MS/MS), molecules (gas-phase ions) of interest are selected and fragmented, then products specific to the analyte are again selected for detection. Thus, the tandem mass spectrometer adds levels of selection with each process. Experimental sequences can be derived that allow simultaneous measurement of similar analytes from a single sample. The whole process is controlled by addition of fixed amounts of an internal standard. Optimally, in isotope dilution mass spectrometry, the standard is the same molecule as the target analyte except that heavy stable isotopes, such as deuterium or carbon-13, are inserted.[19] Because the mass is different, the internal standard is read separately, and the analysis is based on the ratio of analyte to internal standard. The specificity of HPLC and isotope dilution mass spectrometry theoretically offers a means for sensitive, robust assays.

The specificity of mass spectrometry methodology is not derived from an antibody-antigen interaction, thus it does not depend on potentially limited antibody reagent. Still, just as there are potential pitfalls in immunoassay measurements, there can be barriers to accurate HPLC mass spectrometry measurements (Table 4-4). Extraction and good HPLC chromatography solve many of the issues for steroid tests, reducing interference and suppression of ionization.[20] Review of molecular fragments can often reveal interference; these are called qualifier ions.

Protein mass spectrometry has been slower to displace immunoassay techniques because the immunoassays remain effective tools and mass spectrometry may be challenging. Large proteins are difficult to measure by HPLC tandem mass spectrometry because of instrument limitations and because variations in posttranslational

TABLE 4-3 **Performance Characteristics of Endocrine Methods**

Method	Analytic Sensitivity	Analytic Precision	Analytic Specificity	Interference	Remarks
Competitive Immunoassay —RIA —EIA —FIA	Poor to excellent µg to pg	Poor to good 10% to 30% CV	Poor to good Polyclonal Ab	Nonspecific—pH, ionic strength, degradative enzymes, matrix Specific—cross reactants	Poor specificity maybe clinically advantageous in some cases (e.g., RIA method may capture all biologically active forms of growth hormone), whereas highly specific method may only measure selected isoform of growth hormone and can exclude polymorphism with LCMS method.
Two-site "sandwich" immunoassay —IRMA —ELISA —ICMA	Good to excellent ng to pg	Good to excellent < 5% to 20%	Good to excellent Monoclonal Ab Affinity purified poly Ab	Same as above in addition to: —Autoantibodies —HAMA —High dose hook effect	Automated methods exhibit excellent precision but may suffer accuracy and sensitivity.
LCMSMS	Good to excellent ng to pg	Good to excellent < 10% to 20%	Excellent	Matrix Isomers	Performance characteristic highly dependent on performing laboratory, as most methods are laboratory developed and manual.

RIA, radio immunoassay (RIA); EIA, enzyme immunoassay; FIA, fluorescence immnoassay; IRMA, immunoradiometric; ELISA, enzyme-linked immunosorbent assay; ICMA, immunochemiluminometric; LCMSMS, high pressure liquid chromatography with tandem mass spectrometry; HAMA, human antimouse antibody; LCMS, liquid chromatography with mass specrometry.

TABLE 4-4 **Common Problems and Solutions for HPLC Mass Spectrometric Assays**

Potential Problem	Effect	Detection and Solution
Interference	High results if analyte is affected Low results if internal standard is affected Isobaric compounds Ions that have the same mass to charge, as the analyte can cause high results	Peak review and review of alternant qualifier fragment
Poor standardization	Inaccuracy	Various governmental and industry agencies have standardization programs
Matrix effects	Interference with ionization	Identification of and HPLC separation of interferences Heavy isotope internal standards
Use of nonspecific derivative to increase sensitivity	Interference if the derivative becomes the measured ion	Avoid measurement of derivative fragment ions
Noise	Background ions from nonspecific interferents such as serum separator can reduce precision	Avoid or improve extraction and chromatography

HPLC, high-pressure liquid chromatography.

modification and natural abundance of heavy stable isotopes creates groups of mass species to measure that are difficult to discriminate. Several types of mass spectrometers are available and may be used clinically in the future. One type is matrix-assisted laser desorption/ionization coupled with time of flight mass (MALDI-TOF) spectrometry, which has been used largely as a discovery tool and has not found general utility in quantitative experiments. Protein capture with antibodies followed by digestion into peptides has been used to develop commercial tests. Thyroglobulin, for example, can be measured in this way to avoid autoantibody interference; however, currently the sensitivity does not quite match immunoassay. These techniques are new and less is known about their reliability.[21]

INTERPRETATION OF TEST RESULTS

Laboratory studies should provide reliable information to aid in the diagnosis and management of patients. It is crucial that potential confounders that can lead to misinterpretation be promptly and correctly recognized. Method validation is the responsibility of the laboratory and includes components mandated by regulatory agencies such as the Clinical Laboratory Improvement Amendments (CLIA), Food and Drug Administration (FDA), and Clinical Laboratory Standards Institute (CLSI) in the United States. Many laboratories have internal programs that assure that their assays are performing within stringently defined limits.[22, 23] Meanwhile, preanalytic variables, defined as factors related to the subject and specimen collection and delivery but independent of the assay itself, can also lead to erroneous interpretation.[23, 24] Finally, one must always recognize that assay validation and clinical validation of assay utility are not interchangeable concepts. Clinical validation lies at the interface of the laboratory and the clinician. Frequent communication between clinical and laboratory personnel facilitates optimal care.

Preanalytic Variables

Normal endocrine physiology, nonendocrine illness,[25] sample collection and handling, and drugs or interference factors influence measured hormone levels (Table 4-5).

TABLE 4-5 **Preanalytic Physiologic Variables Affecting Hormonal Measurements**

Variable	Hormone
Episodic secretion	Pituitary hormones, cortisol, testosterone
Exercise (acute)	Adrenocorticotropic hormone (ACTH), antidiuretic hormone (ADH), aldosterone, cortisol, epinephrine, glucagon, growth hormone (GH), prolactin, norepinephrine, testosterone
Circadian rhythm, diurnal variation	ACTH, cortisol, dehydroepiandrosterone sulfate (DHEAS), epinephrine, estradiol, follicle-stimulating hormone (FSH), GH, luteinizing hormone (LH), norepinephrine, prolactin, testosterone
Seasonal variation	Estradiol, prolactin, testosterone
Postural change	Aldosterone, epinephrine, norepinephrine, renin
Nutrition	C peptide, estradiol, glucagon, insulin-like growth factor-I (IGF-I), insulin-like growth factor binding protein-1 (IGFBP-1), insulin, proinsulin, thyroxine-binding globulin (TBG), renin, aldosterone

Many hormones are secreted in an episodic manner. Chronologic age, pubertal stage, emotional and physical stress, nutritional status, and postural effects contribute to variation. Consequently, some hormones, growth factors, or surrogate markers may have very wide normal basal ranges and large intrasubject variability. Intrasubject variability can be demonstrated with diurnal variation, for example, in levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol, and testosterone.[26] Variability has also been verified for 24-hour integrated concentrations[26, 27] and for dynamic responses.[28]

Valid test results are dependent on proper patient preparation, specimen handling and transport, and timely accessioning into the laboratory system.[29, 30] Quality management stipulates that the laboratory provide a collection manual, and current electronic online availability allows almost any location ready availability to this information at any time. When stimulation and timed testing are desired, access to the variables of specimen collection is essential: the details of patient preparation, attention to the time and site of specimen collection, specifics of testing protocols, and influences of concomitant medications should all be clearly stated in collection manuals. Container specifications and acceptable preservatives must be listed for each analyte, and minimal and ideal volumes to allow retesting or add-on of additional tests should be stipulated. Immediate postcollection handling, such as the time until serum separation from a clot and intervals until refrigeration or freezing must occur, should be specifically mentioned and easily located in the instructions. Clinical data required for proper interpretation may be included in a test description. Should any questions arise, the clinician should consult with the specific laboratory to understand collection and handling requirements for a particular assay.

Analytic Validation, Quality Assurance, and Quality Control

Clinical Scenario 1

An investigator inadvertently runs two tubes containing nothing but water on a peptide RIA and is nonplussed when the results show a significant level of the peptide in these tubes.

Clinical Scenario 2

A laboratory declines to run a tumor marker immunoassay ordered on viscous cyst fluid due to a lack of analytic validation data, but relents when the physician insists that the laboratory run the sample with a disclaimer "nonvalidated sample type; interpret with caution." Despite the disclaimer, the laboratory is later sued successfully for inappropriate diagnosis and unnecessary treatments based on what turns out to be a falsely positive result.

Both of these brief clinical vignettes illustrate why regulatory agencies and anyone concerned with quality laboratory testing place such emphasis on analytic method validation. A peptide RIA may give accurate results in serum, but totally inaccurate results in a protein-free fluid; perhaps the tracer in scenario 1 stuck to the sides of the plastic tube, leading to decreased tracer binding and an apparent detectable level of peptide where none was actually present. Scenario 2 represents a situation of misdiagnosis; the viscosity of the solution might have affected the interaction of the assay components, with substantial impact on the patient and legal consequences for all involved.

Analytic validation is meant to ensure that an assay method is accurate for its intended use.[31] Components of an analytic validation include the following: (1) linearity/reportable range; (2) precision; (3) analytic sensitivity; (4) analytic specificity, interferences, and recovery; (5) accuracy/method comparison; (6) sample types and matrix effects; (7) stability; and (8) carryover. Determining reference intervals is an important part of many analytic validations and crosses over to clinical validation. Note that even though not all of these components are always required from a regulatory point of view, all represent good-quality laboratory practice.

Linearity/Reportable Range

Also referred to as the "analytic measurement range" (AMR), this is the range of concentrations over which the assay is known to be reliable. Standards of known concentration (calibrators) are assayed and plotted against the signal generated in the assay. For the hypothetical study shown in Figure 4-8, the upper limit of the AMR would likely be at the concentration represented by calibrator D, because the higher concentration represented by calibrator E does not result in a similar degree of increased signal. However, it may be possible to dilute the sample so that one can make a measurement within the AMR, thereby allowing for assay of concentrations above the upper limit of the

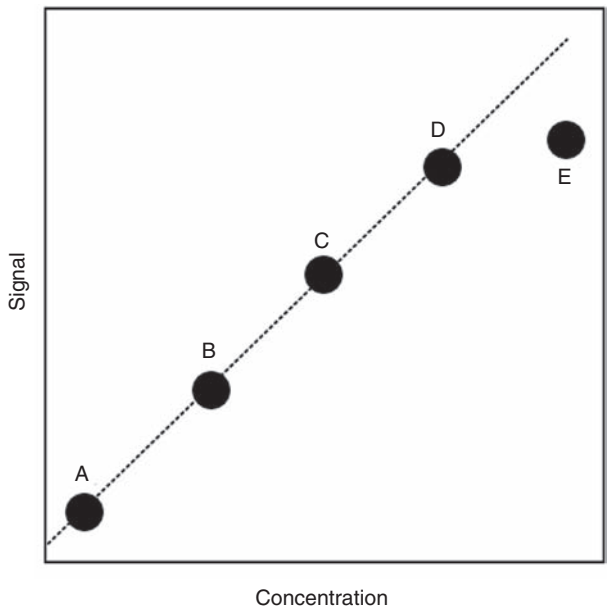


FIGURE 4-8 ■ Linearity study used to determine analytic measurement range. The concentrations represented by points A and D represent the likely minimum and maximum concentrations of the analytic measurement range. Higher concentrations such as that represented by point E may still be measured on a diluted sample if it can be proven that the response remains linear when a dilution is performed.

AMR. The calibrator choice may alter the absolute value reported, particularly with peptides and proteins where the standard may represent only one of a mixture of differentially modified (e.g., glycosylated or cleaved) forms present in the circulation.

Precision

Also known as reproducibility or replicability, this defines whether the random error of the assay is small enough to make the assay clinically useful. A commonly used analogy is that of the target shooter: how close together are the bullet holes? Note that precision is distinct from accuracy (a different part of the validation study); precision addresses only if the shots are close together, not whether they are actually hitting the bulls-eye. Both intra-assay (e.g., 20 measurements done on the same assay run) and inter-assay (1 measurement done daily for 20 days) precision are studied and the standard deviations (SDs) of the replicate measurements calculated. Generally precision is presented as the *coefficient of variation* (CV), which is the SD divided by the mean, expressed as a percentage. For example, at a mean value of 100 ng/mL, an assay with an SD of 5 ng/mL would have a CV of 5%. Note that the concentration of the analyte factors into the assay's precision, as CV values tend to be higher at either limit of the AMR (Figure 4-9).

Clinical Scenario

A 14-year-old boy is referred to the pediatric endocrinology clinic for evaluation of delayed puberty. A serum testosterone level drawn 3 weeks prior to the visit was reported as 36 ng/dL by the hospital lab, which had been taken as evidence that the boy had indeed initiated puberty. Repeat testing in the clinic returns a value of <30 ng/dL. The pediatric endocrinologist becomes concerned that the patient is demonstrating regression in his testosterone production and contacts the laboratory director, who explains the functional sensitivity for the testosterone assay on the platform is indeed 30 ng/dL.

Analytic Sensitivity

This part of a validation study determines how low an analyte concentration can be measured with acceptable precision. It is distinct from "diagnostic sensitivity" (how often a result is positive in a patient with disease), which is a general part of a clinical validation study done well after the analytic validation has been completed. A frequent problem is the use of many different terms for analytic sensitivity, for example *minimal detectable concentration*, *limit of detection (LOD)*, or *limit of absence*, all of which describe the lowest possible concentration that can be confidently distinguished from zero. Although an assay developer might cite the LOD to make the assay look as sensitive as possible, the clinician should realize that values down near the LOD are hugely variable and not really quantitative. A more conservative and clinically useful analytic sensitivity limit is the limit of quantitation (LOQ), also known as functional sensitivity and typically defined as the lowest concentration that can be measured with a CV of less than 20%. In the previous scenario, a CV of 20% at 30 ng/dL equates to a SD of 6 ng/dL. Therefore, it is inappropriate to conclude the change in testosterone from 36 ng/dL to < 30 ng/dL is absolutely indicative of diminished testosterone production. Here, the clinician should specify that the testosterone assay include extraction and chromatography, rather than simply a direct measurement on an automated laboratory platform, which should improve the assay performance at lower testosterone levels.

Analytic Specificity, Interferences, and Recovery

Specificity in this context refers to the ability of the assay to measure a specific analyte without cross-reacting with other substances in the sample. Analytic specificity studies may involve the addition of known amounts of similar analytes to a sample; for example, a cortisol assay may be tested

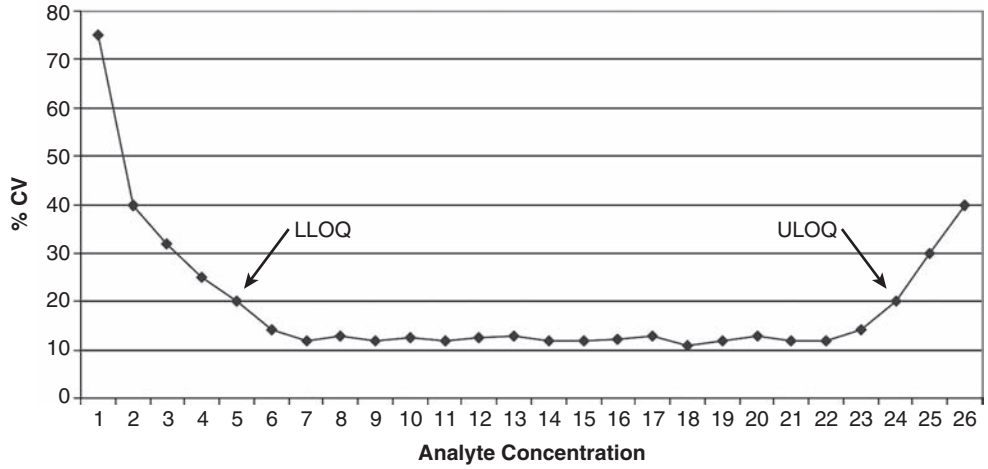


FIGURE 4-9 ■ Estimating the Precision profile over a range of analyte concentration. Lower limit of quantitation (LLOQ) is defined as the lowest concentration at which interassay precision is 20%. Upper limit of quantitation (ULOQ) is the highest measuring concentration at which interassay precision is 20%.

for cross-reactivity with cortisone, prednisone, prednisolone, dexamethasone, 17-hydroxyprogesterone, and other steroids. Closely related are interference studies to see if commonly encountered situations such as hemolysis, hyperbilirubinemia, and lipemia affect the test results. Recovery studies are less often performed; here, a standard of known concentration is added into a sample, and the sample assayed to see what percentage of the added standard is detected (ideally 100%, but frequently less).

Accuracy/Method Comparison

Determining the accuracy of an assay is a multistep process, not all of which can be addressed in a typical analytic validation study. Accuracy may be part of the original test development decision-making process—for example, including an extraction and chromatography step to avoid otherwise problematic cross-reactions. Full determination of the clinical accuracy of a test may not be possible until the analytic validation is completed and the test released to investigators for clinical validation studies. Therefore, so-called accuracy studies in an analytic validation are by necessity limited to just a small portion of the whole accuracy process. The interference and recovery studies mentioned earlier are pertinent to test accuracy, but the most common approach is to compare the new test method to another comparator method. Ideally, the method used for comparison will be some type of gold standard reference method, but often such a method is not available. As a proxy, the method under validation is compared to a well-accepted method, and the results shown (new method result on the y-axis, comparison method result on the x-axis) on a correlation plot (Figure 4-10, left panel). Also frequently used is a difference plot (Figure 4-10, right panel), which better shows intermethod differences that may escape notice on a simple correlation plot.

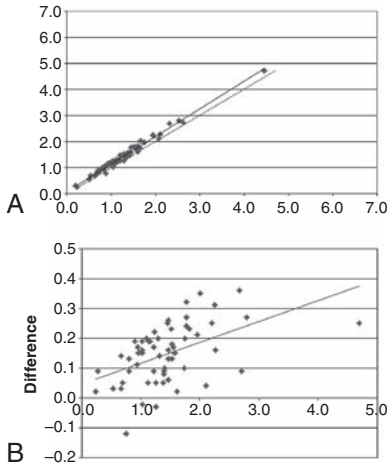


FIGURE 4-10 ■ A correlation plot compared with a difference plot. For the correlation plot (**A**), values for free T4 from a new assay (y-axis) are compared with values from an established free T4 assay (x-axis) showing a best fit (solid line) deviating slightly from the line of identity (dotted line). For the difference plot (**B**), the difference between the values from the new versus the old assay is plotted on the y-axis, whereas the mean value of the results from both assays is plotted on the x-axis. The systematic upward bias of the new assay is seen more clearly on the difference plot.

Sample Types and Matrix Effects

Sample types are not automatically interchangeable. EDTA plasma from a lavender top tube may perform fine in an assay, whereas heparin plasma from a green top tube may not. One parathyroid hormone assay may give comparable results from both EDTA plasma and red top tube serum, whereas a different parathyroid hormone assay may not. As noted in our vignettes, the matrix (e.g., protein-free, viscous, high salt, containing high levels of paraproteins) may have a profound effect on the accuracy of a laboratory assay. Whatever sample types/matrices will be used should be studied to ensure the accuracy of results.

Stability

Typically, sample stability is studied at ambient (about 22° to 26° C), refrigerated (about 2° to 6° C), and regular frozen (around −18° to −20° C) temperatures. For assays used in clinical studies where specimens may be banked for prolonged periods, stability studies should also be performed at deeper frozen (e.g., −70° C) temperatures. Aliquots stored at these temperatures for varying periods of time are recovered and assayed to see if results are stable relative to baseline. Stability limits are an inherent property of the assay rather than the analyte: an osteocalcin assay designed to pick up only full-length molecules may have a short stability limit for samples at room temperature, whereas another assay that detects osteocalcin molecule fragments as well as full-length protein may have a much longer stability limit. Stability limits for common hormone assays are listed in Table 4-6, but consultation with the specific laboratory is recommended. It is also worthwhile to understand the routine laboratory protocol for freezing aliquots for add-on studies.

Carryover

Another important quality process is to ensure that there is no cross-contamination between samples, and that a sample with very high values will not "carry over" and falsely elevate results in the next sample to be assayed. Assays should be designed to minimize this problem, but there should also be awareness on the part of the laboratory staff to check for carryover whenever a sample with an extremely elevated value is encountered.

CLINICAL VALIDATION

Analytic validation assures that the laboratory assay accurately reflects the true quantitative value of an analyte. The issue of whether the laboratory value reflects a pathophysiologic process and can be used in diagnosis and management of an endocrine disease requires clinical validation. To provide context to laboratory values, reference intervals are an absolute requirement in clinical

TABLE 4-6 **Hormone Assays: Preanalytic and Other Considerations**

Hormone	Patient Preparation, Sample Collection, and Hormone Stability	Assay Notes
Adrenocorticotropic hormone (ACTH)	EDTA 6-hour RT, 4° C Ship frozen Avoid FT	1-24 ACTH, used for stimulation studies, and pro-opiomelanocortin (POMC) fragments can lower ACTH levels as measured by two-site methods.
Antidiuretic hormone (ADH)	EDTA Avoid FT Ship frozen	Solid-phase purification required as extraction before assay.
Aldosterone	Patient: posture and sodium Stability: 6 hours at RT, 2 days at 4° C	Spironolactone and tetrahydroaldosterone 3-glucuronide may cross-react in some assays.
Calcitonin	1 day at RT 1 day at 4° C	Large calcitonin and others cross-react in some radioimmunoassay (RIA) methods. High dose suppression of values possible with two-site methods. Heterophile antibodies and human antianimal antibodies may give falsely elevated results in two-site methods.
Cortisol saliva	Sampling time important Stable refrigerated or RT 6 days Avoid tooth brushing and eating	Any blood in the sample invalidates the salivary cortisol result due to much higher cortisol levels in blood.
Cortisol, urine-free cortisol	With boric acid 5 days at RT and 14 days at 4° C	Prednisolone and 6-beta-hydroxycortisol may cross-react.
C peptide	EDTA: 1 day at RT RT: 1 day at 4° C Ship frozen	C peptide immunoreactivity may be unstable. Some immunoassays may be affected by anti-insulin antibodies.
DHEA, serum	4 hours RT 12 days 4° C	Delta 5 steroids unstable at RT.
Dopamine, epinephrine, norepinephrine—plasma	Patient preparation: posture and activity Stability: 0 days at 4° C Must freeze sample ASAP	Very unstable analytes present in low concentrations.
Dopamine, epinephrine, norepinephrine—urine	Stable if acidified below pH3 Stability: 14 days at 4° C Must freeze sample ASAP	Unstable at neutral or basic pH.
Estradiol	2 days at RT 6 days at 4° C	RU-486, Efavirenz, and steroid-binding proteins may interfere with some assays.
Free IGF-I	Avoid FT Ship frozen	Direct methods controversial.
Growth hormone (GH)	2 days at RT 2 days at 4° C	20-kD GH react in certain RIA methods. Human placental lactogen (hPL) may augment or suppress hGH levels. 44-191 GH in RIA methods. High hGH concentrations may require sample dilution if accurate results are needed. After treatment, anti-GH antibodies may suppress GH levels in sandwich assays.
Glucagon	EDTA Avoid FT Ship frozen	Unstable; keep frozen.
Insulin-like growth factor (IGF)-I	2 days at RT 2 days at 4° C	IGF binding proteins react in methods that do not exclude or block. Variable reactivity with therapeutic IGF-I and IGF-I/IGF-BP3 complex with some assays.
Insulin	6 hours at RT 1 day at 4° C Ship frozen	Variable reactivity with various recombinant insulins. On treatment, hook effect possible for two-site assays. Human anti-insulin antibodies may interfere (use free and total measurements to avoid this issue). Assays typically most reactive with human insulin, less reactive with porcine insulin, and least reactive with bovine insulin.

Continued

TABLE 4-6 **Hormone Assays: Preanalytic and Other Considerations—cont'd**

Hormone	Patient Preparation, Sample Collection, and Hormone Stability	Assay Notes
IPTH, intact parathyroid hormone	EDTA 4 hours at RT 1 day at 4° C	Assay terminology: second-generation N-terminal anti-1-24, third-generation N-terminal anti-1-6 Second- and third-generation typically use C-terminal anti-39-84. Midrange parathyroid hormone RIAs (MPTH) recognize parathyroid hormone (PTH) molecules containing amino acid sequences 44-68. C-terminal (CPTH) assays recognize molecules containing amino acids 39-84. Circulating PTH fragments are not biologically active unless they have key N-terminal residues (1-24) and can lower or raise results from two-site methods. Third-generation assays do not detect PTH 7-84 that is not biologically reactive. Fragments are especially high with renal failure. Human anti-PTH antibodies may interfere with assays.
LH and FSH	2 days at RT 2 days at 4° C	HCG for stimulation or due to tumor may augment RIA values and suppress two-site methods.
Osteocalcin	EDTA Avoid FT	Some RIAs more sensitive to degradation.
Proinsulin	1 day at RT 1 day at 4° C	High insulin levels associated with treatment may suppress proinsulin levels in two-site assays. Human antibodies specific to insulin or proinsulin.
Prolactin	2 days at RT 2 days at 4° C	Macroprolactin or big prolactin a complex of prolactin and IgG lacks biologic activity because not freely available to tissues. Two-site assays may demonstrate suppression (high-dose hook) at extremely high prolactin levels.
PTH-rP, parathyroid-hormone-related protein	Plasma with protease inhibitor Avoid FT Ship frozen	Unstable; collect with protease inhibitor and keep frozen. PTHrp N terminal contains key PTH sequences and is biologically active. Also known as the humoral hypercalcemia of malignancy factor.
Renin	EDTA Process at RT and snap freeze Ship frozen Do not leave cold	Process ambient and freeze rapidly to avoid plasma renin activity (PRA) increases due to cold-activated proteases. Activity assays known as PRA. Also direct renin assays.
Testosterone	2 days at RT 6 days at 4° C	Use assays certified by Centers for Disease Control and Prevention for accuracy
Thyroglobulin	2 days at RT 2 days at 4° C	Circulating anti-TG antibodies significantly suppress two-site methods. RIA methods are less likely to be impacted.
TSH, thyroid-stimulating hormone	2 days at RT 2 days at 4° C	bHCG or alpha subunit with some methods causing falsely lower results. Heterophile antibodies have been reported as causing falsely high values. Controversy about TSH reference ranges.
Vitamin D 25 OH	Ship frozen	Some assays may or may not efficiently measure D2 form and C-3 epimer.

Notes: Stability: RT = room temperature. FT = freeze/thaw cycle. Stability studies using normal sera have shown that all hormone levels except ACTH, ADH, DHEA, dopamine, epinephrine, glucagon, 17-hydroxypregnenolone, insulin, norepinephrine, osteocalcin, IPTH, pregnenolone, and renin are relatively stable when kept at ambient temperature (20° to 25° C) for 1 day. Stability may be worse for patient samples and for samples kept at elevated temperatures, and may vary with the method. For most reliable hormone data, patient samples generally should be separated as soon as possible and kept frozen until measurement. Consult your laboratory for proper handling. Circulating antihormone antibodies will usually suppress two-site assay data. Hormone assay precision, the 95% confidence interval for a hormone level, ranges from ±10% to 20% to ±15% to 30%, depending on the absolute hormone concentration and the method used.

practice. Because most tests are not standardized, values vary by method and by laboratory. Reference intervals are key to applying test results from different sources. Laboratories use one of several methods to develop or validate a set of reference data for each analyte.

Clinical Scenario

A 13-year-9-month-old boy presents for evaluation of short stature and delayed puberty. He reports that he has always been among the shortest kids in his class, but the difference has become magnified in the past 1 to 2 years. His parents are of average height, and his father states that he grew well past his high school graduation. On exam, the patient appears younger than his stated age and his height is at the second percentile for his age. He shows no evidence of puberty. His bone age is read as closest to the 11-year-6-month-standard. The pediatric endocrinology fellow notes that his insulin-like growth factor (IGF)-1 level returns at a Z score of −2.3 for his age. On the other hand, his IGF-1 level is at a Z score of only −0.6 for his bone age and −0.5 for his height age. She asks her attending which reference range is most appropriate to interpret the IGF-1 level and whether this influences the suspicion for growth hormone deficiency.

Healthy individuals are tested and the results are evaluated using statistics. Published reference intervals also can reflect the population norm but not reflect physiologic health; for instance, obesity or vitamin D insufficiency may be the population norm. To generate a reference interval, a minimum of 120 individuals should be tested[32]; however, in practice the numbers are often lower. For pediatric-age patients, nearly all hormone values must be interpreted with regard to age. Moreover, several hormones will also change significantly during the course of puberty independently of chronologic age. Therefore, depending on the hormone assay, reference intervals that are partitioned to capture specific populations should be provided. Generating reference intervals for multiple subpopulations poses a challenge to the clinical laboratory, and in the absence of laboratory-derived data, practitioners must rely on the literature for comparison.

Evaluation of results of a reference interval study may be accomplished using simple or more complex statistics. A frequency plot of the results shows the number of individual results at each concentration range (Figure 4-11). If the smoothed curve describing the frequency distribution is bell shaped, the curve is "normal" or Gaussian. More commonly the distribution is skewed, and results tail more on the high or low side. Results may be power transformed to reduce the skewness and then analyzed. When this approach is used, the standard deviation on each side of the mean may be different. Increasingly, as an adjunct to the reference interval, the laboratory will also report a Standard Deviation Score (SDS) or Z score that may aid in interpretation, which is simply the difference of the result from the mean divided by the standard deviation. Whether the distribution is normal or skewed, the reference interval, by definition, should encompass the central 95% of results.

For existing analytes, a reference method with an extensive normal data set allows for a less costly and more accessible way of establishing reference intervals. A subset of normal samples can be used to confirm the reference database if the results utilizing the newly implemented method are similar to those used to establish the reference

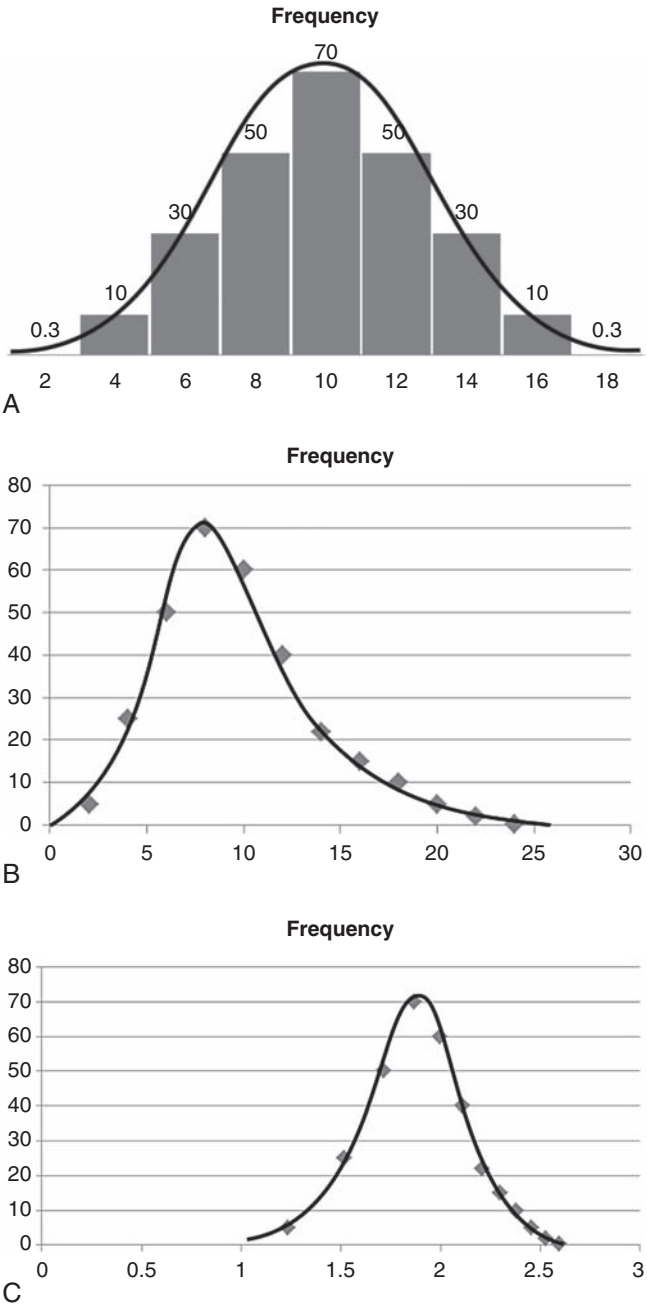


FIGURE 4-11 ■ **A,** Normal distribution has a symmetric frequency distribution. Quantities are indicated on the x-axis, and the frequency of individuals with any given quantity is indicated on the y-axis. **B,** A skewed distribution has an asymmetric frequency distribution. This picture is frequently found. A skewed distribution power transformed (0.3 power). **C**, After the curve is fitted, the central 95% are selected, and then the limits are "untransformed" by taking the (3.333 power) to return to the usual units.

population.[32] Ideally, the laboratory would also test specimens in subjects with disease to demonstrate that the test appropriately distinguishes them from the normal range. Laboratories now increasingly rely on device manufacturers to provide this base data set against which the smaller internally derived normal data are statistically evaluated for transference.[33] Current practice may fall short of these requirements because method differences and bias between

methods are sometimes unrelated to the differences in reference intervals.

A few tests for specific analytes, such as hemoglobin A1c or cholesterol, are standardized, and reference intervals and treatment goals are established by professional societies.[34] The laboratory must use a certified method to be certain that the test results meet the standards that allow interpretation according to guidelines. Standardization for testosterone by the Centers for Disease Control and Prevention (CDC) with the support of the Pediatric Endocrine Society, the Endocrine Society, and others is a current reality.[2] A standardized assay reference interval using large international population-based studies is being developed. Standardization of estradiol, vitamin D, growth hormone, and IGF-1 is likely to follow.

SUMMARY

Arriving at the correct diagnosis and management pathway for most pediatric endocrinology conditions involves a comprehensive history, careful physical examination, and laboratory investigations. The intent of this chapter is to help pediatric endocrinologists choose the appropriate tests and to aid in correctly interpreting the results obtained by the method used. The decisions of when and what tests to request, and how to correctly place the obtained data within the complex context of clinical decision making, remain the province of the physician at the center of the child's care team.

The clinical laboratory has an important collaborative and supportive role in optimizing patient care. A better understanding of the clinical laboratory's procedures, requirements, and limitations, can only improve the vital ongoing communication that should occur routinely between the clinicians and the personnel of the testing laboratory.

REFERENCES

1. 2012 Automated immunoassay analyzers. (July 2012). *CAP Today*, 18–54.
2. Rosner, W., Auchus, R. J., Azziz, R., et al. (2007). Position statement: Utility, limitations, and pitfalls in measuring testosterone: an Endocrine Society position statement. *J Clin Endocrinol Metab*, *92*, 405–413.
3. Thienpont, L. M., Van Uytfanghe, K., Blincko, S., et al. (2008). State-of-the-art of serum testosterone measurement by isotope dilution-liquid chromatography-tandem mass spectrometry. *Clin Chem*, *54*, 1290–1297.
4. Neely, E. K., Wilson, D. M., Lee, P. A., et al. (1995). Spontaneous serum gonadotropin concentrations in the evaluation of precocious puberty. *J Pediatr*, *127*, 47–52.
5. Wheeler, M. J. (2006). Immunoassay techniques. *Methods Mol Biol*, *324*, 1–23.
6. Juppner, H., & Potts, J. T., Jr. (2002). Immunoassays for the detection of parathyroid hormone. *J Bone Miner Res*, 17(suppl 2): N81–N86.
7. Davies, C. (2005). Introduction to immunoassay principles. In O.Wild (Ed.). *The immunoassay handbook* (3rd ed.). Boston: Elsevier.
8. Frieze, T. W., Mong, D. P., & Koops, M. K. (2002). "Hook effect" in prolactinomas: case report and review of literature. *Endocr Pract*, *8*, 296–303.
9. Klee, G. G. (2004). Interferences in hormone immunoassays. *Clin Lab Med*, *24*, 1–18.
10. Spencer, C. A., & Wang, C. C. (1995). Thyroglobulin measurement: techniques, clinical benefits, and pitfalls. *Endocrinol Metab Clin North Am*, *24*, 841–863.
11. Spencer, C. A. (2011). Clinical review: clinical utility of thyroglobulin antibody (TgAb) measurements for patients with differentiated thyroid cancers (DTC). *J Clin Endocrinol Metab*, *96*, 3615–3627.
12. Fuqua, J. S., Sher, E. S., Migeon, C. J., & Berkovitz, G. D. (1995). Assay of plasma testosterone during the first six months of life: importance of chromatographic purification of steroids. *Clin Chem*, *41*, 1146–1149.
13. Ekins, R. (1992). The free hormone hypothesis and measurement of free hormones. *Clin Chem*, *38*,1289–1293.
14. Pardridge, W. M., & Landaw, E. M. (1984). Tracer kinetic model of blood-brain barrier transport of plasma protein-bound ligands: empiric testing of the free hormone hypothesis. *J Clin Invest*, *74*, 745–752.
15. Vermeulen, A., Verdonck, L., & Kaufman, J. M. (1999). A critical evaluation of simple methods for the estimation of free testosterone in serum. *J Clin Endocrinol Metab*, *84*, 3666–3672.
16. Hackbarth, J. S., Hoyne, J. B., Grebe, S. K., & Singh, R. J. (2011). Accuracy of calculated free testosterone differs between equations and depends on gender and SHBG concentration. *Steroids*, *76*, 48–55.
17. Chandler, D. W., & Grant, R. P. (2007). A new approach to steroid analysis: mass spectrometry. *Highlights*, *15*, 7–10.
18. Soldin, S. J., & Soldin, O. P. (2009). Steroid hormone analysis by tandem mass spectrometry. *Clin Chem*, *55*, 1061–1066.
19. Ciccimaro, E., & Blair, I. A. (2010). Stable-isotope dilution LC-MS for quantitative biomarker analysis. *Bioanalysis*, *2*, 311–341.
20. Annesley, T. M. (2003). Ion suppression in mass spectrometry. *Clin Chem*, *49*, 1041–1044.
21. Hoofnagle, A. N., Becker, J. O., Wener, M. H., & Heinecke, J. W. (2008). Quantification of thyroglobulin, a low-abundance serum protein, by immunoaffinity peptide enrichment and tandem mass spectrometry. *Clin Chem*, *54*, 1796–1804.
22. Sharkey, F. E. (Ed.). (2011). *CAP laboratory accreditation manual.* College of American Pathologists.
23. Valenstein, P. N., Raab, S. S., & Walsh, M. K. (2006). Identification errors involving clinical laboratories: a College of American Pathologists Q-Probes study of patient and specimen identification errors at 120 institutions. *Arch Pathol Lab Med*, *130*, 1106–1113.
24. Narayanan, S. (2000). The preanalytic phase: an important component of laboratory medicine. *Am J Clin Pathol*, *113*, 429–452.
25. Kempers, M. J., Lanting, C. I., van Heijst, A. F., et al. (2006). Neonatal screening for congenital hypothyroidism based on thyroxine, thyrotropin, and thyroxine-binding globulin measurement: potentials and pitfalls. *J Clin Endocrinol Metab*, *91*, 3370–3376.
26. Albertsson-Wikland, K., Rosberg, S., Lannering, B., et al. (1997). Twenty-four-hour profiles of luteinizing hormone, follicle-stimulating hormone, testosterone, and estradiol levels: a semi-longitudinal study throughout puberty in healthy boys. *J Clin Endocrinol Metab*, *82*, 541–549.
27. Albertsson-Wikland, K., & Rosberg, S. (1992). Reproducibility of 24-h growth hormone profiles in children. *Acta Endocrinol (Copenh)*, *126*, 109–112.
28. Cacciari, E., Tassoni, P., Parisi, G., et al. (1992). Pitfalls in diagnosing impaired growth hormone (GH) secretion: retesting after replacement therapy of 63 patients defined as GH deficient. *J Clin Endocrinol Metab*, *74*, 1284–1289.
29. Flower, L., Ahuja, R. H., Humphries, S. E., & Mohamed-Ali, V. (2000). Effects of sample handling on the stability of interleukin 6, tumour necrosis factor-alpha and leptin. *Cytokine*, *12*, 1712–1716.
30. Wenk, R. E. (1998). Mechanism of interference by hemolysis in immunoassays and requirements for sample quality. *Clin Chem*, *44*, 2554.
31. Rambla-Alegre, M., Esteve-Romero, J., & Carda-Broch, S. (2012). Is it really necessary to validate an analytical method or not? That is the question. *J Chromatogr A*, *1232*, 101–109.
32. Sasse, E. A., Doumas, B. T., Miller, W. G., et al. (2000). How to define and determine reference intervals in the clinical laboratory. *NCCLS*, *20*, 1–37.
33. Linnet, K. (2000). Nonparametric estimation of reference intervals by simple and bootstrap-based procedures. *Clin Chem*, *46*, 867–869.
34. Sacks, D. B., Arnold, M., Bakris, G. L., et al. (2001). Position statement executive summary: guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. *Diabetes Care*, *34*, 1419–1423.

QUESTIONS

1. Which of the following terms is defined as the measurement of the replication of repeated measurements of the same specimen?
   a. Accuracy
   b. Linearity
   c. Precision
   d. Sensitivity
   e. Specificity

Answer: c

2. The presence of heterophile antibodies can lead to interference in immunometric (sandwich) antibody assays and false results. Which of the following is true regarding heterophile antibodies in immunoassay?
   a. Competitive immunoassays can have a false high report from a heterophile antibody mimicking antigen and bridging an interaction between the capture and signal antibodies.
   b. Immunometric assays can be subjected to a false low report because of blocking antibody preventing antibody-antigen interaction.
   c. Competitive immunoassays cannot be impacted by heterophile antibodies.
   d. All of the above
   e. None of the above

Answer: b

3. Which of the following is commonly measured via a receptor technique for biologic activity?
   a. TSH
   b. TSH receptor autoantibodies (TRAb)
   c. Thyroid-stimulating immunoglobulins (TSI)
   d. Thyroid peroxidase antibody (TPOAb)
   e. Total T4

Answer: c

4. A laboratory reports the analytic measurement range for a hormone assay as 10 to 550 units/mL. The lab technicians test blood samples in 180 normal individuals and establish the normal reference interval as 150 to 200 ng/mL. They perform replicate measurements at the lower limit of the reference interval and determine a mean of 150 ng/mL with a standard deviation of 10 ng/mL. What is the coefficient of variation at 150 units/mL for the assay?
   a. 6.7%
   b. 10%
   c. 15%
   d. 16%
   e. 20%

Answer: a

5. Several automated immunoassay systems that measure free thyroxine employ a labeled analog of thyroxine. How does the analog function in this assay?
   a. Competes with thyroxine for binding sites on thyroxine binding globulin
   b. Competes with free thyroxine for binding sites on a thyroxine antibody
   c. Competes with HAMA to negate interference
   d. Produces a light flash when it binds thyroxine binding globulin
   e. None of the above

Answer: b

6. Mass spectrometric assays often utilize a chromatographic step before measurement of steroids. The chromatographic step improves specificity of mass spectrometry. What physical property is the basis for the separation of steroids by mass spectrometry?
   a. Weight
   b. Polarity of the steroid
   c. Mass to charge ratio
   d. The functional groups on the steroid molecule
   e. None of the above

Answer: c

7. Isobars are distinct structures that have the same mass, which creates a challenge to assay specificity. For example, with mass spectrometric assays a chromatographic separation must be utilized, unless the two structures fragment uniquely. Which of the following pairs are isobars?
   a. Corticosterone and 11-desoxycortisol
   b. DHEA and testosterone
   c. 17-Hydroxyprogesterone and deoxycorticosterone
   d. All of the above
   e. None of the above

Answer: d

8. Immunometric assays offer some advantages over competitive immunoassays, leading them to become the preferred format of measurement for many hormones. Which of the following is *not* an inherent advantage of immunometric assays over competitive immunoassays?
   a. Potential for increased sensitivity with improved signal detection technology
   b. Increased specificity because of recognition of two distinct epitopes on the analyte
   c. Less prone to "prozone" or "hook" effect
   d. Readily utilize monoclonal antibodies, which have more predictable characteristics than polyclonal antibodies
   e. None of the above

Answer: c

SECTION **II**

ENDOCRINE DISORDERS IN THE NEONATE

CHAPTER 5

Ambiguous Genitalia

Selma Feldman Witchel, MD • Peter A. Lee, MD, PhD

CHAPTER OUTLINE

CHAPTER OUTLINE—cont'd

INTRODUCTION

Normal gonadal differentiation and sex development depend on the meticulous choreography and synchrony of a network of endocrine, paracrine, and autocrine signaling pathways reflecting the actions and interactions of specific genes, transcription factors, and hormones. Perturbations of this intricate network of gene regulation and gene expression governing fetal gonadal development result in disorders of sex development (DSD). Approximately 1 in 4000 infants is born with a DSD.[1]

These disorders include a spectrum of abnormalites in which chromosomal, genetic, gonadal, hormonal, or anatomic aspects of sex are atypical. Understanding the developmental biology and embryology of the urogenital system is crucial to categorizing and identifying the molecular basis of the disorder, and its treatment where possible in an individual patient. Knowledge of the many genes, proteins, and hormones involved in gonadal sex determination and sexual differentiation is rapidly increasing. Clarity in terminology provides a framework to approach the differential diagnosis in a patient. The pediatric endocrinologist's role in the care of a child with ambiguous genitalia begins at diagnosis or earlier for infants identified through prenatal testing. In such circumstances the physician's responsibility for the management of this child is only starting and will continue until transition to adult care providers is deemed appropriate.

TALKING WITH THE PARENTS

For parents, the birth of their child is a long-anticipated and exciting event. Due to the increased frequency of prenatal ultrasound examinations, parents have usually been told the sex of their child and have selected their infant's name. In some instances, abnormal genital development has been identified through ultrasound and the parents have been informed of this situation and educated regarding the likely diagnostic and treatment plans. In the absence of prior knowledge, the parents are suddenly confronted with a newborn with a birth defect involving the external genitalia and uncertainty regarding their infant's gender. The apprehension regarding the baby's health and gender are particularly traumatic if the parents had no prior awareness of the abnormal genital development.

Initially, the parents need to be congratulated on the birth of their child. They need to hear that their child has a problem of sex development and that this condition will be carefully and completely addressed. They need to learn that abnormalities of sex development involve the complex system that directs the development of reproductive system including external genital development. Explanation that it may be impossible to tell the sex of their child simply by examining the external genitalia is essential. It is important to emphasize that the atypical development is not the parents' fault and that they should not feel guilty. Physician and healthcare professionals should not offer guesses or presumptive diagnoses. The parents can be reassured that necessary studies will be obtained to provide information to ascertain their child's gender. These studies may also identify the etiology of the DSD. The infant's care should involve a multidisciplinary team that includes pediatric endocrinologists, pediatric urologists/surgeons, geneticists, neonatalogists, radiologists, behavioral health providers, and pediatric endocrine nurse educators. One team member needs to serve as the prinicipal communicator with the family.

Although contemporary society includes overt references to sex and sexuality, parents may have difficulty thinking of their child as a sexual being and feel embarrassed discussing their child's sex and future sexuality. Cultural attitudes, preexisting expectations, and family support systems impact the parent's responses. The medical team needs to promote an open and caring network to provide support for the parents and engage the parents in the medical decision-making process progressively as information becomes available.

The initial treatment goal is to determine if there is an underlying or associated life-threatening condition that requires specific urgent treatment. If the child's gender remains unclear, information needs to be obtained to assist the parents in determining the most appropriate sex of rearing. Usually, this can be accomplished within a matter of hours or days. In more complex instances, the diagnostic process may take longer. In situations in which

it is impossible to identify the specific etiology, the general DSD category (see later) provides a basis for decision making.

Factors to be considered in the medical decision-making process include the extent of external and internal reproductive system development, evidence of gonadal functionality (potential for pubertal hormone secretion and fertility), and hormone responsiveness. In some instances, these factors are more relevant than the karyotype. Genes and gene products mapped to diverse autosomal areas of the genome influence the sex development of the developing fetus and child. When consensus has been reached regarding a diagnostic category, available outcome information for that diagnosis should be reviewed. Knowledge of the specific etiology, including details of the diagnosis, enables planning therapeutic interventions and genetic counseling for future pregnancies. Although the exact details are unclear, the extent, timing, and duration of prenatal androgen exposure likely influences CNS differentiation and affects multiple functions. Hence, healthcare providers need to be cognizant that available outcome data to assist in the decision-making processes are limited. Currently accessible information in published reports is largely based on retrospective studies obtained using diverse methodologies.[2]

The first interview with the parents should set a positive and optimistic tone to promote parental bonding with their infant. Indeed, the emotional tone of this initial interaction is usually more meaningful than the factual information provided and is recalled by parents for many years. Respect for the family and individual perspectives together with a willingness to repeat or defer detailed explanations are crucial. In the midst of the emotional distress associated with the uncertainty of their infant's gender, parents cannot be expected to assimilate the vast amount of information that eventually needs to be shared. Repeated discussions with the parents will enable them to deal emotionally and intellectually with their concerns regarding their infant and to allow them to appropriately interact with their infant, family members, friends, and colleagues.

Factual explanations regarding the process of sexual differentiation with a focus on their infant's situation should be initially outlined. The primary goal at this point is to provide the parents with a basic understanding that the internal and external genital structures for both boys and girls develop from the same primordial tissues. It is also helpful to explain that there are not exclusively male and female hormones. Rather, the environments in which male and female fetuses develop are characterized by differing relative amounts of these hormones. The use of simple sketches and provision of pictures and diagrams can be helpful to explain the embryology of genital development to the parents. Some parents may benefit from practicing the words they will use to discuss the infant's health with other family members. Detailed explanations and discussion can be repeated multiple times as the child ages.

During the early dialogues, examining the infant with the parents to identify the specific physical findings of their infant is often beneficial. This can reduce their apprehension, increase their comfort when viewing their infant's genitalia, and reinforce their perception that their infant's needs are similar to those of all infants. Information can be presented in a manner that will minimize anxiety and better equip parents to participate in the decision-making process. To provide the best support for their infant, each parent must reach an individual resolution with a commitment to a positive perspective concerning this situation. Discussion of the many concerns (particularly those related to gender identity, pubertal development, sexual orientation, sexual function, and fertility) may be helpful. Honest discussions will engender positive feelings that enhance positive interactions and enable the parents to promote their child's self-esteem.

Unless the gender assignment is clear at this point, the healthcare team should recommend that the parents delay naming the infant, announcing the baby's birth, and registering the birth until more information becomes available. The message should be clear that they will be involved in deciding the appropriate gender of rearing for their child since it is their privilege and responsibility to participate in the process leading to a gender assignment. Until sex of rearing is established, it is best to refer to the infant as "your baby" or "your child." Terms such as *he*, *she*, and *it* should be avoided.

TERMINOLOGY

Under the auspices of the Pediatric Endocrine Society (North America) and the European Society for Pediatric Endocrinology, an international consensus statement was formulated that recommended a revised classification of the medical terminology used for disorders of sex development to avoid confusing and derogatory terms.[1] This descriptive classification attempts to be sensitive to concerns of parents and flexible enough to incorporate novel molecular genetic information. The updated classification system integrates molecular genetic considerations into the nomenclature for "disorders of sexual differentiation (DSD)"[1] and provides an approach to the diagnostic evaluation.

Terms such as *pseudohermaphrodite*, *intersex*, and *gender labeling* in the diagnosis should be avoided.[3] To accommodate all types of DSD, the classification system is broad and includes some conditions that do not present with obvious abnormalities of genital development (Box 5-1). The primary goal of this classification system is to provide a framework for diagnosis, assessment, and care management based largely on sex chromosome status. Currently, microarray and candidate gene analyses are increasingly available and utilized.The DSD categories include sex chromosome DSDs such as 45,X/46,XY (formerly mixed gonadal dysgenesis); ovotesticular DSD (formerly true hermaphroditism); 46,XY DSDs such as disorders of testicular development, disorders of androgen synthesis and action (replacing and expanding the former category of male pseudohermaphroditism), and XY sex reversal; and 46,XX DSDs such as masculinization of the XX individual (replacing female pseudohermaphrodite) and XX sex reversal. Because of the complexities of

BOX 5-1 Summary of Disorders Associated with Ambiguous Genitalia

Sex Chromosome DSD
- 45,X Turner syndrome
- 45,X/46,XY gonadal dysgenesis
- 46,XX/46,XY gonadal dysgenesis
- 46,XX gonadal dysgenesis

Disorders of Gonadal Differentiation
- Denys-Drash syndrome (WT1)
- Frasier syndrome (WT1)
- Meacham syndrome (WT1)
- WNT4
- SF1/NR5A1
- CBX2

46,XY DSD
- SRY
- Campomelic dwarfism (SOX9)
- Dosage sensitive sex reversal (duplications of DAX1)
- Desert hedgehog (DHH) mutations
- GATA4
- FOG2
- MAP3K1
- ATR-X syndrome
- Vanishing testes

Multiple Congenital Anomalies
- IMAGe syndrome (CDKN1C)
- Pallister-Hall syndrome (GLI3)
- ARX
- Genitopatellar syndrome (KAT6B)
- CHARGE syndrome (CHD7)

Ovotesticular Disorder

46,XX Testicular Disorder
- SRY positive
- SRY negative

46,XX Ovarian Disorder
- RSPO1
- FOXL2
- NOBOX
- FIGLA

Disorders of Cholesterol and Steroid Biosynthesis
- Leydig cell hypoplasia (LHCGR)
- Smith-Lemli-Opitz syndrome (DHCR7)
- Lipoid adrenal hyperplasia (StAR)
- Side chain cleavage deficiency (CYP11A1)

Virilizing Congenital Adrenal Hyperplasias
- 21-Hydroxylase deficiency (CYP21A2)
- 11β-Hydroxylase deficiency (CYP11B1)
- 3β-Hydroxysteroid dehydrogenase deficiency (HSD3B2)

Disorders of Sex Steroid Synthesis
- 17α-Hydroxylase/17,20-lyase deficiency (CYP17)
- Cytochrome b_5 Deficiency (CYP5)
- 3α-Hydroxysteroid Dehydrogenase Isozyme Deficiency (AKR1C2 and AKR1C4)
- P450 oxidoreductase deficiency (POR)
- 17β-Hydroxysteroid dehydrogenase deficiency (HSD17B3)
- 5α-reductase deficiency (SRD5A2)
- Placental aromatase deficiency (CYP19)
- Maternal hyperandrogenism

Disorders of Androgen Action
- Androgen insensitivity (AR)

Persistent Mullerian Duct Syndrome
- AMH mutation
- AMH-R mutation

Mullerian Duct Abnormalities
- Mayer-Rokitansky-Kuster-Hauser
- MKKS mutation

Other
- Hand-foot-genital syndrome (HOXA13)
- Environmental endocrine disrupters
- VACTERL syndrome
- MURCs syndrome
- Cloacal extrophy
- Aphallia

chromosomal and gonadal development, some diagnoses can be included in more than one of the three major categories. The number of genes identified to be involved in sex development continues to increase. Nevertheless, despite many recent advances the specific molecular etiology of the genital ambiguity in an individual cannot always be identified.

SEX DETERMINATION

Sex determination is the binary switch that launches the developmental destiny of the embryonic gonads to become testes or ovaries. Sexual differentiation refers to the process through which male or female phenotype develops. The gonads, internal genital ducts, and external genital structures all develop from bipotential embryologic tissues. Each cell in the developing gonad has the potential to differentiate into either a testicular or ovarian cell. How the transcriptome of the undifferentiated cell realizes its pathway to develop into an ovary or testis provides an opportunity to elucidate cell fate decision making. However, "the fate decisions in individual cells are highly coordinated such that cells of discordant fate are rarely seen."[4] Thus, male and female development depends on the regulated orchestration of the expression and interaction of specific genes and gene products. Sex determination is largely influenced through transcriptional regulation whereas secreted hormone and hormone receptors influence sex differentiation.

Through Alfred Jost's experiments with fetal rabbits in the 1940s and 1950s, the critical requirements for a testis

and testosterone for male sexual differentiation were established.[5] Chromosomal composition of the human embryo, XX or XY, determines gonadal sex. The genetic locus primarily responsible for this binary switch, the sex-determining region on the Y *(SRY)* gene on the Y chromosome, was identified through studies of patients with disorders of sexual differentiation. Studies involving creation of transgenic SRY+ mice confirmed the essential role of SRY and provided further molecular understanding of testicular differentiation.[6,7]

In the usual situation, the karyotype (46,XY or 46,XX) of the primordial gonad determines whether it differentiates into a testis or ovary, respectively. Local factors (such as hormones secreted by the developing gonads or tissue-specific transcription factors) influence the ensuing differentiation of the internal and external genital structures. This process integrates sex-specific pathway signals that appear to antagonize each other. Divergence from the normal sequence of events leads to disorders of sex differentiation that can manifest as abnormal gonadal differentiation, inconsistent internal genital differentiation, or ambiguity of the external genitalia. Although genital ambiguity is usually not considered to be a medical emergency, it may be highly distressing to the parents and extended family. Hence, prompt referral and evaluation by a multidisciplinary team with expertise in disorders of sex differentiation is strongly recommended. Information regarding our current knowledge of sex determination is presented, followed by a discussion of the various causes and treatments for ambiguous genital development.

DEVELOPMENT OF THE REPRODUCTIVE SYSTEM

Urogenital Development

The gonads are derived from intermediate mesoderm. In humans, at 4 to 6 weeks of gestation, the urogenital ridges develop as paired outgrowths of coelomic epithelium (mesothelium). The gonads, adrenal cortex, kidney, and reproductive tract derive from the urogenital ridge (Figure 5-1). Several genes are requisite for the development of the bipotential gonad. The Wilms tumor (WT1) gene codes a zinc transcription factor that is expressed in embryonic mesodermal tissues and appears to influence mesodermal-epithelial interactions.[8] GATA4 is expressed in the somatic cells of the urogenital ridge and bipotential gonad before showing sex specific expression. Chromobox homolog 2 (CBX2) appears to play a role in early gonadal development and may promote transactivation of steroidogenic factor-1 (NR5A1), which is encoded by NR5A1/SF1. NR5A1 is expressed in the urogenital ridge and appears to upregulate SRY expression. In addition to transcription factors and specific secreted factors (hormones), physical contact

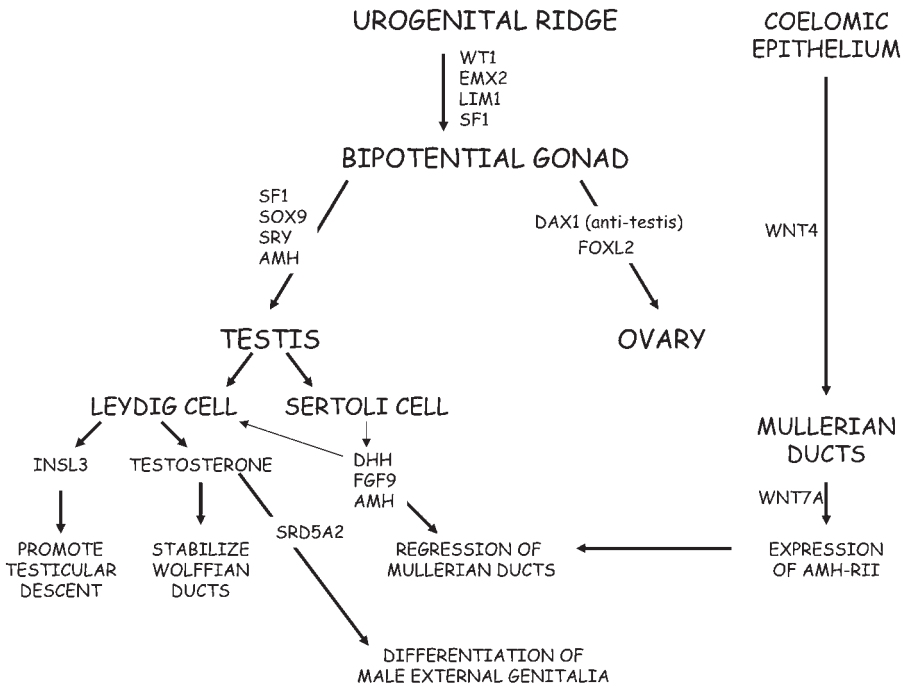


FIGURE 5-1 ■ Cartoon of the genes involved in the process of sexual differentiation. Wilms tumor (WT1), EMX2, LIM1, and steroidogenic factor-1 (SF1) play roles in differentiation of gonad from urogenital ridge. Genes involved in testicular differentiation include SF-1, SOX9, sex-determining region on Y (SRY), and anti-Mullerian hormone (AMH). The dosage-sensitive sex-adrenal hypoplasia congenital critical region on X (DAX1) appears to function as an anti-testis factor. Wnt4 promotes development of the Mullerian ducts, whereas Wnt7a promotes expression of the receptor for AMH (AMH-RII). Sertoli cells secrete AMH, which, acting through its cognate receptor (AMH-RII), promotes regression of the Mullerian ducts. Leydig cells secrete testosterone and insulin-like hormone-3 (INSL3). Testosterone stabilizes the Wolffian ducts and is converted to DHT by 5α-reductase in target tissues to promote differentiation of the prostate and development of male external genitalia. INSL3 is involved in transabdominal testicular descent.

with the mesonephros appears to be important for subsequent gonadal differentiation.[9]

Due to their origin as part of the developing urogenital system, ovaries and testes are initially located high in the abdomen near the kidneys. One of the earliest morphologic changes is increased proliferation and size of developing 46,XY gonads. The bipotential gonad consists of at least four cell lineages, which are germ cells, supportive cells, steroidogenic cells, and connective tissue. Although sex determination has been equated with testis differentiation, recent data challenge this dogma. Rather, specific signaling molecules activate or repress gonadal determination for both testes and ovaries. Competition between specific genes and proteins influences cell fate decisions in gonadal development.[10] Specific examples include Forkhead transcription factor 2 (FOXL2) vs. SRY-box 9 (SOX9) and SOX9 vs. Wingless-type MMMTV integration site family member (WNT)/β-catenin, which are discussed later.[11]

Germ Cell Development

Germ cells are not required for the initial development of ovaries or testes. Rather, the local environment directs the fate of the primordial germ cells. Until approximately 6 weeks of gestation in the human, primordial germ cells proliferate and migrate from the hindgut to colonize the genital ridges. This migration depends on intrinsic motility and external guidance cues as specified by attractive and repulsive signals.[12] When this migration process goes awry, the gonadal germ cell population is lacking. Factors important for germ cell migration and colonization of the genital ridges include WNT5A, NANOG, stromal cell–derived factor 1 (SDF1, also known as CXCL12) and its receptor CXCR4.

Meiosis is a sexually dimorphic regulated step governing terminal differentiation of germ cells with meiosis favoring oocytes and active inhibition of meiosis being essential for male germ cells.[13] The RNA binding protein Deleted in azoospermia-like (DAZL) plays a role in this branding of germ cells as male or female.[14] Factor in germline alpha (FIGLA) and newborn ovary homeobox (NOBOX) are oocyte-specific proteins that appear to repress male specific genes.[15] Subsequent stages of germ cell differentiation into a sperm or an egg are closely linked to the cell cycle decision between mitosis and meiosis.[16] Meiosis is dependent on retinoic acid, a morphogen, primarily synthesized in the developing gonad.[17] Expression of Stimulated by Retinoic Acid (*STRA8)* increases in the human fetal ovary concomitant with the initiation of meiosis in this tissue, but remains low in the testis. STRA8 is required for pre-meiotic DNA replication and progression through meiosis. In mice, the expression of CYP26B1, a cytochrome P450 enzyme that degrades retinoic acid, prevents meiosis in male germ cells. In human male fetuses, expression of CYP26B1 does not fully account of inhibition of meiosis in the germ cell.[18] Expression for NANO2 appears to be restricted to fetal testes.

The Sertoli cells envelope the germ cells to form seminiferous cords at approximately 7 to 9 weeks of gestation in the human XY gonad. The germ cells in the developing testis enter a state of mitotic arrest. In a human XX gonad, germ cell meiosis begins at 10 to 11 weeks of gestation. In the developing ovary, the germ cells initially form clusters connected by intracellular bridges. Selected oogonia enter meiosis and progress through meiotic prophase I (MPI) to arrest at the diplotene stage. The fetal ovary is characterized by the existence of multiple subpopulations of germ cells at different developmental stages. By approximately 20 weeks of gestation, the oogonia clusters break down to form primordial follicles. Primordial follicles destined for future ovulation remain quiescent. By the 24th week of gestation, most oogonia are surrounded by supporting cells. However, apoptosis is the fate for many oogonia. From a peak of 6.8 million oocytes at approximately 5 months of gestation, approximately 2 million are present at birth due to follicular atresia.[19] Accelerated follicular atresia contributes to the follicular depletion characteristic of streak gonads in X monosomy.

The internal environment throughout fetal ovary development has the potential to directly influence the fertility of the developing fetus (controlling the size of the ovarian reserve) and the quality of the oocyte that will eventually become her child (by influencing the extent of selection and apoptosis).[20] Although not associated with genital ambiguity, mutations in genes governing oocyte and ovarian development cause disorders of sex development characterized by delayed puberty or premature ovarian failure.

During gestation, maternal and paternal alleles are differentially imprinted such that monoallelic expression of specific genes occurs. During this process of imprinting, mature oocytes and sperm are differentially marked reflecting "parent-of-origin" specific methylation patterns. In the primordial immature germ cells, inherited imprints are erased shortly after the germ cells enter the gonadal ridge. Sexually dimorphic methylation imprinting is subsequently reestablished in male and female gametes. This process occurs late in fetal development in the male and postnatally in female germ cells.[21,22] The importance of this imprinting process has been elucidated through study of parent-of-origin–dependent gene disorders such as Beckwith-Wiedemann, Prader-Willi, and Angelman syndromes and some forms neonatal diabetes mellitus.

Human Testicular Development

Testicular differentiation occurs earlier than ovarian development. The testis consists of five cell types: supporting or Sertoli cells, endothelial cells, peritubular myoid cells, steroid-secreting Leydig cells, and germ cells. The first evidence of testicular differentiation is the appearance of primitive Sertoli cells at 6 to 7 weeks gestation in the human fetal testis. Cells, mostly endothelial cells, migrate from the mesonephros and interact with the pre-Sertoli cells to promote development of the testicular cords.[23] The testicular cords are precursors of the seminiferous tubules that will contain Sertoli and germ cells. Interactions between endothelial and mesenchymal cells appear to influence development of the testicular cords.[24]

The binary switch responsible for testicular development is the *SRY* gene located on the short arm of the Y chromosome. The SRY protein contains a high-mobility group (HMG) domain and is encoded by a single exon gene. Two nuclear localization signals are located in the HMG domain. The SRY protein is expressed in pre-Sertoli cells, where it triggers a molecular switch to induce Sertoli cell differentiation, thus, initiating the process of male sexual differentiation. The HMG domain of the SRY protein binds to the minor DNA groove, where it functions as a transcription factor by bending DNA to presumably permit other proteins access to regulatory regions and to promote assembly of nucleoprotein transcription complexes. A threshold SRY level must be achieved at a critical time during gestation to establish male sexual differentiation. otherwise, the ovarian differentiation pathway is activated.[25] Available data suggest that NR5A1 promotes SRY expression.

SRY expression is independent of the presence of germ cells. SRY increases the expression of the SRY-related HMG box-containing-9 (SOX9) gene. Phenotype-genotype studies of humans and mice demonstrate that SOX9 expression is a crucial step, downstream of SRY, in testis development. Upstream from the SOX9 transcription start site, there appears to be a testis specific enhancer element (hTES). Available data suggest that initially phosphorylated NR5A1 and SRY cooperate to activate the hTES leading to increased SOX9 expression; subsequently SRY, NR5A1, and SOX9 maintain SOX9 expression through actions at hTES.[26] In addition to SRY, SOX9, and NR5A1, sequential expression of several other genes is required for normal male sexual differentiation. These genes include fibroblast growth factor 9 (FGF9), anti-Mullerian hormone (AMH), dosage-sensitive sex reversal adrenal hypoplasia congenita critical region on X (DAX1),GATA-binding-4 (GATA4), desert hedgehog (DHH), patched (PTCH1), and WNT7A.

Using immunohistochemistry, NR5A1 and SOX9 proteins can be detected in human embryonic gonadal tissue at 6 to 7 weeks of gestation. At this time, SOX9 expression becomes limited to nuclei of Sertoli cells in a 46,XY fetus but remains cytosolic in a 46,XX fetus. NR5A1 and SOX9 protein expression precede AMH expression. After AMH protein expression increases, Wilms tumor (WT1) and GATA-4 protein expression increase in the fetal testis.[27] GATA4 belongs to a family of zinc finger transcription factors known as GATA-binding proteins because they bind to a consensus sequence in the promoter and enhancer regions of target genes. Sexually dimorphic expression of Doublesex- and MAB3-Related Transcription Factor 1 (DMRT1) was found in 6- and 7-week-old human fetuses, with expression limited to male fetuses.[28]

SOX9 induces expression of prostaglandin D synthase (Pgds), an enzyme involved in prostaglandin synthesis.[29] In a positive feedback loop, developing Sertoli cells secrete prostaglandin D2, which binds to its cognate receptor to up-regulate SOX9 expression and recruit additional Sertoli cells.[30] In addition, SOX9 promotes expression of fibroblast growth factor 9 (FGF9). FGF9 acting through the its receptor, FGFR2, helps to maintain SOX9 expression and promote Sertoli cell differentiation.[31] Normal testicular development appears to involve diffusion of FGF9 from the center to the poles of the undifferentiated gonad.[32] Through direct and indirect mechanisms, SOX9 interferes with genes promoting ovarian differentiation.

Vascular development in the gonad is sexually dimorphic with the endothelial cells in the developing testis, forming a characteristic pattern consisting of a prominent coelomic vessel on the antimesonephric surface with branches between the testis cords. This blood vessel is absent in the ovary. Peritubular myoid cells are testis-specific smooth muscle–like cells important to structural integrity and development of the testis cords. Factors relevant to peritubular myoid cell differentiation include desert hedgehog (DHH), which is secreted by Sertoli cells and its receptor Patched (Ptch1).[33] The peritubular myoid cells surround the Sertoli cells, separating them from the Leydig cells, which are then sequestered in the interstitium. Downstream of SRY, hedgehog signaling plays a role in cell-cell communication and cell fate determination to influence sex dimorphic development in gonads, reproductive tracts, and external genitalia.[33]

Leydig cell differentiation depends on paracrine signals, including platelet-derived growth factor receptor-alpha (PDGFR-α), DHH, PTCH1, and Aristaless-related homeobox (ARX). NR5A1 is expressed in Leydig cells to promote steroidogenic enzyme genes expression. The number of fetal Leydig cells reflects gonadotropin stimulation because the number is decreased in anencephalic male fetuses and increased in 46,XY fetuses, with elevated gonadotropin concentrations secondary to complete androgen insensitivity.[34] Differentiation of adult Leydig cells occurs postnatally.[34]

By 11 weeks of gestation, the testicular compartments, tubular and interstitial components, and the cell types of interest (Leydig, Sertoli, and germ cells) can be visualized. In human fetal testes, *HSD17B3*, *CYP11A1*, and *PTCH1* mRNA levels increased significantly through the second trimester without significant changes in *CYP17A1*, *LHR*, or *INSL3* levels. The most rapid growth in Sertoli cell number appears to occur during the latter half of the first trimester and the second trimester.[35]

Human Ovary Development

Although ovarian differentiation has long been considered the default pathway that occurs in the absence of *SRY* gene expression, accumulating evidence indicates that specific genes influence ovarian differentiation. Genes implicated in ovarian differentiation include wingless-related MMTV integration site 4 (WNT4), forkhead L2 (FOXL2), follistatin (FST), Iroquois-3 (IRX3), bone morphogenic protein-2 (BMP2), and R-spondin-1 (RSPO1).[36] FOXL2 functions to repress male-specific genes especially SOX9 beginning in the fetus and continuing through adulthood. FOXL2 and SOX9 expression appear to be mutually exclusive in developing gonads and in gonadal tissue obtained from patients with DSD conditions.[37]

In the absence of SRY, between 6 to 9 weeks of gestation, R-spondin-1(RSPO1) expression increases in the

developing human ovary. RSPO1 is a secreted factor that activates the β-catenin WNT signaling pathway.[38] WNT4 expression also increases. RSPO1 and WNT4 appear to stabilize and amplify β-catenin signaling to activate target gene transcription.[39] LIN28A, an RNA-binding protein is differentially expressed in the fetal ovary particularly in the germ cells; its expression appears to be restricted to primordial and premeiotic germ cells.[40] LIN28 blocks the production of mature *let*-7, which is a microRNA that interferes with translation of cell cycle regulators and metabolic pathway components.[41,42] In the absence of LIN28, *let*-7 inhibits production of BLIMP1, a factor involved with primordial germ cell development.[41] Expression of both let-7 and LIN28A decreases when germ cells transition from the stem cell state to meiosis.[41]

Persistent DAX1 expression appears to play a role in ovarian differentiation. The functions of WNT4 include suppression of the androgen-secreting interstitial cells, inhibition of coelomic vascularization, and support of Mullerian derivatives. FOXL2 is a forkhead transcription factor expressed in the ovary.

Two genes, factor in FIGLA and NOBOX, are expressed in fetal or neonatal germ cells where they recruit developing granulosa cells to form primordial and primary follicles. As noted previously, many oocytes degenerate through apoptosis. GATA 4 is expressed in the granulosa cells and is speculated to prevent granulosa cell apoptosis.[43] The second trimester human fetal ovary expresses proteins necessary to synthesize and respond to estrogenic, progestogenic, and androgenic signaling.[44]

Development of Internal Genital Structures

The Wolffian duct originates as the excretory duct of the mesonephros and develops into the epididymis, vas deferens, ejaculatory duct, and seminal vesicle. The epididymis consists of four functional portions: initial segment, caput, corpus, and cauda. Sperm mature in the caput and corpus, whereas the cauda is primarily for storage. The Mullerian or paramesonephric duct originates as an invagination of the coelomic epithelium and develops into the Fallopian tubes, uterus, and upper third of the vagina.

In the male fetus, the Sertoli cells secrete anti-Mullerian hormone (AMH), also known as Mullerian inhibitory hormone (MIH). In human 46,XY fetuses, AMH expression can be detected by 7 weeks of gestation, is not dependent on the presence of germ cells within the testis, and promotes regression of the Mullerian ducts. AMH, a member of the transforming growth factor-β (TGF-β) family, undergoes proteolytic cleavage to become biologically active. AMH binds to its receptor, AMH-RII, on the surface of the Mullerian duct mesenchymal cells to induce increased matrix metalloproteinase 2 expression.[45,46] The net result is degeneration and loss of basement membrane integrity of the epithelial and mesenchymal Mullerian cells, leading to regression of the Mullerian ducts.

AMH expression is highly regulated because inappropriate expression in a 46,XX fetus would lead to uterine agenesis. In the 46,XX fetus with absence of both AMH and testosterone, the Mullerian duct derivatives persist and the Wolffian ducts regress. When a female fetus is inappropriately exposed to AMH (as in freemartin cattle), Mullerian duct regression and ovarian masculinization occur. By 12 weeks in the XX fetus, the uterine corpus and cervix have begun to differentiate.[47]

The fetal hypothalamic-pituitary-gonadal (HPG) axis is active by mid-gestation, with peak fetal testosterone concentrations occurring at approximately 15 to 16 weeks of gestation. Prior to this time, placental hCG stimulates testosterone production by the fetal Leydig cells. Secretion of testosterone by the fetal Leydig cells stabilizes the Wolffian ducts in 46,XY fetuses. Region-specific signaling molecules such as bone morphogenic proteins (BMPs), homeobox genes (HOXA10 and HOXA11), growth differentiation factor 7 (GDF7), relaxin, an orphan G-protein–coupled receptor (LGR4), platelet-derived growth factor A (PDGFA), and its cognate receptor (PDGFRA) influence the development of the epididymis and seminal vesicle.

The prostate, a male accessory sex gland, contributes to seminal fluid plasma and develops from the urogenital sinus. After the initial testosterone-dependent induction of prostate differentiation, subsequent development involves epithelial-mesenchymal interactions that lead to cell differentiation and branching morphogenesis. The requisite signaling molecules FGFs, sonic hedgehog (SHH), BMPs, HOXA13, and HOXD13 are similar to those required for external genital differentiation.[48,49]

Development of External Genital Structures

The genital tubercle, urethral folds, and labioscrotal swellings give rise to the external genitalia. Under the influence of circulating androgens that are converted to dihydrotestosterone in target tissues, the urethral folds fuse to form the corpus spongiosum and penile urethra, the genital tubercle develops into the corpora cavernosa of the penis, and the labioscrotal folds fuse to form the scrotum.

In the normal 46,XY human fetus, a cylindrical 2-mm phallus with genital swellings has developed by 9 weeks of gestation. By 12 to 14 weeks of gestation, the urethral folds have fused to form the cavernous urethra and corpus spongiosum. By 14 weeks, the external genitalia are clearly masculine apart from testicular location. The high incidence of hypospadias in humans suggests that urethral fusion is a delicate and finely regulated process.

In the 46,XX fetus, in the absence of androgens the urethral folds and labioscrotal swellings do not fuse and develop into the labia minora and labia majora, respectively. The genital tubercle forms the clitoris, and canalization of the vaginal plate creates the lower portion of the vagina. By 11 weeks of gestation, the clitoris is prominent and the lateral boundaries of the urogenital sulcus have separated. Minimal clitoral growth, well-defined labia majora, hypoplastic labia minora, and separate vaginal and urethral perineal openings are present by 20 weeks of gestation.

By 33 days' postconception, the human fetal adrenal cortex is distinct from the developing gonad. Due to its role as the source of DHEAS for placental estrogen biosynthesis, the fetal adrenal cortex grows rapidly. By 50 to 52 days' postconception, expression of several steroidogenic enzymes, steroidogenic acute regulatory protein (StAR), 11β-hydroxylase (CYP11B1), 17α-hydroxylase/17,20-lyase (CYP17), and 21-hydroxylase (CYP21) in the fetal adrenal cortex have been demonstrated immunohistochemically.[50] Recent data indicate that transitory cortisol biosynthesis peaks at 8 to 9 weeks' gestation.[50] This early cortisol biosynthesis coincides with transient adrenal expression of both nerve growth factor IB-like (NGFI-B) and 3β-hydroxysteroid dehydrogenase-2 (HSD3B2).[50] At the same time, ACTH can be detected in the anterior pituitary—suggesting the presence of negative feedback inhibition during the first trimester.[50] During the time male sexual differentiation begins, this negative feedback inhibition may serve to prevent virilization of female fetuses to ensure normal female sexual differentiation.[50,51]

Anogenital Distance

The anogenital distance (AGD), the distance from the posterior aspect of the scrotum to the anal verge, shows sexually dimorphism. Longitudinal assessment of AGD defined as measurement from the center of the anus to the base of the scrotum in males and to the posterior fourchette in females showed that mean AGD (+/- SD) at birth was 19.8 +/- 6.1 mm in males and 9.1 +/- 2.8 mm in females. In both sexes, AGD increased up to 12 months and maintained the sex dimorphic pattern. AGD also showed positive correlation with penile length at birth and with the increase in AGD from birth to 3 months.[52] AGD provides an index of early fetal androgen exposure and masculinization.

Androgens play a time-dependent role in formation, differentiation, and growth of the fetal external genitalia; a role that is especially relevant for male reproductive organ development. Studies among rats suggest that there is a limited time during early fetal development known as the masculinization programming window (MPW). During the MPW the potential for masculinization is determined. Deficiency of androgen or androgen action in the MPW results in reduced penis length, which can not be rescued by postnatal T therapy.[53] The MPW predetermines potential organ size, while post-natal androgen action is required to realize normal potential.[54]

Indirect evidence suggests that the MPW occurs in the human. This window would likely precede or occur between 8 and 13 weeks of fetal life when the penis is forming. Thereafter, the penis grows at a rate of 0.7 mm/week from 14 weeks to term.[55] If, as in studies of rats, the MPW determines penis growth potential assuming normal androgen exposure subsequently during fetal and postnatal life,[56] this has implication for potential of human male genitalia. Thus, the AGD may indicate early in utero androgen exposure and can be used as a diagnostic tool. Measurement of AGD has also been used to indicate impact of potential environmental endocrine disruptors on external genital development.[57] AGD at birth predicts adult anogenital distance and has been found to be correlated with serum testosterone levels, sperm density, and paternity.[58,59]

Sexual Differentiation of the Brain

Clinical investigations suggest that the brain is sexually dimorphic and that testosterone is a masculinizing hormone in human. Males with aromatase deficiency manifest male psychosexual behavior and gender identity. Alternatively, 46,XY individuals with complete androgen insensitivity syndrome (CAIS) develop female gender identity.[60] However, preliminary data implicate genetic differences, independent of sex steroid exposure, as the molecular basis for some aspects of sexual dimorphism of the brain.[61] Nevertheless, the prenatal hormone milieu in conjunction with genetic factors and, perhaps postnatal environmental factors, may influence sexual orientation.[62] Postmortem histologic examination demonstrated that women have more synapses in the neocortex, whereas men have more neurons in this region.[63,64]

MOUSE MODELS

Sexual differentiation is a complex process in which precise spatiotemporal coordination and regulation of gene expression are crucial to achieve full reproductive capability. Despite expanded knowledge of the molecular details of sexual differentiation, additional factors and the precise sequence of biologic events in the human fetus remain to be elucidated. Investigation of animal models, especially mice, and transgenic mouse models has provided insight into the processes involved in sex development (Table 5-1). Investigation of normal and transgenic mice confirmed the crucial role of the *SRY* gene in male differentiation when XX mice carrying only a 14-kb fragment of the Y chromosome showed a male phenotype.[65] The caveat is that mouse and human developmental processes and phenotypes may differ; humans with a 45,X karyotype develop gonadal dysgenesis associated with infertility whereas XO mice are fertile. Despite this limitation, information gleaned from the developmental processes in mice may be applicable to the human situation.

The outcome of cell fate decisions involves antagonism between the male and female developmental programs. During the bipotential phase, both male and female specific genes are expressed with an over-representation of female genes.[66] Abnormal gonadal development has been described in mice homozygous for targeted deletions of genes involved in urogenital differentiation (i.e., Wt1, Sf1, Emx2, M33 [Cbx2], and Lim1). In mice, these genes are expressed earlier in gestation than Sry, which is expressed transiently at 10.5 to 11 days postcoitum (d.p.c.).[67] Wnt9b is expressed throughout the Wolffian duct epithelium from E9.5 to E14.5 where it plays a role in development of mesonephric tubules, metanephric tubules, and caudal extension of the Mullerian ducts.[68] Wt1 is expressed

TABLE 5-1 **Consequences of Loss-of-Function Mutations in Genes Associated with DSD in Humans and Mice**

Gene	Human Locus	Human Phenotype	Mouse Phenotype
WT1	11p13	Denys-Drash, Frasier, and Mecham syndromes	Homozygous: embryonic lethal with absence of kidney and gonads.
SRY	Yp11.3	46,XY gonadal dysgenesis	"Knock-in" female-to-male sex reversal.
SOX9	17q24-25	Campomelic dysplasia and gonadal dysgenesis	Overexpression in XX mice results in female-to-male sex reversal. Heterozygous null associated with perinatal death, cleft palate, and skeletal abnormalities.
DHH	12q13.1	Gonadal dysgenesis, with or without minifascicular neuropathy	Abnormal peripheral nerves, infertility due to impaired spermatogenesis in males.
ATRX/XH2	Xq13.3	α-Thalassemia, mental retardation, genital abnormalities, short stature, hypotonic facies	Phenotype of null is embryonic lethal. Overexpression associated with growth retardation, neural tube defects, and embryonic death.
ARX	Xp22.13	Lissencephaly, absence of the corpus callosum, microcephaly	Males have abnormal CNS development and abnormal testicular differentiation.
SF1/NR5A1	9q33	46,XY sex reversal with or without adrenal insufficiency 46,XX adrenal insufficiency, POF	Absent adrenal glands, absent gonads, abnormal pituitary differentiation.
EMX2	10q26.1	Schizencephaly	Absence of kidneys, ureters, gonads, and genital tracts.
FGF9	13q11-q12	Autosomal dominant multiple synostoses	Male-to-female sex reversal, lung hypoplasia.
WNT4	1p35	46,XX, Uterine agenesis 46,XY, Male-to-female sex reversal	Males are normal. Females manifest female-to-male sex reversal.
WNT7A	3p25	Limb malformation syndromes	Persistent Mullerian duct derivatives in males. Abnormal Mullerian duct differentiation in females.
DAX1/ NROB1	Xp21.3	Adrenal hypoplasia congenita; duplication associated with male-to-female sex reversal	Male infertility.
GATA4	8p23.2-p22	Congenital heart disease	Embryonic lethal, heart defects.
FOG2	8q23.1	Tetralogy of Fallot, diaphragmatic hernia	Embryonic lethal, heart defects.
MAP3K1	5q11.2	46,XY gonadal dysgenesis	Failure of eyelid development
CDKN1C	11p15.4	IMAGE syndrome	N/A
GLI3	7p14.1	Pallister-Hall syndrome, polydactyly	Polydactyly, fetal lethal
FOXL2	3q23	Blepharophimosis/ptosis/epicanthus inversus syndrome	Small, absence of eyelids, craniofacial anomalies, female infertility.
LHGCR	2p21	Leydig cell hypoplasia	N/A
DHCR7	11q12-q13	Smith-Lemli-Opitz syndrome	IUGR, cleft palate, neurologic abnormalities.
StAR	8p11.2	Congenital lipoid adrenal hyperplasia	All with female external genitalia. Neonatal death due to adrenal insufficiency.
CYP11A1	15q23-q24	Male-to-female sex reversal, adrenal insufficiency	Male-to-female sex reversal. Neonatal death due to adrenal insufficiency.
CYP19A1	15q21.1	Aromatase deficiency	Immature infertile female. Males are initially fertile, but develop disrupted spermatogenesis.
POR	7q11.2	Antley-Bixler syndrome	Embryonic lethal.
AMH	19p13.3-p13.2	Persistent Mullerian duct syndrome	Male has persistent Mullerian duct derivatives and infertility.
AMH-RII	12q13	Persistent Mullerian duct syndrome	Male has persistent Mullerian duct derivatives and infertility.
INSL3	19p13.2	Associated with cryptorchidism	Cryptorchidism in males. Overexpression in females associated with development of gubernaculum and aberrant ovarian location.
LGR8	13q13.1	Associated with cryptorchidism	Cryptorchidism.

Key: N/A, not available; POF, premature ovarian failure

throughout the intermediate mesoderm at 9 d.p.c. Subsequently, Wt1 is expressed in the developing gonad. The phenotype of Wt1 knockout mice includes embryonic lethality, failure of gonadal and kidney development, and abnormal development of the mesothelium, heart, and lungs.[69] Homozygous deletion of Emx2, a homeodomain transcription factor, results in an embryonic lethal phenotype associated with absence of kidneys, ureters, gonads, and genital tracts.[70,71] As Wt1 expression is initially normal in the metanephric blastema of Emx2 knockout mice, Emx2 is likely downstream of Wt1. Interestingly, adrenal gland and bladder development are normal in Emx2 knockout mice.

The polycomb group proteins function to establish imprinting patterns in developmental control genes and appear to be involved in recognition of methylated histones. One such protein is Cbx2 also known as M33. The phenotype of Cbx2 knockout mice includes male to female sex reversal and hypoplastic gonads for both sexes.[72] Based on available animal studies, Cbx2 appears to regulate Sry expression, modulate expression of Sf1/Nr5a1 and Lhx9 in both genders, and influence gonad proliferation and size. Lim1, also known as Lhx1, encodes a homeobox protein that is important in the differentiation of intermediate mesoderm and the urogenital system.[73] Homozygous deletion of Lim1 is associated with absence of kidneys and gonads and with anterior head structures.[73]

Steroidogenic factor 1 (Sf1), also known as Nr5a1, is an orphan nuclear hormone receptor that functions as a transcription factor. In mice, Sf1 is expressed from the earliest stages of gonadogenesis at 9 days post conception (d.p.c.) and regulates expression of steroidogenic enzyme genes in gonads and adrenals. At the onset of testicular differentiation, Sf1 expression becomes sexually dimorphic with increased expression in fetal testis, in both Sertoli and Leydig cells, and decreased expression in fetal ovaries.[74] In Sertoli cells, Sf1 and Sry act synergistically to promote Sox 9 expression. In Leydig cells, Sf1 promotes transcription of steroidogenic enzyme genes. The pathologic findings in mice homozygous for targeted deletion of Sf1 include absence of gonads, adrenal glands, and ventromedial hypothalamus with decreased number of gonadotropes in the anterior pituitary.[75] Sf1 knockout mice have female internal and external genitalia irrespective of genetic sex and die shortly after birth secondary to adrenal insufficiency.[75] Pituitary-specific Sf1 knockout mice manifest hypogonadotropic hypogonadism, confirming the essential role of Sf1 in pituitary function.[75]

Sf1 and Sry bind to a gonad specific enhancer (Tesco) to induce Sox-9 expression. Following the onset of testicular differentiation, Sox9 expression increases in the testis and decreases in the ovary by 11.5 d.p.c. Subsequently, Sox-9 and Sf1 bind to Tesco to maintain Sox9 expression. Thus, Sox9 binds to its own promoter to maintain its expression. Continued Sox9 expression is also dependent on Fgf9 and its receptor, Fgfr2, to maintain Sox9 expression and repress Wnt4 expression. Ectopic expression of Sox9 in XX mice leads to testicular differentiation. Mice with homozygous targeted deletion of the Sox9 gene die during mid-gestation.[76] Sox9 also plays a major role in chondrocyte differentiation and cartilage formation.

Dax1 is first expressed in the genital ridge at 10.5 to 11 d.p.c. With differentiation of the testicular cords, Dax1 expression becomes sexually dimorphic characterized by decreased expression in the fetal testis and continued expression in the fetal ovary where it appears to inhibit gonadal steroidogenesis. Dax1 may interfere with steroidogenesis by inhibiting StAR expression and/or Sf1-mediated transactivation.[77] Dax1 functions as an adaptor molecule to recruit the nuclear receptor corepressor N-CoR to the Sf1 promoter, thus interfering with transactivation.[78] Rather than functioning to promote ovarian differentiation, it appears that Dax1 acts as an anti-testis factor. In male mice with targeted disruptions of the Dax1 gene, abnormalities of testicular germinal epithelium and male infertility develop despite normal testicular appearance at birth. Female mice homozygous for targeted Dax1 mutation showed normal adult reproductive function. Testicular development is delayed in XY mice carrying extra copies of mouse Dax1, but sex reversal is not observed unless the mouse also carries weak alleles of the Sry gene.

At 11.5 days, Fgf9 is expressed in gonads of both sexes. By day 12.5, Fgf9 is detected only in testes. Mice homozygous for Fgf9-targeted deletions show male-to-female sex reversal with disruption of testis differentiation.[79] Loss of Fgf9 does not interfere with Sry expression, but its absence is associated with a premature decline in Sox9 expression leading to arrested Sertoli cell differentiation, upregulation of Wnt4 expression, and male-to-female sex reversal of germ cells.[80] Targeted deletion of Fgfr2 is associated with partial XY sex reversal.[81] Fgf9 signaling through Fgfr2 plays a major role in testicular differentiation by repressing Wnt4 expression to prevent activation of the female developmental program.[82]

At 11.5 to 12.5 d.p.c., in both sexes, Mullerian ducts arise from coelomic epithelium in the mesonephric region under the influence of Wnt4.[83] Male mice with homozygous targeted deletions of Wnt4 show normal testicular and Wolffian duct development, but Mullerian ducts never develop. At 11.5 d.p.c., Gata4 is expressed in the somatic cells of the bipotential gonad. By E13.5, Gata4 expression is up-regulated in XY Sertoli cells, down-regulated in XY interstitial cells, and down-regulated in XX gonads. The GATA4 protein interacts with several proteins such as Sf1/NR5A1 and friend of GATA protein 2 (Fog2). Mice with missense mutation in Gata4, that interfere with its interaction with Fog2, showed absence of testicular cord formation, abnormal Sertoli and Leydig cell development, and decreased expression of Sox9.[84]

Ovarian fate can no longer be considered as the default pathway caused by the absence of the Sry gene. Rather, specific genes influence development of the ovary. Gata2 is expressed in XX gonads with expression restricted to germ cells in XX gonads by E13.5.[85] By E13.5, Gata4 is down-regulated in XX gonads. The phenotype of female mice with homozygous deletions of Wnt4 includes absence of Mullerian duct derivatives, retention of Wolffian duct derivatives, and decreased oocyte development. In addition, the large coelomic

blood vessel (typical of testicular differentiation) develops in the ovaries of female mice homozygous for targeted deletion of Wnt4.[86] Wnt4 appears to be involved in differentiation of Mullerian ducts, repression of endothelial migration from the mesonephros into the gonad, and maintenance of oocyte development. Another ovary-specific gene is Foxl2; its expression begins at 12.5 d.p.c. Female mice with targeted Foxl2 deletions manifest hypoplastic uterine tubes, defective granulosa cell differentiation, premature depletion of the follicle pool, oocyte atresia, and infertility.[87] Follistatin and bone morphogenetic protein 2 (Bmp2) show similar patterns of expression becoming detectable at 11.5 d.p.c. Foxl2 and Wnt4 promote follistatin expression. Bmp2 appears to act in cooperation with Foxl2 during fetal ovary development.[88] Female-to-male sex reversal was observed in XX mice with targeted Rspo1 deletion. Activation of β-catenin in XY mice results in male-to-female sex reversal suggesting that β-catenin functions as a pro-ovarian and anti-testis signaling molecule.[89] Rspo1 appears to be upstream of Wnt4 in the signaling cascade and its actions are mediated by β-catenin.[90] In the adult female mouse, conditional loss of Foxl2 leads to post-natal ovary to testis transdifferentation with granulosa cells becoming Sertoli cells and increased Sox9 expression.[91]

Persistence of Mullerian duct derivatives and infertility were noted in male mice homozygous for targeted deletion of Wnt7a. One consequence of Wnt7a deletions is absence of Mullerian hormone receptor (Amh-rII) expression by Mullerian ducts. In females, although Mullerian duct derivatives develop, they are abnormal showing loss of uterine glands, reduction in uterine stroma, and deficient coiling and elongation of the Mullerian duct. Thus, Wnt7a appears to function as an epithelial-to-mesenchymal signal important in the sexually dimorphic differentiation of Mullerian duct derivatives.

The phenotype associated with targeted disruption of c-kit ligand, a receptor tyrosine kinase also known as Steel factor, is complete lack of germ cells in the gonads.[92] Affected animals are sterile, but show normal sexual differentiation.[93] In the Dazl knockout, germ cells migrate to the developing gonad, but genes required for male-specific and female-specific differentiation are not activated.[14] Both male and female mice homozygous for targeted deletion of the Stra8 gene show no overt phenotype apart from infertility.[94]

Mullerian inhibitory hormone (Amh) is first expressed at 12 d.p.c. Transcriptional regulation of Amh appears to involve both protein-protein and protein-DNA interactions with Sf1 and Sox9 being two of the proteins involved. Amh action is mediated by a heteromeric-signaling complex consisting of type I and type II serine/threonine kinase receptors. The Amh type II receptor binds Amh and recruits a type I receptor. Sf1 appears to regulate expression of the Amh type II receptor (Amh-rII) gene. The phenotypes of the Amh and Amh-rII knockout mice are identical. In XY mice, both male and female internal genital structures are found. Male mice are infertile because the retained uterus blocks passage of sperm through the vas deferens.

The phenotype of male mice with targeted deletion of desert hedgehog (Dhh) includes infertility and impaired spermatogenesis.[95] These mice also showed abnormal peripheral nerves with extensive minifascicles within the endoneurium.[96] During development, expression of Dhh is limited to Sertoli cells and Schwann cells in peripheral nerves.[97] In mice, other key molecules in the development of male external genital structures include sonic hedgehog (Shh), fibroblast growth factors, Wnts, Bmps, Hoxa13, and Hoxad13. Shh increases expression of Bmp4, Hoxa13, Hoxd13, and Ptc expression. Shh signaling, mediated through its membrane receptor complex consisting of Patched (Ptc) and Smoothed (Smo), plays a key role in regulation of mesenchymal-epithelial interactions and genital tubercle outgrowth. Expression of 5α-reductase in the genital tubercle mesenchyme occurs. Male mice homozygous for targeted deletions of the insulin-like hormone-3 (Insl3), Hoxa10, or leucine-rich repeat-containing Lgr8/Rxfp2 genes show bilateral cryptorchidism.[98-101]

Investigations involving normal and transgenic mice have provided much information about the genes and gene products involved in sexual differentiation. Nevertheless, differences do exist between rodents and humans. Thoughtful examination of patients with disorders affecting sexual differentiation has elucidated many of the factors involved in human sexual differentiation.

DISORDERS OF GONADAL DIFFERENTIATION

Wilms Tumor Gene

The Wilms tumor suppressor (WT1) gene, located at chromosome 11p13, plays an important role in both kidney and gonadal differentiation. Through alternative splicing, multiple translation start sites, and post-translational RNA editing, multiple isoforms can be derived from this one gene. The protein contains four zinc finger domains. The two major isoforms differ by the inclusion or exclusion of three amino acids, lysine, threonine, and serine (KTS), between the third and fourth zinc finger domains. Subnuclear localization studies have shown that the –KTS form colocalizes predominantly with transcription factors, whereas the +KTS form colocalizes mainly with splicing factors and plays a role in RNA processing.[102] The ratio of the +KTS/–KTS isoforms appears to be tightly regulated.

Depending on cellular context, WT1 can function as a transcriptional activator, a transcriptional repressor, or tumor suppressor. WT1 plays a role in the balance between mesenchymal-epithelial transitions.[103] The carboxyl terminal domain of the WT1 protein contains four zinc fingers that serve as the nucleic acid binding domain. Downstream target genes include WNT4 and AMHRII.[104] Distinct isoforms may have unique functions.

Phenotypic heterogeneity occurs among patients found to have mutations of the WT1 gene. Although Wilms tumor and genitourinary abnormalities can be associated with heterozygous WT1 deletions, only 6% to 15% of sporadic Wilms tumors are associated with WT1 mutations. Heterozygous deletions at chromosome 11p13 can be part of a contiguous gene deletion syndrome known as WAGR syndrome (Wilms tumor, aniridia, genitourinary anomalies, gonadoblastoma, and mental retardation). In

general, missense mutations in exons 6-9 are associated with severe gonadal dysgenesis and early-onset nephropathy. The −KTS form appears to be protective from development of Wilms tumor whereas mutations located in the N terminal repression domain are associated with development of Wilms tumor.

Denys-Drash syndrome is characterized by genitourinary anomalies, Wilms tumor, and nephropathy. Typically, the nephropathy begins during the first few years of life, manifests with proteinuria, and results in end-stage renal failure due to focal or diffuse mesangial sclerosis.[105,106] Among affected 46,XY individuals, the external genitalia can range from ambiguous to normal female. Affected 46,XX individuals show normal female external genital development. Internal genital differentiation varies because persistence of Wolffian and/or Mullerian structures is inconsistent. Typically, the gonads are dysgenetic in 46,XY individuals. The heterozygous missense mutations associated with Denys-Drash syndrome are believed to act in a dominant negative fashion.[107]

The features of Frasier syndrome include gonadal dysgenesis, progressive glomerulopathy, and an increased risk for gonadoblastoma. Wilms tumor is extremely rare in Frasier syndrome.[108] The typical renal lesion is focal glomerular sclerosis. The majority of cases are associated with a specific point mutation in intron 9 of WT1 associated with altered splicing and decreased amounts of the +KTS isoform.

Meacham syndrome is characterized by genital anomalies, cyanotic congenital heart defects, and pulmonary hypoplasia secondary to diaphragmatic abnormalities. In 46,XY infants, the spectrum of internal genital anomalies extends from presence of a uterus to a blind ending vaginal pouch. External genitalia have been described as ranging from normal female to ambiguous.[109]

In general, mutations in exons 1-5 appear to be associated with gonadal dysgenesis, Wilms tumor, and no nephropathy. Patients with exons 6-9 missense mutation manifest gonadal dysgenesis and early onset nephropathy; this phenotype has been attributed to a dominant negative mechanism whereas nonsense mutations are associated with haploinsufficiency. Analysis for WT1 mutations should be considered in patients with 46,XY DSD, complex hypospadias, and kidney disease.[110]

WNT4 Gene

WNT4 is a secreted molecule that binds to members of the frizzled family of receptors, resulting in transcriptional regulation of target genes. WNT4 increases follistatin expression, which inhibits formation of the coelomic vessel (anti-testis action) and supports ovarian germ cell survival (pro-ovarian action).[111] Duplication of the WNT4 gene, located at chromosome 1p31-1p35, has been associated with 46,XY male-to-female sex reversal. One such patient presented with ambiguous external genitalia accompanied by severe hypospadias, fibrous gonads, remnants of both Mullerian and Wolffian structures, cleft lip, microcephaly, and intrauterine growth retardation.[112] A female-to-male sex reversed patient with kidney, adrenal, and lung dysgenesis was found to have a homozygous loss-of-function WNT4 mutation.[113] Loss-of-function WNT 4 mutations have been detected in 46, XX women with primary amenorrhea secondary to Mullerian duct abnormalities and androgen excess.[1114-116] The phenotypes of these patients support the hypothesis that WNT4 plays a role in ovarian differentiation.

SF1/NR5A1 Gene

The SF1/NR5A1 gene, located at chromosome 9q33, codes for a 461-amino-acid protein. This protein, also known as Ad4BP is involved in adrenal, gonadal, and hypothalamic development and plays an essential role in the regulation of steroidogenesis. Two distinct domains, a DNA-binding domain and a highly conserved Ftz-F1 box, are present in the protein. Genetic changes identified in the NR5A1 gene have included nonsense, frameshift, and missense changes that have affected DNA-binding and gene transcription. Most mutations are heterozygous. In some instances, these genetic changes have been inherited from the mother. Phenotype-genotype-functional correlations have yet to be established due to the phenotypic and genetic heterogeneity. Analyses of gonadal tissue obtained from 46,XY individuals shows intracellular lipid accumulation. In vitro functional studies indicate defective transactivation of CYP17A1. Individuals with SF1/NR5A1 mutations require longitudinal assessments with attention to changing gonadal and adrenal function.[117]

Two 46,XY patients with female external genitalia, Mullerian structures, gonadal dysgenesis, and adrenal insufficiency have been described. One patient was heterozygous for a de novo G35E mutation, which appears associated with decreased transactivation of NR5A1 responsive reporter genes. The second patient was homozygous for a R92Q change inherited in an autosomal recessive manner.[118] A 46,XX female with adrenal insufficiency, adrenal hypoplasia, and an NR5A1 mutation showed normal ovarian morphology.[119] Haploinsufficiency of NR5A1 can also manifest as a predominantly gonadal phenotype characterized by hypergonadotropic hypogonadism associated with delayed puberty or premature ovarian failure in 46,XX individuals.

The phenotypic spectrum associated with NR5A1 mutations has greatly expanded. Heterozygous mutations associated with 46,XY gonadal dysgenesis in the absence of adrenal insufficiency have been reported. These patients can present with ambiguous genitalia, urogenital sinuses, and small inguinal testes. Mullerian structures are generally absent. Penoscrotal hypospadias, bilateral anorchia, and testicular regression syndrome have been described. The phenotypic spectrum extends to include 46,XY girls who present with delayed puberty, primary amenorrhea, and low testosterone concentrations in the absence of adrenal insufficiency.[120]

Chromobox Homolog 2 (CBX2)

The CBX2 gene, mapped to chromosome 17q25, is a subunit of the chromatin-associated polycomb repressive

complex 1. As noted earlier, the polycomb group proteins function to establish imprinting patterns in developmental control. Loss of function missense mutations in CBX2 were identified in a 46,XY infant who had normal female external genitalia and a normal uterus; ovarian biopsy showed normal ovarian tissue with primordial follicles. The phenotype of 46,XY sex reversal in this patient suggests that this protein functions upstream of *SRY* and that it may act to repress ovarian development in developing XY gonads.[121]

46,XY Disorders of Sex Development (Gonadal Dysgenesis)

Phenotype/genotype studies of 46,XY sex-reversed patients played a major role in the localization of the Y chromosome gene responsible for the initial signal-promoting testicular differentiation. Subsequently, this gene was identified to be the *SRY* gene located at Yp11.3 near the pseudoautosomal region on Yp. Identification of mutations in SRY in 46,XY females confirmed the vital role of SRY in testicular differentiation.

Individuals with sex chromosome DSDs due to partial or mixed gonadal dysgenesis usually present with asymmetrical genital ambiguity (Figure 5-2). Somatic features of Turner syndrome such as short stature, webbed neck, cubitus valgus, and gonadal failure may be present. Multiple cell lines, including a monosomic X cell line, may be detected. The most common karyotype is 45,X/46,XY. However, there is much phenotypic heterogeneity associated with 45,X/46,XY karyotype in that internal and external genital differentiation ranges from normal male to ambiguous to female. Whereas the typical histologic features consist of poorly developed seminiferous tubules surrounded by wavy ovarian stroma, gonadal differentiation can range from normal testis to streak gonads. At the time of puberty, virilization can occur.

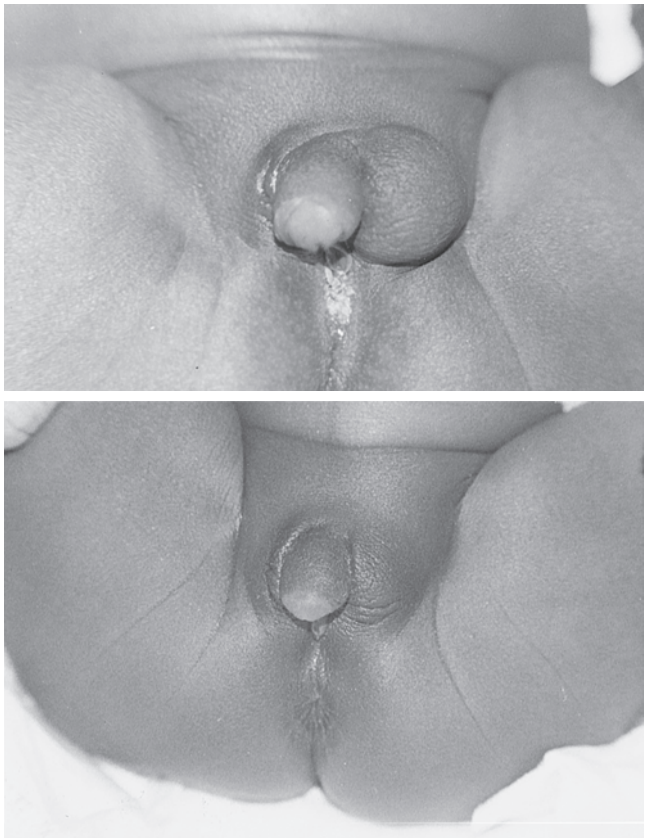


FIGURE 5-2 ■ Genital ambiguity with asymmetry in patient with 46,XY disorder of sexual differentiation (mixed gonadal dysgenesis).

Individuals with sex chromosome DSDs due to gonadal dysgenesis have an increased risk of developing gonadal tumors such as gonadoblastoma or dysgerminoma because a dysgenetic gonad carrying a Y chromosome has an increased risk for neoplastic changes.[121] Although gonadal tumors typically do not develop until the second decade of life, they can occur earlier.[122]

SRY

SRY is a single exon gene encoding a 204-amino-acid protein. The protein contains an HMG DNA-binding domain flanked by nuclear localization signals (NLS). The majority of sex-reversing SRY mutations are located in the HMG/NLS domain and affect DNA-binding affinity, DNA bending ability, or nuclear localization. Mutations located in the N-terminal and C-terminal domains have been identified in 46,XY sex reversed individuals. Mutations in the non-HMG domains may influence transcriptional activation, DNA binding, or protein-protein interactions.[123] Paternal mosaicism for SRY mutations in which different cells carry different *SRY* genes has been described.[124,125] More puzzling are the pedigrees in which fathers and unaffected brothers carry the identical mutant SRY allele as the propositus.[126,127] These paradoxical findings implicate involvement of other genes, gene-gene interactions, and gene-environment interactions in the process of sexual differentiation. Nevertheless, only 15% to 20% of cases of 46,XY DSD due to gonadal dysgenesis can be attributed to SRY mutations. Mutations in the *SRY* gene have also been reported in patients with stigmata typical of Turner syndrome and 45,X/46XY karyotype.[128]

SOX9

SOX9 is a member of the SRY-related HMG domain gene family located at chromosome 17q24.3-17q25.1. It is a 508-amino-acid protein containing an 80-amino-acid HMG domain involved in DNA binding and bending; a 41-amino-acid proline, glutamine, and alanine motif; and a C-terminal transactivation domain. SOX9 is highly expressed in Sertoli cells where it functions to promote Sertoli cell differentiation. Mutations in SOX9 can affect DNA-binding affinity, DNA bending ability, nuclear import, transactivation, and nuclear export. Haploinsufficiency is the mechanism responsible for the many of the consequences of SOX9 mutations. Somatic cell mosaicism, de novo germline mutations, and mitotic gene conversion events have been described. Patients have been reported with balanced chromosome rearrangements involving the

17q24 region and with deletions upstream of SOX9 implicating cis-regulatory elements upstream and/or downstream of the gene as additional mechanisms responsible for this disorder.

Heterozygous loss of function mutations in the SOX9 gene are associated with autosomal-dominant campomelic dwarfism and male-to-female sex reversal.[130] Features of campomelic dwarfism include congenital bowing of long bones, hypoplastic scapulae, 11 pairs of ribs, narrow chest, congenital dislocated hips, and clubfeet. Facial features include micrognathia, cleft palate large head, flat nasal bridge, and low-set malformed ears. Although the severity of the bone malformations varies, most affected individuals die shortly after birth due to respiratory failure. Approximately 75% of affected 46,XY fetuses show sex reversal, with external genital differentiation ranging from ambiguous to female. Gonadal dysgenesis and persistence of Mullerian duct derivatives are typical. Phenotypic heterogeneity with differing phenotypes including ovotesticular DSD and complete sex reversal has been described in affected siblings.[131] Acampomelic dysplasia lacks the typical limb anomalies, but manifests other features associated with the syndrome such a small thorax, limb shortening, and respiratory distress. Patients with acampomelic dwarfism and sex reversal have been reported.[132] Some affected individuals show longer survival presumably due to milder phenotype. Familial campomelic dysplasia associated with a deletion upstream of SOX9 has been described in a mother and 46,XY child with female external genitalia, normal uterus, and streak gonads.[133]

DAX1

DAX1 is an orphan nuclear receptor that lacks a typical zinc finger DNA-binding domain. The gene (NROB1) coding for DAX1 is located on the short arm of the X chromosome and consists of two exons. The N terminus of the 470-amino-acid protein contains a novel DNA-binding domain whereas the C terminus shows characteristics of a nuclear hormone receptor ligand-binding domain. DAX1/NROB1 is expressed throughout the HPG axis. The DAX1 protein functions as a transcriptional repressor of many genes, including NR5A1 and some steroidogenic enzyme genes.

Duplication of the DAX1/NROB1 locus is associated with male-to-female sex reversal.[134] External genital differentiation ranges from female to ambiguous. Descriptions of internal genitalia include the presence of Mullerian and Wolffian structures. Gonads are typically described as streaks. Using high-resolution array comparative genome hybridization (CGH), a submicroscopic interstitial duplication of the DAX1/NROB1 gene was discovered during thorough evaluation of two sisters.This 637-kb duplication included DAX1, four MAGEB genes, CXorf21, glycerol kinase (GK), and part of the MAP3K7IP3 gene. The older sister had presented with primary amenorrhea, female external genitalia, 46,XY karyotype, and gonadal dysgenesis; the younger phenotypic female sister was prepubertal, but found to have 46,XY karyotype.[135]

Loss-of-function mutations are associated with X-linked adrenal hypoplasia congenita (AHC). In this disorder, development of the fetal adrenal cortex is normal. However, the adult or definitive adrenal cortex fails to develop. Adrenal insufficiency may not be evident in the immediate neonatal period, but may become obvious during early infancy. Although adrenal insufficiency generally manifests in infancy or early childhood, phenotypic heterogeneity in severity and age at presentation occurs.[136] Unilateral or bilateral cryptorchidism can also occur. At the age of expected puberty, hypogonadotropic hypogonadism due to hypothalamic and pituitary dysfunction may occur among affected males.[137] Delayed puberty has been recognized in heterozygous females.[138] One female homozygous for DAX1 mutations has been reported. Her phenotype was hypogonadotropic hypogonadism.[139] As part of a contiguous gene deletion syndrome, X-linked AHC can be associated with glycerol kinase deficiency, Duchenne muscular dystrophy, ornithine transcarbamylase deficiency, and mental retardation.[140]

Nonsense mutations have been identified throughout the gene. Missense mutations account for 20% of mutations associated with AHC. These mutations tend to cluster in the carboxyl terminal of the protein corresponding to the putative ligand-binding domain and impair the transcriptional repression activity of the protein. One missense point mutation, located in the hinge region of the protein, was identified in an 8-year-old girl with clinical and laboratory features indicative of adrenal insufficiency. Additional studies showed that this mutation hindered nuclear localization of the protein. Curiously, the hemizygous father and heterozygous younger sister of the proband did not manifest the AHC phenotype.[141]

Desert Hedgehog

The desert hedgehog (DHH) gene is located on chromosome 12q12-q13.1 and encodes a protein consisting of 396 amino acids. DHH is a member of the hedgehog family. Hedgehog family proteins play roles in regulating morphogenesis. One patient's phenotype consisted of female external genitalia, polyneuropathy, a testis on one side, and a streak gonad on the other side. Additional evaluation of this patient revealed 46,XY karyotype, and homozygosity for a single nucleotide substitution at the initiation codon, ATG→ACG, of the DHH gene. This variation was predicted to abolish initiation of translation at the normal start site. Histologic analysis of the sural nerve revealed extensive formation of minifascicles within the endoneurium.[142] Mutations in the DHH gene have been reported in several additional 46,XY patients with gonadal dysgenesis and hypoplastic Mullerian structures.[143,144]

GATA4

GATA proteins comprise a family of organ-specific transcription factors and contain two zinc finger domains. Mutations in GATA4, mapped to 8p23.1, are typically associated with congenital heart disease. Three males in one family were reported to manifest congenital heart disease and varying abnormalities in male sex

development were identified to carry a heterozygous missense GATA4 mutation; the female carrier had congenital heart disease, but no ovarian phenotype was noted. This mutation abrogated the ability of the GATA4 protein to interact with the FOG2 protein.[145]

FOG2

The friends of GATA (FOG) protein are transcriptional cofactors that modulate the activity of GATA proteins. The FOG2 gene maps to chromosome 8q23.1. One boy with a balanced translocation and breakpoint with intron 4 of the FOG2 gene had congenital heart disease, retractile testes, and hypergonadotropic hypogonadism. At 11.5 years, he was noted to have early pubertal changes in increased penile size and pubic hair development. Subsequently, he showed no spontaneous pubertal development and was noted to have small firm testes.[146]

MAP3K1

The MAP3K1 gene is located on the long arm of chromosome 5. This gene encodes a protein involved in mitogen-activated protein kinase (MAPK) signaling. genetic mapping studies were used to identify mutations in this gene in two families with multiple affected members. Phenotypes of affected individuals include 46,XY complete gonadal dysgenesis, perineoscrotal hypospadias, and cryptorchidism. Inheritance appears to be sex-limited autosomal dominant.[147]

Chromosome 9p Monosomy

Monosomy for distal chromosome 9p has been reported in male-to-female sex reversal. Most deletions associated with sex reversal have involve the region 9p24.3 where the three DMRT genes are located.[148,149] DMRT1 appears to be involved in Sertoli and cell differentiation. External genitalia have been described as ambiguous or female. The external genitalia may appear symmetric or asymmetric. Differentiation of internal genitalia is highly variable, with the presence of Mullerian and Wolffian remnants being reported. In addition to sex reversal, clinical features include mental retardation, low-set ears, trigonocephaly, wide nasal bridge, single palmar creases, heart defects, epilepsy, and scoliosis. Genital anomalies reported in XY individuals include gonadal dysgenesis, ovotestis, hypospadias, penoscrotal inversion, and cryptorchidism. Gonadoblastoma have been reported.[150] Although phenotype/genotype correlations are not apparent, haploinsufficiency for DMRT1 appears to be sufficient to cause gonadal dysgenesis.[151]

ATR-X Syndrome

ATR-X (α-thalassemia, mental retardation, X-linked protein) syndrome is an X-linked disorder characterized by mild alpha-thalassemia, severe mental retardation, and genital abnormalities. This disorder is due to mutations in the ATRX (also known as XH2 or XHP) gene located at Xq13.3.[152] The ATRX gene product is a member of the SWI/SNF DNA helicase family and contains functional domains involved in protein-protein and protein-DNA interactions. The protein appears to function as an epigenetic factor involved in transcriptional regulation, nuclear architecture, and chromosome stability.[153] The ATRX and DMRT1 expression patterns overlap in germ and somatic cells.[154]

Urogenital anomalies occur in approximately 80% of patients and include ambiguous genitalia, cryptorchidism, hypoplastic scrotum, hypospadias, shawl scrotum, and small penis.[155] The urogenital anomalies are associated with mutations that truncate the protein and with mutations located in the plant homeodomain-like domain. Other typical features include short stature, psychomotor retardation, microcephaly, seizures, talipes equinovarus, and gastrointestinal problems. The facies are described as coarse with midface hypoplasia, short nose, and widely spaced incisors. The hemoglobin H inclusions can be demonstrated on brilliant cresyl blue stained peripheral blood smears.[156] Typically, Wolffian duct structures are present whereas Mullerian duct structures and germ cells are absent indicating at least partial Sertoli and Leydig cell function. Histologic studies of testes suggest aberrant Leydig cell development.[157] Phenotype-genotype correlations are inconsistent. Most carrier females show preferential inactivation of the X chromosome carrying ATR-X mutations. Greater than 75% of cases are inherited from carrier mothers. Carpenter-Waziri, Juber-Marsidi, and Smith-Fineman-Myers syndromes and X-linked mental retardation with spastic paraplegia are also associated with mutations in the ATRX gene.[158]

Vanishing Testes

The terms *testicular regression syndrome* and *vanishing testes* are used to describe testicular absence in boys with undescended testes. In some instances, this situation is associated with ambiguous genitalia and undervirilization which presumably represents regression of testicular tissue occurring between 8 and 14 weeks of gestation. Physical findings reflect duration of testicular function. At operation, a rudimentary spermatic cord and nubbin of testicular tissue may be identified. Histologic examination of the testicular nubbins often reveals hemosiderin-laden macrophages and dystrophic calcification.[159] It has been suggested that an antenatal vascular accident associated with antenatal testicular torsion is a cause of testicular regression.[160] Although usually sporadic, familial testicular regression has been described.[161]

Multiple Congenital Anomalies

CDKN1C

In affected 46,XY infants, the IMAGe syndrome is characterized by intrauterine growth retardation, metaphyseal dysplasia, adrenal hypoplasia, cryptorchidism, and micropenis in the absence of DAX1 or NR5A1/SF1 mutations.[162] Both sporadic and familial cases have been described. Heterozygous missense mutations in the

CDKN1C gene have been detected in some cases. This gene is located at chromosome 11p15, encodes a protein involved in inhibiting cell cycle progression, and is imprinted with expression of the maternal allele. Mutations associated with the IMAGe syndrome appear to be localized to the PCNA-binding domain resulting in gain of function with excessive inhibition of growth and differentiation. Curiously, mutations affecting the cyclin-dependent kinase binding domain of this protein are associated with overgrowth syndromes such as Beckwith-Wiedemann syndrome.[163]

GLI3

Autosomal dominant Pallister-Hall syndrome, mapped to chromosome 7p13, is associated with micropenis, hypospadias, hypothalamic hamartoma, postaxial polydactyly, and imperforate anus. Mutations in the GLI3 gene are associated with Pallister-Hall syndrome.[164] In male patients, hypospadias, micropenis, and bifid or hypoplastic scrotum have been described; affected female patients had hydrometrocolpos and/or vaginal atresia.[165]

ARX

Mutations in the Aristaless-related homeobox gene (ARX), mapped to the X chromosome, are associated with intellectual disability.[166] Additional features in affected XY individuals include genital ambiguity, X-linked lissencephaly, absence of the corpus callosum, and hypothalamic dysfunction including temperature instability.[167]

KAT6B

The genitopatellar syndrome is a rare disorder characterized by skeletal dysplasia, genital anomalies, craniofacial defects, and developmental delay. The skeletal features include hypoplastic or absent patellae, flat temporal bones, and brachydactyly. Genital anomalies include hypoplastic labia, clitoromegaly, scrotal hypoplasia, and cryptorchidism. Cardiac anomalies and hydronephrosis have been described in several patients. Mutations in the lysine acetyltransferase 6 gene, KAT6B, located at chromosome 10q22have been identified in affected patients.[168] The KAT6B protein is the catalytic subunit of a complex involved in chromatin modeling. The majority of defective alleles are de novo mutations that generate truncated proteins that disrupt histone acetylation.[169]

CHD7

The CHARGE syndrome is associated with mutations in the chromodomain helicase DNA binding protein-7 (CHD7). Features of this syndrome include eye coloboma, heart malformations, choanal atresia, short stature, genital anomalies, ear abnormalities, and hearing loss. Micropenis and cryptorchidism are found in males. Hypogonadotropic hypogonadism can occur. Although usually sporadic, autosomal dominant cases have been described. The CHD7 gene, located at chromosome 8q12.1-q12.2, codes for a large protein that participates in chromatin remodeling and transcription.

Ovotesticular Disorder of Sex Development

Ovotesticular DSD is defined as presence of ovarian tissue with follicles and testicular tissue with seminiferous tubules in the same individual. Although an ovotestis is the most commonly identified gonad in ovotesticular DSD, there can be an ovary on one side and a testis on the other. In most ovotestes, ovarian and testicular tissue show distinct separation in an end-to-end arrangement. Karyotypes are usually 46,XX. Mosaic karyotypes (46,XX/46,XY and 46,XX/47,XXY) have been described.[170] In some instances, Y chromosomal material such as the *SRY* gene can be detected by PCR amplification. However, ovotesticular DSD in the absence of Y chromosomal material has been reported.[171] In one patient in whom the peripheral blood karyotype was 46,XX, molecular genetic analysis showed a deletion of the promoter region of the *SRY* gene in the testicular tissue of an ovotestis.[172]

Several pedigrees in which both XX males and XX individuals with ovotesticular disorder coexist have been described.[173,174] These families likely represent incomplete penetrance of mutations of genes involved in sexual differentiation.[175] Although most patients present in infancy or childhood, phenotypic males can present with bilateral gynecomastia.[176] Available outcome data indicate rare fertility and only among women. In a series of 33 patients followed longitudinally, germ cells identified in the testicular tissue during infancy degenerated—resulting in azoospermia. Although normal menstrual cycles have been reported in a few females, no pregnancies were documented in one series.[171] Yet, another report indicated that pregnancies can occur in some females with ovotesticular DSD.[177]

46,XX Testicular Disorder of Sex Development

Testicular DSD is characterized by a male phenotype with a 46,XX karyotype. The frequency of the XX male syndrome is approximately 1 in 25,000 males.[178] This form of DSD can be sub classified as SRY-positive and SRY-negative groups. The SRY-positive 46,XX males generally have normal male external genitalia, small azoospermic testes, hypergonadotropic hypogonadism, and often present with infertility.[179] In most instances, the *SRY* gene is located on an X chromosome due to recombination between the X and Y chromosomes. However, translocation to an autosome can occur. One example was the incidental finding small testes, azoospermia, and translocation of the *SRY* gene onto the terminal end of chromosome 16q in a 61-year-old 46,XX man.[180]

Approximately 10% of these patients are SRY negative. The phenotypic spectrum ranges from genital ambiguity to normal male external genitalia[181] Molecular etiologies are more diverse for the SRY-negative 46,XX patient. Overexpression of the SOX10 gene at 22q13was found in one patient with 46,XX sex reversal

in association with multiple congenital anomalies.[182] Duplication of SOX9 was described in one family; all affected family members had normal male secondary sexual characteristics and azoospermia.[183] The SOX3 gene maps to Xq27, a highly conserved region of the X chromosome. Gain-of-function SOX3 mutations have been associated with XX male sex reversal.[184,185] Hypoplasia of the anterior pituitary and ectopic position of the posterior pituitary has been described in two patients.[186] It has been speculated that this group of disorders is due to impaired action of other genes involved in male sexual diffentiation.[187]

RSPO1

The R-spondin 1 (RSPO1) gene codes for a secreted furin-like domain protein that stabilizes β-catenin in the Wnt-signaling pathway. Mutations in this gene, located at chromosome 1p34, are associated with 46, XX sex reversal. This gene was initially identified by investigating individuals with palmoplantar hyperkeratosis, squamous cell carcinoma of skin, and sex reversal in one family.[188] Affected XX, sex-reversed individuals lack Mullerian structures.[189] A 46,XY individual, homozygous for mutations in RSPO1, fathered 2 children. An XX individual with ovotesticular disorder and palmoplantar keratoderma was found to be homozygous for a splicing mutation in the RSPO1 gene.[190]

XX Disorder of Sex Development/ Premature Ovarian Failure

46,XX gonadal dysgenesis is a rare disorder associated with delayed puberty and premature menopause associated with hypergonadotropic hypogonadism. Affected individuals typically present with lack of spontaneous pubertal development, primary amenorrhea, and uterine hypoplasia. Typically, external genital development is female.

More recently, it has been recognized that development of the ovary is an active process involving multiple genes. However, mutations of genes involved in ovarian development are not typically associated with ambiguous genitalia. Rather, these disorders typically present with delayed puberty, primary amenorrhea, and premature ovarian failure.[15] In these disorders, follicles may fail to develop or may undergo premature atresia. Mutations in genes involved in ovarian development such as FOXL2, NOBOX, and FIGLA have been described. Mutations in the FSH receptor gene have been described as an autosomal recessive etiology. A heterozygous missense mutation in BMP15 was associated with X-linked XX gonadal dysgenesis.[191] Ovarian leukodystrophy is a leukoencephalopathy characterized by vanishing white matter and ovarian dysgenesis associated with mutations in the eukaryotic initiation factor genes, EIF2B2, EIF2B4, and EIF2B5.[192] A mutation in the proteasome 26 subunit, ATPase, 3-interacting protein (PSMC3IP) gene has been described in autosomal recessive 46,XX gonadal dysgenesis; this loss of function mutation reduces estrogen-induced transcriptional activation of the protein encoded by this gene.[193]

FOXL2

Forkhead box L2 (FOXL2) is a member of the winged helix/forkhead transcription family. The protein contains a DNA-binding domain, which is primarily located in the nucleus. The protein contains three α-helices. Mutations in FOXL2, located at chromosome 3q23, are associated with the autosomal dominant blepharophimosis ptosis epicanthus inversus syndromes (BPES).[194] BPES is characterized by eyelid dysplasia consisting of small palpebral fissures (blepharophimosis), ptosis, epicanthus inversus, and a broad nasal bridge. The phenotype of BPES type 1 consists of eye findings and premature ovarian failure. Patients with BPES type 2 manifest only the eye features. Patients heterozygous for FOXL2 mutations generally experience spontaneous pubertal development culminating in menarche, but undergo premature follicle depletion leading to premature ovarian failure. The histologic appearance of ovaries in patients with BPES type 1 is reported to vary from the presence of primordial follicles with some atretic follicles to the complete absence of follicles.[195] Mutations generating nonsense mutations, predicted to generate truncated proteins lacking the transrepression domain, are associated with BPES type 1. Expansion of the polyalanine tract is associated with BPES type 2.[194-196]

One major role of FOXL2 in the ovary appears to be transrepression of genes involved in steroidogenesis such as StAR, P450scc, and aromatase to prevent premature differentiation and proliferation of granulosa cells.[197] Loss of the transrepressor activity due to nonsense mutations likely contributes to the premature depletion of ovarian follicles and POF.[198] One patient with ovotesticular disorder was found to have expression of both FOXL2 and SOX9.[37] However, in general, expression of these proteins is mutually exclusive.

Curiously, in goats mutations in this locus are responsible for the autosomal dominant phenotype characterized by the absence of horns in male and female goats (polledness) and XX female-to-male sex reversal in a recessive manner.[199]

NOBOX

The newborn ovary homeobox (NOBOX) gene, expressed in the ovary, encodes a transcription factor involved in the transition from primordial to primary follicles. The NOBOX gene is located at chromosome 7q35. The phenotype of patients with NOBOX mutations includes delayed puberty with primary amenorrhea and secondary amenorrhea.[200]

FIGLA

Factor in germline alpha (FIGLA) is a germ cell specific transcription factor that is located at chromosome 2p13.3. FIGLA is expressed in the human fetal ovary. Women with FIGLA mutations have been reported to have secondary amenorrhea and ovaries devoid of follicles.[201]

DISORDERS OF CHOLESTEROL AND STEROID BIOSYNTHESIS (also discussed in Chapter 13)

Genital ambiguity can be due to abnormalities in the biosynthetic pathways involved in cholesterol, cortisol, and sex steroid synthesis. Steroidogenesis refers to the multiple enzyme processes through which cholesterol is converted to biologically active steroid hormones. This process depends on the coordinated regulation of cholesterol uptake into cells, transfer into mitochondria, and actions of tissue specific enzymes.[202] Many of the steroidogenic enzymes are cytochrome P450 heme-containing proteins that absorb light at 450 nm in their reduced state or hydroxysteroid dehydrogenases. The P450 enzymes receive electrons from the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) and use their heme center to mediate catalysis. The type 1 enzymes are located in mitochondria and receive electrons from NADPH from a flavoprotein, ferredoxin reductase, and an iron-sulfur protein, ferredoxin. The type 2 enzymes are located in the endoplasmic reticulum and receive electrons from NADPH from another protein, P450 oxidoreductase.

The hydroxysteroid dehydrogenase enzymes utilize NAD+ and NADP+ as cofactors; these enzymes do not contain heme groups. The hydroxysteroid dehydrogenases are classified into two groups. One group is the short-chain dehydrogenase reductase family. The other group is the aldo-keto reductase family. These enzymes function as dehydrogenases and reductases. Although many of these enzymes show both dehydrogenase and reductase activities in vitro, most function unidirectionally.[203]

During fetal life, steroidogenic enzymes are expressed in placenta, testis, and adrenal (Figure 5-2). The fetal testis secretes testosterone that is converted to dihydrotestosterone (DHT) in target tissues such as the prostate and external genitalia. Inborn errors of testosterone biosynthesis can lead to undervirilization and ambiguous genitalia in 46,XY fetuses. Specific proteins necessary for testosterone biosynthesis include NR5A1, LH receptor, steroidogenic acute regulatory peptide (StAR), 17α-hydroxylase/17,20-lyase, 3β-hydroxysteroid dehydrogenase type 2, 17β-hydroxysteroid dehydrogenase type 3, P450-oxidoreductase, and 5β-reductase type 2 (Figure 5-3). Inborn errors of glucocorticoid biosynthesis are often associated with the virilizing congenital adrenal hyperplasias.

The fetal adrenal cortex is derived from coelomic epithelium and consists of two major zones: the fetal zone and the adult zone. The fetal zone is primarily responsible for DHEA synthesis, which is then sulfated to

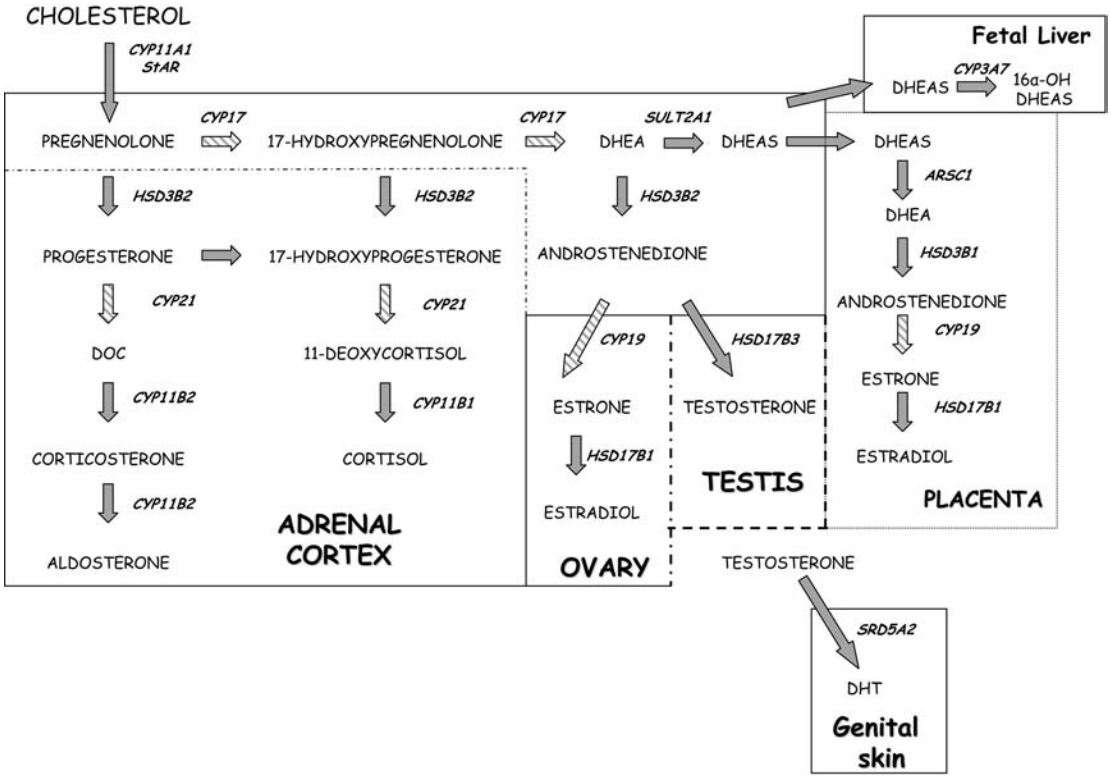


FIGURE 5-3 ■ Diagram of classical steroidogenic pathways. Substrates, products, and genes involved in adrenal, ovarian, testicular, and placental steroidogenesis are indicated. Genes are 17α-hydroxylase/17,20-lyase (CYP17), 3β-hydroxysteroid dehydrogenase (HSD3B2), 21-hydroxylase (CYP21), 11β-hydroxylase (CYP11B1), aldosterone synthase (CYP11B2), aromatase (CYP19), 17β-hydroxysteroid dehydrogenase type 1 (HSD17B1), 17β-hydroxysteroid dehydrogenase type 3 (HSD17B3), 5α-reductase type 2 (SRD5A2), sulfotransferase (SULT2A1), and steroid sulftase/arylsulfatase C (ARSC1). CYP3A7 is a cytochrome P450 enzyme expressed in fetal liver, where it catalyzes the 16α-hydroxylation of estrone (E1) and DHEA. Its expression decreases postnatally. Steroidogenic enzymes that utilize P450 oxidoreductase, a flavoprotein encoded by POR, to transfer electrons are indicated by hatched arrows.

provide substrate for placental estrogen biosynthesis (see Figure 5-3). The adult zone, which after birth differentiates into the three zones of the adult adrenal cortex, is primarily responsible for cortisol biosynthesis. By 10 weeks of gestation, the adrenal is secreting DHEAS and the hypothalamic-pituitary-adrenal axis is functional. Prior studies indicated that CYP17A1 was not expressed in the human placenta.[204] Newer in vitro data indicate CYP17A1 is expressed in the trophoblast and can generate androgens.[205]

Through investigations of the tammar wallaby and patients with disordered steroidogenesis, the presence of another pathway leading to dihydrotestosterone synthesis was uncovered.[206] In this "backdoor pathway", 17-hydroxyprogesterone (17-OHP) undergoes 3α- and 5α-reduction followed by 17,20-lyase, 17β-hydroxysteroid dehydrogenase, and 3α-oxidation steps to generate dihydrotestosterone in the absence of the intermediates, DHEA, androstenedione, and testosterone, requisite to the classical pathway. In humans, since 17-OHP is not a favored substrate for the 17,20-lyase reaction, this pathway acquires functional importance in disorders of steroidogenesis associated with increased 17-OHP concentrations.

Luteinizing Hormone Choriogonadotropin Receptor Gene

Leydig cell hypoplasia is an autosomal recessive disorder characterized by failure of testicular Leydig cell differentiation secondary to inactivating LHCGR mutations and target cell resistance to LH.[207] The LHCGR gene is mapped to chromosome 2p21. The 674-amino-acid protein is a seven-transmembrane domain G-protein–coupled receptor. Specific mechanisms through which the loss-of-function mutations induce LH resistance include decreased receptor protein, decreased ligand binding, altered receptor trafficking, and impaired ability to activate G_s. Exon 6A in the LHCGR gene can generate three splice variants; the consequence of one specific mutation in exon 6 was aberrant gene transcription which altered ratios of the LHCGR transcripts.[208]

Complete inability to respond to hCG or LH results in substantially decreased Leydig cell testosterone biosynthesis. The phenotype of affected 46,XY infants ranges from undervirilization to female external genitalia. Affected individuals raised as females often seek medical attention for delayed breast development. Mullerian duct derivatives are absent because AMH is secreted by the unaffected Sertoli cells. Testes are typically inguinal or intra-abdominal. Laboratory studies show elevated LH, low testosterone, and normal FSH concentrations. There is no significant testosterone response to hCG stimulation. Testicular histology reveals absence of Leydig cells, but relatively preserved seminiferous tubules and testicular volume due to normal FSH secretion and responsiveness. Undervirilization with hypospadias, micropenis, and cryptorchidism can occur with incomplete loss-of-function missense mutations. Genetic females, sisters of affected 46,XY individuals, who carry the identical mutations show normal female genital differentiation and normal pubertal development but have amenorrhea and infertility.[209-211] The uterus is small to normal in size. Ovarian cysts may develop. Estrogen concentrations generally fail to achieve the threshold required for ovulation. Genetic analysis may be helpful to distinguish LHCGR mutations from other disorders affecting testosterone biosynthesis.[212]

Smith-Lemli-Opitz Syndrome

Several enzymes catalyze the conversion of lanosterol to cholesterol. Decreased activity of these enzymes leads to cholesterol deficiency. The enzyme, 7-dehydrocholesterol reductase, encoded by the 7-dehydrocholesterol reductase (DHCR7) gene catalyzes the last step in cholesterol biosynthesis. Smith-Lemli-Opitz (SLO) syndrome is due to mutations in the 7-dehydrocholesterol reductase (DHCR7) gene located at chromosome 11q12-q13. Mutations in the DHCR7 gene are associated with elevated 7-dehydroxycholesterol concentrations. Demonstration of elevated 7-dehydroxycholesterol concentrations is required to confirm the diagnosis.

As anticipated, mutations in this cholesterol biosynthetic pathway are associated with decreased cholesterol and accumulation of sterol intermediates proximal to the defective enzyme. Decreased cholesterol concentrations lead to decreased steroid concentrations because cholesterol serves as the precursor for glucocorticoid, mineralocorticoid, and sex steroid biosynthesis. In addition to its role as the precursor for steroid biosynthesis, cholesterol modification of sonic hedgehog protein (SHH) is necessary for normal signaling through its cognate receptor, Patched (PTCH1), which contains a sterol sensing domain. In fibroblasts from patients with SLO, SHH signaling is impaired. Available data indicate that accumulation of sterol intermediates rather than cholesterol deficiency interferes with midline fusion of facial structures. These observations shed light on the molecular pathophysiology responsible for the cleft palate associated with SLO.[213]

Clinical features of this autosomal recessive disorder include multiple malformations, urogenital anomalies, mental retardation, failure to thrive, facial abnormalities, developmental delay, and behavioral abnormalities. The most common urogenital abnormalities include male-to-female sex reversal, hypospadias, and cryptorchidism. The facial abnormalities consist of a broad nose, upturned nares, micrognathia, short neck, and cleft palate.[213,214] Typical limb anomalies comprise short thumbs, syndactyly of the second and third toes, and post-axial polydactyly.

Infants often have hypotonia, feeding problems, and failure to thrive. Tube feedings may be necessary due to the poor weight gain. Although the efficacy remains unclear, dietary cholesterol supplementation is often prescribed.[214] The limited cholesterol biosynthesis may be associated with adrenal insufficiency, especially during stress. Thus, stress glucocorticoid dosing may be beneficial.

Prenatal diagnosis can be performed by measurement of amniotic fluid 7-dehydrocholesterol concentrations.[215] Low plasma estriol, elevated blood 16α-hydroxyestrogens

concentrations, and elevated urinary Δ^7 and Δ^8 unsaturated C_{18}, C_{19}, and C_{21} dehydrosteroid concentrations are found in women pregnant with affected fetuses, presumably due to impaired fetal cholesterol production.[215] The incidence of biochemically confirmed SLO is estimated at 1/20,000 to 1/60,000 live births.[216] With the identification of the molecular basis for this disorder, a surprisingly high heterozygote carrier rate for DHCR7 mutations has been found. Because the prevalence of SLO at 16 weeks of gestation is comparable to the prevalence at birth, early fetal loss and/or reduced fertility of carrier couples may be occurring.[216]

Congenital Lipoid Adrenal Hyperplasia

This autosomal-recessive disorder is characterized by a severe defect in the conversion of cholesterol to pregnenolone, leading to impaired steroidogenesis of all adrenal and gonadal steroid hormones. Impaired testosterone biosynthesis in utero prevents male sexual differentiation. Hence, all affected fetuses (46,XY or 46,XX) have female external genitalia. In the XY fetus, Sertoli cells are intact, AMH secretion is unaffected, and Mullerian duct elements regress. Low or undetectable steroid hormone concentrations, elevated ACTH concentrations, and elevated plasma renin activity are consistent with this diagnosis. Elevated 17-hydroxypregnenolone or pregnenolone concentrations distinguish 3β-hydroxysteroid dehydrogenase deficiency from congenital lipoid adrenal hyperplasia.

Following cloning of the gene for steroidogenic acute regulatory protein (StAR), mutations in the StAR gene were identified among patients with congenital lipoid adrenal hyperplasia.[217-219] The StAR protein facilitates cholesterol transport across the mitochondria to P450scc.[220] In congenital lipoid adrenal hyperplasia, the impaired mitochondrial cholesterol transport leads to accumulation of cholesterol esters and sterol auto-oxidation products. Ultimately, the lipid accumulation alters the cell cytostructure provoking cell destruction and complete loss of StAR-dependent steroidogenesis.[221] Hence, the pathogenesis of congenital lipoid adrenal hyperplasia can be characterized as having "two hits," one is defective steroidogenesis and the other is destruction of the steroidogenic cell. Since the definitive zone of the adrenal cortex is relatively quiescent during gestation, manifestations of aldosterone deficiency may not be apparent in the immediate newborn period. This two-hit mechanism allows for spontaneous pubertal development in affected girls because significant estrogen synthesis does not take place until puberty. Subsequently, the follicular cells are damaged resulting in hypergonadotropic hypogonadism.[222]

Nonclassic lipoid congenital adrenal hyperplasia (CAH) is a milder form with variable adrenal and gonadal function presenting in childhood and adolescence.[223,224] Nonclassic lipoid CAH may present with isolated glucocorticoid deficiency and resembles familial glucocorticoid deficiency due to mutations in the ACTH receptor (MC2R) gene.[225]

Side Chain Cleavage Cytochrome P450 Enzyme

The side chain cleavage enzyme (also known as cholesterol desmolase) is a cytochrome P450 enzyme encoded by the CYP11A1 gene mapped to chromosome 15q23-q24. This enzyme converts cholesterol to pregnenolone and is essential to steroidogenesis. This enzyme plays a crucial role in placental progesterone synthesis. Thus, mutations in this gene would be expected to be incompatible with term gestation. However, children with mutations in this enzyme have been reported. The children affected with this autosomal-recessive disorder have adrenal insufficiency and female external genitalia irrespective of the karyotype. Yet, the phenotypic spectrum has been extended to include adrenal insufficiency with or without hypospadias.[226-228] Postnatally, no enlargement of the adrenals and gonads is seen on ultrasound or magnetic resonance imaging.[229] The absence of adrenal/gonadal tissue has been speculated to result from lipid accumulation similar to the cell destruction observed in patients with StAR mutations. Genetic analysis may be necessary to distinguish this disorder from congenital lipoid adrenal hyperplasia.[230]

Virilizing Congenital Adrenal Hyperplasias

The virilizing congenital adrenal hyperplasias are a group of disorders due to mutations in the steroidogenic enzyme genes involved in cortisol biosynthesis. These genes are 3β-hydroxysteroid dehydrogenase type 2 (HSD3B2), 21-hydroxylase (CYP21A2), and 11β-hydroxylase (CYP11B1). These three disorders share a common pathophysiology. Insufficient cortisol production leads to decreased negative feedback inhibition, increased ACTH production, accumulation of steroid intermediates proximal to the deficiency enzyme, and increased androgen concentrations. The specific manifestations and laboratory abnormalities vary depending on which enzyme gene is involved. Indeed, the magnitude of glucocorticoid and mineralocorticoid deficiencies varies—generally in proportion to the severity of the enzyme deficiency.

Accumulation of steroid intermediates such as 17-OHP results in increased androgen concentrations. In the affected female, the increased androgen exposure promotes virilization of the external genitalia. Excessive 17-OHP can be converted through an alternate pathway to DHT. This alternate route (the "backdoor pathway") involves 5α and 3α-reduction of 17-OHP to 5α-pregnane-3α,17α-diol-20-one (pdiol), ultimately generating androstanediol, which is the substrate for 3α-oxidation and conversion to DHT[206] (Figure 5-4). During fetal life, accumulation of 17-OHP due to mutations in CYP21A2, CYP11B2, or P450-oxidoreductase (POR) may increase flux through this "backdoor pathway" leading to elevated DHT concentrations.[231]

21-Hydroxylase Deficiency

The most common type of CAH (accounting for 90% to 95% of cases) is 21-hydroxylase deficiency due to mutations in the 21-hydroxylase (CYP21A2) gene located at chromosome 6p21.3 in the HLA class III region.[232] The reported incidence of 21-hydroxylase deficiency ranges from 1 in 5,000 to 1 in 15,000, with

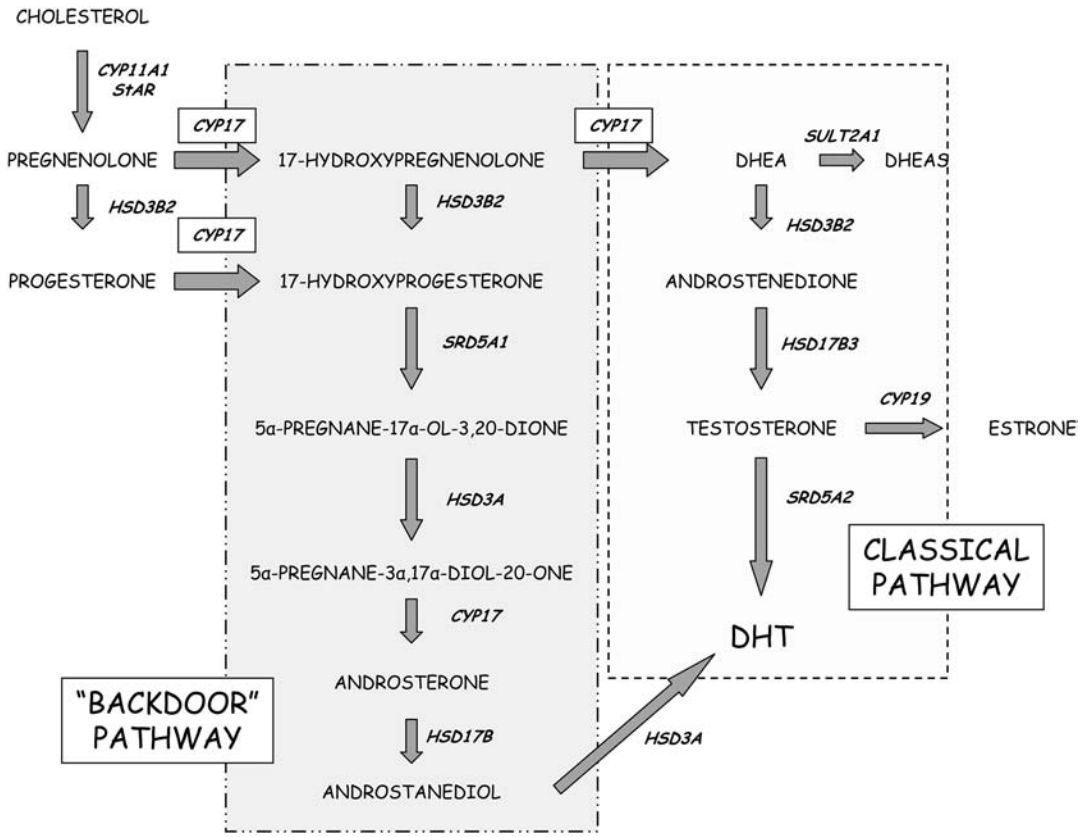


FIGURE 5-4 ■ Steroidogenic pathways relevant to fetal androgen biosynthesis. The classic steroidogenic pathway relevant to the testis (light background) and the "backdoor pathway" (dark background) are indicated. Both pathways can generate DHT through the actions of target tissue enzymes capable of converting substrates, testosterone, and androstanediol into DHT. In the presence of elevated ACTH and 17-OHP concentrations due to CYP21, CYP11B1, or POR mutations, the backdoor pathway may contribute to the excessive androgen concentrations responsible for virilization of XX fetuses.

variation among ethnic/racial backgrounds.[233,234] Decreased 21-hydroxylase activity impairs conversion of 17-hydroxyprogesterone to 11-deoxycortisol in the zona fasciculata (the primary site of cortisol biosynthesis) and conversion of progesterone to deoxycorticosterone in the zona glomerulosa, the primary site of aldosterone biosynthesis.

Infant girls with classic salt-losing 21-hydroxylase deficiency usually present in the immediate neonatal period due to genital ambiguity (Figure 5-5).[235] When the diagnosis is delayed, affected girls develop dehydration, hyponatremia, and hyperkalemia due to glucocorticoid and mineralocorticoid deficiencies. In affected females, the spectrum of genital virilization varies from clitoromegaly to perineal hypospadias to complete fusion of labiourethral and labioscrotal folds giving rise to a phallus with chordee and urethral meatus at the tip of phallus. The magnitude of external genital virilization may be so extensive that affected female infants appear to be males with bilateral undescended testes.[236,237] Unless identified by neonatal screening, infant boys typically present at 2 to 3 weeks of age with failure to thrive, poor feeding, lethargy, dehydration, hypotension, hyponatremia, and hyperkalemia. When the diagnosis is delayed or missed, congenital adrenal hyperplasia is potentially fatal. Newborn screening programs decrease the morbidity and mortality associated with acute adrenal insufficiency.

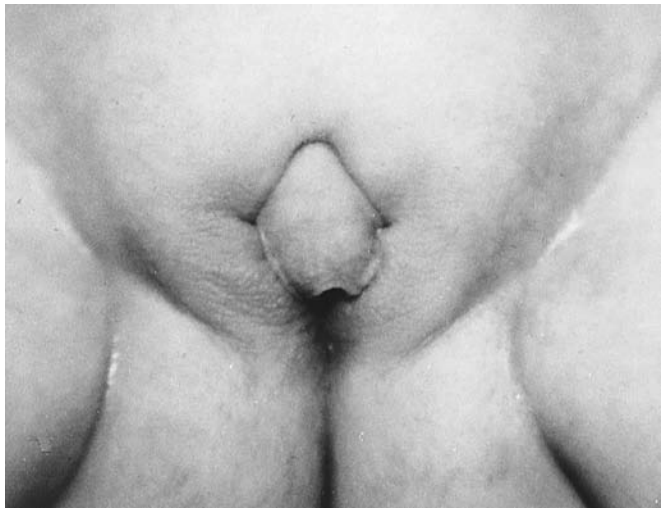


FIGURE 5-5 ■ Genital ambiguity in virilized female with 21-hydroxylase deficiency. The labioscrotal folds are fused, and the clitoris is enlarged.

In affected infants, random 17-OHP concentrations are usually elevated. Concentrations are greater than 5,000 ng/dL and may be much higher.[238] Androstenedione and progesterone concentrations are also typically elevated. In some instances, plasma renin activity (PRA) can be helpful to assess mineralocorticoid status.

Measurement of 21-deoxycortisol is extremely helpful, but availability of this hormone assay is limited. For female infants, a normal uterus is present and can be identified on ultrasound. Ovaries may be too small to be readily identified on ultrasound. Despite excessive antenatal androgen exposure, ovarian position is normal and internal Wolffian structures are not retained.

The spectrum of impaired 21-hydroxylase activity ranges from complete glucocorticoid and mineralocorticoid deficiencies to mild deficiencies manifested principally by compensatory excessive adrenal androgen secretion. Infants capable of adequate aldosterone synthesis do not usually manifest overt salt loss. Female infants capable of adequate aldosterone synthesis may still have sufficient androgen exposure in utero to virilize their external genitalia. In the absence of newborn screening programs, affected males capable of aldosterone biosynthesis may not be identified until they present with genital overgrowth or premature pubarche. Infants with the milder forms of congenital adrenal hyperplasia are generally not identified by most newborn screening programs.[239] Urinary steroid hormone GC/MS analyses showed increased ratios of 5α-pregnane-3α,17α-20-one (pdiol) to metabolites of the Δ^4 and Δ^5 pathways, which indicates postnatal activity of the backdoor pathway especially during early infancy.[240]

CYP21A2 is located approximately 30 kilobases from a highly homologous pseudogene, CYP21A1P. The tenascin-XB (TNXB) gene encoding an extracellular matrix protein is located on the DNA strand opposite CYP21A2.[241] At this time, more than 100 CYP21A2 mutations have been reported. However, only a few mutations account for the majority of affected alleles. Most of the common mutations represent gene conversion events in which CYP21A2 has acquired deleterious CYP21A1P sequences. The frequency of specific mutations varies among ethnic groups.[242] Molecular genotyping can be a useful adjunct to newborn screening. Caveats to bear in mind are that multiple mutations can occur on a single allele and that different CYP21A2 mutations can occur in one family.[243,244]

11β-Hydroxylase Deficiency

Congenital adrenal hyperplasia due to 11β-hydroxylase deficiency is characterized by glucocorticoid deficiency, excessive androgen secretion, and hypertension. This form of CAH is due to mutations in the 11β-hydroxylase (CYP11B1) gene. The enzyme is expressed in the zona fasciculata, where it converts 11-deoxycortisol to cortisol. Congenital adrenal hyperplasia, due to CYP11B1 mutations, is rare (3% to 5% of cases) apart from the high incidence among Moroccan Jews, for whom the incidence approaches 1 in 6,000.[245] Despite the presence of the identical mutation (R448H), phenotypic heterogeneity for the magnitude of virilization and hypertension occurs even in a single family. Affected females may present with ambiguous genitalia. The typical laboratory finding is an elevated 11-deoxycortisol concentration. Serum concentrations of 17-hydroxyprogesterone, androstenedione, and testosterone may be mildly elevated. PRA concentrations are low or suppressed. Nevertheless, infants may experience salt loss presumably due to mineralocorticoid resistance.[246] Patients with non-classic forms have been identified.[3] Although ACTH-stimulated hormone responses among heterozygotic carriers are usually normal, elevated 11-deoxycortisol and 11-deoxycorticosterone have been reported.

The CYP11B1 gene is located at chromosome 8q22 in close proximity to a highly homologous gene CYP11B2, which codes for aldosterone synthase. Novel mutations associated with classical and non-classic 11β-hydroxylase deficiency have been identified.[247-249] CYP11B1 is expressed in the zona fasciculata, whereas CYP11B2 is expressed primarily in the zona glomerulosa.

3β-Hydroxysteroid Dehydrogenase Deficiency

Congenital adrenal hyperplasia due to 3β-hydroxysteroid dehydrogenase type 2 deficiency leads to virilization of the external genitalia of 46,XX fetuses due to increased DHEA synthesis. Affected 46,XY fetuses have ambiguous genitalia characterized by undervirilization of the external genitalia secondary to testosterone deficiency. Despite decreased testosterone synthesis, affected 46,XY fetuses usually have intact Wolffian duct structures (including vas deferens). The NAD+-dependent enzyme 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4-isomerase catalyzes the conversion of the Δ^5 steroid precursors, pregnenolone, 17-hydroxypregnenolone, and DHEA into the respective Δ^4-ketosteroids, progesterone, 17-OHP, and androstenedione.[250]

Two isozymes encoded by two different highly homologous genes have been identified and mapped to chromosome 1p13.1. The type 1 (HSD3B1) gene is expressed primarily in skin, placenta, prostate, and other peripheral tissues. The type 2 (HSD3B2) gene is the form primarily expressed in the adrenal cortex and gonads. Mutations in HSD3B2, but not HSD3B1, have been detected in patients with 3β-hydroxysteroid dehydrogenase deficiency congenital adrenal hyperplasia.[251] Acute adrenal insufficiency occurs in the newborn period when complete loss of function mutations impair biosynthesis of mineralocorticoids, glucocorticoids, and sex steroids. Typical presentations for the non–salt-losing forms include premature pubarche and (in 46,XY infants) perineal hypospadias. Confirmatory laboratory findings include elevated pregnenolone, 17-hydroxypregnenolone, and DHEA concentrations with elevated ratios of Δ^5 to Δ^4 steroids. Because enzymatic activity of the type 1 isozyme is unimpaired, elevated 17-OHP and androstenedione concentrations may be found.

Defects in Sex Steroid Biosynthesis

17α-Hydroxylase/17,20-Lyase Deficiency

Synthesis of mineralocorticoids, glucocorticoids, and sex steroids is modulated by the enzyme, 17α-hydroxylase/17-20-lyase. This enzyme is encoded by a single gene,

CYP17A1 mapped to chromosome 10q24.3 and catalyzes two steps in steroidogenesis, pregnenolone is 17α-hydroxylated to 17α-hydroxypregnenolone, which then undergoes 17,20-lyase activity to form DHEA. Factors favoring the 17,20-lyase reaction include availability of P450 oxidoreductase and cytochrome b_5 and serine/threonine phosphorylation of the P450c17 protein.[232]

Loss-of-function mutations interfere with glucocorticoid and sex steroid biosynthesis. Affected males present with undervirilization of the external genitalia. Affected females have normal genital development and present with delayed puberty. Patients are often hypertensive due to the increased ACTH-stimulated mineralocorticoid synthesis. Serum levels of progesterone, deoxycortisone, corticosterone, 18-hydroxycorticosterone, and 18-hydroxy DOC are elevated and can be suppressed glucocorticoid replacement. Low or absent glucorticoids and sex steroids with poor ACTH-stimulated responses are consistent with this diagnosis. Mutations solely affecting the 17,20-lyase activity are extremely rare.[252,253] The phenotype of apparent isolated 17,20-lyase deficiency can be due to mutations in other genes such a cytochrome b_5, P450 oxidoreductase, and several aldo-keto reductase enzymes as described next.

Cytochrome b_5 Deficiency

Cytochrome b_5 participates in electron transfer for some cytochrome P450 reactions. Although it is not an effective electron donor to P450c17, this protein enables the interactions between P450c17 and oxidoreductase to promote the 17,20-lyase reaction essential for sex steroid synthesis. One 46,XY infant found to be homozygous for a loss of function mutation in the cytochrome b_5 (CYP5) gene was described to have micropenis, bifid scrotum, scrotal hypospadias, undetectable DHEAS concentration, low testosterone concentration, and elevated methemoglobin concentration.[254] A missense mutation associated with mild methemoglobinemia and undervirilized 46,XY siblings has been described.[255]

3α-Hydroxysteroid Dehydrogenase Isozyme Deficiencies

Re-evaluation of the families originally reported to have isolated 17,20-lyase deficiency led to identification of alternative pathways for androgen synthesis.[256,257] Investigation of one family revealed no mutations in CYP17A1, NR5A1, POR, or CYB5. Rather, mutations were detected in the 3α-hydroxysteroid dehydrogenase type III (AKR1C2) gene, which is mapped to chromosome 10p15.1. The phenotype, undervirilization of affected 46,XY individuals, followed a sex-linked recessive inheritance pattern. A splicing mutation in another closely related 3α-HSD gene, AKR1C4, was found in a second family. Digenic inheritance involving mutations in both AKR1C2 and AKR1C4 were found in this second family. Thus, both the classic and backdoor pathways appear to be necessary for normal male external genital development.[257] However, details regarding the specific roles of these enzymes remain to be elucidated.

Cytochrome P450 Oxidoreductase Deficiency

In 1985, a disorder with biochemical evidence suggesting decreased 17α-hydroxylase and 21-hydroxylase activity was initially reported.[258] This disorder was found to be associated with mutations in the cytochrome P450 oxidoreductase (POR) gene. The POR gene codes for a protein that functions as a mandatory electron donor to microsomal steroidogenic and hepatic P450 enzymes. The POR gene, mapped to chromosome 7q11-12, plays a major role in glucocorticoid and sex steroid synthesis. Loss-of-function mutations appear to be scattered throughout the gene.

Clinical features included genital ambiguity, craniosynostosis, midface hypoplasia, and radiohumeral synostosis. At birth, genital ambiguity was noted in both male and female infants. However, progressive postnatal virilization does not occur. Insufficient testosterone synthesis likely causes the undervirilization of male infants. Virilization of female fetuses is attributed to shunting of the excessive 17-OHP to the "backdoor pathway" resulting in increased DHT synthesis. During pregnancy, some mothers developed signs associated with androgen excess such as acne, hirsutism, and clitoromegaly.[259] Typical laboratory findings include elevated 17-OHP, low sex steroid, and normal mineralocorticoid concentrations. Some affected individuals may benefit from daily glucocorticoid replacement therapy. Others may need glucocorticoid treatment only for stress dosing.[260]

The skeletal malformations resemble those found in the Antley-Bixler syndrome, which is an autosomal dominant disorder associated with mutations in the fibroblast growth factor receptor 2 (FGFR2) gene. Patients with Antley-Bixler syndrome due to FGFR2 mutations have normal steroidogenesis, whereas patients with POR mutations have abnormal steroidogenesis.[261] The molecular basis of the skeletal anomalies is unclear but is suspected to be due to impaired activity of enzymes involved in sterol biosynthesis such as 14α-lanosterol demethylase (CYP51A1) and squalene epoxidase or retinoic acid metabolism.[262,263]

17β-Hydroxysteroid Dehydrogenase Deficiency

This disorder is due to mutations of the 17β-hydroxysteroid dehydrogenase type 3 gene (HSD17B3) located at chromosome 9q22. The enzyme is expressed almost exclusively in the testis where it converts androstenedione to testosterone. Loss-of-function mutations result in testosterone deficiency and subsequent undervirilization of 46,XY fetuses.[264] In this autosomal recessive disorder, external genitalia range from female with perineoscrotal hypospadias and a blind-ending vaginal pouch to ambiguous with labio-scrotal fusion to hypospadias. Testes are present and may be palpable in the labio-scrotal folds or incompletely descended. Despite the presence of female external genitalia, Wolffian structures are typically present.[265] In The Netherlands, incidence was estimated to be 1:147,000.[266]

When unrecognized, patients are usually considered to be female at birth. At puberty, progressive virilization

occurs. This virilization is attributed to extratesticular conversion of androstenedione to testosterone and may lead affected individuals to change gender identity from female to male. Increased conversion of androstendione to estrogens may cause gynecomastia.

Appropriate male gender assignment can be made in infancy when the diagnosis is suspected and confirmed. Diagnostic laboratory features include increased basal and hCG-stimulated androstenedione to testosterone ratios. The clinical features are similar to those of 5α-reductase deficiency and androgen insensitivity; molecular genetic analysis may be beneficial to confirm the diagnosis.[265] Affected 46,XX girls are usually asymptomatic, have normal female internal and external genitalia, and normal pubertal development; infertility has been reported for some.[267]

5α-Reductase Deficiency

This autosomal-recessive disorder is due to mutations in the 5α-reductase type 2 gene (SRD5A2) which is is located at chromosome 2p23. This gene is expressed primarily in androgen target tissues where it converts testosterone to dihydrotestosterone (DHT). Clusters of individuals with SRD5A2 mutations have been described in regions of the Dominican Republic, Papua New Guinea, Turkey, and the Middle East.

Affected 46,XY individuals have genital ambiguity characterized by differentiation of Wolffian structures, absence of Mullerian-derived structures, small phallus, urogenital sinus with perineoscrotal hypospadias, and blind vaginal pouch.[268] Four patients were reported who were raised as females and presented with primary amenorrhea; all had genital ambiguity.[269] Deletions, missense mutations, and uniparental disomy have been reported.[270] Due to the extensive heterogeneity in the clinical features, phenotype/genotype correlations are not well established. One study reported that T/DHT ratio greater than 10 detected almost 75% of the patients; hCG-stimulated T/DHT ratios were also used.[271] Caveats regarding this diagnosis include the need for hCG stimulation in prepubertal patients and accurate hormone determinations in which cross-reactivity between testosterone and DHT is minimized.[272]

At puberty, progressive virilization occurs with muscular development, voice change, and phallic enlargement. These features have been attributed to the actions of 5α-reductase type 1 (SRD5A1). SRD5A1, located at chromosome 5p15, is expressed in post-pubertal skin and scalp. Phenotypic heterogeneity occurs frequently. Some 46,XY individuals with this disorder who were raised as girls, develop a male gender identity and changed gender role in adolescence or adulthood. Despite the virilization, facial hair tends to be scanty, the prostate is hypoplastic, semen tends to be viscous, and the amount of ejaculate is low. Affected men often have azospermia or oligospermia. Intrauterine insemination with sperm from an affected male has resulted in pregnancy.[273]

Placental Aromatase Deficiency

Placental aromatase deficiency is a rare autosomal recessive disorder due to mutation in the aromatase gene, CYP19A1, which is located at chromosome 15q21.2 and codes for a 503-amino-acid protein.[274] Inactivating mutations of CYP19A1 impair conversion of androgens to estrogens, leading to increased androgens.[232] During pregnancies with affected fetuses, progressive maternal virilization characterized by hirsutism, clitoral hypertrophy, acne, and frontal balding occurs. During pregnancy, testosterone, DHT, and androstenedione concentrations are elevated and estradiol, estrone, and estriol concentrations are low. In the postpartum period, some clinical features of androgen excess regress and the elevated androgen concentrations return to normal levels.

At birth, 46,XX infants are variably virilized with labioscrotal fusion, clitoromegaly, and perineal scrotal hypospadias. Affected 46,XX individuals generally manifest delayed puberty characterized by minimal or absent breast development, primary amenorrhea, hypergonadotropic hypogonadism, multi-cystic ovaries, and decreased bone mineral density.[275,276] At birth, affected 46,XY infants have normal internal and external genital development. Affected males have generally presented after puberty with tall stature, skeletal pain, delayed skeletal maturation, and infertility.[277] Investigation of aromatase-deficient men suggests that estrogen deficiency is associated with abdominal obesity, insulin resistance, dyslipidemia, and relative infertility. Patients with less severe phenotypic features have been described.[278]

Aromatase is a cytochrome P450 enzyme that plays an important role in the biosynthesis of estrogens (C18 steroids) from androgens (C19 steroids). In addition to its role in estrogen biosynthesis in adolescents and adults, aromatase located in the human placenta converts fetal adrenal androgens to estrogens and protects the mother from the potential virilizing effects of the fetal androgens. Tissue-specific aromatase expression is governed by several different promoters associated with alternative first exon usage.[279,280]

MATERNAL HYPERANDROGENISM

During pregnancy, maternal hyperandrogenism can occur secondary to luteomas of pregnancy, androgen secreting tumors, and exposure to exogenous androgen. The excessive maternal androgen concentrations can cause virilization of the external genitalia of 46,XX fetuses. Endocrine disruptors are exogenous chemicals or mixtures of chemicals that interfere with any facet of hormone action.[281] Organochlorine pesticides, polychlorinated biphenyls (PCBs), and alkylpolyethoxylates are considered to be "endocrine disruptors" because of their estrogenic and/or antiandrogenic properties.

DISORDERS OF ANDROGEN ACTION

During the process of sexual development, androgen action is essential to promote retention of Wolffian duct derivatives, development of the prostate, and differentiation of male external genitalia. Androgen action is mediated by the androgen receptor (AR, also known as NR3C4), a member of the steroid/thyroid hormone

family of hormone receptors. Similar to other members of this receptor family, the androgen receptor is a ligand-dependent transcription factor with a characteristic modular structure. The major modules of the protein include the amino-terminus transactivation (AF1), DNA-binding, and ligand-binding domains. Other features include a nuclear localization signal, another transactivation domain (AF2) in the carboxy terminus ligand-binding domain, and the hinge region.[282] The AR has two polymorphic trinucleotide repeat regions located in the amino terminal domain encoding the polyglutamine (CAG) and polyglycine (GCN) repeat regions.[283,284]

Androgen insensitivity is an X-linked recessive disorder secondary to mutations in AR, which is located near the centromere at Xq11-12.[285,286] Approximately 30% of cases represent de novo mutations. Somatic cell mosaicism can occur when the mutation arises in the post-zygotic stage and is associated with a lower recurrence risk.[287] Complete androgen insensitivity (CAIS) is characterized by female external genitalia, absence of Mullerian duct derivatives, sparse sexual hair, inguinal masses, and primary amenorrhea in the adolescent girl. Among patients with CAIS, Wolffian duct derivatives (e.g., vas deferens and epididymides) are absent because of deficient androgen action. Although rare exceptions have been described, Mullerian-derived structures are usually absent because Sertoli cell function is normal with in utero AMH secretion. It has been suggested that 1% to 2% of girls with bilateral inguinal herniae may have androgen insensitivity. The finding of a gonad within the hernia sac should prompt cytogenetic studies.[288] The expected LH surge in testosterone concentrations during the first few months of life may be absent in some infants with CAIS.[289] Spontaneous pubertal breast development due to aromatization of androgens to estrogens occurs if the gonads are in situ.

Partial androgen insensitivity syndrome (PAIS) is characterized by clinical features suggestive of a partial biologic response to androgens.[290] Typical features include ambiguous genitalia with perineoscrotal hypospadias, microphallus, and bifid scrotum. Testicular position is variable, ranging from undescended to palpable in the scrotum. Infants with PAIS generally manifest the expected neonatal testosterone surge, suggesting that prenatal androgen responsiveness plays a role in imprinting of the HPG axis. Features of mild androgen insensitivity syndrome (MAIS) include gynecomastia and infertility in otherwise normal males. Older chronologic age at presentation is typical. In all instances, karyotype is 46,XY.

Typical laboratory findings are elevated LH and testosterone concentrations because testicular testosterone synthesis is unimpeded. Yet, there is loss of negative feedback inhibition of gonadotropins. LH concentrations are usually higher than FSH concentrations because testicular inhibin secretion is not impeded. FSH concentrations may be elevated or normal. Infants with PAIS may require dynamic endocrine tests to assess hCG-stimulated Leydig cell testosterone secretion and, more importantly, end-organ responsiveness to androgens. The risk for gonadal tumors is increased in the presence of a Y chromosome. Tumors associated with AIS include carcinoma-in-situ (CIS), seminoma, leiomyoma, and testicular stromal cell tumors.[291] In one series, only 2 of 44 subjects with CAIS had CIS. Both subjects were postpubertal.[292]

In the absence of ligand, the AR protein is located primarily in the cytoplasm where it is bound to chaperone proteins. Upon ligand binding, the conformation of the androgen receptor changes. The ligand-receptor complexes dimerize and move to the nucleus.[293] A key feature of androgen receptor dimerization is the intramolecular interaction between the N-terminal and C-terminal domains. Binding of ligand stabilizes the androgen receptor and slows its degradation.[294] The increased potency of dihydrotestosterone is attributed to the greater stability of the dihydrotestosterone-receptor complex compared to the testosterone-receptor complex. In the nucleus, the complex binds to androgen response elements (AREs) and alters target gene transcription. Nucleotide sequences of AREs in conjunction with specific AR amino acids confer greater specificity for transcriptional regulation of specific genes.[295]

Over 500 different AR mutations have been described in affected individuals.[296] In general, the phenotype correlates with degree of impaired androgen action. However, clinical features can vary despite the presence of the identical mutation (even within the same family). Complete and partial androgen insensitivity associated with the same AR mutation can occur in siblings.[297] Different missense mutations at the same position can also be associated with differing phenotypes.[298,299] Complete loss-of-function mutations and premature termination codons are typically associated with complete androgen insensitivity.[300] Partial loss-of-function mutations are typically missense mutations. Receptors with mutations in the DBD bind ligand normally but fail to transactivate target genes. Mutations in the LBD of the AR gene can be associated with decreased affinity for ligand, increased instability of the hormone-receptor complex, or increased susceptibility of the receptor to thermal denaturation. In addition to hormone determinations, diagnostic evaluation may include DNA sequence analysis of the AR gene (www.genetests.org and http://www.androgendb.mcgill.ca).

Phosphorylation, acetylation, ubiquitylation, and sumoylation are post-translational modifications that influence AR transactivational function. The DNA-binding domain (DBD) contains two zinc fingers that interact with DNA. X-ray crystallographic studies indicate that the three-dimensional structure of the ligand-binding domain (LBD) consists of 12 α-helixes that form the ligand-binding pocket. Kinetic and biochemical assays with molecular dynamic simulations of mutations identified in patients with CAIS indicate that the position of helix 12 is crucial to AR function.[301]

AR transcriptional activity also depends on other proteins including coactivators and corepressors. These other proteins presumably modulate physical interactions linking the basal transcription machinery, the ligand-receptor complex, and chromatin. For example, a missense AR mutation altered the interaction of AR with the melanoma

antigen A-11 (MAGE-11) protein by interfering with the stimulatory effects of the coregulator.[302,303]

In addition to androgen sensitivity, Kennedy disease (also known as spinal and bulbar muscular dystrophy) is mapped to the AR locus. Kennedy disease is a progressive neurodegenerative disorder with onset in the thirties or forties. This disorder is associated with excessive expansion of the CAG polyglutamine trinucleotide repeat region in exon 1 of AR.[304] Repeat lengths greater than 35 are associated with spinal and bulbar muscular atrophy. In Kennedy disease, aberrant degradation of misfolded AR generates insoluble aggregates leading to cellular toxicity. However, the precise mechanism responsible for the neuropathy remains elusive.[305] Mild symptoms of androgen insensitivity can be detected with slight decreases in AR mRNA and protein concentrations.

MULLERIAN DUCT ABNORMALITIES

Persistent Mullerian Duct Syndrome

Persistent Mullerian Duct Syndrome is an autosomal recessive disorder due to mutations in AMH or its receptor (AMH-RII).[306] The phenotypes of patients with AMH or AMH-RII mutations are comparable. AMH is a member of the TGF-β family and signals through two different interacting membrane-bound serine/threonine receptors. The ligand, AMH, binds to the type II receptor which leads to recruitment and phosphorylation of a type I receptor. The type II receptor is specific for AMH, whereas there are multiple subtypes of the type 1 receptors. AMH concentrations are low among patients with mutations in the AMH gene. Among patients with AMH-RII mutations, AMH concentrations are normal or elevated. Females who carry mutations on both AMH alleles appear to have normal fertility.

The typical clinical features of PMDS include cryptorchidism, testicular ectopia associated with inguinal hernia, and hernia uteri inguinalis. Testicular differentiation is usually normal, but the male excretory ducts may be embedded in the Mullerian duct remnants or incompletely developed. Infertility may ensue secondary to cryptorchidism, intertwining of vas deferens and uterine wall, or lack of proper communication between the testes and excretory ducts. Testicular torsion is not uncommon because the testes may not be anchored properly to the bottom of the processus vaginalis.

Mullerian Duct Abnormalties in 46,XX Individuals

Mayer-Rokitansky-Küster-Hauser syndrome refers to congenital absence of the vagina associated with uterine hypoplasia or aplasia. Primary amenorrhea is the typical presentation. Magnetic resonance imaging (MRI) is helpful in the diagnosis and management.[307] Renal anomalies and skeletal malformations may be present. Unilateral renal agenesis was found in 29.8% of cases of Mullerian duct anomalies on MRI.[308] No single gene defect has been identified. Rather, mutations in WNT4, LHX1, and several chromosome regions have been detected.[309] The aggregation of Mullerian duct aplasia, renal aplasia, and cervico-thoracic somite dysplasia has been labeled MURCS syndrome. Mullerian duct hypoplasia has been associated with facio-auriculo-vertebral anomalies such as Goldenhar syndrome.[310]

Transverse vaginal septa can occur sporadically or in association with other features, such as polydactyly in the McKusick-Kaufman syndrome, which is associated with mutations in the MKKS gene located at chromosome 20p12.[311] Mutations in the MKKS gene are also associated with Bardet-Biedel type 6.[311] Because of the high frequency associated anomalies, careful physical examination for skeletal malformations and renal sonography should be included in the diagnostic evaluation of women with abnormal development of the Mullerian duct system.

HOXA GENES

Hand-foot-genital syndrome is characterized by genitourinary tract malformations and distal limb anomalies. Genital anomalies include Mullerian duct anomalies in females and hypospadias with or without chordee. The typical limb abnormalities are hypoplasia of the first digit, shortening of the carpal and tarsal bones, and clinodactyly of the fifth digit. The inheritance pattern is autosomal dominant. Mutations have been identified in the HOXA13 gene in association with this syndrome.[312]

MICROPHALLUS, HYPOSPADIAS, AND CRYPTORCHIDISM

Hypospadias

Hypospadias is a congenital hypoplasia of the penis characterized by ventral displacement of the urethral meatus. It is often associated with chordee, cryptorchidism, and additional congenital anomalies. The reported incidence ranges from 1 in 100 to 1 in 1000. Familial hypospadias has been described. Genes involved in early genital tubercle patterning include BMP4, BMP7, HOXA4, HOXB6, FGF8, FGF10, and FGFR2. Deletions involving 19q13 are associated with intrauterine growth retardation, microcephaly, postnatal growth retardation, clinodactyly, and hypospadias. The Wilms tumor interacting protein (WTIP) is located in the deleted region.[313]

Cryptorchidism

During sexual differentiation, the gonads are positioned between two structures: the cranial suspensory ligament and the gubernaculum. Testicular descent is divided into two phases: intraabdominal and inguinoscrotal. Factors involved in gubernacular development during the intraabdominal phase include INSL3 and its receptor, LGR8/RXFP2. INSL3 is secreted by Leydig cells. Its receptor, LGR8, is a leucine-rich G-protein–coupled receptor expressed by the gubernaculum. By 13 or 14 weeks of gestation, the gubernaculum anchors the testis to the internal inguinal ring.[314] At approximately 22 to 25 weeks of gestation, the testes and epididymides are located at the internal rings of the inguinal canal. Androgen action during the intraabdominal phase

appears to be limited to regression of the cranial suspensory ligament. In females, the cranial suspensory ligament persists as the suspensory ligament of the ovary. Testicular descent through the inguinal canal is usually accomplished by the end of seventh month of gestation, with completion of the inguinoscrotal phase by the end of week 35.[315]

Cryptorchidism (undescended testes) is the most common disorder of sexual differentiation, affecting 3% of male infants.[316] Cryptorchidism can be associated with decreased number of germ cells, impaired germ cell maturation, and decreased number of Leydig cells.[317] In some instances of unilateral cryptorchidism, abnormal histology is apparent in the contralateral normally descended testis.[318] However, it is unclear if these features represent consequences or causes of cryptorchidism. In a prospective randomized study, orchidopexy at 9 months of age results in greater testicular volume and increased number of germ cells compared to orchidopexy at 3 years of age.[319]

Because spontaneous descent often occurs during infancy, the prevalence decreases to 1% by 6 months of age.[319] Cryptorchidism has been associated with hypothalamic hypogonadism, aberrant testicular differentiation, impaired testosterone biosynthesis, androgen insensitivity, holoprosencephaly, abnormal AMH production or action, abnormalities affecting INSL3/LGR8 function, and possibly environmental factors.[4] Other associations include prune belly syndrome, bladder exotrophy, and renal anomalies. Cryptorchidism is also a feature of many syndromes (Table 5-2). Maternal diabetes mellitus, including gestational diabetes, may be a risk factor.

Heterozygous missense INSL3 mutations have been identified in patients with cryptorchidism.[320] Mutations have also been identified in the LGR8 gene in males with cryptorchidism. Sequence variants have been identified in the HOXA10 gene in boys with cryptorchidism.[321] Yet, mutations in INSL3, LGR8, or HOXA 10 are rare causes of isolated cryptorchidism. Typically,

TABLE 5-2 **Disorders Associated with Small Penis or Cryptorchidism in Males**

Disorder	Gene	Human Phenotype
Aarskog-Scott syndrome	FGD1	Mental retardation, shawl scrotum, short stature
Börjeson-Forssman-Lehman syndrome	PHF6	Mental retardation, epilepsy, hypogonadism
Carnevale syndrome	COLEC11	Blepharophimosis, blepharoptosis
Cornelia de Lange syndrome	NIPBL	Low anterior hairline, arched eyebrows, synophrys, mental retardation, growth retardation
Holoprosencephaly		Holoprosencephaly, seizures, hypopituitarism, diabetes insipidus
• HPE1	21q22.3	Holoprosencephaly, seizures, hypopituitarism, diabetes insipidus
• HPE2	SIX3	Holoprosencephaly, seizures, hypopituitarism, diabetes insipidus
• HPE3	SHH	Holoprosencephaly, seizures, hypopituitarism, diabetes insipidus
• HPE4	TGIF	Holoprosencephaly, seizures, hypopituitarism, diabetes insipidus
IFAP Syndrome	MBTPS2	Ichthyosis follicularis, atrichia, photophobia
Johanson-Blizzard syndrome	UBR1	Short stature, SGA, microcephaly, congenital heart disease
Lenz-Majewski hyperostosis syndrome	NK	Mental retardation, progressive skeletal sclerosis, severe growth retardation
Lowe syndrome	OCRL	Congenital cataracts, mental retardation, renal failure
McKusick-Kaufman syndrome	MKKS	Congenital heart disease, renal anomalies, vaginal anomalies in females
Meckel-Gruber	MKS1	Cystic renal disease, CNS malformation, liver anomalies
Miller-Dieker syndrome	17p13.3	IUGR, microcephaly, lissencephaly
Multiple pterygium syndrome, Escobar variant	CHRNG	Webbing, arthrogryposis
Myotonic dystrophy	DMPK	Muscular dystrophy, cataracts
Noonan syndrome	PTPN11	Short stature, hypertelorism, congenital heart disease, bleeding diathesis
Pfeiffer	FGFR1, FGFR2	Craniosynostosis, skeletal anomalies
Popliteal pterygium syndrome	IRF6	Cleft lip, cleft palate, lower lip cysts, spina bifida occulta
Prader-Willi syndrome	NDN, SNRPN	Decreased fetal activity, hypotonia, obesity, short stature
Robinow syndrome	WNT5A, ROR2	Mesomelic limb shortening, hypertelorism
Rubinstein-Taybi syndrome	CREBBP	Microcephaly, short stature, broad thumbs, dysmorphic facial features
Seckel syndrome, type 1	ATR	Bird-headed facial appearance, IUGR, ataxia-telangiectasia
Simpson-Golabi-Behmel, type 1	GPC3	Pre- and postnatal overgrowth, coarse facies, congenital heart disease
Townes-Brocks syndrome	SALL1	Microcephaly, large ears, congenital heart disease, renal anomalies
VATER/VACTERL syndrome		Spectrum of anomalies including vertebral defects, anal atresia, tracheoesophageal fistula, renal anomalies
Weaver	NSD1	Overgrowth, developmental delay

the testes of patients with androgen insensitivity have completed the intraabdominal phase but fail to undergo inguineoscrotal descent because this second phase is androgen dependent. However, the more complete the androgen insensitivity the greater likelihood of finding abdominal testes. Exposure of XX fetuses to androgen does not promote significant ovarian descent in humans, as evidenced by normal ovarian position in females with congenital adrenal hyperplasia.[322]

HYPOGONADOTROPIC HYPOGONADISM

Hypogonadotropic hypogonadism associated with GnRH deficiency is characterized by a spectrum of reproductive, olfactory, and non-reproductive clinical features. Inheritance patterns include X-linked, autosomal dominant, and autosomal recessive. Nevertheless, sporadic cases are more common than inherited forms. Males infants with hypogonadotropic hypogonadism may present in the newborn period with microphallus and/or cryptorchidism. Additional anterior pituitary hormone deficiencies may be present. Genital ambiguity would not be anticipated because placental hCG secretion is unaffected. Decreased gonadotropin secretion presumably results in decreased testosterone secretion such that microphallus and cryptorchidism can occur.

The 1000 to 2000 GnRH neurons that reside in the postnatal hypothalamus originate in the olfactory placode and migrate during early fetal development from the nose through the forebrain to the hypothalamus. Investigation of families with inherited hypogonadotropin hyponadism has led to identification of specific genes involved in this process (Table 5-3). Kallmann syndrome is the eponym used for the X-linked recessive form of hypogonadotropic hypogonadism associated with anosmia due to failed migration of GnRH neurons from the olfactory placode into the forebrain along branches of the vomeronasal nerve.[323] Olfactory tract hypoplasia or aplasia has been found on MRI. The molecular basis of this X-linked form is mutations in the Kallmann (KAL) gene (located at Xp22.3). This gene escapes X-inactivation, codes for a 680-amino-acid protein, and helps target GnRH neurons to the hypothalamus.

Mutations in the GnRH receptor (GNRHR), fibroblast growth factor receptor 1 (FGFR1), FGF8, KISS1, KISS1R, NELF, GNRH1, and GPR54 genes are also associated with hypogonadotropic hypogonadism.[324-327] More recently, mutations in the genes coding for prokineticin receptor-2 (PROKR2) and its ligand [prokineticin-2 (PROK2)] have been associated with hypogonadotropic hypogonadism.[327] Mutations in TAC3 and its receptor TACR3 are associated with hypogonadotropic hypogonadism; the prevalence of microphallus among males carrying TACR3 mutations is high.[328] The Charge syndrome is another disorder associated with genital hypoplasia; addition features include ocular coloboma, heart defects, choanal atresia, short stature, and hearing loss. The Charge syndrome is associated with mutations in the chromodomain helicase DNA binding protein 7 (CHD7) gene.[329] GnRH deficiency can be due to mutations in several genes (an oligogenic disorder).[330,331]

TABLE 5-3 **Monogenic Disorders Associated with Hypothalamic Hypogonadism**

Gene	Location	Associated Phenotype
KAL1	Xp22.3	Anosmia, renal anomalies
FGFR1	8p11.2-p11.1	Anosmia, cleft lip/palate
FGF8	10q24	Anosmia, cleft lip/palate
NELF	9q34.3	Anosmia
PROK2	3p21.1	Anosmia/normosmia
PROKR2	20p13	Anosmia/normosmia
GNRH1	8p21-p11.2	Normosmia
KISS1	1q32	Normosmia
KISS1R	19p13.3	Normosmia
TAC3	12q13-q21	Normosmia
TACR3	4q25	Normosmia
SOX2	3q26.33	Optic nerve hypoplasia
HESX1	3p21.2-p21.2	Hypopituitarism (septo-optic dysplasia)
PITX2	4q25-q26	Hypopituitarism (Rieger syndrome)
PROP1	5q	Hypopituitarism
LHX3	9q34.4	Hypopituitarism

ROBINOW SYNDROME

Robinow syndrome is characterized by short stature, mesomelic limb shortening, hypertelorism, mandibular hypoplasia, irregular dental alignment, and hypoplastic external genitalia. Both autosomal dominant and autosomal recessive inheritance patterns have been observed. Heterozygous loss of function mutations in WNT5A have been identified in patients with the autosomal dominant form.[332] Loss-of-function mutations in the gene encoding the tyrosine kinase-like orphan receptor 2, ROR2, have been identified in the more severe autosomal recessive form. Hemivertebrae and scoliosis are found more often in patients with the recessive form whereas supernumerary teeth are found almost exclusively in the dominant form.[333] ROR2 has been identified as a putative receptor for WNT5A.

WARBURG-MICRO SYNDROME

Warburg-Micro syndrome is a heterogenous disorder associated with congenital cataracts, microphthalmia, postnatal microcephaly, developmental delay, micropenis, hypoplasia of the corpus callosum, and cryptorchidism. Mutations have been identified in several genes, including RAB3GAP1, RAB3GAP2, and RAB18.[334-336]

MAMLD1

The mastermind-like domain containing 1 (MAMLD1) gene was identified during studies to identify the genetic basis of X-linked myotubular myopathy. This gene is also known as chromosome X open reading frame 6

(CXorf6).[337] Mutations and polymorphisms have been identified in some patients with micropenis, bifid scrotum, and hypospadias.[338,339]

ENVIRONMENTAL DISRUPTORS

Prenatal treatment with diethylstilbestrol (DES), a nonsteroidal synthetic estrogen, has been associated with urogenital abnormalities of both male and female fetuses with cryptorchidism occurring in 46,XY fetuses.[340,341] It has been speculated that the frequency of cryptorchidism and poor semen quality is increasing because exposure to endocrine disruptors in the environment has increased.[342] The genital ambiguity, described in three 46,XY infants born in heavily agricultural areas, was attributed to fetal exposure to endocrine disruptors especially because no mutations were detected in the *SRY* or SRD5A2 genes.[342] Although no definite causation has been found, various environmental substances such as herbicides, fungicides, pesticides, and plasticizers have been considered to be potential endocrine disruptors. Potential mechanisms include binding to nuclear hormone receptors modulating gene expression or epigenetic changes. Reliable evidence confirming detrimental environmental effects on genital development is lacking.[343]

OTHER DISORDERS

The VACTERL association (VA) is characterized by several anomalies. These abnormalities include vertebral anomalies, anal atresia, cardiovascular malformations, tracheoesophageal fistula and/or esophageal atresia, renal anomalies, limb anomalies, and/or genital ambiguity. Mullerian hypoplasia/aplasia, renal agenesis, and cervicothoracic somite dysplasia (MURCS association) is characterized by primary amenorrhoea. Features of this disorder can include absence of uterus and fallopian tubes, cervical spine abnormalities, and renal anomalies. Complete absence of penis also known as aphallia is rare and may be associated with additional congenital anomalies of the genitourinary and gastrointestinal systems.

EXSTROPHY OF BLADDER

Bladder exstrophy is a primary field defect involving the pelvis, urinary tract, and external genitalia and should not be considered a disorder of sex development. Exstrophy of the cloaca is characterized by persistence of a common cloaca associated with failure of fusion of the genital tubercles. Spinal abnormalities may be present.

DIAGNOSIS

History

A detailed family history should be obtained. The family history should include ascertainment of unexplained infant deaths, consanguinity, and infertility. Infants with congenital adrenal hyperplasia may have died prior to diagnosis. Many DSDs are inherited as autosomal-recessive disorders. Infertility and gynecomastia may represent milder phenotypes for some DSDs. For X-linked disorders such as androgen insensitivity, there may be affected maternal family members (i.e., either amenorrheic or infertile aunts or partially virilized uncles). Pertinent questions include prenatal exposure to exogenous or endogenous androgens, estrogens, or potential endocrine disruptors. Maternal virilization during pregnancy should be queried.

Physical Examination

DSDs encompass a spectrum of physical findings. The specific physical findings range from micropenis, hypospadias, undescended testes, minimal clitoromegaly, and scrotalized labia to more extensive forms of genital ambiguity. Severe clitoromegaly with posterior labial fusion in an 46,XX patient may be difficult to distinguish from perineal hypospadias, undescended testes, and a bifid scrotum in a 46,XY individual. During the physical examination, attention should be focused on phallic size, symmetry of the external genitalia, presence and location of palpable gonads, and any additional anomalies.

The extent of virilization should be carefully documented, recording the configuration, stretched dorsal length, and diameter of the phallus (including the glans penis). The location of the urethral opening, degree of labiourethral fold fusion, and extent of labioscrotal fold fusion should also be noted. Labioscrotal folds fuse from posterior to anterior such that the appearance extends from posterior labial fusion, a partially fused hemiscrota, to completely fused scrotum with labiourethral fusion extending to a midline urethral opening. The position of the urethra should be noted, as well as whether one or two perineal openings are present.

Gonadal or adnexal structures may be identified upon careful palpation for content of the labioscrotal structures, scrotum or labia majora, inguinal region, and the lower abdomen. The groin area may be "milked" to maneuver the testis into the scrotum. The absence of palpable testes may indicate a genetic female with virilization, as occurs with adrenal hyperplasia or a genetic male with undescended or absent testes. Although structures palpated within the labioscrotal folds are usually testes, ovaries or even the uterine cervix can be found within the labioscrotal folds. Testes typically have a characteristic ovoid structure.

Symmetry or asymmetry of external genital differentiation may provide clues to the etiology of the genital ambiguity (Figure 5-6). Unilateral structures with asymmetry of other genital structures suggests ovotesticular or 45X/46,XY DSDs and is often associated with unilateral gonadal maldescent. Asymmetry implies differing local influences, which often reflect abnormalities in gonadal differentiation (Figures 5-6 and 5-7). Repeated examinations may be beneficial for diagnostic precision.

Penile length measurements extend from the tip of stretched penis from the pubic ramus. Normal length depends on gestational age, the lower limit (approximately –2.5 SD) at term being 2.0 cm. An isolated micropenis can be a consequence of decreased testosterone exposure in the second half of gestation due to Leydig cell failure, LH

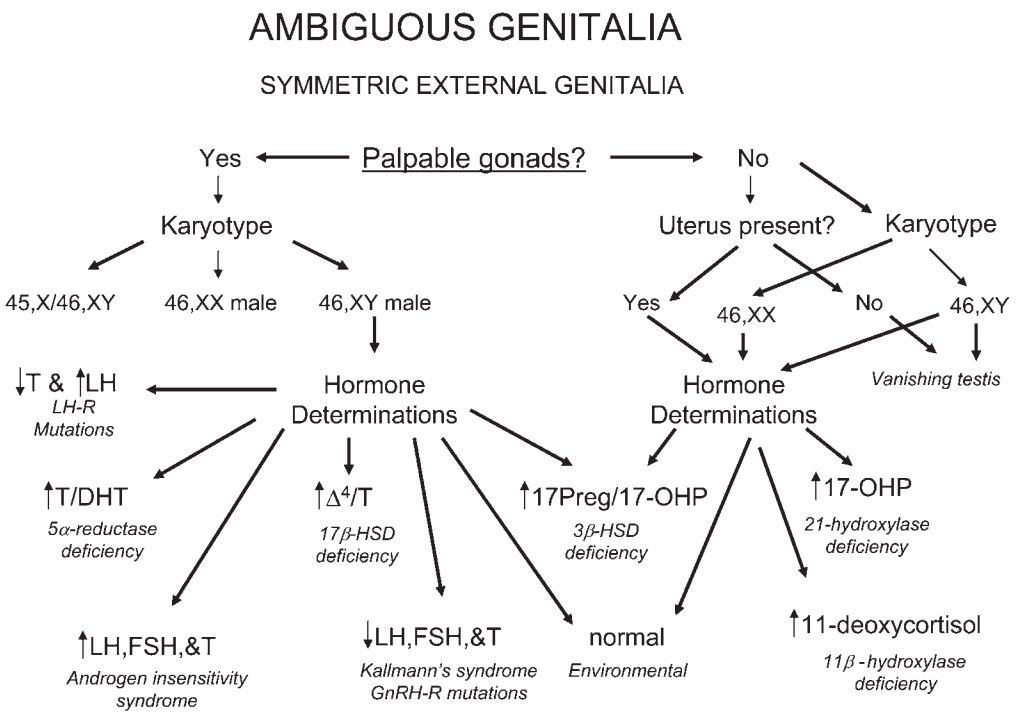


FIGURE 5-6 ■ Algorithm for the approach to the child with symmetric genital ambiguity. The configuration of the labioscrotal folds and presence/absence of palpable gonads is comparable on both sides. The presence or absence of palpable gonads directs the initial laboratory evaluation. Ultrasound examination to determine whether a uterus is present is helpful. For example, symmetric fusion of the labioscrotal folds, nonpalpable gonads, and presence of a uterus provide strong circumstantial evidence for the diagnosis of a virilized female with congenital adrenal hyperplasia.

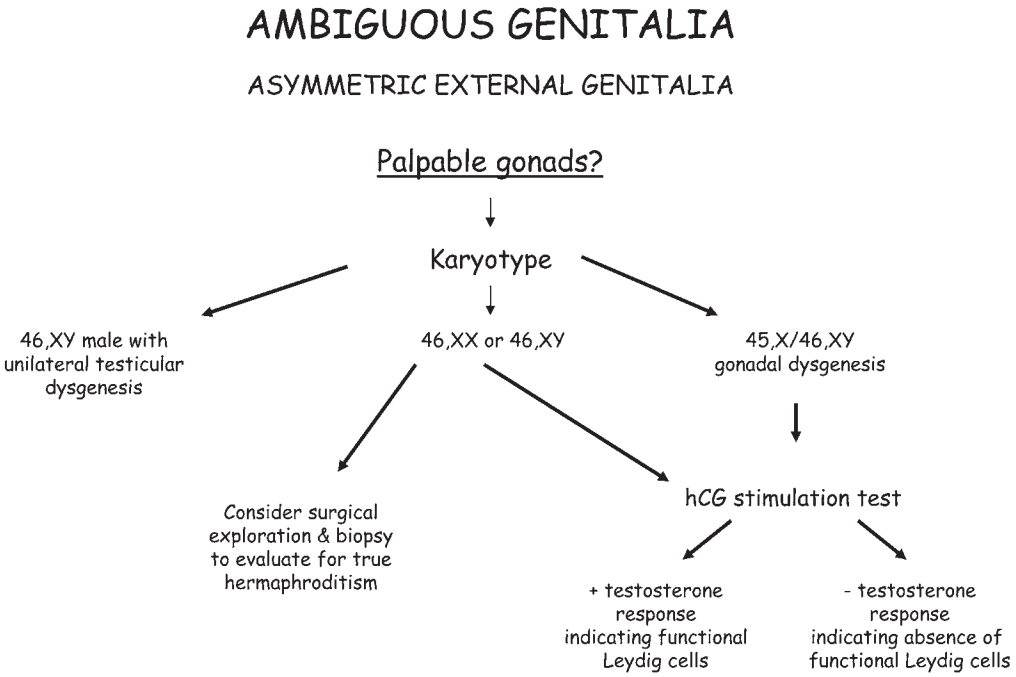


FIGURE 5-7 ■ Algorithm for the approach to the child with asymmetric genital ambiguity. In this instance, the labioscrotal folds may appear different or a gonad is palpable only on one side.

deficiency, androgen insensitivity syndrome, LHCGR mutations, or GH deficiency. A micropenis with hypospadias suggests a broader range of DSDs.

Clitoral length is usually less than 1.0 cm, although rare variations exist. Measurement of the clitoris requires a careful estimate of the proximal end and careful exclusion of overlying skin. The location of the urethral opening should be ascertained by visualization, witnessing the urinary stream, or with careful insertion of a firm catheter. If urination is observed, force, diameter, and direction of the urinary stream should be noted. The position of the inserted catheter may also provide crucial initial information.

If directed toward the anal opening and palpable under the perineal skin, the catheter is likely in a urogenital sinus as occurs often with virilization of a 46,XX fetus secondary to 21-hydroxylase deficiency. However, a penile urethra is anticipated if the catheter is directed anteriorly and is nonpalpable.

The anogenital ratio is measured as the distance between the anus and the posterior fourchette divided by the distance between the anus and the base of the phallus. If the ratio is >0.5, this suggests a component of female differentiation and hence virilization with posterior labial fusion.[344] Because pelvic ultrasound is part of the initial laboratory assessment, a rectal examination may not be necessary. If present, a midline uterine cervix can often be palpated upon rectal exam.

The Prader scale is often used to classify the appearance of the external genitalia: (1) normal female genitalia with clitoromegaly, (2) partial labial fusion and clitoromegaly, (3) labioscrotal fusion so that there is a single opening from the urogenital sinus and clitoromegaly, (4) fusion of labioscrotal folds so that the single opening is at the base of the phallic structure, and (5) complete male virilization with penis-size phallus, complete labial fusion, and meatus on the glands. A recent description extends this traditional Prader classification to include urogenital sinus characteristics by defining the vaginal confluence in relation to the bladder neck and the meatus.[345]

In addition to the genital examination, the examination should include weight, length, and other features to ascertain whether findings are consistent with gestational age particularly in the apparent female because the clitoris is more prominent in preterm infants in that there is scant subcutaneous fat and clitoral growth is completed before the last trimester of fetal life.[346] A careful examination includes inspection for additional dysmorphic features because genital ambiguity may occur in association with other anomalies. These include midline facial defects, head size, and ear and digital anomalies. Bladder exstrophy and epispadias represent a non-endocrine malformation of the urologic system. Infants with congenital adrenal hyperplasia, both male and female, may manifest hyperpigmentation of the genitalia and nipples due to ACTH hypersecretion.

Laboratory Studies

Initial laboratory studies to assess genital ambiguity should include an abdominal/pelvic ultrasound and karyotype. The ultrasound will provide information regarding the presence or absence of a uterus. Information regarding the size and location of the gonads and adrenals may be obtained on ultrasound. However, lack of visualization of gonads on ultrasound examination does not indicate absence of gonads. When gonads are not palpable, the external genitalia are symmetrically ambiguous, and a uterus and possibly ovaries are present, the most likely diagnosis is a virilized 46,XX fetus with congenital adrenal hyperplasia. However, the possibility of markedly dysgenetic testes cannot be excluded. If the differential diagnosis based on the presentation includes congenital adrenal hyperplasia, the initial laboratory studies should include electrolytes, plasma renin activity, and serum 17-hydroxyprogesterone and cortisol levels.

The karyotype is essential to determine chromosomal sex even if prenatal chromosome testing was performed. In general, peripheral blood karyotypes are sufficient. However, the patient may be a mosaic with one or more additional cell lines restricted to gonadal tissue.[347] Chromosomal microarray analyses are increasingly available and may identify submicroscopic genomic changes.[348]

Other initial studies depend on the physical findings. If the external genitalia are symmetrically virilized to any degree in the absence of palpable gonads, particularly if a normal uterus is present, additional studies should be directed toward causes of virilization of a female infant. Because 21-hydroxylase deficiency is the most common cause of virilization and genital ambiguity in 46,XX infants, initial laboratory studies should include determination of 17- hydroxyprogesterone concentrations. Generally, 17-OHP concentrations should be measured no earlier than 48 to 72 hours of age because of the potential for false positive results which may be due to cross-reacting steroid sulfates.[349] Nevertheless, extremely elevated 17-OHP concentrations may be detected at earlier time points in infants with classical CAH. If one or both gonads can be palpated, the intent of screening studies is to determine the adequacy of androgen synthesis and androgen action in a male infant. Determination of LH, FSH, and testosterone concentrations in infancy provides information regarding the function of the testes and the HPG axis (Table 5-3).

The pattern of steroid hormone concentrations provides evidence for specific defects in steroidogenesis (Table 5-3). The diagnosis of congenital adrenal hyperplasia due to 21-hydroxylase deficiency is confirmed by finding elevated 17-hydroxyprogesterone concentrations. Typically, 17-hydroxyprogesterone concentrations are greater than 10,000 ng/dL (300 nmol/L) in the affected neonate. For 11β-hydroxylase deficiency, 11-deoxycortisol and 17-hydroxyprogesterone concentrations are elevated. For 3β-hydroxysteroid dehydrogenase deficiency, pregnenolone, 17-hydroxypregnenolone, and DHEA concentrations are typically elevated. Steroid concentrations are low for patients with congenital lipoid adrenal hyperplasia and CYP11A1 mutations. When salt-losing forms of congenital adrenal hyperplasia are included in the differential diagnosis, serum electrolytes and plasma renin activity should be monitored. Typically, hyponatremia and hyperkalemia are not present at birth and develop during the first week of life.

Newborn screening programs have been established in all states and many countries to identify infants with classic congenital adrenal hyperplasia. Many screening programs measure whole-blood 17-hydroxyprogesterone concentrations eluted from a dried filter paper blood spot.[350] Whereas false negative 17-hydroxyprogesterone results are uncommon, slightly increased whole-blood 17-OHP concentrations are detected often enough (especially in preterm infants) to complicate clinical decision making regarding affected status and need to initiate glucocorticoid therapy. Etiologies of slightly increased 17-OHP concentrations include prematurity,

cross-reacting steroids, sampling prior to 48 to 72 hours of age, heterozygosity for 21-hydroxylase deficiency, and late-onset congenital adrenal hyperplasia.

To avoid an excessive number of false-positive screening results, the cutoff levels are typically selected to identify all infants with classic salt-losing or simple virilizing forms—often missing those infants with late-onset congenital adrenal hyperplasia. Improved specificity can be achieved by use of additional procedures such as organic extraction, chromatography, or LC/MS analysis.[351] The caveat for LC/MS is the need for precise quality control. Prenatal or neonatal treatment with glucocorticoids can result in a false negative screening result.[351] If the differential diagnosis includes CAH, specific laboratory testing is warranted even if the newborn screen results are reported as negative for 21-hydroxylase deficiency. GC/MS analysis of urinary steroids can also be used to diagnose other disorders of steroidogenesis.[352,353] In the future, GC/MS urine analyses may become standard of care in the management of patients with CAH.[353]

For the milder forms of congenital adrenal hyperplasia or other disorders affecting adrenal steroidogenesis, ACTH stimulation testing may be necessary to confirm the diagnosis. After a basal blood sample has been drawn, synthetic ACTH (0.25 mg) can be administered by intravenous bolus or intramuscular injection. A second blood sample to measure ACTH-stimulated hormone response can be obtained 30 or 60 minutes later. The milder forms of congenital adrenal hyperplasia generally do not affect external genital differentiation and are therefore not usually associated with genital ambiguity. Infants with late-onset congenital adrenal hyperplasia are generally not detected through newborn screening programs, presumably because the whole-blood 17-OHP concentrations determined from the newborn screening filter paper are lower than the values used as cutoffs.

In addition to the diagnostic evaluation for disorders of steroidogenesis, hormone measurements in the immediate neonatal period provide an index to the function of the HPG axis. Low testosterone and elevated gonadotropin concentrations in a 46,XY infant with ambiguous genitalia suggest inadequate testosterone biosynthesis. Elevated testosterone and gonadotropin concentrations in an infant with female external genitalia, bilateral labial masses, and 46,XY karyotype are consistent with the diagnosis of androgen insensitivity.

Measurement of AMH provides another assessment of testicular function because AMH concentrations reflect Sertoli cell function. AMH concentrations are sexually dimorphic, with high values in boys (20-80 ng/mL) during the first six years of life and low values in girls.[354] Thus, in the patient with nonpalpable gonads and absence of Mullerian structures AMH concentrations may help distinguish between anorchia and cryptorchidism.[355] In addition, AMH concentrations can be helpful in disorders of testicular dysgenesis or in virilized females to determine the presence of testicular tissue.[356] Inhibin B concentrations, lower in females than in males, provide another marker of Sertoli cell function.[356,357]

Assessment of the ability of a gonad to secrete testosterone may be helpful, especially for patients with evidence of testicular tissue by palpation or ultrasound and AMH levels indicating testicular tissue. This can be done by administering hCG and measuring hormone responses. To assess hormone responses, doses of 1,000 to 1,500 units can be injected subcutaneously either daily or every other day for one to five days—with blood sampling on the day after the last injection. Hormone determinations should include androstenedione, testosterone, and dihydrotestosterone.[358] Testosterone concentrations should more than double, and the T/DHT ratio should be $<10{:}1$.[359] Intermittent injections can be given for up to three weeks to stimulate penile growth to demonstrate both testosterone secretion and target tissue responsiveness. Total dosage should not exceed 15,000 units of hCG.

Endoscopic studies can be used to locate the vaginal-urethral confluence in relation to the bladder neck and the single opening of the urogenital sinus. Using a cystoscope and catheter placement, these distances can be determined before or in conjunction with retrograde contrast studies performed to outline the urethra and to demonstrate (if present) the vagina, uterus, cervix, and uterine cavity. Such information is needed to plan for reconstructive surgery, to assess the risk of medical complications, and in certain instances to provide information to determine the sex of rearing. If such procedures are unhelpful, laparoscopy may be necessary to visualize and biopsy gonadal and internal genital structures. MRI may be helpful to define anatomic relationships.

Some patients with DSDs present at puberty with delayed puberty or primary amenorrhea. The triad of clitoromegaly, hirsutism, and primary amenorrhea suggests 17β-hydroxysteroid dehydrogenase, 5α-reductase, P450 oxidoreductase, aromatase, or mild virilizing forms of CAH.

Molecular genetic analyses have become increasingly available to determine and confirm the molecular basis of the genital ambiguity. Knowledge regarding the specific mutation enables more accurate genetic counseling for inherited disorders of sexual differentiation (http://www.genecards.org/). The genetics laboratory can often perform fluorescent in situ hybridization (FISH), for the *SRY* gene, or other detailed chromosomal tests such as CGH microarrays. For some DSDs, molecular genetic analysis of specific genes is available through commercial laboratories. Information regarding the particular details can be obtained from an NIH-funded web-based resource, Genetests (http://www.genetests.org). In some instances, genetic testing is available through research laboratories.

TREATMENT

While awaiting results of the initial laboratory studies and imaging studies if necessary, attention is focused on the main decision: the gender of rearing. In the ideal situation, there is a specific DSD team comprised of a pediatric surgeon or pediatric urologist with expertise in urogenital reconstructive surgery, a behavioral science

professional (psychologist, social worker, or psychiatrist), a pediatric endocrinologist, and a neonatalogist.[360,361] Using all available data, the DSD team educates the family and helps them decide on the gender of rearing. Guessing is not appropriate.[362] In many instances, gender assignment is apparent based on the initial physical examination and laboratory findings.

Initially, the neonatalogist may facilitate coordination of care and communication with the parents. Long-term management needs to be delineated. In addition to the appointments necessary to the patient's medical, surgical, and psychological needs, additional sessions may benefit the parents to address their questions and concerns about their child.[363] These evaluations should be performed promptly and often necessitate transfer of the infant to a tertiary care facility.

The parents need to participate in discussions and decision making regarding the options for sex of rearing and possible surgical interventions. Honest, sensitive, and candid discussions with the parents can only benefit the child. As the child matures, honest explanations regarding the medical condition are essential. The child should progress from a silent partner to a full participating member in this decision-making process, with the child's wishes taking precedence where appropriate.

Once the diagnosis is confirmed as specifically as possible, therapy is instituted. When a specific diagnosis has been confirmed, decision making and therapy can be guided accordingly. Considerations for medical care include sex of rearing, possible need for surgery and a timeline for planned surgery, plan for medical treatment, and timely and appropriate psychological counseling and support.

Sex of Rearing

Decisions regarding the appropriate sex of rearing are based on the specific pathophysiology, external genital features, internal reproductive system anatomy, prognosis for spontaneous pubertal development, capacity for sexual activity and orgasm, and potential for fertility.[364] Using modern techniques to assist fertility, the potential for fertility is more likely than in the past. Although current surgical techniques spare more of the neurovascular bundle of the phallus, surgery should be avoided except in those instances of severe ambiguity or markedly discordant genitalia to preserve sexual responsiveness.

In instances where a decision regarding sex of rearing is necessary, each child evaluated for genital ambiguity warrants careful consideration of the physical findings, laboratory information, and available outcome data. In most instances, children are raised in the gender consistent with the karyotype, but acknowledging that there are rare exceptions that favor sex reversal (gender reassignment). Limited longitudinal outcome studies are available regarding adult function and gender identity. Most patients who are 46,XY with CAIS identify themselves as female.

About a quarter of patients with PAIS are dissatisfied with the gender assignment, regardless of whether they are raised as male or female. The majority of patients with 5α-reductase deficiency identify as male, whereas about half of those diagnosed with 17β-hydroxysteroid dehydrogenase self-reassign from female to male.[365,366] The majority of virilized 46,XX individuals with CAH identify as female. Thus, in spite of the accumulating evidence that androgen exposure to the CNS alters general and cognitive behavior, much remains to be learned about the influences of nature and nurture on gender identity.[62,367,368]

Because the majority of 46,XX virilized females (primarily those with CAH) identify as female, this sex of rearing is usually recommended if the question arises in infancy. For virilized 46,XX patients with Prader stage 4 or 5 genitalia, the recent consensus conference felt that the outcome data was insufficient to support a sex of rearing as male. Yet, a recent report suggests that this possibility warrants consideration and may be an appropriate option for some situations.[369] For most virilized females with CAH, the primary issue is not gender assignment but questions regarding genital surgery.

To date, more information and knowledge is available about physical sexual development than about psychosexual cognitive development. The primary factors, critical steps, and sequence of psychosexual development are unclear. Traditionally, psychosexual development has been viewed as having three components: gender identity, gender role, and sexual orientation. The development of gender identity begins as an infant with the self-recognition that one is a boy or girl. Gender role develops during childhood as a result of society's expectations concerning behavior and is influenced by prenatal hormonal exposure.

Gender roles are defined in part by the messages society relays concerning appropriate and inappropriate male and female behavior. Gender role may subsequently shift and even become fluid, depending on the individual's reaction to the shifting expectations of society. How prenatal factors such as hormones and environmental exposures impact gender role has been difficult to ascertain. Sexual orientation may be apparent prior to puberty or not expressed until late in adult life, underlying the unknown influencing factors.

The effects of prenatal androgen exposure on gender identity are uncertain.[370,371] Prenatal androgen levels apparently influence gender-related behavior and cognitive function during childhood.[370] Yet, the impact on degrees of femininity and masculinity and cognitive function (spatial and verbal abilities) and handedness does not appear to persist into adult life.[372,373] Among virilized 46,XX individuals with CAH, an increased rate of homosexual orientation has been reported primarily based on self-reported sexual imagery and sexual attraction, with reports of actual homosexual involvement being less well documented.[374-377]

Some females with classic CAH are more likely to question their female gender,[378] and the reported incidence of sex reassignment from female to male is greater than in the general population.[379] Among 46,XX CAH patients with gender self-reassignment, it was judged that factors contributing to this change were not genotype or phenotype, but rather gender-atypical behavioral, self-image and body image, and development

of erotic attraction to women.[380] Other studies concluded that psychological adjustment was comparable between females with CAH and their unaffected siblings.[381] In another study, gender identity of girls with CAH was comparable to the control group.[382] In these studies, the impact of environmental influences cannot be separated from the effects of prenatal hormonal exposures.

Female gender of rearing is appropriate for 46,XY individuals with complete androgen insensitivity because virilization and fertility are unattainable. The lack of androgen responsiveness limits androgen effect on the developing CNS of individuals with CAIS. Conversely, the decision regarding gender of rearing for individuals with partial androgen insensitivity may be problematic because the extent of androgen impact on CNS development and the androgen responsiveness of the genital development are uncertain.[383] The extent of clinical response to exogenous testosterone may benefit this decision-making process. Testosterone responsiveness may be ascertained by assessing penile growth following one or more intramuscular injections of 25-mg testosterone depot formulations.

Among individuals with DSD associated with gonadal dysgenesis, hCG-stimulated testosterone secretion and clinical response facilitate the decision regarding gender of rearing. The genital phenotype, ability of the Leydig cell to secrete testosterone, and extent of genital virilization in response to androgen stimulation are indicators regarding the potential for spontaneous pubertal development and need for hormone replacement therapy. Nevertheless, in gonadal dysgenesis the impact of prenatal androgen exposure on gender development processes is often indeterminate and cannot be reliably predicted from routine diagnostic studies in the newborn period.

Generally, with the exception of CAIS, 46,XY individuals with fetal androgen exposure manifested as partial genital masculinization should be raised as males unless there are extenuating individual circumstances. Ideally, 46,XY infants with 17β-HSD3 or 5α-reductase deficiency will be diagnosed in the newborn period. Caution, reflection, and dialog are essential before those with a 46,XY, karyotype are assigned female because fetal androgen exposure appears to impact strongly on self-concept as male despite compromised external genital development.

Medical Treatment

With the exception of disorders affecting glucocorticoid and mineralocorticoid biosynthesis, most DSD conditions do not require specific medical therapy in infancy. At the time of expected puberty, patients with hypogonadism will need appropriate hormone replacement therapy. In general, hormone replacement therapy is initiated using low doses of the appropriate sex steroid hormone with incremental increases designed to mirror spontaneous pubertal development. It is helpful to review with families the anticipated frequency of outpatient visits and how the adequacy of replacement therapy will be assessed.

For females, induction of puberty involves low-dose estrogen therapy—usually initiated between 10.5 to 12 years of age to avoid excessive acceleration of skeletal maturation. The initial estrogen dosage is usually the lowest available, such as 0.3 mg of conjugated estrogens every other day or 5 μg of ethinyl estradiol daily. Transdermal estrogen preparations which provide 0.007-0.025 mg daily can be utilized. Transdermal patches may be used only at night in an effort to mimic spontaneous puberty.[384] Matrix transdermal patches can also be cut into smaller pieces to provide a lower estrogen dosage. Based on clinical response and the patient's perception, the dose of estrogen can be increased in 6- to 12-month intervals such that complete replacement doses and development are achieved within 3 years.

Therapy involves the addition of a progestational agent after 12 months of estrogen therapy or when withdrawal bleeding occurs, whichever occurs sooner. Thereafter, cyclic estrogen-progesterone therapy should be used. Once full pubertal development has been reached, the estrogen dosage should be the minimum that will maintain normal menstrual flow and prevent calcium bone loss (equivalent to 0.625 conjugated estrogen or 20 μg ethinyl estradiol). Options for cyclic estrogen-progesterone therapy include low-dosage estrogen birth control pills or estrogen-progestin transdermal patches.

Another regimen involves a daily oral estrogen regimen or the transdermal form for 21 days with the addition of progesterone, 5 to 10 mg of medroxyprogesterone acetate, or 200 mg of micronized progesterone daily added for 12 days (day 10 to day 21). This is followed by a week of no hormones. At this point, the replacement regimen may be extended so that less frequent withdrawal bleeding occurs. In the absence of a uterus, progesterone therapy becomes optional. Among patients with low circulating androgen levels, sexual hair growth and libido may be improved by administering small doses of DHEA or methyltestosterone. Gonadotropins hCG and/or hMG (human menopausal gonadotropin/recombinant FSH) are only used to stimulate ovulation or during assisted fertility attempts.

For males, testosterone hormone replacement typically begins at 12.5 to 14 years. In instances where the psychological impact is felt to be needed, therapy may be initiated one or two years earlier. If therapy is begun earlier to assure the patient concerning physical changes, the rate of skeletal maturation should be carefully monitored. Conversely, treatment may be delayed to allow for psychological or emotional maturity or catch-up growth. Testosterone therapy may be given by depot intramuscular injections or topically by a patch or gel. Depot testosterone (such as enanthate or cyprionate) is begun at a dosage 50 mg IM every four weeks, followed by increased dosing and frequency over about 3 years to a full replacement dosage of approximately 200 mg every 2 weeks. Availability of the gel in metered-dose pumps allows gradual increases of dosage from 1.25 g daily upward. Among those with differentiated testes and gonadotropin deficiency, assisted fertility techniques may involve intratesticular germ cell retrieval or hFSH/hLH stimulation.

For patients with CAH, carefully monitored hormone replacement therapy is essential.[385,386] The goal of glucocorticoid therapy is suppression of excessive adrenal androgen secretion while maintaining normal growth and development. Typically, oral cortisol doses range from 8 to 20 mg/m^2/day. This range is based on provision of 1.5 to 2 times the daily cortisol production rate, 7 to 12 mg/m^2/day. Oral fludrocortisone (e.g., Florinef) is commonly used for mineralocorticoid replacement for patients with salt-losing CAH. Patients with simple virilizing CAH may benefit from mineralocorticoid replacement. The typical dose is 0.1 mg administered as a single daily dose. Neonates and infants may require higher fludrocortisone doses as well as salt supplementation because of relative mineralocorticoid resistance, higher aldosterone production rates, and relatively lower sodium intakes. Topics of discussion with parents include how to crush and administer tablets, what to do if doses are accidentally missed, use of medical alert identification IDs, and when to administer "stress" dosages.

For children with CAH, adequacy of replacement therapy is monitored by periodic reassessment of growth velocity, extent of virilization, and salt craving. Laboratory monitoring may include serum androgen and 17-OHP concentrations, skeletal maturation, and 24-hour urinary 17-ketosteroid excretion. Androstenedione concentrations are useful to assess adequacy of glucocorticoid replacement, whereas 17-OHP concentrations are useful to assess for overtreatment. Determination of testosterone concentrations is helpful in girls and prepubertal boys. In pubertal and postpubertal girls, menstrual cyclicity is a sensitive indicator of hormone replacement therapy. Adequacy of mineralocorticoid replacement can be judged using plasma renin activity. Boys with CAH need to be regularly assessed by ultrasound for the development of testicular adrenal rest tumors; these rest tumors may be too small to palpate on clinical examination.[387]

At times of physiological stress (such as fever greater than 101° F, persistent vomiting, significant trauma, and surgical procedures), the glucocorticoid dose should be increased. In general, two to three times the usual dose is sufficient to prevent adrenal insufficiency. Higher doses may be necessary for surgical procedures. All families should have at home and be able to administer injectable hydrocortisone (e.g., Solu-Cortef) intramuscularly in case of medical emergencies. Recommended intramuscular doses are 25 mg for infants, 50 mg for children less than 4 years of age, and 100 mg for all others.

During surgical procedures, additional hydrocortisone can be administered either as continuous intravenous infusion or intramuscular injection. Intravenous normal saline (0.9% NaCl) can be used if oral mineralocorticoid replacement is not tolerated by the patient. While receiving glucocorticoid replacement therapy (and occasionally in the newborn period), physiologically stressed individuals with 11β-hydroxylase deficiency may develop hyponatremia and hyperkalemia and benefit from mineralocorticoid therapy. At times of physiologic stress, individuals with Smith-Lemli-Opitz syndrome or P450 oxidoreductase deficiency may benefit from glucocorticoid treatment.

Considerations with Regard to Surgery

For the virilized female patient with female sex of rearing, the extent of ambiguity in combination with the magnitude of clitoromegaly and posterior fusion must be carefully evaluated to determine whether genitoplasty, including clitoral reduction or clitoroplasty, should be considered. Another factor in the decision-making process is the location of the urethral outlet. If located high in the urogenital sinus, early surgery may be indicated to decrease the risk of recurrent urinary tract infections by providing a direct urinary outflow path. The current perspective is that girls with mild to moderate degrees of clitoromegaly do not need surgery because of the potential risk of compromising genital sensitivity. Currently, there is no consensus regarding clitoral size requiring surgery.[388] Despite surgery, lower urinary tract symptoms such as stress incontinence and incomplete bladder emptying may persist among female DSD patients and among males with hypospadias.[5]

One important point to share with parents of a virilized girl with CAH is that the stimulated genital tissues will regress after glucocorticoid therapy is begun. On one hand, the parents need to be informed that some (including patient advocacy) groups discourage genital surgery until the child is old enough to make her own decision. On the other hand, they must be empowered to make the choice with which they will be comfortable. This must be done with the understanding that their daughter may criticize them for this decision when she is older. Many parents of daughters with severe ambiguity still choose surgery.

If there is agreement between the interdisciplinary medical team and the parents for surgery, the surgery is generally performed as soon as feasible. If surgery is anticipated, the operation should be described in a manner understandable to the parents. An experienced surgeon should discuss the options, risks, and benefits of surgery including the innervation of the clitoris or penis and the surgical approach to be used to attempt to spare the neurovascular supply.[389,390] Although opinions vary as to whether surgery should be done in one or more stages and whether vaginal reconstruction should be attempted during infancy, the parents need to be informed that subsequent vaginoplasty or vaginal dilatation may be required after puberty. Among patients who underwent vaginal reconstructive surgery during infancy, the frequency of postoperative vaginal stenosis has been reported to range from 0% to 77%.[391] Therapeutic goals for vaginal reconstruction surgery include adequate sexual function with minimal need for continual dilatation or lubrication.[392] Girls with Mayer-Rokitansky-Kuster-Hauser syndrome and individuals with CAIS can achieve increased vaginal size with dilator treatment.[393]

For the undervirilized male, decisions concerning genital surgery and when it occurs belong to the parents until the patient reaches adolescence and adulthood. Most decide for surgery, except in the instances of minimal to moderate hypospadias. When discussing surgery with the parents, pertinent topics include common male concerns about the importance of being able to stand to urinate, adequacy of genital development, and capability

for sexual activities. With hypospadias, the location of the meatus does not necessarily indicate that fusion is complete to that point since there may be more proximal inadequate development of the corpus spongiosum and surrounding tissues. Thus, the extent of surgery may not be known until labiourethral fusion is better visualized at surgery. Because correction of hypospadias and chordee is generally performed in stages, this discussion also needs to cover the details of the surgical approach, the proposed schedule for follow-up visits, the likely number of surgical procedures, and the optimal age for each surgical stage. The surgeon should review the options, risks, and anticipated outcomes. If the precise location and differentiation status of the testes are unknown, the parents should be aware that exploration and biopsy may be performed. As would be anticipated, individuals with 46,XY DSD have sexual concerns regardless of gender of rearing. In addition to reconstructive genital surgery, potential confounding factors include self-esteem, genital appearance, infertility, need for hormone replacement therapy, and lack of adequate counseling and support likely influence adult outcome.[394]

Outcome studies reflect the results of the type of surgery and timing of surgery. While former surgical techniques resulted in impaired genital sensitivity,[395] it is as yet unclear whether current surgical techniques sparing the neurovascular supply to the clitoris will result in better outcomes. While it is anticipated (and hoped) that current surgical techniques will have better outcome, it will be years before this information is available. In the meantime, available outcome studies can be informative.

The goals of genital reconstruction surgery have been and continue to be a good cosmetic appearance and the potential for functionality involving sensitivity for sexual responsiveness. Function after surgery is related to severity of genital ambiguity at birth, extent of surgery, and complications of surgery. For example, among females diminished vaginal diameter correlated with greater prenatal virilization. Among 21-hydroxylase deficiency CAH females, the outcome of surgery appears to be related to the specific mutations. Those with the null genotype have more surgical complications and poorer scores regarding sexual function.[396] Available outcome data indicate that most postsurgical patients have a female gender identity. Yet, studies show that a greater portion of women with CAH, even those with non-classic CAH (NCAH), are sexually attracted to other women compared to the general population. However, long-term outcomes are not well documented. Among 46,XX DSD patients, those living as females indicated that they preferred early surgery and felt that a satisfactory sex life was possible.[397] Nonetheless, decreased sexual desire, difficulty achieving orgasm, and later sexual debut were common concerns. Fertility is possible for women with CAH; reduction of progesterone concentrations is one important factor in fecundity for women with classical forms of CAH.[398-400]

Most males with 46,XY DSDs are satisfied with long-term results of genitoplasty. Recurrent complaints include penile size, sexual activity, and urinary symptoms. There is considerable variation in masculinization and virilization.[401] Despite improved understanding of DSDs and openness with patients and families, quality of life issues have not been fully resolved.[401-403]

Risks for Gonadal Tumors

The aberrant fetal gonadal environment and subsequent anomalous germ cell differentiation is associated with the development of germ cell tumors. The presence of Y chromosome material increases the propensity risk for gonadal tumors. Thus, another consideration regarding the need for and timing of surgery is the relative risk for gonadal tumors.

In general, CIS or gonadoblastoma are the most common germ cell tumors. The prevalence reported for gonadoblastoma ranges from 15% to 30%, depending on age of the patient, gonadal histology, and diagnostic criteria used for CIS/gonadoblastoma. Gonadoblastomas are mixed germ cell sex cord-stromal tumors that arise in dysgenetic gonads, are composed of immature germ cells and sex cord-stromal cells of indeterminate differentiation, and precede the development of the more invasive neoplasms such as dysgerminoma, seminoma, and nonseminoma. Assessment of FOXL2 and SOX9 expression in the tumor tissue can help to discriminate between those with a Sertoli cell component from those with a granulosa cell component.[404]

Increased and prolonged expression of immunohistochemical markers, OCT3/4 and testis-specific protein Y-encoded (TSPY), is common in CIS and gonadoblastoma.[404] In one series, TSPY was abundantly expressed in germ cells within dysgenetic testes and undifferentiated gonadal tissue suggesting upregulation when germ cells are located in an unfavorable environment.[404] It is hypothesized that the normal germ cell maturation process is interrupted, resulting in prolonged expression of OCT3/4, erased genomic imprinting, and subsequent immortalization of the cell.[405,406]

In one series of patients with Turner syndrome, 14/171 (8%) were positive for Y-chromosomal material. Among these 14 patients, the prevalence of gonadoblastoma was 33%.[407] A meta-analysis of 11 studies, all using PCR methodology, found that 5% of patients with Turner syndrome showed positive results for Y chromosome material.[407] Frasier syndrome is also associated with gonadoblastoma.

The prevalence of germ cell tumors is lower in androgen insensitivity and disorders of androgen biosynthesis. Since the risk for malignancy appears to be low in childhood and early adolescence, patients with CAIS and their parents often choose to retain the gonads. However, accurate details regarding long term risk for malignancy are unknown at this time.[408] Limited sample size, ascertainment bias, inconsistent diagnostic criteria for malignant cells, and confusing terminology leave many questions to be answered. For example, which patients with DSD benefit from gonadal biopsy to assess risk for neoplasia? Another consideration is how many biopsies are necessary to be representative of the histology of the gonad. Laparoscopy and video-assisted gonadectomy are invaluable techniques when the risk for malignancy is high. Ultimately, the decision regarding gonadectomy

involves consideration of the patient's phenotype (internal and external genital anatomy), karyotype, gender of rearing, psychosocial factors, and gonadal histology.[409]

Transition from Pediatrics to Adult Care

Parents and pediatric healthcare providers envision that children with DSD will become productive adults who will find fulfillment and gratification in their lives. In view of the report from England regarding loss of follow-up of patients with CAH, a planned transition program from the pediatric healthcare team to the adult healthcare team is essential.[410] This planned transition process needs to consider the medical, psychosocial, educational, emotional, cognitive, and vocational needs of the young adult. Differences in expectations between pediatric and adult healthcare situations should be reviewed. Explanation of the specific details of his/her diagnosis with the young adult is essential.[411] For many of these patients, the specific details of their disorder were reviewed with the parents at the time of diagnosis when the individual was too young to participate in these discussions. The young adult needs to know the etiology, basic genetics, pathophysiology of his/her disorder, recurrence risk, and fertility status. The young adult, especially those requiring glucocorticoid replacement therapy, needs to understand the rationale for treatment.[411,412]

Psychological and Genetic Counseling and Support and Ethical Considerations

Longitudinal continuity of care and provision for support systems are essential because of the medical and psychological aspects of DSDs. If an experienced social worker, psychologist, or psychiatrist is available, careful assessment and counseling are invaluable throughout childhood and adolescence. Although most such cases of 46,XY DSD may not require medical therapy during early childhood, intermittent visits with the pediatric endocrinologist are helpful to address the patient's and parents' concerns, update parents concerning new therapy and outcome data, and ensure that appropriate psychological support needs are being met. These visits can foster a positive relationship between the child and the physician. Some parents may find disorder- specific support groups (e.g., AIS, www.aissg.org; CAH, www.caresfoundation.org; Turner syndrome, www.turnersyndrome.org) to be helpful.

Many DSD conditions are inherited. The most common inheritance patterns are autosomal recessive or X-linked traits. Genetic counseling is indicated because parents are often interested to learn about recurrence risks. Phenotypic heterogeneity occurs in some DSD disorders, so it may be appropriate to perform hormone determinations and genetic analyses (when available) for other family members.

Ethical principles and recommendations have been published in a effort to provide a comprehensive view of the perspective of clinicians, patients and families, based on the principles of fostering the well-being of the child and future adult, uphold their rights to participate in decisions that impact them now and later, and to respect family and parent-child relationships.[413]

CONCLUSIONS

Identification of genes involved in sexual differentiation has elucidated some of the molecular events responsible for normal and abnormal sexual differentiation. Knowledge of the genetic, hormonal, and environmental factors that influence sexual differentiation benefits the affected children, their parents, and their healthcare providers. This information enables better parental education regarding the etiology, natural history, and prognosis for their child. With understanding of factors that affect sexual differentiation, recurrence risks can be estimated.

The evaluation and subsequent management of a child with ambiguous genitalia is based on history, physical examination, and laboratory data. Understanding the family's background enables the medical team to discuss the child's sex development using terminology that they can understand at the time of diagnosis as well as during the following years. Initial diagnosis and management are the beginning of a long term relationship between the family and healthcare providers.

Despite the advances in characterizing the molecular basis of ambiguous genitalia, sensitivity and compassion demonstrated by the team of healthcare professionals in their interactions with the patient and family remain an essential aspect in the management of an infant with genital ambiguity. The initial evaluation and diagnostic processes are merely the beginning of an extended relationship with the child and family ultimately culminating in successful transition to adult care providers. Respectful and empathetic interactions foster development of the infant with genital ambiguity to become an adult able to live a contented life. We, the healthcare providers, need to remain cognizant that knowledge regarding all aspects of sex determination, sex development, and gender identity determinants remains to be elucidated such that management of individuals with DSDs will still involve uncertainties and likelihoods.[362]

Glossary of Genes

Gene	Name	Locus
AKR1C2	Aldo-keto reductase family 1 member 2	10p15.1
AKR1C4	Aldo-keto reductase family 1 member 4	10p15.1
AMH	Anti-Mullerian hormone	19p13.3
AMH-RII	Anti-Mullerian hormone receptor	12q13.13
AR	Androgen receptor	Xq11-q12
ARX	Aristaless-related homeobox, X-linked	Xp21.3
BLIMP1/ PRDM1	B lymphocyte–induced maturation protein	6q21
ATR-X	Alpha-thalassemia/mental retardation, X-linked	Xq13
BMP2	Bone morphogenetic protein-2	20p12.3
BMP15	Bone morphogenetic protein-15	Xp11.22
CBX2/M33	Chromobox homolog 2	17q25
CDKN1C	Cyclin-dependent kinase inhibitor 1C	11p15.4
CYB5	Cytochrome b_5	18q22.3
CHD7	Chromodomain helicase DNA binding protein 7	8q12.1-12.2
CYP21A2	21-hydroxylase	6p21.33
CYP11B1	11β-Hydroxylase	8q21
CYP19A1	Aromatase	15q21.2
CYP11A1	Cholesterol side chain cleavage	15q23-q24
CYP17A1	17α-hydroxylase/17,20-lyase	10q24.3
CYP26B1	Cytochrome P450, subfamily XXVIB, polypeptide 1	2p13.2
CXCL12	Stromal cell-derived factor 1	10q11.21
CXCR4	Chemokine, CXC motif, receptor 4	2q22.1
DAX1/ NR0B1	DSS-AHC critical region on the X chromosome 1	Xp21.2
DAZL	Deleted in azoospermia-like	3p24.2
DHCR7	7-dehydrocholesterol reductase	11q13.4
DHH	Desert hedgehog	12q13.1
DMRT1	Doublesex- and MAB3-related transcription factor 1	9p24.3
EIF2B2	Eukaryotic translation initiation factor 2B, subunit 2	14q24.3
EIF2B4	Eukaryotic translation initiation factor 2B, subunit 4	2p23.3
EIF2B5	Eukaryotic translation initiation factor 2B, subunit 5	3q27.1
EMX2	Empty spiracles, drosophila, 2, homolog of	10q26.11
FIGLA	Factor in germline alpha, mouse, homolog of	2p12
FGF8	Fibroblast growth factor 8	10q24.32
FGF9	Fibroblast growth factor 9	13q12.11
FGFR1	Fibroblast growth factor receptor 1	8p11.23-p11.22
FGFR2	Fibroblast growth factor receptor 2	10q26.13
FOG2/ ZFPM2	Friend of GATA 2	8q23
FOXL2	Forkhead transcription factor 2	3q23
GATA4	GATA binding protein 4	8p23.1-p22
GDF7	Growth differentiation factor 7	2p24.1
GLI3	Gli-Kruppel family member 3	7p14.1
GNRHR	GnRH receptor	4q13.2
HOXA10	Homeobox A10	7p15.2
HOXA11	Homeobox A11	7p15.2
HOXA13	Homeobox A13	7p15.2
HSD3B2	3β Hydroxysteroid dehydrogenase 2	1p13.1
HSD17B3	17β Hydroxysteroid dehydrogenase 3	9q22
INLS3	Insulin like 3	19p13.11
IRX3	Iroquis homeobox protein 3	16q12.2
KAL1	Anosmin 1	Xp22.31
KAT6B	Lysine acetyltransferase 6B	10q22
LGR4	G-protein–coupled receptor 48	11p14.1
LGR8/RXFP2	Relaxin/insulin-like peptide receptor 2	13q13.1
LIM1/ LHX1	Lim homeobox gene 1	17q12
LHX9	Lim homeobox gene 1	1q31.3
LIN28A/ZCCHC1	Zing finger CCHC domain-containing protein 1	1p36.11
LHR/LHCGR	LH receptor	2p16.3

Glossary of Genes—cont'd

Gene	Name	Locus
MAMLD1/ CXORF6	Mastermind-like domain-containing protein 1	Xq28
MAP3K1	Mitogen-activated kinase kinase kinase 1	5q11.2
MKKS	McKusick-Kaufman syndrome	20p12.2
NANOG	Homeobox transcription factor nanog	12p13.31
NGFI-B/ NR4A1	Nuclear receptor subfamily 4, group A, member 4	12q13.13
NKB/TAC3	Neurokinin B/tachykinin 3	12q13.3
NK3R/TACR3	Neurokinin B receptor/tachykinin receptor 3	4q24
NOBOX	Newborn ovary homeobox, mouse, homolog of	7q35
PDGFA	Platelet-derived growth factor, alpha polypeptide	7p22.3
PDGFRA	Platelet-derived growth factor receptor, alpha	4q12
POR	P450 oxidoreductase	7q11.2
PROK2	Prokineticin 2	3p13
PROKR2	Prokineticin receptor 2	20p12.3
PTC/ PTCH1	Patched	9q22.32
ROR2	Receptor tyrosine kinase-like orphan receptor, 2	9q22.31
RSPO1	R-spondin family member 1	1p34.3
SHH	Sonic hedgehog	7q36.3
SMO/SMOH	Smoothened, Drosophila, homolog of	7q32.1
SOX3	SRY Box 3	Xq27.1
SOX9	SRY Box 9	17q24.3-q25.1
SOX10	SRY Box 10	22q13.1
SRD5A2	5α-Reductase 2	2p23
SRY	Sex determining region on Y	Yp11.3
SF1/NR5A1	Steroidogenic factor 1/nuclear receptor subfamily 5, group A, member 1	9q33
STAR	Steroidogenic acute regulatory protein	8p11.23
STRA8	Stimulated by retinoic acid	7q33
WNT4	Wingless-type MMTV integration site family, member 4	1p36.12
WNT5	Wingless-type MMTV integration site family, member 5A	3p14.3
WNT7A	Wingless-type MMTV integration site family, member 7A	3p25.1
WNT9B	Wingless-type MMTV integration site family, member 9B	17q21.32
WT1	Wilms tumor 1	11p13
WTIP	WT1-interacting protein	19q13.11

REFERENCES

1. Lee, P. A., Houk, C. P., & Ahmed, S. F. (2006). Consensus statement on management of intersex disorders. *Pediatrics*, *118*, e488–e500.
2. Schober, J., Nordenström, A., & Hoebeke, P. (2012). Disorders of sex development: summaries of long-term outcome studies. *J Pediatr Urol*, *8*, 616–623.
3. Vilain, E., Achermann, J. C., Eugster, E. A., et al. (2007). We used to call them hermaphrodites. *Genet Med*, *9*, 65.
4. Kim, Y., & Capel, B. (2006). Balancing the bipotential gonad between alternative organ fates: a new perspective on an old problem. *Dev Dynamics*, *235*, 2292.
5. Jost, A. (1947). Recherches sur la différenciation sexuelle de l'embryon de lapin. *Arch Anat Microsc Morph Exp*, *36*, 271.
6. Sinclair, A. H., Berta, P., Palmer, M. S., et al. (1990). A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. *Nature*, *346*, 240.
7. Gubbay, J., Collignon, J., Koopman, P., et al. (1990). A gene mapping to the sex-determining region of the mouse Y chromosome is a member of a novel family of embryonically expressed genes. *Nature*, *346*, 245.
8. Miller-Hodges, E., & Hohenstein, P. (2012). WT1 in disease: shifting the epithelial-mesenchymal balance. *J Pathol*, *226*, 229–240.
9. Parker, K. L., & Schimmer, B. P. (2006). Embryology and genetics of the mammalian gonads and ducts. In *Knobil and Neill's Physiology of Reproduction* (3rd ed.) (pp. 313–336). St. Louis: Elsevier.
10. Capel, B. (1998). Sex in the 90s: SRY and the switch to the male pathway. *Annu Rev Physiol*, *60*, 497.
11. Bernard, P., Ryan, J., Sim, H., et al. (2012). Wnt signaling in ovarian development inhibits Sf1 activation of Sox9 via the Tesco enhancer. *Endocrinology*, *153*, 901–912.
12. Richardson, B. E., & Lehmann, R. (2010). Mechanisms guiding primordial germ cell migration: strategies from different organisms. *Nat Rev Mol Cell Biol*, *11*, 37–49.
13. Baltus, A. E., Menke, D. B., Hu, Y. C., et al. (2006). In germ cells of mouse embryonic ovaries, the decision to enter meiosis precedes premeiotic DNA replication. *Nat Genet*, *38*, 1430.
14. Gill, M. E., Hu, Y. C., Lin, Y, & Page, D. C. (2011). Licensing of gametogenesis, dependent on RNA binding protein DAZL, as a gateway to sexual differentiation of fetal germ cells. *Proc Natl Acad Sci USA*, *108*, 7443–7448.
15. Jagarlamudi, K., & Rajkovic, A. (2012). Oogenesis: transcriptional regulators and mouse models. *Mol Cell Endocrinol*, *356*, 31–39.
16. Kimble, J., & Page, D. C. (2007). The mysteries of sexual identity: the germ cell's perspective. *Science*, *316*, 400.
17. Le Bouffant, R., Guerquin, M. J., Duquenne, C., et al. (2010). Meiosis initiation in the human ovary requires intrinsic retinoic acid synthesis. *Hum Reprod*, *25*, 2579–2590.
18. Childs, A. J., Cowan, G., Kinnell, H. L., et al. (2011). Retinoic acid signalling and the control of meiotic entry in the human fetal gonad. *PLoS One*, *6*, e20249.
19. Baker, T. G. (1963). A quantitative and cytological study of germ cells in human ovaries. *Proc R Soc Med Lond*, *158*, 417.

20. Hartshorne, G. M., Lyrakou, S., Hamoda, H., et al. (2009). Oogenesis and cell death in human prenatal ovaries: what are the criteria for oocyte selection? *Mol Hum Reprod*, *15*, 805–819.
21. Schaefer, C. B., Ooi, S. K., Bestor, T. H., & Bourc'his, D. (2007). Epigenetic decisions in mammalian germ cells. *Science*, *316*, 398.
22. Allegrucci, C., Thurston, A., Lucas, E., & Young, L. (2005). Epigenetics and the germline. *Reproduction*, *129*, 137.
23. Capel, B., Albrecht, K. H., Washburn, L. L., & Eicher, E. M. (1999). Migration of mesonephric cells into the mammalian gonad depends on Sry. *Mech Dev*, *84*, 127.
24. Cool, J., DeFalco, T. J., & Capel, B. (2011). Vascular-mesenchymal cross-talk through Vegf and Pdgf drives organ patterning. *Proc Natl Acad Sci USA*, *108*, 167–172. Advance online publication.
25. Bullejos, M., & Koopman, P. (2005). Delayed Sry and Sox9 expression in developing mouse gonads under lies B6-Y(DOM) sex reversal. *Dev Biol*, *278*, 473.
26. Knower, K. C., Kelly, S., Ludbrook, L. M., et al. (2011). Failure of SOX9 regulation in 46XY disorders of sex development with SRY, SOX9 and SF1 mutations. *PLoS One*, *6*, e17751.
27. De Santa Barbara, P., Moniot, B., Poulat, F., et al. (2000). Expression and subcellular localization of SF-1, SOX9, WT1, and AMH proteins during early human testicular development. *Dev Dyn*, *217*, 293.
28. Moniot, B., Berta, P., Scherer, G., et al. (2000). Male specific expression suggests role of DMRT1 in human sex determination. *Mech Dev*, *91*, 323.
29. Wilhelm, D., Hiramatsu, R., Mizusaki, H., et al. (2007). SOX9 regulates prostaglandin D synthase gene transcription in vivo to ensure testis development. *J Biol Chem*, *282*, 10553.
30. Wilhelm, D., Martinson, F., Bradford, S., et al. (2005). Sertoli cell differentiation is induced both cell-autonomously and through prostaglandin signaling during mammalian sex determination. *Dev Biol*, *287*, 111.
31. Kim, Y., Bingham, N., Sekido, R., et al. (2007). Fibroblast growth factor receptor 2 regulates proliferation and Sertoli differentiation during male sex determination. *Proc Natl Acad Sci USA 104*, *104*, 16558–16563.
32. Hiramatsu, R., Harikae, K., Tsunekawa, N., et al. (2010). FGF signaling directs a center-to-pole expansion of tubulogenesis in mouse testis differentiation. *Development*, *137*, 303–312.
33. Franco, H. L., & Yao, H. H. (2012). Sex and hedgehog: roles of genes in the hedgehog signaling pathway in mammalian sexual differentiation. *Chromosome Res*, *20*, 247–258.
34. Wilhelm, D., Palmer, S., & Koopman, P. (2007). Sex determination and gonadal development in mammals. *Physiol Rev*, *87*, 1.
35. O'Shaughnessy, P. J., Baker, P. J., Monteiro, A., et al. (2007). Developmental changes in human fetal testicular cell numbers and messenger ribonucleic acid levels during the second trimester. *J Clin Endocrinol Metab*, *92*, 4792–4780.
36. Nef, S., Schaad, O., Stallings, N. R., et al. (2005). Gene expression during sex determination reveals a robust female genetic program at the onset of ovarian development. *Dev Biol*, *287*, 361.
37. Hersmus, R., Kalfa, N., de Leeuw, B., et al. (2008). FOXL2 and SOX9 as parameters of female and male gonadal differentiation in patients with various forms of disorders of sex development (DSD). *J Pathol*, *215*, 31–38.
38. Chassot, A. A., Gregoire, E. P., Magliano, M., et al. (2008). Genetics of ovarian differentiation: Rspo1, a major player. *Sex Dev*, *2*, 219–227.
39. Tomaselli, S., Megiorni, F., Lin, L., et al. (2011). Human RSPO1/R-spondin1 is expressed during early ovary development and augments β-catenin signaling. *PLoS One*, *6*, e16366.
40. El-Khairi, R., Parnaik, R., Duncan, A. J., et al. (2012). Analysis of LIN28A in early human ovary development and as a candidate gene for primary ovarian insufficiency. *Mol Cell Endocrinol*, *351*, 264–268.
41. Childs, A. J., Kinnell, H. L., He, J., & Anderson, R. A. (2012). LIN28 is selectively expressed by primordial and pre-meiotic germ cells in the human fetal ovary. *Stem Cells Dev*, *21*, 2343–2349.
42. Thornton, J. E., & Gregory, R. I. (2012). How does Lin28 let-7 control development and disease? *Trends Cell Biol*, *22*, 474–482.
43. Vaskivuo, T. E., Anttonen, M., Herva, R., et al. (2001). Survival of human ovarian follicles from fetal to adult life: apoptosis, apoptosis-related proteins, and transcription factor GATA-4. *J Clin Endocrinol Metab*, *86*, 3421–3429.
44. Fowler, P. A., Anderson, R. A., Saunders, P. T., et al. (2011). Development of steroid signaling pathways during primordial follicle formation in the human fetal ovary. *J Clin Endocrinol Metab*, *96*, 1754–1762.
45. Roberts, L. M., Visse,r J. A., & Ingraham, H. A. (2002). Involvement of a matrix metalloproteinase in MIS-induced cell death during urogenital development. *Development*, *129*, 1487.
46. Zhan, Y., Fujino, A., MacLaughlin, D. T., et al. (2006). Mullerian inhibiting substance regulates its receptor/SMAD signaling and causes mesenchymal transition of the coelomic epithelial cells early in Mullerian duct regression. *Development*, *133*, 2359.
47. Spencer, T. E., Dunlap, K. A., & Filant, J. (2012). Comparative developmental biology of the uterus: insights into mechanisms and developmental disruption. *Mol Cell Endocrinol*, *354*, 34–53.
48. Marker, P. C., Donjacour, A. A., Dahiya, R., & Cunha, G. R. (2003). Hormonal, cellular, and molecular control of development. *Dev Biol*, *253*, 156.
49. Zhang, T. J., Hoffman, B. G., Ruiz de Algara, T., & Helgason, C. D. (2006). SAGE reveals expression of Wnt signalling pathway members during mouse prostate development. *Gene Expr Patterns*, *6*, 310.
50. Goto, M., Piper Hanley, K., Marcos, J., et al. (2006). In humans, early cortisol biosynthesis provides a mechanism to safeguard female sexual development. *J Clin Invest*, *116*, 953.
51. White, P. C. (2006). Ontogeny of adrenal steroid biosynthesis: why girls will be girls. *J Clin Invest*, *116*, 872.
52. Thankamony, A., Ong, K. K., Dunger, D. B., et al. (2009). Anogenital distance from birth to 2 years: a population study. *Environ Health Perspect*, *117*, 1786–1790.
53. Welsh, M., MacLeod, D. J., Walker, M., et al. (2010). Critical androgen-sensitive periods of rat penis and clitoris development. *Int J Androl*, *33*, e144–e152.
54. MacLeod, D. J., Sharpe, R. M., Welsh, M., et al. (2010). Androgen action in the masculinization programming window and the development of the male reproductive system. *Androl*, *33*, 279–287.
55. Zalel, Y., Pinhas-Hamiel, O., Lipitz, S., et al. (2001). The development of the fetal penis an in utero sonographic evaluation. *Ultrasound Obstet Gynecol*, *17*, 129–131.
56. Hayley, M., Scott, J., Mason I., & Sharpe, R. M. (2009). Steroidogenesis in the fetal testis and its susceptibility to disruption by exogenous compounds. *Endocr Rev*, *30*, 883–925.
57. Vafeiadi, M., Agramunt, S., Papadopoulou, E., et al. (2013). In utero exposure to dioxins and dioxin-like compounds and anogenital distance in newborns and infants. *Environ Health Perspect*, *121*, 125–130. Advance online publication.
58. Hsieh, M. H., Walters, R. C., Walters, R. C., et al. (2011). The relationship between anogenital distance, fatherhood, and fertility in adult men. *PLoS One*, *6*, e18973.
59. Eisenberg, M. L., Jensen, T. K., Chanc Walters, R., & Skakkebaek, N. E. (2012). The relationship between anogenital distance and reproductive hormone levels in adult men. *J Urol*, *187*, 594–598.
60. Wilson. J. D. (2001). Androgens, androgen receptors, and male gender role behavior. *Horm Behav*, *40*, 358.
61. Arnold, A. P., & Burgoyne, P. S. (2004). Are XX and XY brain cells intrinsically different? *Trends Endocrinol Metab*, *15*, 16.
62. Balthazart, J. (2011). Minireview: hormones and human sexual orientation. *Endocrinology*, *152*, 2937–2047.
63. de Courten-Myers, G. M. (1999). The human cerebral cortex: gender differences in structure and function. *J Neuropathol Exp Neurol*, *58*, 217.
64. Rabinowicz, T., Dean, D. E., Petetot, J. M., & de Courten-Myers, G. M. (1999). Gender differences in the human cerebral cortex: more neurons in males: More processes in females. *J Child Neurol*, *14*, 98.
65. Koopman, P., Gubbay, J., Vivian, N., et al. (1991). Male development of chromosomally female mice transgenic for Sry. *Nature*, *351*, 117.
66. Jameson, S. A., Natarajan, A., Cool, J., et al. (2012). Temporal transcriptional profiling of somatic and germ cells reveals biased lineage priming of sexual fate in the fetal mouse gonad. *PLoS Genet*, *8*, e1002575.
67. Hacker, A., Capel, B., Goodfellow, P., et al. (1995). Expression of Sry, the mouse sex determining gene. *Development*, *121*, 1603.
68. Carroll, T. J., Park, J. S., Hayashi, S., et al. (2005). Wnt9b plays a central role in the regulation of mesenchymal to epithelial transitions underlying organogenesis of the mammalian urogenital system. *Dev Cell*, *9*, 283–292.

69. Kreidberg, J. A., Sariola, H., Loring, J. M., et al. (1993). WT-1 is required for early kidney development. *Cell*, *74*, 679.
70. Miyamoto, N., Yoshida, M., Kuratani, S., et al. (1997). Defects of urogenital development in mice lacking Emx2. *Development*, *124*, 1653.
71. Pellegrini, M., Pantano, S., Lucchini, F., et al. (1997). Emx2 developmental expression in primordia of the reproductive and excretory systems. *Anat Embryol*, *196*, 427.
72. Katoh-Fukui, Y., Miyabayashi, K., Komatsu, T., et al. (2012). Cbx2, a polycomb group gene, is required for Sry gene expression in mice. *Endocrinology*, *153*, 913–924.
73. Shawlot, W., & Behringer, R. R. (1995). Requirement for Lim1 in head-organizer function. *Nature*, *374*, 425.
74. Ikeda, Y. Shen, W.-H., Ingraham, H. A., et al. (1994). Developmental expression of mouse steroidogenic factor-1, an essential regulator of the steroid hydroxylases. *Mol Endocrinol*, *8*, 654.
75. Zhao, L., Bakke, M., Hanley, N. A., et al. (2004). Tissue-specific knockouts of steroidogenic factor 1. *Mol Cell Endocrinol*, *215*, 289.
76. Chaboissier, M. C., Kobayashi, A., Vidal, V. I. P., et al. (2004). Functional analysis of Sox8 and Sox9 during sex determination in the mouse. *Development*, *131*, 1891.
77. Zazopoulos, E., Lalli, E., Stocco, D. M., et al. (1997). DNA binding and transcriptional repression by DAX-1 blocks steroidogenesis. *Nature*, *390*, 311.
78. Hanley, N. A., Rainey, W. E., Wilson, D. I., et al. (2001). Expression profiles of SF-1, DAX1, and CYP17 in the human fetal adrenal gland: potential interactions in gene regulation. Mol *Endocrinol*, *15*, 57.
79. DiNapoli, L., Batchvarov, J., & Capel, B. (2006). FGF9 promotes survival of germ cells in the fetal testis. *Development*, *133*, 1519.
80. Kim Y., Kobayashi, A., Sekido, R., et al. (2006). Fgf9 and Wnt4 act as antagonistic signals to regulate mammalian sex determination. *PLoS Biol*, *4*, 1000.
81. Bagheri-Fam, S., Sim, H., Bernard, P., et al. (2008). Loss of Fgfr2 leads to partial XY sex reversal. *Dev Biol*, *314*, 71–83.
82. Jameson, S. A., Lin, Y. T., & Capel, B. (2012). Testis development requires the repression of Wnt4 by Fgf signaling. *Dev Biol*, *370*, 24–32.
83. Vainio, S., Heikkila, M., Kispert, A., et al. (1999). Female development in mammals is regulated by Wnt-4 signalling. *Nature*, *397*, 405.
84. Tevosian, S. G., Albrecht, K. H., Crispino, J. D., et al. (2002). Gonadal differentiation, sex determination and normal Sry expression in mice require direct interaction between transcription partners GATA4 and FOG2. *Development*, *129*, 4627–4634.
85. Siggers, P., Smith, L., & Greenfield, A. (2002). Sexually dimorphic expression of Gata-2 during mouse gonad development. *Mech Dev*, *111*, 159–162.
86. Jeays-Ward, K., Hoyle, C., Brennan, J., et al. (2003). Endothelial and steroidogenic cell migration are regulated by WNT4 in the developing mammalian gonad. *Development*, *130*, 3663.
87. Uda, M., Ottolenghi, C., Crisponi, L., et al. (2004). Foxl2 disruption causes mouse ovarian failure by pervasive blockage of follicle development. *Hum Mol Genet*, *13*, 1171–1181.
88. Kashimada, K., Pelosi, E., Chen, H., Schlessinger D., et al. (2011). FOXL2 and BMP2 act cooperatively to regulate follistatin gene expression during ovarian development. *Endocrinology*, *152*, 272–280.
89. Maatouk, D. M., DiNapoli, L., Alvers, A., et al. (2008). Stabilization of beta-catenin in XY gonads causes male-to-female sex-reversal. *Hum Mol Genet*, *17*, 2949–2955.
90. Chassot, A. A., Ranc, F., Gregoire, E. P., et al. (2008). Activation of beta-catenin signaling by Rspo1 controls differentiation of the mammalian ovary. *Hum Mol Genet*, *17*, 1264–1277.
91. Uhlenhaut, N. H., Jakob, S., Anlag, K., et al. (2009). Somatic sex reprogramming of adult ovaries to testes by FOXL2 ablation. *Cell*, *139*, 1130–1142.
92. Besmer, P., Manova, K., Duttlinger, R., et al. (1993). The kit-ligand (steel factor) and its receptor c-kit/W: pleiotropic roles in gametogenesis and melanogenesis. *Dev* (suppl), 125–137.
93. Brannan, C. I., Lyman, S. D., Williams, D. E., et al. (1991). Steel-Dickie mutation encodes a c-kit ligand lacking transmembrane and cytoplasmic domains. *Proc Nat Acad Sci U S A*, *88*, 4671.
94. Anderson, E. L., Baltus, A. E., Roepers-Gajadien, H. L., et al. (2008). Stra8 and its inducer, retinoic acid, regulate meiotic initiation in both spermatogenesis and oogenesis in mice. *Proc Natl Acad Sci U S A*, *105*, 14976–14980.
95. Bitgood, M. J., Shen, L., & McMahon, A. P. (1996). Sertoli cell signaling by desert hedgehog regulates the male germline. *Curr Biol*, *6*, 298.
96. Parmantier, E., Lynn, B., Lawson, D., et al. (1999). Schwann cell-derived desert hedgehog controls the development of peripheral nerve sheath. *Neuron*, *23*, 713.
97. Bitgood, M. J., & McMahon, A. P. (1995). Hedgehog and Bmp genes are coexpressed at many diverse sites of cell-cell interaction in the mouse embryo. *Dev Biol*, *172*, 126.
98. Zimmermann, S., Steding, G., Emmen, J. M. A., et al. (1999). Targeted disruption of the Insl3 gene causes bilateral cryptorchidism. *Mol Endocrinol*, *13*, 681.
99. Nef, S., & Parada, L. (1999). Cryptorchidism in mice mutant for Insl3. *Nature Genetics*, *22*, 295.
100. Rijli, F. M., Matyas, R., Pellegrini, M., et al. (1995). Cryptorchidism and homeotic transformations of spinal nerves and vertebrae in Hoxa-10 mutant mice. *Proc Natl Acad Sci*, *92*, 8185.
101. Gorlov, I. P., Kamat, A., Bogatcheva, N. V., et al. (2002). Mutations of the GREAT gene cause cryptorchidism. *Hum Mol Genet*, *11*, 2309.
102. Larsson, S. H., Charlieu, J.-P., Miyagawa, K., et al. (1995). Subnuclear localization of WT1 in splicing or transcription factor domains is regulated by alternative splicing. *Cell*, *81*, 391.
103. Miller-Hodges, E., & Hohenstein, P. (2012). WT1 in disease: nephropathie parenchymateusemal balance. *J Pathol*, *226*, 229–240.
104. Klattig, J., Sierig, R., Kru0spe, D., et al. (2007). The Wilms tumor protein Wt1 is an activator of the anti-Mullerian hormone receptor gene Amhr2. *Mol Cell Biol*, *27*, 4355.
105. Denys, P., Malvaux, P., van den Berghe, H., et al. (1967). Association d'un syndrome anatomo-pathologique de pseudo-hermaphroditisme masculin d'une tumeur de Wilms, d'une nephropathie parenchymateuse et d'un mosaicisme XX/XY. *Arch Fr Pediatr*, *24*, 729.
106. Drash, A., Sherman, F., Hartmann, W. H., et al. (1979). A syndrome of pseudohermaphroditism, Wilms tumor, hypertension, and degenerative renal disease. *J Pediatr*, *76*, 585.
107. Bardeesy, N., Zabel, B., Schmitt, K., et al. (1994). WT1 mutations associated with incomplete Denys-Drash syndrome define a domain predicted to behave in a dominant-negative fashion. *Genomics*, 21, 663.
108. Barbosa, A. S., Hadjuatgabasiou, C. G., Theodoridis, C., et al. (1999). The same mutation affecting the splicing of WT1 gene is present on Frasier syndrome patients with or without Wilms tumor. *Hum Mutat*, *13*, 146.
109. Suri, M., Kelehan, P., O'neill, D., et al. (2007). WT1 mutations in Meacham syndrome suggest a coelomic mesothelial origin of the cardiac and diaphragmatic malformations. *Am J Med Genet A*, *143A*, 2312–2320.
110. Köhler, B., Biebermann, H., Friedsam, V., et al. (2011). Analysis of the Wilms tumor suppressor gene (WT1) in patients 46,XY disorders of sex development. *J Clin Endocrinol Metab*, *96*, E–131–E1136.
111. Yao, H. H., Matzuk, M. M., Jorgez, C. J., et al. (2004). Follistatin operates downstream of Wnt4 in mammalian ovary organogenesis. *Dev Dyn*, *230*, 210.
112. Elejalde, B. R., Opitz, J. M., de Elejalde, M. M., et al. (1984). Tandem dup (1p) within the short arm of chromosome 1 in a child with ambiguous genitalia and multiple congenital anomalies. *Am J Med Genet*, *17*, 723.
113. Mandel, H., Shemer, R., Borochowitz, Z. U., et al. (2008). SERKAL syndrome: an autosomal-recessive disorder caused by a loss-of-function mutation in WNT4. *Am J Hum Genet*, *82*, 39–47.
114. Philibert, P., Biason-Lauber, A., Gueorguieva, I., et al. (2011). Molecular analysis of WNT4 gene in four adolescent girls with mullerian duct abnormality and hyperandrogenism (atypical Mayer-Rokitansky-Küster-Hauser syndrome). *Fertil Steril*, *95*, 2683–2686.
115. Biason-Lauber, A., De Filippo, G., Konrad, D., et al. (2007). WNT4 deficiency: a clinical phenotype distinct from the classic Mayer-Rokitansky-Kuster-Hauser syndrome: a case report. *Hum Reprod*, *22*, 224.
116. Biason-Lauber, A., Konrad, D., Navratil, F., & Schoenle, E. J. (2004). A WNT4 mutation associated with Mullerian-duct

regression and virilization in a 46,XX woman. *N Engl J Med*, *351*, 792.
117. Camats, N., Pandey, A. V., Fernández-Cancio, M., et al. (2012). Ten novel Mutations in the NR5A1 gene cause disordered sex development in 46,XY and ovarian insufficiency in 46,XX individuals. *J Clin Endocrinol Metab*, *97*, E1294–E1306.
118. Ferraz-de-Souza, B., Lin, L., & Achermann, J. C. (2011). Steroidogenic factor-1 (SF-1, NR5A1) and human disease. *Mol Cell Endocrinol*, *336*, 198–205.
119. Biason-Lauber, A., & Schoenle, E. J. (2000). Apparently normal ovarian differentiation in a prepubertal girl with transcriptionally inactive steroidogenic factor 1 (NR5A1/SF-1) and adrenocortical insufficiency. *Am J Hum Genet*, *67*, 1563.
120. Philibert, P., Leprieur, E., Zenaty, D., et al. (2010). Steroidogenic factor-1 (SF-1) gene mutation as a frequent cause of primary amenorrhea in 46,XY female adolescents with low testosterone concentration. *Reprod Biol Endocrinol*, *8*, 28.
121. Biason-Lauber, A., Konrad, D., Meyer, M., et al. (2009). Ovaries and female phenotype in a girl with 46,XY karyotype and mutations in the CBX2 gene. *Am J Hum Genet*, *84*, 658–663.
122. Hersmus, R., de Leeuw, B. H., Wolffenbuttel, K. P., et al. (2008). New insights into type II germ cell tumor pathogenesis based on studies of patients with various forms of disorders of sex development (DSD). *Mol Cell Endocrinol*, *291*, 1–10.
123. Zhao, L., & Koopman, P. (2012). SRY protein function in sex determination: thinking outside the box. *Chromosome Res*, *20*, 153–162.
124. Schnitt-Ney, M., Thiele, H., Kaltwasser, P., et al. (1995). Two novel SRY missense mutations reducing DNA binding identified in XY females and their mosaic father. *Am J Hum Genet*, *56*, 862.
125. Hines, R. S., Tho, S. P., Zhang, Y. Y., et al. (1997). Paternal somatic and germ-line mosaicism for a sex-determining region on Y (SRY) missense mutation leading to recurrent 46,XY sex reversal. *Fertil Steril*, *67*, 675.
126. Hawkins, J. R., Taylor, A., Goodfellow, P. N., et al. (1992). Evidence for increased prevalence of SRY mutations in XY females with complete rather than partial gonadal dysgenesis. *Am J Hum Genet*, *51*, 979.
127. Jager, R. J., Harley, V. R., Pfeiffer, R. A., et al. (1992). A familial mutation in the testis-determining gene SRY shared by both sexes. *Hum Genet*, *90*, 350.
128. Shahid, M., Dhillon, V. S., Aslam, M., & Husain, S. A. (2005). Three new novel point mutations localized within and downstream of high-mobility group-box region in SRY gene in three Indian females with Turner syndrome. *J Clin Endocrinol Metab*, *90*, 2429–2435.
129. Foster, J. W., Dominguez-Steglich, M. A., Guioli, S., et al. (1994). Campomelic dysplasia and autosomal sex reversal caused by mutations in an SRY-related gene. *Nature*, *372*, 525.
130. Cameron, F. J., Hageman, R. M., Cooke-Yarborough, C., et al. (1996). A novel germ line mutation in SOX9 causes familial campomelic dysplasia and sex reversal. *Hum Molec Genet*, *5*, 1625.
131. Chen, S. Y., Lin, S. J., Tsai, L. P., & Chou, Y. Y. (2012). Sex-reversed acampomelic campomelic dysplasia with a homozygous deletion mutation in SOX9 gene. *Urology*, *79*, 908–911.
132. Corbani, S., Chouery, E., Eid, B., et al. (2011). Mild campomelic dysplasia: report on a case and review. *Mol Syndromol*, *1*, 163–168.
133. Lecointre, C., Pichon, O., Hamel, A., et al. (2009). Familial acampomelic form of campomelic dysplasia caused by a 960 kb deletion upstream of SOX9. *Am J Med Genet A*, *149A*, 1183–1189.
134. Bardoni, B., Zanaria, E., Guioli, S., et al. (1994). A dosage sensitive locus at chromosome Xp21is involved in male to female sex reversal. *Nature Genetics*, *7*, 497.
135. Barbaro, M., Oscarson, M., Schoumans, J., et al. (2007). Isolated 46,XY gonadal dysgenesis in two sisters caused by a Xp21.2 interstitial duplication containing the DAX1 gene. *J Clin Endocrinol Metab*, *92*, 3305–3313.
136. Bernard, P., Ludbrook, L., Queipo, G., et al. (2006). A familial missense mutation in the hinge region of DAX1 associated with late-onset AHC in a prepubertal female. *Mol Genet Metab*, *88*, 272.
137. Jadhav, U., Harris, R. M., & Jameson, J. L. (2011). Hypogonadotropic hypogonadism in subjects with DAX1 mutations. *Mol Cell Endocrinol*, *346*, 65–73.
138. Seminara, S. B., Achermann, J. C., Genel, M., et al. (1999). X-linked adrenal hypoplasia congenita: a mutation in DAX1 expands the phenotypic spectrum in males and females. *J Clin Endocrinol Metab*, *84*, 4501.
139. Merke, D. P., Tajima, T., Baron, J., et al. (1999). Brief report: hypogonadotropic hypogonadism in a female caused by an X-linked recessive mutation in the DAX1 gene. *N Engl J Med*, *340*, 1248.
140. Francke, U., Harper, J. F., Darras, B. T., et al. (1987). Congenital adrenal hypoplasia, myopathy, and glycerol kinase deficiency: molecular genetic evidence for deletions. *Am J Hum Genet*, *40*, 212.
141. Bernard, P., Ludbrook, L., Queipo, G., et al. (2006). A familial missense mutation in the hinge region of DAX1 associated with late-onset AHC in a prepubertal female. *Mol Genet Metab*, *88*, 272.
142. Umehara, F., Tate, G., Itoh, K., et al. (2006). A novel mutation of desert hedgehog in a patient with 46,XY partial gonadal dysgenesis accompanied by minifascicular neuropathy. *Am J Hum Genet*, *67*, 1302.
143. Das, D. K., Sanghavi, D., Gawde, H., et al. (2011). Novel homozygous mutations in Desert hedgehog gene in patients with 46,XY complete gonadal dysgenesis and prediction of its structural and functional implications by computational methods. *Eur J Med Genet*, *54*, e529–e534.
144. Canto, P., Vilchis, F., Soderlund, D., et al. (2005). A heterozygous mutation in the desert hedgehog gene in patients with mixed gonadal dysgenesis. *Mol Hum Reprod*, *11*, 833.
145. Lourenço, D., Brauner, R., Rybczynska, M., et al. (2011). Loss-of-function mutation in GATA4 causes anomalies of human testicular development. *Proc Natl Acad Sci USA*, *108*, 1597–1602.
146. Finelli, P., Pincelli, A. I., Russo, S., et al. (2007). Disruption of friend of GATA 2 gene (FOG-2) by a de novo t(8;10) chromosomal translocation is associated with heart defects and gonadal dysgenesis. *Clin Genet*, *71*, 195–204.
147. Pearlman, A., Loke, J., Le Caignec, C., et al. (2010). Mutations in MAP3K1 cause 46,XY disorders of sex development and implicate a common signal transduction pathway in human testis determination. *Am J Hum Genet*, *87*, 898–904.
148. Ogata, T., Muroya, K., Matsuo, N., et al. (1997). Impaired male sex development in an infant with molecularly defined partial 9p monosomy: implication for a testis forming gene(s) on 9p. *J Med Genet*, *34*, 331.
149. McDonald, M. T., Flejter, W., Sheldon, S., et al. (1997). XY sex reversal and gonadal dysgenesis due to 9p24 monosomy. *Am J Med Genet*, *73*, 321.
150. Onesimo, R., Orteschi, D., Scalzone, M., et al. (2012). Chromosome 9p deletion syndrome and sex reversal: novel findings and redefinition of the critically deleted regions. *Am J Med Genet A*, *158A*, 2266–2271.
151. Ledig, S., Hiort, O., Wünsch, L., & Wieacker, P. (2012). Partial deletion of DMRT1 causes 46,XY ovotesticular disorder of sexual development. *Eur J Endocrinol*, *167*, 119–124.
152. Wada, T., Kubota, T., Fukushima, Y., et al. (2000). Molecular genetic study of Japanese patients with X-linked alpha-thalassemia/mental retardation syndrome (ATR-X). *Am J Med Genet*, *94*, 242.
153. De La Fuente, R., Baumann, C., & Viveiros, M. M. (2011). Role of ATRX in chromatin structure and function: implications for chromosome instability and human disease. *Reproduction*, *142*, 221–234.
154. Huyhn, K., Renfree, M. B., Graves, J. A., & Pask, A. J. (2011). ATRX has a critical and conserved role in mammalian sexual differentiation. *BMC Dev Biol*, *11*, 39.
155. Reardon, W., Gibbons, R. J., Winter, R. M., et al. (1995). Male pseudohermaphroditism in sibs with the alpha-thalassemia/mental retardation (ATR-X) syndrome. *Am J Med Genet*, *55*, 285.
156. McPherson, E. W., Clemens, M. M., Gibbons, R. J., et al. (1995). X-linked alpha-thalassemia/mental retardation (ATR-X) syndrome: a new kindred with severe genital anomalies and mild hematologic expression. *Am J Med Genet*, *55*, 302.
157. Tang, P., Park, D. J., Marshall Graves, J. A., & Harley, V. R. (2004). ATRX and sex differentiation. *Trends Endocrinol Metab*, *15*, 339.

158. Gibbons, R. J., & Higgs, D. R. (2000). Molecular-clinical spectrum of the ATR-X syndrome. *Am J Med Genet*, *97*, 204.
159. Antic, T., Hyjek, E. M., & Taxy, J. B. (2011). The vanishing testis: a histomorphologic and clinical assessment. *Am J Clin Pathol*, *136*, 872–880.
160. Law, H., Mushtaq, I., Wingrove, K., et al. (2006). Histopathological features of testicular regression syndrome: relation to patient age and implications for management. *Fetal Pediatr Pathol*, *25*, 119.
161. Josso, N., & Briard, M. L. (1980). Embryonic testicular regression syndrome: variable phenotypic expression in siblings. *J Pediatr*, *97*, 200.
162. Vilain, E., Le Merrer, M., Lecointre, C., et al. (1999). IMAGe, a new clinical association of intrauterine growth retardation, metaphyseal dysplasia, adrenal hypoplasia congenita, and genital anomalies. *J Clin Endocrinol Metab*, *84*, 4335.
163. Arboleda, V. A., Lee, H., Parnaik, R., et al. (2012). Mutations in the PCNA-binding domain of CDKN1C cause IMAGe syndrome. *Nat Genet*, *44*, 788-792.
164. Kang, S., Graham, J. M., Jr., Olney, A. H., & Biesecker, L. G. (1997). GLI3 frameshift mutations cause autosomal dominant Pallister-Hall syndrome. *Nat Genet*, *15*, 266–268.
165. Narumi, Y., Kosho, T., & Tsuruta, G. (2010). Genital abnormalities in Pallister-Hall syndrome: report of two patients and review of the literature. *Am J Med Genet A*, *152A*, 3143–3147.
166. Shoubridge, C., Fullston, T., & Gécz, J. (2010). ARX spectrum disorders: making inroads into the molecular pathology. *Hum Mutat*, *31*, 889–900.
167. Uyanik, G., Aigner, L., Martin, P., et al. (2003). ARX mutations in X-linked lissencephaly with abnormal genitalia. *Neurology*, *61*, 232.
168. Campeau, P. M., Kim, J. C., & Lu, J. T. (2012). Mutations in KAT6B, encoding a histone acetyltransferase, cause Genitopatellar syndrome. *Am J Hum Genet*, *90*, 282–289.
169. Simpson, M. A., Deshpande, C., Dafou, D., et al. (2012). De novo mutations of the gene encoding the histone acetyltransferase KAT6B cause Genitopatellar syndrome. *Am J Hum Genet*, *90*, 290–294.
170. Verkauskas, G., Jaubert, F., Lortat-Jacob, S., et al. (2007). The long-term followup of 33 cases of true hermaphroditism: a 40-year experience with conservative gonadal surgery. *J Urol*, *177*, 726.
171. Aleck, K. A., Argueso, L., Stone, J., et al. (1999). True hermaphroditism with partial duplication of chromosome 22 and without SRY. *Am J Med Genet*, *85*, 2.
172. Jimenez, A. L., Kofman-Alfaro, S., Berumen, J., et al. (2000). Partially deleted SRY gene confined to testicular tissue in a 46,XX true hermaphrodite without SRY in leukocytic DNA. *Am J Med Genet*, *93*, 417.
173. Kuhnle, U., Schwarz, H. P., Lohrs, U., et al. (1993). Familial true hermaphroditism: paternal and maternal transmission of true hermaphroditism (46,XX) and XX maleness in the absence of Y-chromosomal sequences. *Hum Genet*, *92*, 571.
174. Ramos, E. S., Moreira-Filho, C. A., Vicente, Y. A., et al. (1996). SRY-negative true hermaphrodites and an XX male in two generations of the same family. *Hum Genet*, *97*, 596–598.
175. Sarafoglou, K., & Ostrer, H. (2000). Familial sex reversal: a review. *J Clin Endocrinol Metab*, *85*, 483–493.
176. Alonso, G., Pasqualini, T., Busaniche, J., et al. (2007). True hermaphroditism in a phenotypic male without ambiguous genitalia: an unusual presentation at puberty. *Horm Res*, *68*, 261.
177. Talerman, A., Verp, M. S., Senekjian, E., et al. (1990). True hermaphrodite with bilateral ovotestes, bilateral gonadoblastomas and dysgerminomas, 46,XX/46,XY karyotype, and a successful pregnancy. *Cancer*, *66*, 2668.
178. de la Chapelle, A., Hastbacka, J., Korhonen, T., & Maenpaa, J. (1990). The etiology of XX sex reversal. *Reprod Nutr Dev*, *1*, 39S.
179. Kadandale, J. S., Wachtel, S. S., Tunca, Y., et al. (2000). Localization of SRY by primed in situ labeling in XX and XY sex reversal. *Am J Med Genet*, *95*, 71.
180. Dauwerse, J. G., Hansson, K. B., Brouwers, A. A., et al. (2006). An XX male with the sex-determining region Y gene inserted in the long arm of chromosome 16. *Fertil Steril*, *86*, 463.e1.
181. Alves, C., Braid, Z., Coeli, F. B., & Mello, M. P. (2010). 46,XX male—testicular disorder of sexual differentiation (DSD): hormonal, molecular and cytogenetic studies. Arq Bras Endocrinol Metabol, 54, 685–689.
182. Seeherunvong, T., Perera, E. M., Bao, Y., et al. (2004). 46,XX sex reversal with partial duplication of chromosome arm 22q. *Am J Med Genet A*, *127A*, 149–151.
183. Cox, J. J., Willatt, L., Homfray, T., & Woods, C. G. (2011). A SOX9 duplication and familial 46,XX developmental testicular disorder. *N Engl J Med*, *364*, 91–93.
184. Sutton, E., Hughes, J., White, S., et al. (2011). Identification of SOX3 as an XX male sex reversal gene in mice and humans. *J Clin Invest*, *121*, 328–341.
185. Moalem, S., Babul-Hirji, R., Stavropolous, D. J., et al. (2012). XX male sex reversal with genital abnormalities associated with a de novo SOX3 gene duplication. *Am J Med Genet A*, *158A*, 1759–1764.
186. Woods, K. S., Cundall, M., Turton, J., et al. (2005). Over- and underdosage of SOX3 is associated with infundibular hypoplasia and hypopituitarism. *Am J Hum Genet*, *76*, 833–849.
187. Benko, S., Gordon, C. T., Mallet, D., et al. (2011). Disruption of a long distance regulatory region upstream of SOX9 in isolated disorders of sex development. *J Med Genet*, *48*, 825–830.
188. Radi, O., Parma, P., Imbeaud, S., et al. (2005). XX sex reversal, palmoplantar keratoderma, and predisposition to squamous cell carcinoma: genetic analysis in one family. *Am J Med Genet A*, *138A*, 241–246.
189. Parma, P., Radi, O., Vidal, V., et al. (2006). R-spondin1 is essential in sex determination, skin differentiation and malignancy. *Nat Genet*, *38*, 1304–1309.
190. Tomaselli, S., Megiorni, F., De Bernardo, C. et al. (2008). Syndromic true hermaphroditism due to an R-spondin1 (RSPO1) homozygous mutation. *Hum Mutat*, *29*, 220–226.
191. Di Pasquale, E., Beck-Peccoz, P., & Persani, L. (2004). Hypergonadotropic ovarian failure associated with an inherited mutation of human bone morphogenetic protein-15 (BMP15) gene. *Am J Hum Genet*, *75*, 106–111.
192. Fogli, A., Rodriguez, D., Eymard-Pierre, E., et al. (2003). Ovarian failure related to eukaryotic initiation factor 2B mutations. *Am J Hum Genet*, *72*, 1544–1550.
193. Zangen, D., Kaufman, Y., Zeligson, S., et al. (2011). XX ovarian dysgenesis is caused by a PSMC3IP/HOP2 mutation that abolishes coactivation of estrogen-driven transcription. *Am J Hum Genet*, *89*, 572–579.
194. Pisarska, M. D., Barlow, G., & Kuo, F. T. (2011). Minireview: roles of the forkhead transcription factor FOXL2 in granulosa cell biology and pathology. *Endocrinology*, *152*, 1199–1208.
195. Uhlenhaut, N. H., & Treier, M. (2006). Foxl2 function in ovarian development. *Mol Genet Metab*, *88*, 225.
196. Méduri, G., Bachelot, A., Duflos, C., et al. (2010). FOXL2 mutations lead to different ovarian phenotypes in BPES patients: case report. *Hum Reprod*, *25*, 235–243.
197. Benayoun, B. A., Caburet, S., Dipietromaria, A., et al. (2008). The identification and characterization of a FOXL2 response element provides insights into the pathogenesis of mutant alleles. *Hum Mol Genet*, *17*, 3118–3127.
198. Bentsi-Barnes, I. K., Kuo, F. T., Barlow, G. M., & Pisarska, M. D. (2010). Human forkhead L2 represses key genes in granulosa cell differentiation including aromatase, P450scc, and cyclin D2. *Fertil Steril*, *94*, 353–356.
199. Vaiman, D., Koutita, O., Oustry, A., et al. (1996). Genetic mapping of the autosomal region involved in XX sex-reversal and horn development in goats. *Mamm Genome*, *7*, 133–137.
200. Bouilly, J., Bachelot, A., Broutin, I., et al. (2011). Novel NOBOX loss-of-function mutations account for 6.2% of cases in a large primary ovarian insufficiency cohort. *Hum Mutat*, *32*, 1108–1113.
201. Zhao, H., Chen, Z. J., Qin, Y., et al. (2008). Transcription factor FIGLA is mutated in patients with premature ovarian failure. *Am J Hum Genet*, *82*, 1342–1348.
202. Miller, W. L., & Bose, H. S. (2011). Early steps in steroidogenesis: intracellular cholesterol trafficking. *J Lipid Res*, *52*, 2111–2135.
203. Auchus, R. J., & Miller, W. L. (2012). Defects in androgen biosynthesis causing 46,XY disorders of sexual development. *Semin Reprod Med*, *30*, 417–426.
204. Voutilainen, R., & Miller, W. L. (1986). Developmental expression of genes for the stereoidogenic enzymes P450scc (20,22-desmolase),

P450c17 (17 alpha-hydroxylase/17,20-lyase), and P450c21 (21-hydroxylase) in the human fetus. *J Clin Endocrinol Metab*, *63*, 1145–1150.
205. Escobar, J. C., Patel, S. S., Beshay, V. E., et al. (2011). The human placenta expresses CYP17 and generates androgens de novo. *J Clin Endocrinol Metab*, *96*, 1385–1392.
206. Auchus, R. J. (2004). The backdoor pathway to dihydrotestosterone. *Trends Endocrinol Metab*, *15*, 432–438.
207. Kremer, H., Kraaij, R., Toledo, S. P. A., et al. (1995). Male pseudohermaphroditism due to a homozygous missense mutation of the luteinizing hormone receptor gene. *Nat Genet*, *9*, 160.
208. Kossack, N., Troppmann, B., Richter-Unruh, A., et al. (2013). Aberrant transcription of the LHCGR gene caused by a mutation in exon 6A leads to Leydig cell hypoplasia type II. *Mol Cell Endocrinol*, *366*, 59–67.
209. Toldeo, S. P. A., Brunner, H. G., Kraaij, R., et al. (1996). An inactivation mutation of the luteinizing hormone receptor causes amenorrhea in a 46,XX female. *J Clin Endocrinol Metab*, *81*, 3850.
210. Arnhold, I. J. P., Latronico, A. C., Batista, M. C., et al. (1997). Ovarian resistance to luteinizing hormone: a novel cause of amenorrhea and infertility. *Fertil Steril*, *67*, 394.
211. Latronico, A. C., & Arnhold, I. J. (2012). Inactivating mutations of the human luteinizing hormone receptor in both sexes. *Semin Reprod Med*, *30*, 382–386.
212. Richter-Unruh, A., Korsch, E., Hiort, O., et al. (2005). Novel insertion frameshift mutation of the LH receptor gene: problematic clinical distinction of Leydig cell hypoplasia from enzyme defects primarily affecting testosterone biosynthesis. *Eur J Endocrinol*, *152*, 255.
213. Porter, F. D. (2008). Smith-Lemli-Opitz syndrome: pathogenesis, diagnosis and management. *Eur J Hum Genet*, *16*, 535–541.
214. DeBarber, A. E., Eroglu, Y., Merkens, L. S., et al. (2011). Smith-Lemli-Opitz syndrome. *Expert Rev Mol Med*, *13*, e24.
215. Shackleton, C. H. L., Roitman, E., Kratz, L. E., et al. (1999). Equine type estrogens produced by a pregnant woman carrying a Smith-Lemli-Opitz fetus. *J Clin Endocrinol Metab*, *84*, 1157.
216. Nowaczyk, M. J., Waye, J. S., & Douketis, J. D. (2006). DHCR7 mutation carrier rates and prevalence of the RSH/Smith-Lemli-Opitz syndrome: where are the patients? *Am J Med Genet A*, *140*, 2057.
217. Lin, D., Sugawara, T., Strauss, J. F., III, et al. (1999). Role of steroidogenic acute regulatory protein in adrenal and gonadal steroidogenesis. *Science*, *267*, 1828.
218. Tee, M. K., Lin, D., Sugawara, T., et al. (1995). T→A transversion 11 bp from a splice acceptor site in the gene for steroidogenic acute regulatory protein causes congenital lipoid adrenal hyperplasia. *Hum Mol Genet*, *4*, 2299.
219. Bose, H. S., Sato, S., Aisenberg, J., et al. (2000). Mutations in the steroidogenic acute regulatory protein (StAR) in six patients with congenital lipoid adrenal hyperplasia. *J Clin Endocrinol Metab*, *85*, 3636.
220. Miller, W. L. (2007). StAR search: what we know about how the steroidogenic acute regulatory protein mediates mitochondrial cholesterol import. *Mol Endocrinol*, *21*, 589.
221. Bose, H. S., Sugawara, T., Strauss, J. F., III, et al. (1996). The pathophysiology and genetics of congenital lipoid adrenal hyperplasia. *N Engl J Med*, *335*, 1870.
222. Bose, H. S., Pescovitz, O. H., & Miller, W. L. (1997). Spontaneous feminization in a 46,XX female patient with congenital lipoid adrenal hyperplasia caused by a homozygous frame shift mutation in the steroidogenic acute regulatory protein. *J Clin Endocrinol Metab*, *82*, 1511.
223. Sahakitrungruang, T., Soccio, R. E., Lang-Muritano, M., et al. (2010). Clinical, genetic, and functional characterization of four patients carrying partial loss-of-function mutations in the steroidogenic acute regulatory protein (StAR). *J Clin Endocrinol Metab*, *95*, 3352–3359.
224. Flück, C. E., Pandey, A. V., Dick, B., et al. (2011). Characterization of novel StAR (steroidogenic acute regulatory protein) mutations causing non-classic lipoid adrenal hyperplasia. *PLoS One*, *6*, e20178.
225. Metherell, L. A., Naville, D., Halaby, G., et al. (2009). Nonclassic lipoid congenital adrenal hyperplasia masquerading as familial glucocorticoid deficiency. *J Clin Endocrinol Metab*, *94*, 3865–3871.
226. Rubtsov, P., Karmanov, M., Sverdlova, P., et al. (2009). A novel homozygous mutation in CYP11A1 gene is associated with late-onset adrenal insufficiency and hypospadias in a 46,XY patient. *J Clin Endocrinol Metab*, *94*, 936–939.
227. Sahakitrungruang, T., Tee, M. K., Blackett, P. R., & Miller, W. L. (2011). Partial defect in the cholesterol side-chain cleavage enzyme P450scc (CYP11A1) resembling nonclassic congenital lipoid adrenal hyperplasia. *J Clin Endocrinol Metab*, *96*, 792–798.
228. Parajes, S., Kamrath, C., Rose, I. T., et al. (2011). A novel entity of clinically isolated adrenal insufficiency caused by a partially inactivating mutation of the gene encoding for P450 side chain cleavage enzyme (CYP11A1). *J Clin Endocrinol Metab*, *96*, E1798–E1806.
229. Kim, C. J., Lin, L., Huang, N., et al. (2008). Severe combined adrenal and gonadal deficiency caused by novel mutations in the cholesterol side chain cleavage enzyme, P450scc. *J Clin Endocrinol Metab*, *93*, 696–702.
230. Gucev, Z. S., Tee, M. K., Chitayat, D., et al. (2013). Distinguishing deficiencies in the steroidogenic acute regulatory protein and the cholesterol side chain cleavage enzyme causing neonatal adrenal failure. *J Pediatr*, *162*, 819–822.
231. Hanley, N. A., & Arlt, W. (2006). The human fetal adrenal cortex and the window of sexual differentiation. *Trends Endocrinol Metab*, *17*, 391.
232. Miller, W. L., & Auchus, R. J. (2011). The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. *Endocr Rev*, *32*, 81–151.
233. Speiser, P. W., Dupont, B., Rubinstein, P., et al. (1985). High frequency of nonclassical steroid 21-hydroxylase deficiency. *Am J Hum Genet*, *37*, 650.
234. Pang, S., & Clark, A. (1993). Congenital adrenal hyperplasia due to 21-hydroxylase deficiency: newborn screening and its relationship to the diagnosis and treatment of the disorder. *Screening*, *2*, 105.
235. Witchel, S. F., & Azziz, R. (2011). Congenital adrenal hyperplasia. *J Pediatr Adolesc Gynecol*, *24*, 116–126.
236. Prader, A. (1954). Der genitalbefund beim Pseudohermaphroditismus femininus der kengenitalen adrenogenitalen Syndroms. *Helv Paediatr Acta*, *9*, 231.
237. Grumbach, M. M., Ducharme, J. R. (1960). The effects of androgens on fetal sexual development: androgen-induced female pseudohermaphroditism. *Fertil Steril*, *11*, 157.
238. Witchel, S. F., Nayak, S., Suda-Hartman, M., et al. (1997). Newborn screening for 21-hydroxylase deficiency: results of CYP21 molecular genetic analysis. *J Pediatr*, *131*, 328.
239. Votava, F., Torok, D., Kovacs, J., et al. for the Middle European Society for Paediatric Endocrinology. (2005). Congenital adrenal hyperplasia (MESPE-CAH) study group: estimation of the false-negative rate in newborn screening for congenital adrenal hyperplasia. *Eur J Endocrinol*, *152*, 869.
240. Kamrath, C., Hochberg, Z., Hartmann, M. F., et al. (2012). Increased activation of the alternative "backdoor" pathway in patients with 21-hydroxylase deficiency: evidence from urinary steroid hormone analysis. *J Clin Endocrinol Metab*, *97*, E367–E375.
241. Bristow, J., Tee, M. K., Gitelman, S. E., et al. (1993). Tenascin-X: a novel extracellular matrix protein encoded by the human XB gene overlapping P450c21B. *J Cell Biol*, *122*, 265.
242. Wilson, R. C., Nimkarn, S., Dumic, M., et al. (2007). Ethnic-specific distribution of mutations in 716 patients with congenital adrenal hyperplasia owing to 21-hydroxylase deficiency. *Mol Genet Metab*, *90*, 414–421.
243. Witchel, S. F. Smith, R., Crivellaro, C. E., et al. (2000). CYP21 mutations in Brazilian patients with 21-hydroxylase deficiency. *Hum Genet*, *106*, 414.
244. Finkielstain, G. P., Chen, W., Mehta, S. P., et al. (2011). Comprehensive genetic analysis of 182 unrelated families with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *J Clin Endocrinol Metab*, *96*, E161–E172.
245. Rösler, A., Lieberman, E., & Cohen, T. (1992). High frequency of congenital adrenal hyperplasia (classic 11β-hydroxylase deficiency) among Jews from Morocco. *Am J Med Genet*, 42, 827.
246. Zachmann, M., Tassinari, D., & Prader, A. (1983). Clinical and biochemical variability of congenital adrenal hyperplasia due to

11 beta-hydroxylase deficiency: a study of 25 patients. *J Clin Endocrinol Metab*, *56*, 222-229.
247. Parajes, S., Loidi, .L, Reisch, N., et al. (2010). Functional consequences of seven novel mutations in the CYP11B1 gene: four mutations associated with nonclassic and three mutations causing classic 11{beta}-hydroxylase deficiency. *J Clin Endocrinol Metab*, *95*, 779–788.
248. Chabraoui, L., Abid, F., Menassa, R., et al. (2010). Three novel CYP11B1 mutations in congenital adrenal hyperplasia due to steroid 11Beta-hydroxylase deficiency in a Moroccan population. *Horm Res Paediatr*, *74*, 182–189.
249. Simard, J., Ricketts, M. L., Gingras, S., et al. (2005). Molecular biology of the 3beta-hydroxysteroid dehydrogenase/delta5-delta4 isomerase gene family. *Endocr Rev*, *26*, 525.
250. Welzel, M., Wüstemann, N., Simic-Schleicher, G., et al. (2008). Carboxyl-terminal mutations in 3beta-hydroxysteroid dehydrogenase type II cause severe salt-wasting congenital adrenal hyperplasia. *J Clin Endocrinol Metab*, *93*, 1418–1425.
251. Miller, W. L. (2012). The syndrome of 17,20 lyase deficiency. *J Clin Endocrinol Metab*, *97*, 59–67.
252. Geller, D. H., Auchus, R. J., Mendonça, B. B., & Miller, W. L. (2001). The genetic and functional basis of isolated 17,20-lyase deficiency. *Nat Genet*, *17*, 201–205.
253. Kok, R. C., Timmerman, M. A., Wolffenbuttel, K. P., et al. (2010). Isolated 17,20-lyase deficiency due to the cytochrome b5 mutation W27X. *J Clin Endocrinol Metab*, *95*, 994–999.
254. Idkowiak, J., Randell, T., Dhir, V., et al. (2012). A missense mutation in the human cytochrome b5 gene causes 46,XY disorder of sex development due to true isolated 17,20 lyase deficiency. *J Clin Endocrinol Metab*, *97*, E465–E475.
255. Zachmann, M., Völlmin, J. A., Hamilton, W., & Prader, A. (1997). Steroid 17,20-desmolase deficiency: a new cause of male pseudohermaphroditism. *Clin Endocrinol* (Oxf), *1*, 369–385.
256. Flück, C. E., Meyer-Böni, M., Pandey, A. V., et al. (2011). Why boys will be boys: two pathways of fetal testicular androgen biosynthesis are needed for male sexual differentiation. *Am J Hum Genet*, *89*, 201–218.
257. Peterson, R. E., Imperato-McGinley, J., Gautier, T., & Shackleton, C. (1985). Male pseudohermaphroditism due to multiple defects in steroid-biosynthetic microsomal mixed-function oxidases: a new variant of congenital adrenal hyperplasia. *N Engl J Med*, *313*, 1182.
258. Shackleton, C., Marcos, J., Arlt, W., & Hauffa, B. P. (2004). Prenatal diagnosis of P450 oxidoreductase deficiency (ORD): a disorder causing low pregnancy estriol, maternal and fetal virilization, and the Antley-Bixler syndrome phenotype. *Am J Med Genet A*, *129*, 105.
259. Krone, N., Reisch, N., Idkowiak, J., et al. (2012). Genotype-phenotype analysis in congenital adrenal hyperplasia due to P450 oxidoreductase deficiency. *J Clin Endocrinol Metab*, *97*, E257–E267.
260. Fluck, C. E., Tajima, T., Pandey, A. V., et al. (2004). Mutant P450 oxidoreductase causes disordered steroidogenesis with and without Antley-Bixler syndrome. *Nat Genet*, *36*, 228.
261. Porter, F. D., & Herman, G. E. (2011). Malformation syndromes caused by disorders of cholesterol synthesis. *J Lipid Res*, *52*, 6–34.
262. Ribes, V., Otto, D. M., Dickmann, L., et al. (2007). Rescue of cytochrome P450 oxidoreductase (Por) mouse mutants reveals functions in vasculogenesis, brain and limb patterning linked to retinoic acid homeostasis. *Dev Biol*, *303*, 66–81.
263. Andersson, S., Geissler, W. M., Wu, L., et al. (1996). Molecular genetics and pathophysiology of 17-beta-hydroxysteroid dehydrogenase 3 deficiency. *J Clin Endocr Metab*, *81*, 130.
264. Lee, Y. S., Kirk, J. M., Stanhope, R. G., et al. (2007). Phenotypic variability in 17beta-hydroxysteroid dehydrogenase-3 deficiency and diagnostic pitfalls. *Clin Endocrinol* (Oxf), *67*, 20–28.
265. Boehmer, A. L. M., Brinkmann, A. O., Sandkuijl, L. A., et al. (1999). 17β-hydroxysteroid dehydrogenase 3 deficiency: diagnosis, phenotypic variability, population genetics, and worldwide distribution of ancient and de novo mutations. *J Clin Endocrinol Metab*, *84*, 4713.
266. Mendonca, B. B., Arnhold, I. J., Bloise, W., et al. (1999). 17Beta-hydroxysteroid dehydrogenase 3 deficiency in women. *J Clin Endocrinol Metab*, *84*, 802–804.
267. Fernández-Cancio, M., Audí, L., Andaluz, P., et al. (2011). SRD5A2 gene mutations and polymorphisms in Spanish 46,XY patients with a disorder of sex differentiation. *Int J Androl*, *34*(6 Pt. 2), e526–e535.
268. Maimoun, L., Philibert, P., Bouchard, P., et al. (2011). Primary amenorrhea in four adolescents revealed 5α-reductase deficiency confirmed by molecular analysis. *Fertil Steril*, *95*, 804.e1–e5.
269. Chávez, B., Valdez, E., & Vilchis, F. (2000). Uniparental disomy in steroid 5alpha-reductase 2 deficiency. *J Clin Endocrinol Metab*, *85*, 3147–3150.
270. Maimoun, L., Philibert, P., Cammas, B., et al. (2011). Phenotypical, biological, and molecular heterogeneity of 5α-reductase deficiency: an extensive international experience of 55 patients. *J Clin Endocrinol Metab*, *96*, 296–307.
271. Costa, E. M., Domenice, S., Sircili, M. H., et al. (2012). DSD due to 5α-reductase 2 deficiency: from diagnosis to long term outcome. *Semin Reprod Med*, *30*, 427–431.
272. Marumudi, E., & Ammini, A. C. (2011). Fertility in patients with 5-alpha-reductase-2 deficiency. *Fertil Steril*, *95*, e45.
273. Belgorosky, A., Guercio, G., Pepe, C., et al. (2009). Genetic and clinical spectrum of aromatase deficiency in infancy, childhood and adolescence. *Horm Res*, *72*, 321–330.
274. Lin, L., Ercan, O., Raza, J., et al. (2007). Variable phenotypes associated with aromatase (CYP19) insufficiency in humans. *J Clin Endocrinol Metab*, *92*, 982.
275. Mullis, P. E., Yoshimura, N., Kuhlmann, B., et al. (1997). Aromatase deficiency in a female who is compound heterozygote for two new point mutations in the P450(arom) gene: impact of estrogens on hypergonadotropic hypogonadism, multicystic ovaries, and bone densitometry in childhood. *J Clin Endocr Metab*, *82*, 1739.
276. Morishima, A., Grumbach, M. M., Simpson, E. R., et al. (1995). Aromatase deficiency is male and female siblings caused by a novel mutation and the physiological role of estrogens. *J Clin Endocrinol Metab*, *80*, 3689.
277. Jones, M. E., Boon, W. C., McInnes, K., et al. (2007). Recognizing rare disorders: aromatase deficiency. *Nat Clin Pract Endocrinol Metab*, *3*, 414.
278. Lin, L., Ercan, O., Raza, J., et al. (2007). Variable phenotypes associated with aromatase (CYP19) insufficiency in humans. *J Clin Endocrinol Metab*, *92*, 982.
279. Jones, M. E., Boon, W. C., Proietto, J., & Simpson, E. R. (2006). Of mice and men: the evolving phenotype of aromatase deficiency. *Trends Endocrinol Metab*, *17*, 55.
280. Zoeller, R. T., Brown, T. R., Doan, L. L., et al. (2012). Endocrine-disrupting chemicals and public health protection: a statement of principles from The Endocrine Society. *Endocrinology*, *153*, 4097–4110.
281. Clinckemalie, L., Vanderschueren, D., Boonen S., & Claessens, F. (2012). The hinge region in androgen receptor control. *Mol Cell Endocrinol*, *358*, 1–8.
282. Brinkman, A. O., Faber, P. W., van Rooy, H. C. J., et al. (1989). The human androgen receptor: domain structure, genomic organization and regulation of expression. *J Steroid Biochem Molec Biol*, *34*, 307–310.
283. Lumbroso, R., Beitel, L. K., Vasiliou, D. M., et al. (1997). Codon-usage variants in the polymorphic (GGN)n trinucleotide repeat of the human androgen receptor gene. *Hum Genet*, *101*, 43.
284. Jääskeläinen, J. (2012). Molecular biology of androgen insensitivity. *Mol Cell Endocrinol*, *352*, 4–12.
285. Hughes, I. A., Davies, J. D., Bunch, T. I., et al. (2012). Androgen insensitivity syndrome. *Lancet*, *380*, 1419–1428. Advance online publication.
286. Kohler, B., Lumbroso, S., Leger, J., et al. (2005). Androgen insensitivity syndrome: somatic mosaicism of the androgen receptor in seven families and consequences for sex assignment and genetic counseling. *J Clin Endocrinol Metab*, *90*, 106.
287. Deeb, A., & Hughes, I. A. (2005). Inguinal hernia in female infants: a cue to check the sex chromosomes. *BJU International*, *96*, 401.
288. Bouvattier, C., Carel, J. C., Lecointre, C., et al. (2002). Postnatal changes of T, LH, and FSH in 46,XY infants with mutations in the AR gene. *J Clin Endocrinol Metab*, *87*, 29.
289. Quigley, C. A. (2002). The postnatal gonadotropin and sex steroid surge-insights from the androgen insensitivity syndrome. *J Clin Endocrinol Metab*, *87*, 24.

290. Siminas, S., Kokai, G., & Kenny, S. E. (2013). Complete androgen insensitivity syndrome associated with bilateral Sertoli cell adenomas and paratesticular leiomyomas: case report and review of the literature. *J Pediatr Urol*, *9*, e31–e34. Advance online publication.
291. Hannema, S. E., Scott, I. S., Rajpert-De Meyts, E., et al. (2006). Testicular development in the complete androgen insensitivity syndrome. *J Pathol*, *208*, 518.
292. He, B., Gampe, R. T., Jr., Kole, A. J., et al. (2004). Structural basis for androgen receptor interdomain and coactivator interactions suggests a transition in nuclear receptor activation function dominance. *Mol Cell*, *16*, 425.
293. Kemppainen, J. A., Lane, M. V., Sar, M., et al. (1992). Androgen receptor phosphorylation, turnover, nuclear transport, and transcriptional activation: specificity for steroids and antihormones. *J Biol Chem*, *267*, 968.
294. Schoenmakers, E., Verrijdt, G., Peeters, B., et al. (2000). Differences in DNA binding characteristics of the androgen and glucocorticoid receptors can determine hormone-specific responses. *J Biol Chem*, *275*, 12290.
295. Gottlieb, B., Beitel, L. K., Nadarajah, A., et al. (2012). The androgen receptor gene mutations database: 2012 update. *Hum Mutat*, *33*, 887–894.
296. Rodien, P., Mebarki, F., Moszowicz, I., et al. (1996). Different phenotypes in a family with androgen insensitivity caused by the same M708I point mutation in the androgen receptor gene. *J Clin Endocrinol Metab*, *81*, 2994.
297. Elhaji, Y. A., Wu, J. H., Gottlieb, B., et al. (2004). An examination of how different mutations at arginine 855 of the androgen receptor result in different androgen insensitivity phenotypes. *Mol Endocrinol*, *18*, 1876.
298. Kazemi-Esfarjani, P., Beitel, L. K., Trifiro, M., et al. (1993). Substitution of valine-865 by methionine or leucine in the human androgen receptor causes complete or partial androgen insensitivity, respectively with distinct androgen receptor phenotypes. *Mol Endocrinol*, *7*, 37.
299. Quigley, C. A., Friedman, K. J., Johnson, A., et al. (1992). Complete deletion of the androgen receptor gene: definition of the null phenotype of the androgen insensitivity syndrome and determination of carrier status. *J Clin Endocr Metab*, *74*, 927.
300. Elhaji, Y. A., Stoica, I., Dennis, S., et al. (2006). Impaired helix 12 dynamics due to proline 892 substitutions in the androgen receptor are associated with complete androgen insensitivity. *Hum Mol Genet*, *15*, 921.
301. Askew, E. B., Bai, S., Hnat, A. T., et al. (2009). Melanoma antigen gene protein-A11 (MAGE-11) F-box links the androgen receptor NH2-terminal transactivation domain to p160 coactivators. *J Biol Chem*, *284*, 34793–34808.
302. Lagarde, W. H., Blackwelder, A. J., Minges, J. T., et al. (2012). Androgen receptor exon 1 mutation causes androgen insensitivity by creating phosphorylation site and inhibiting melanoma antigen-A11 activation of NH2- and carboxyl-terminal interaction-dependent transactivation. *J Biol Chem*, *287*, 10905–10915.
303. La Spada, A. R., Wilson, E. M., Lubahn, D. B., et al. (1991). Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. *Nature*, *352*, 77.
304. Ranganathan, S., & Fischbeck, K. H. (2010). Therapeutic approaches to spinal and bulbar muscular atrophy. *Trends Pharmacol Sci*, *31*, 523–527.
305. Josso, N., Belville, C., di Clemente, N., & Picard, J. Y. (2005). AMH and AMH receptor defects in persistent Mullerian duct syndrome. *Hum Reprod Update*, *11*, 351.
306. Fiaschetti, V., Taglieri, A., Gisone, V., et al. (2012). Mayer-Rokitansky-Kuster-Hauser syndrome diagnosed by magnetic resonance imaging: role of imaging to identify and evaluate the uncommon variation in development of the female genital tract. *J Radiol Case Rep*, *6*, 17–24.
307. Li, S., Qayyum, A., Coakley, F. V., & Hricak, H. (2000). Association of renal agenesis and mullerian duct anomalies. *J Comput Assist Tomogr*, *24*, 829.
308. Ledig, S., Schippert, C., Strick, R., et al. (2011). Recurrent aberrations identified by array-CGH in patients with Mayer-Rokitansky-Küster-Hauser syndrome. *Fertil Steril*, *95*, 1589–1594.
309. Wulfsberg, E. A., & Grigsby, T. M. (1990). Rokitansky sequence in association with the facio-auriculo-vertebral sequence: part of a mesodermal malformation spectrum? *Am J Med Genet*, *37*, 100.
310. Stone, D. L., Slavotinek, A., Bouffard, G. G., et al. (2000). Mutation of a gene encoding a putative chaperonin causes McKusick-Kaufman syndrome. *Nat Genet*, *25*, 79–82.
311. Katsanis, N., Beales, P. L., Woods, M. O., L et al. (2000). Mutations in MKKS cause obesity, retinal dystrophy and renal malformations associated with Bardet-Biedl syndrome. *Nat Genet*, *26*, 67–70.
312. Goodman, F. R., Bacchelli, C., Brady, A. F., et al. (2000). Novel HOXA13 mutations and the phenotypic spectrum of hand-foot-genital syndrome. *Am J Hum Genet*, *67*, 197–202.
313. Gana, S., Veggiotti, P., Sciacca, G., et al. (2012). 19q13.11 cryptic deletion: description of two new cases and indication for a role of WTIP haploinsufficiency in hypospadias. *Eur J Hum Genet*, *20*, 852–856.
314. Virtanen, H. E., Cortes, D., Rajpert-De Meyts, E., et al. (2007). Development and descent of the testis in relation to cryptorchidism. *Acta Paediatr*, *96*, 622.
315. Bay, K., Main, K. M., Toppari, J., Skakkebæk, N. E., & Medscape. (2011). Testicular descent: INSL3, testosterone, genes and the intrauterine milieu. *Nat Rev Urol*, *8*, 187–196.
316. Cortes, D., Thorup, J. M., & Visfeldt, J. (2001). Cryptorchidism: aspects of fertility and neoplasms. A study including data of 1,335 consecutive boys who underwent testicular biopsy simultaneously with surgery for cryptorchidism. *Horm Res*, *55*, 21.
317. Huff, D. S., Fenig, D. M., Canning, D. A., et al. (2001). Abnormal germ cell development in cryptorchidism. *Horm Res*, *55*, 11.
318. Kollin, C., Stukenborg, J. B., Nurmio, M., et al. (2012). Boys with undescended testes: endocrine, volumetric and morphometric studies on testicular function before and after orchidopexy at nine months or three years of age. *J Clin Endocrinol Metab*, *97*, 4588–4595.
319. Virtanen, H. E., Bjerknes, R., Cortes, D., et al. (2007). Cryptorchidism: classification, prevalence and long-term consequences. *Acta Paediatr*, *96*, 611.
320. Tomboc, M., Lee, P. A., Mitwally, M. F., et al. (2000). Insulin-like3/relaxin-like factor gene mutations are associated with cryptorchidism. *J Clin Endocrinol Metab*, *85*, 4013.
321. Kolon, T. F., Wiener, J. S., Lewitton, M., et al. (1999). Analysis of homeobox gene HOXA10 mutations in cryptorchidism. *J Urol*, *161*, 275.
322. Scheffer, I. E., Hutson, J. M., Warne, G. L., et al. (1988). Extreme virilization in patients with congenital adrenal hyperplasia fails to induce descent of the ovary. *Pediatr Surg Int*, *3*, 165.
323. Schwanzel-Fukuda, M., Bick, D., & Pfaff, D. W. (1989). Luteinizing hormone releasing hormone (LHRH)-expressing cells do not migrate normally in an inherited hypogonadal (Kallmann) syndrome. *Mol Brain Res*, *6*, 311.
324. de Roux, N., Genin, E., Carel, J. C., et al. (2003). Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54. *Proc Natl Acad Sci USA*, *100*, 10972.
325. Seminara, S. B., Messager, S., Chatzidaki, E. E., et al. (2003). The GPR54 gene as a regulator of puberty. *N Engl J Med*, *349*, 1614.
326. Dode, C., Levilliers, J., Dupont, J. M., et al. (2003). Loss-of-function mutations in FGFR1 cause autosomal dominant Kallmann syndrome. *Nat Genet*, *33*, 463.
327. Dode, C., Teixeira, L., Levilliers, J., et al. (2006). Kallmann syndrome: mutations in the genes encoding prokineticin-2 and prokineticin receptor-2. *PLoS Genet*, *2*, e175.
328. Gianetti, E., Tusset, C., Noel, S. D., et al. (2010). TAC3/TACR3 mutations reveal preferential activation of gonadotropin-releasing hormone release by neurokinin B in neonatal life followed by reversal in adulthood. *J Clin Endocrinol Metab*, *95*, 2857–2867.
329. Pinto, G., Abadie, V., Mesnage, R., et al. (2005). CHARGE syndrome includes hypogonadotropic hypogonadism and abnormal olfactory bulb development. *J Clin Endocrinol Metab*, *90*, 5621–5626.
330. Sykiotis, G. P., Plummer, L., Hughes, V. A., et al. (2010). Oligogenic basis of isolated gonadotropin-releasing hormone deficiency. *Proc Natl Acad Sci USA*, *107*, 15140–15144.
331. Balasubramanian, R., & Crowley, W. F., Jr. (2011). Isolated GnRH deficiency: a disease model serving as a unique prism into

the systems biology of the GnRH neuronal network. *Mol Cell Endocrinol*, *346*, 4–12.
332. Person, A. D., Beiraghi, S., Sieben, C. M., et al. (2010). WNT5A mutations in patients with autosomal dominant Robinow syndrome. *Dev Dyn*, *239*, 327–337.
333. Mazzeu, J. F., Pardono, E., Vianna-Morgante, A. M., et al. (2007). Clinical characterization of autosomal dominant and recessive variants of Robinow syndrome. *Am J Med Genet A*, *143*, 320–325.
334. Morris-Rosendahl, D. J., Segel, R., Born, A. P., et al. (2010). New RAB3GAP1 mutations in patients with Warburg Micro syndrome from different ethnic backgrounds and a possible founder effect in the Danish. *Eur J Hum Genet*, *18*, 1100–1106.
335. Borck, G., Wunram, H., Steiert, A., et al. (2011). A homozygous RAB3GAP2 mutation causes Warburg Micro syndrome. *Hum Genet*, *129*, 45–50.
336. Bem, D., Yoshimura, S., Nunes-Bastos, R., et al. (2011). Loss-of-function mutations in RAB18 cause Warburg Micro syndrome. *Am J Hum Genet*, *88*, 499–507.
337. Ogata, T., Laporte, J., & Fukami, M. (2009). MAMLD1 (CXorf6): a new gene involved in hypospadias. *Horm Res*, *71*, 245–252.
338. Kalfa, N., Liu, B., Klein, O., et al. (2008). Mutations of CXorf6 are associated with a range of severities of hypospadias. *Eur J Endocrinol*, *159*, 453–458.
339. Kalfa, N., Fukami, M., Philibert, P., et al. (2012). Screening of MAMLD1 mutations in 70 children with 46,XY DSD: identification and functional analysis of two new mutations. *PLoS One*, *7*, e32505.
340. Stillman, R. J. (1982). In vitro exposure to diethylstilbestrol: adverse effects on the reproductive tract and reproductive performance in male and female offspring. *Am J Obstet Gynecol*, *142*, 905.
341. Toppari, J., & Skakkebaek, N. E. (1998). Sexual differentiation and environmental endocrine disrupters. *Bailliere Clin Endocrinol Metab*, *12*, 143.
342. Paris, F., Jeandel, C., Servant, N., & Sultan, C. (2006). Increased serum estrogenic bioactivity in three male newborns with ambiguous genitalia: a potential consequence of prenatal exposure to environmental endocrine disruptors. *Environ Res*, *100*, 139.
343. Kalfa, N., Philibert, P., Baskin, L. S., & Sultan, C. (2011). Hypospadias: interactions between environment and genetics. *Mol Cell Endocrinol*, *335*, 89–95.
344. Callegari, C., Everett, S., Ross, M., & Brasel, J. A. (1987). Anogenital ratio: measure of fetal virilization in premature and full-term newborn infants. *J Pediatr*, *111*, 240.
345. Rink, R. C., Adams, M. C., & Misseri, R. (2005). A new classification for genital ambiguity and urogenital sinus anomalies. *BJU Inter*, *95*, 638–642.
346. Riley, W. J., & Rosenbloom, A. L. (1980). Clitoral size in infancy. *J Pediatr*, *96*, 918.
347. Kocova, M., Siegel, S. F., Wenger, S. L., et al. (1995). Detection of Y chromosome sequences in a 45X/46XXq-patient by Southern blot analysis of PCR-amplified DNA and fluorescent in situ hybridization (FISH). *Am J Med Genet*, *55*, 483.
348. Tannour-Louet, M., Han, S., Corbett, S. T., et al. (2010). Identification of de novo copy number variants associated with human disorders of sexual development. *PLoS One*, *5*, e15392.
349. Janzen, N., Sander, S., Terhardt, M., et al. (2011). Rapid steroid hormone quantification for congenital adrenal hyperplasia (CAH) in dried blood spots using UPLC liquid chromatography-tandem mass spectrometry. *Steroids*, *76*, 1437–1442.
350. Van der Kamp, H. J., & Wit, J. M. (2004). Neonatal screening for congenital adrenal hyperplasia. *Eur J Endocrinol*, *151*, U71.
351. Neres, M. S., Auchus, R. J., Shackleton, C. H., & Kater, C. E. (2010). Distinctive profile of the 17-hydroxylase and 17,20-lyase activities revealed by urinary steroid metabolomes of patients with CYP17 deficiency. *Arq Bras Endocrinol Metabol*, *54*, 826–832.
352. McDonald, J. G., Matthew, S., & Auchus, R. J. (2011). Steroid profiling by gas chromatography-mass spectrometry and high performance liquid chromatography-mass spectrometry for adrenal diseases. *Horm Cancer*, *2*, 324–332.
353. Kamrath, C., Hochberg, Z., Hartmann, M. F., et al. (2012). Increased activation of the alternative "backdoor" pathway in patients with 21-hydroxylase deficiency: evidence from urinary steroid hormone analysis. *J Clin Endocrinol Metab*, *97*, E367–E375.
354. Lee, M. M., Donahoe, P. K., Hasegawa, T., et al. (1996). Mullerian inhibiting substance in humans: normal levels from infancy to adulthood. *J Clin Endocrinol Metab*, *81*, 571.
355. Guibourdenche, J., Lucidarme, N., Chevenne, D., et al. (2003). Anti-Mullerian hormone levels in serum from human foetuses and children: pattern and clinical interest. *Mol Cell Endocrinol*, *211*, 55.
356. Rey, R. A., Belville, C., Nihoul-Fekete, C., et al. (1999). Evaluation of gonadal function in 107 intersex patients by means of serum antimullerian hormone measurement. *J Clin Endocrinol Metab*, *84*, 627.
357. Bergada, I., Milani, C., Bedecarras, P., et al. (2006). Time course of the serum gonadotropin surge, inhibins, and anti-Mullerian hormone in normal newborn males during the first month of life. *J Clin Endocrinol Metab*, *91*, 4092.
358. Honour, J. W. (2009). Diagnosis of diseases of steroid hormone production, metabolism and action. *J Clin Res Pediatr Endocrinol*, *1*, 209–226.
359. Ng, K. L., Ahmed, S. F., & Hughes, I. A. (2000). Pituitary-gonadal axis in male undermasculinization. *Arch Dis Child*, *82*, 54–58.
360. Ahmed, S. F., Achermann, J. C., Arlt, W., et al. (2011). UK guidance on the initial evaluation of an infant or an adolescent with a suspected disorder of sex development. *Clin Endocrinol* (Oxf), *75*, 12–26.
361. Auchus, R. J., Witchel, S. F., Leight, K. R., et al. (2010). Guidelines for the development of comprehensive care centers for congenital adrenal hyperplasia: guidance from the CARES Foundation initiative. *Int J Pediatr Endocrinol*, *2010*, 275213.
362. Wilson, J. D., Rivarola, M. A., Mendonca, B. B., et al. (2012). Advice on the management of ambiguous genitalia to a young endocrinologist from experienced clinicians. *Semin Reprod Med*, *30*, 339–350.
363. Jurgensen, M., Hampel, E., Hiort, O., & Thyen, U. (2006). "Any decision is better than none" decision-making about sex of rearing for siblings with 17beta-hydroxysteroid-dehydrogenase-3 deficiency. *Arch Sex Behav*, *35*, 359.
364. Lerman, S. E., McAleer, I. M., & Kaplan, G. W. (2000). Sex assignment in cases of ambiguous genitalia and its outcome. *Urol*, *55*, 8.
365. Imperato-McGinley, J., Miller, M., Wilson, J. D., et al. (2001). A cluster of male pseudohermaphrodites with 5 alpha-reductase deficiency in Papua New Guinea. *Clin Endocrinol* (Oxford), *34*, 293.
366. Cohen-Kettenis, P. T. (2005). Gender change in 46,XY persons with 5alpha-reductase-2 deficiency and 17beta-hydroxysteroid dehydrogenase-3 deficiency. *Arch Sex Behav*, *34*, 399.
367. Carani, C., Granata, A. R., Rochira, V., et al. (2005). Sex steroids and sexual desire in a man with a novel mutation of aromatase gene and hypogonadism. *Psychoneuroendocrinology*, *30*, 413.
368. Glassberg, K. I. (1999). Gender assignment and the pediatric urologist. *J Urol*, *161*, 1308.
369. Houk, C. P., & Lee, P. A. (2010). Approach to assigning gender in 46,XX congenital adrenal hyperplasia with male external genitalia: replacing dogmatism with pragmatism. *J Clin Endocrinol Metab*, *95*, 4501–4508.
370. Meyer-Bahlburg, H. F., Dolezal, C., Baker, S. W., et al. (2004). Prenatal androgenization affects gender-related behavior but not gender identity in 5-12-year old girls with congenital adrenal hyperplasia. *Arch Sex Behav*, *33*, 97.
371. Berenbaum, S. A., & Bailey, J. M. (2003). Effects on gender identity of prenatal androgens and genital appearance: evidence from girls with congenital adrenal hyperplasia. *J Clin Endocrinol Metab*, *88*, 1102.
372. Long, D. N., Wisniewski, A. B., & Migeon, C. J. (2004). Gender role across development in adult women with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *J Pediatr Endocrinol Metab*, *17*, 1367.
373. Malouf, M. A., Migeon, C. J., Carson, K. A., et al. (2006). Cognitive outcome in adult women affected by congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Horm Res*, *65*, 142.
374. Hines, M., Brook, C., & Conway, G. S. (2004). Androgen and psychosexual development: core gender identity, sexual orientation and recalled childhood gender role behavior in women and men with congenital adrenal hyperplasia (CAH). *J Sex Res*, *41*, 75.

375. Dittmmann, R. W., Kappes, M. E., & Kappes, M. H. (1992). Sexual behavior in adolescent and adult females with congenital adrenal hyperplasia. *Psychoneuroendocrinology*, *17*, 153.
376. Zucker, K. J., Bradley, S. J., Oliver, G., et al. (1996). Psychosexual development of women with congenital adrenal hyperplasia. *Horm Behav*, *30*, 300.
377. Meyer-Bahlburg, H. F. (2001). Gender and sexuality in classic congenital adrenal hyperplasia. Endocrinol *Metab Clin North AM*, *30*, 155.
378. Wisniewski, A. B., Migeon, C. J., Malouf, M. A., & Geargart, J. P. (2004). Psychosexual outcome in women affected by congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *J Urol*, *171*, 2497.
379. Meyer-Bahlberg, H. F., Dolezal, C., Baker, S. W., et al. (2006). Gender development in women with congenital adrenal hyperplasia as a function of disorder severity. *Arch Sex Behav*, *35*, 667.
380. Meyer-Bahlburg, H. F., Gruen, R. S., New, M. I., et al. (1996). Gender change from female to male in classical congenital adrenal hyperplasia. *Horm Behav*, *30*, 319.
381. Berenbaum, S. A., Korman Bryk, K., Duck, S. C., & Resnick, S. (2004). Psychological adjustment in children and adults with congenital adrenal hyperplasia. *J Pediatr*, *144*, 741.
382. Berenbaum, S. A., & Bailey, J. M. (2003). Effects on gender identity of prenatal androgens and genital appearance: evidence from girls with congenital adrenal hyperplasia. *J Clin Endocrinol Metab*, *88*, 1102.
383. Wisniewski, A. B. (2012). Gender development in 46,XY DSD: influences of chromosomes, hormones, and interactions with parents and healthcare professionals. *Scientifica*, *2012*.
384. Ankarberg-Lindgren, C., Elfving, M., Wikland, K. A., & Norjavaara, E. (2001). Nocturnal application of transdermal estradiol patches produces levels of estradiol that mimic those seen at the onset of spontaneous puberty in girls. *J Clin Endocrinol Metab*, *86*, 3039.
385. Speiser, P. W., Azziz, R., Baskin, L. S., et al. (2010). Congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*, *95*, 4133-4160.
386. Trapp, C. M., Speiser, P. W., & Oberfield, S. E. (2011). Congenital adrenal hyperplasia: an update in children. *Curr Opin Endocrinol Diabetes Obes*, *18*, 166–170.
387. Aycan, Z., Bas, V. N., Cetinkaya, S., et al. (2013). Prevalence and long-term follow-up outcomes of testicular adrenal rest tumors in children and adolescent males with congenital adrenal hyperplasia. *Clin Endocrinol* (Oxf), *78*, 667–672.
388. Vidal, I., Gorduza, D. B., Haraux, E., et al. (2010). Surgical options in disorders of sex development (DSD) with ambiguous genitalia. *Best Pract Res Clin Endocrinol Metab*, *24*, 311–324.
389. Baskin, L. S., Erol, A., Li, Y. W., et al. (1999). Anatomical studies of the human clitoris. *J Urol*, *162*, 1015.
390. Akman, Y., Lui, W., Li, Y. W., & Baskin, L. S. (2001). Penile anatomy under the pubic arch: reconstructive implications. *J Urol*, *166*, 225.
391. Creighton, S. M., Minto, C. L., & Steele, S. J. (2001). Objective cosmetic and anatomical outcomes at adolescence of feminising surgery for ambiguous genitalia done in childhood. *Lancet*, *358*, 124.
392. Gollu, G., Yildiz, R. V., Bingol-Kologlu, M., et al. (2007). Ambiguous genitalia: an overview of 17 years' experience. *J Pediatr Surg*, *42*, 840.
393. Ismail-Pratt, I. S., Bikoo, M., Liao, L. M., et al. (2007). Normalization of the vagina by dilator treatment alone in complete androgen insensitivity syndrome and Mayer-Rokitansky-Kuster-Hauser syndrome. *Hum Reprod*, *22*, 2020–2024.
394. Köhler, B., Kleinemeier, E., Lux, A., et al. (2012). DSD Network Working Group. Satisfaction with genital surgery and sexual life of adults with XY disorders of sex development: results from the German clinical evaluation study. *J Clin Endocrinol Metab*, *97*, 577–588.
395. Crouch, N. S., Lao. L. M., Woodhouse, C. R., et al. (2008). Sexual function and genital sensitivity following feminization for congenital adrenal hyperplasia. *J Urol*, *179*, 634–638.
396. Nordenskjöld, A., Holmdahl, G., Frisén, L., et al. (2008). Type of mutation and surgical procedure affect long-term quality of life for women with congenital adrenal hyperplasia. *J Clin Endocrinol Metab*, *93*, 380–386.
397. Fagerholm, R., Santtila, P., Miettinen, P. J., et al. (2011). Sexual function and attitudes toward surgery after feminizing genitoplasty. *J Urol*, *185*, 1900–1904.
398. Hagenfeldt, K., Janson, P. O., Holmdahl, G., et al. (2008). Fertility and pregnancy outcome in women with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Hum Reprod*, *23*, 1607–1613.
399. Bidet, M., Bellanné-Chantelot, C., Galand-Portier, M. B., et al. (2010). Fertility in women with nonclassical congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *J Clin Endocrinol Metab*, *95*, 1182–1190.
400. Casteràs, A., De Silva, P., Rumsby, G., & Conway, G. S. (2009). Reassessing fecundity in women with classical congenital adrenal hyperplasia (CAH): normal pregnancy rate but reduced fertility rate. *Clin Endocrinol* (Oxf), *70*, 833–837.
401. Sircili, M. H., e Silva, F. A., Costa, E. M., et al. (2010). Long-term surgical outcome of masculinizing genitoplasty in large cohort of patients with disorders of sex development. *J Urol*, *184*, 1122–1127.
402. Gupta, D., Bhardwaj, M., Sharma, S., et al. (2010). Long-term psychosocial adjustments, satisfaction related to gender and the family equations in disorders of sexual differentiation with male sex assignment. *Pediatr Surg Int*, *26*, 955–958.
403. Schonbucher, V., Schweizer, K., & Richter-Appelt, H. (2010). Sexual quality of life of individuals with disorders of sex development and a 46,XY karyotype: a review of international research. *J Sex Marital Ther*, *36*, 193–215.
404. Buell-Gutbrod, R., Ivanovic, M., Montag, A., et al. (2011). FOXL2 and SOX9 distinguish the lineage of the sex cord-stromal cells in gonadoblastomas. *Pediatr Dev Pathol*, *14*, 391–395.
405. Cools, M., van Aerde, K., Kersemaekers, A. M., et al. (2005). Morphological and immunohistochemical differences between gonadal maturation delay and early germ cell neoplasia in patients with undervirilization syndromes. *J Clin Endocrinol Metab*, *90*, 5295.
406. Cools, M., Drop, S. L., Wolffenbuttel, K. P., et al. (2006). Germ cell tumors in the intersex gonad: old paths, new directions, moving frontiers. *Endocr Rev*, *27*, 468.
407. Mazzanti, L., Cicognani, A., Baldazzi, L., et al. (2005). Gonadoblastoma in Turner syndrome and Y-chromosome-derived material. *Am J Med Genet A*, *135*, 150.
408. Deans, R., Creighton, S. M., Liao, L. M., & Conway, G. S. (2012). Timing of gonadectomy in adult women with complete androgen insensitivity syndrome (CAIS): patient preferences and clinical evidence. *Clin Endocrinol* (Oxf), *76*, 894–898.
409. Wünsch, L., Holterhus, P. M., Wessel, L., & Hiort, O. (2012). Patients with disorders of sex development (DSD) at risk of gonadal tumour development: management based on laparoscopic biopsy and molecular diagnosis. *BJU Int*, *110*, E958–E965.
410. Arlt, W., Willis, D. S., Wild, S. H., et al. (2010). United Kingdom Congenital Adrenal Hyperplasia Adult Study Executive (CaHASE). Health status of adults with congenital adrenal hyperplasia: a cohort study of 203 patients. *J Clin Endocrinol Metab*, *95*, 5110–5121.
411. Conway, G. S. (2007). Congenital adrenal hyperplasia: adolescence and transition. *Horm Res*, *68*(suppl 5), 155–157.
412. Witchel, S. F. (2010). The medical home concept and congenital adrenal hyperplasia: a comfortable habitat! *Int J Pediatr Endocrinol*, *2010*, 561526.
413. Wiesemann, C., Ude-Koeller, S., Sinnecker, G. H. G., & Thyen, U. (2010). Ethical principles and recommendation for the medical management of differences of sex development (DSD)/intersex in children and adolescents. *Eur J Pediatr*, *169*, 671–667

QUESTIONS

1. What is the most common disorder of sex development?
   a. Gonadal dysgenesis
   b. Cryptorchidism
   c. DAX1 duplication
   d. Congenital adrenal hyperplasia

Answer: b

2. What is the most likely diagnosis for an infant with symmetric external genitalia, fused labioscrotal folds, prominent phallus, and no palpable gonads
   a. Androgen insensitivity syndrome
   b. Mixed gonadal dysgenesis
   c. Persistent Mullerian duct syndrome
   d. Congenital adrenal hyperplasia

Answer: d

3. What is the phenotype associated with mutations in the RSPO1 gene?
   a. 46,XY sex reversal with adrenal insufficiency
   b. No obvious phenotype in 46,XX individuals
   c. 46,XX sex reversal with palmoplantar keratoderma
   d. 46,XY sex reversal with ovotesticular disorder

Answer: c

4. A 14 year old girl is referred for evaluation of primary amenorrhea. Her physical exam shows normal to tall stature for her family. She has Tanner 3 breast development, but lacks pubic and axillary hair. Family history reveals a maternal aunt who had infertility. Which of the following options should be avoided?
   a. Reassure the family that she has delayed puberty. Recommend waiting 6 months and re-evaluate
   b. Obtain pelvic ultrasound to assess for presence of uterus
   c. Measure LH, FSH, and testosterone
   d. Obtain karyotype

Answer: a

5. You are asked to evaluate a newborn infant with an abdominal wall defect, bifid phallic structure, and bilateral palpable gonads. Karyotype is 46,XY. What is this infant's diagnosis?
   a. Ovotesticular disorder
   b. Androgen insensitivity
   c. Hypopituitarism
   d. Exstrophy of the bladder/cloaca

Answer: d

CHAPTER 6

Hypoglycemia in the Newborn and Infant

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CHAPTER OUTLINE

INTRODUCTION

One of the most important genetic and metabolic events to mark the transition from fetal to neonatal life is the adaptation from an environment that has a readily available and continuous source of glucose—maternal blood—to an environment in which glucose is provided in a limited and intermittent supply. The complex events involved in the maintenance of plasma glucose concentration must be coordinated to avoid hypoglycemia and resultant damage to the central nervous system. A newborn or infant with hypoglycemia presents an urgent diagnostic and therapeutic challenge. The clinical features must be rapidly assessed and a plan of action developed based on the infant's age, maternal and parturition history, severity and persistence of hypoglycemic state, and all other relevant clinical clues. In this chapter we will review the normal physiology of glucose metabolism and the pathophysiology of the most common disorders resulting in hypoglycemia in the neonate (first 4 weeks of life) and infant (1 month to 1 year of life) with an emphasis on the approach to the diagnosis and management of these pathologies.

PRINCIPLES OF GLUCOSE METABOLISM

A systematic approach to hypoglycemia in the newborn, infant, or child requires an appreciation of the central role of glucose in the body's fuel economy.[1] Glucose metabolism accounts for approximately half of basal daily energy needs and is the principal metabolic fuel of the human brain. Glucose can be stored for energy in the form of glycogen and fat, and its carbon can be used for synthesis of protein and for structural components (such as cell membranes). The aerobic oxidation of glucose yields high energy by producing 36 mols of adenosine triphosphate (ATP) for each mol of glucose.

All glucose extracted by the brain is oxidized, and thus cerebral glucose utilization parallels cerebral oxygen uptake. In 5-week-old infants, cerebral glucose utilization already represents 71% to 93% of the adult level in most brain regions (ranging from 13 to 25 μmol/100 g/min). At that age, the areas with highest metabolic rates for glucose are the sensorimotor cortex, thalamus, midbrain, brainstem, and cerebellar vermis. By 3 months, metabolic rates for glucose increase in the parietal, temporal, and occipital cortices, as well as in basal ganglia. By 8 months,

subsequent increases occur in the frontal cortex and various associative regions, concordant with the appearance of higher cortical and cognitive functions. Adult levels of cerebral glucose utilization (19 to 33 μmol/100 g/min) are reached by the time one is 2 years of age, and they continue to increase until 3 to 4 years of age—when they reach values ranging from 49 to 65 μmol/100 g/min, which are maintained to approximately 9 years of age. They then begin to decline, reaching adult levels by the end of the second decade.[2]

Glucose uptake by the brain occurs by means of a carrier-mediated facilitated diffusion process that is glucose concentration dependent, as well as energy, Na^+, and insulin independent.[3] This process is mediated by facilitative glucose transporter (GLUT) proteins. The human genome contains 14 members of the GLUT family. Each of these isoforms is expressed in a distinct tissue distribution. In most cells, GLUTs mediate the import of glucose, because hexokinase activity maintains low intracellular glucose concentrations. Characterization of the different members has provided new insights into the regulation and significance of glucose transport and its disorders in various tissues[3,4,5] (Table 6-1). Several members of the GLUT family have been detected in the brain. GLUT1 is located at the blood-brain barrier,[6] and although some neurons express GLUT2 and GLUT4, the majority use GLUT3 as their primary transporter.[7] The main isoform present in insulin-responsive tissues is GLUT4.[8,9]

Of major importance is the confirmation at a molecular level of biochemical evidence that glucose entry into brain cells and its subsequent metabolism are not

TABLE 6-1 **Characterization of Glucose Transporters**

Subfamily	Name	Tissue Distribution	Glucose Transport Activity	Location of Human Gene	Regulation by Insulin
Class I	GLUT1	Erythrocytes, brain (blood-brain barrier), placenta, pancreatic islets	Erythrocyte: asymmetric carrier with exchange acceleration; K_m,5-30 mmol/L (variable): Vmax (influx) < Vmax (efflux)	1p35-p31.3	Zero to minimal
	GLUT2	Liver, islet cells, kidney, small intestine, pancreatic islets	Low-affinity. Liver: simple, symmetric carrier; K_m,60 mmol/L; intestine: asymmetric carrier: Vmax (efflux) < Vmax (influx)	3q26.1-q26.2	Zero to minimal
	GLUT3	Brain (neuronal), testis, placenta, pancreatic islets	High-affinity; exchange K_m,10 mmol/L	12p13.3	Zero
	GLUT4	Adipocytes, muscle	High-affinity; adipocyte: simple, symmetric carrier; K_m,2-5 mmol/L	17p13	Dependent on insulin
	GLUT14	Testis	?	12p13.31	?
Class II	GLUT5	Testis, small intestine, kidney	No glucose transport activity, fructose transporter	1p36.2	?
	GLUT7	Small intestine (enterocytes brush border membrane), colon, testis, prostate	High-affinity transport for glucose (K_m = 0.3 mM)	1p36.2	?
	GLUT9	Liver, kidney. Expressed at preimplantation stage in mouse	Proven glucose transport activity	4p16-p15.3	?
	GLUT11	Pancreas, kidney, placenta, heart, skeletal muscle	Low affinity for glucose (possible fructose transporter)	22q11.2	Mediates insulin-stimulated glucose uptake in embryos
Class III	GLUT6	Brain, spleen, leukocytes	Low affinity for glucose	9q34	?
	GLUT8	Testis, brain (neuronal), adipocytes, preimplantation embryos	High-affinity glucose transporter (possible multifunctional transporter)	9q33.3	?
	GLUT10	Liver, pancreas	Glucose transport activity with K_m,3 mmol/L	20q13.1-q12	?
	GLUT12	Heart, prostate, breast cancer	Proven glucose transporter activity	6q23.2	?
	GLUT13 (HMIT)	Brain	No glucose transport activity, myo-inositol and related isomers transporter	12q12	?

dependent on insulin but rather are dependent on circulating arterial glucose concentration.[10] Therefore, a decrease in arterial glucose concentration or a defect in the glucose transport mechanism of the brain will result in intracerebral glucopenia and low cerebrospinal fluid glucose concentration (hypoglycorrhachia)—with attendant symptoms and signs of cerebral glucopenia as subsequently described.[10-12]

In normal humans, plasma glucose concentrations range from 3.9 to 7.1 mmol/L (70 to 128 mg/dL) and in the brain the range is from 0.8 to 2.3 mmol/L (14 to 41 mg/dL).[13] Brain glucose consumption will outstrip glucose transport at plasma glucose concentration < 2 mmol/L (36 mg/dL) (brain glucose will approach 0 mmol/L).[14] To prevent circulating arterial blood glucose from decreasing precipitously under normal physiologic conditions, and therefore to prevent impairment of vital function that depends on cerebral glucose metabolism, an elaborate defense mechanism has evolved.[1] This defense against hypoglycemia is integrated by the autonomic nervous system and by hormones that act synergistically to enhance glucose production through enzymatic modulation of glycogenolysis and gluconeogenesis while simultaneously limiting peripheral glucose use.[15,16] Thus, hypoglycemia is the result of a defect in one or several of the complex interactions that maintain a normal range of glucose concentration, preventing its fall to less than 3.9 mmol/L (70 mg/dL) during fasting and its rise to more than 7.8 mmol/L (140 mg/dL) during feeding.

These mechanisms are not fully developed at birth, when there is an abrupt transition from intrauterine life to extrauterine life. A neonate delivered prematurely whose enzymatic machinery mechanisms are not yet fully developed and expressed or one whose placental insufficiency resulted in intrauterine growth retardation with limited tissue nutrient deposits may be particularly vulnerable to hypoglycemia,[17] often with consequences to subsequent cerebral development or function.[18,19]

PHYSIOLOGY OF PERINATAL GLUCOSE HOMEOSTASIS

Glucose Metabolism in the Fetus

It is estimated that 80% of fetal energy comes from glucose and that the remaining 20% is derived from metabolism of lactate and amino acids.[20] Studies in human pregnancies as well as in other species suggest that fetal glucose is derived entirely from the mother through placenta transfer.[21-24] Thus, maternal and fetal glucose concentrations behave as a single pool, with no endogenous glucose production in the fetus. There are important clinical consequences implicit in these findings. For example, acute hypoglycemia in a mother with diabetes will result in acute hypoglycemia in the fetus—with no ability to acutely compensate for the abrupt reduction in blood glucose supply.

The transfer of glucose across the placenta to the fetus is mediated by sodium-independent facilitated diffusion along a concentration gradient. This process is dependent on specific isoforms of the glucose transporters. GLUT1 has been identified as the major isoform in the human placenta,[25] although other members of the family, such as GLUT3, are also expressed.[26] Of note is the fact that the placental GLUTs are not regulated by insulin, suggesting that maternal glucose concentration and (more specifically) the maternal-fetal glucose gradient is the major determinant of placental glucose transfer independent of the maternal insulin concentration perfusing the placenta.[27]

The fetus, from early gestation to time of delivery, has only slightly lower concentrations of plasma glucose than maternal concentrations with a mean fetal glucose concentration of approximately 4.4 mmol/L (79 mg/dL). This maternal-fetal gradient allows greater transfer of glucose from the maternal circulation.[28] There is a linear relationship between the maternal and fetal glucose concentration with glucose transported by facilitated carrier-mediated diffusion along the concentration gradient.[29] As gestation proceeds this maternal-fetal gradient increases, perhaps reflecting the increased utilization of the fetal placenta unit.[28] When maternal glucose rises > 20 mmol/L (360 mg/dL), glucose transport changes to simple diffusion. Of the maternal glucose taken up by the placenta, approximately 50% to 60% is used by the placenta and 40% to 50% transported to the fetus.[30]

In the second half of gestation, the fetal–placental unit secretes large quantities of human placental lactogen, progesterone, and estrogen, which cause increased maternal insulin resistance. Maternal insulin does not significantly cross the placenta unless it is bound to antibody.[31] Fetal insulin secretion cannot rapidly respond to rapidly changing glucose levels. In utero, the raised insulin-to-glucagon ratio drives metabolism toward anabolism, as evidenced by the rapid rate of growth of the fetus. The insulin resistance of the mother results in an increased availability of glucose, amino acids, and lipids to meet the ever-increasing energy demands of the fetus, which is in a constant anabolic state with glucose utilized for both energy production and the formation of triglycerides stores and protein anabolism. Throughout gestation, the balance of glycogen metabolism is toward anabolism and the building of glycogen stores. As gestation advances, the fetal glycogen content in the liver increases to about 24.6 mg/g liver by 120 days. At 36 weeks, gestation there is a steep increase in accumulation of glycogen, with levels rising to 50 mg/g liver at term.

As gestation progresses, the increasing demand of the fetus for glucose is accomplished by increasing uterine blood flow. There is excess capacity in the uterine blood flow, and it may be reduced as much as 50% without causing any fetal harm. However, reducing blood flow from the placenta to the fetus causes significant impact on the fetus with resultant intrauterine growth restriction (IUGR). In IUGR fetuses, the maternal fetal gradient is increased.

Changes at Birth: Transition Phase

The abrupt interruption of maternal glucose transfer to the fetus at delivery imposes an immediate need to mobilize endogenous glucose and to rapidly adjust insulin secretion to glucose concentration. Following birth and clamping of the cord, the newborn has to transition

from a state of glucose excess and anabolism to a rapidly fluxing state of glucose deficiency and excess. This is due to the change from a constant supply of glucose and amino acids from the mother to variable and intermittent oral intake. In addition, in breast-fed infants the colostrum is low in carbohydrates and high in fat. This transition is regulated by the interplay of hormones and enzyme induction. At birth, plasma epinephrine and norepinephrine rise three- to tenfold,[32] and may be responsible for changes in the insulin-to-glucagon ratio, which in utero is high and favors anabolism. Within the first 2 hours after birth, glucagon concentrations rise and continue to increase during the first 3 days of life; insulin, on the other hand, falls initially and remains in the basal range for several days.[33] These changes induce both pyruvate carboxylase (PC) and phosphoenolpyruvate carboxykinase (PEPCK).[34] By 8 to 12 hours, gluconeogenesis becomes fully effective.[35] The ability to develop significant ketosis is impaired for the first 12 hours of life, as shown by a study in appropriate for gestational age (AGA) infants fasted up to 8 hours after birth. In these infants, free fatty acid (FFA) levels averaged 1.41 mmol/L (appropriately elevated) and total ketones were approximately 0.36 mmol/L (low relative to the FFA); thus, although insulin levels fall and lipolysis occurs, fatty acid oxidation with ketone generation does not occur.[35] However, after 12 hours of life, ketone production and utilization occur at accelerated rates and equal those in adults starved for 48 to 72 hours.[36] Thus, ketone utilization can provide up to 25% of the energy needs of the neonate after the first 12 hours of life.

With the rise in glucagon and epinephrine and the fall in insulin, glycogen is mobilized to produce glucose by activation of glycogen phosphorylase. Indeed, levels of glycogen decline from a peak of 50 mg/g liver just prior to birth, to very low concentrations (< 10 mg/g liver) in the first 24 hours of life, and glycogen contributes to almost 50% of the newborn's glucose requirements during this time. Gluconeogenesis from pyruvate contributes approximately 20% to 30% of glucose needs and glycerol produced by lipolysis contributes approximately 20%.[24,37]

In summary, glucose concentration during intrauterine life is constant and > 3.9 mmol/L (70 mg/dL). Following birth, glucose concentration falls to a mean glucose of 3.1 mmol/L (56 mg/dL) at 2 hours (fifth percentile level 28 mg/dL), and as glycogenolysis and gluconeogenesis become established, glucose concentration rises to a mean of 3.5 mmol/L (63 mg/dL) (fifth percentile level 40 mg/dL) by 2 to 24 hours of life. By 24 hours of life, mean glucose concentrations are 3.8 mmol/L (68 mg/dL) (fifth percentile level 41 mg/dL) and by 48 hours they rise to a mean of 3.7 mmol/L (67 mg/dL) (fifth percentile level 48 mg/dL).[38] As a result of these changes, 30% of normal newborns will have glucose concentrations < 2.8 mmol/L (50 mg/dL) in the first 24 hours of life and thereafter the frequency of glucose concentrations < 50 mg/dL in newborns older than 24 hours of age is 0.5%.[17] This physiologic drop in glucose levels in the first 24 hours of life is *transitional hypoglycemia*, which by definition occurs in normal healthy newborns. It should be differentiated from both transient and persistent pathologic causes of hypoglycemia.

Abnormalities of Transition

Because low plasma glucose concentrations in normal neonates during this transitional hypoglycemia period are common, it can be difficult to identify newborn infants who have a pathologic hypoglycemia disorder. Thus, in neonates who are at risk for a pathologic hypoglycemia problem (e.g., genetic risk, large for gestational age [LGA], IUGR, infant of a diabetic mother, or perinatal stress), it is appropriate to monitor glucose concentrations closely and not merely treat any hypoglycemia expectantly. If such infants are treated and stabilized, formal evaluation or decisions about need for long-term treatment may be delayed until 2 to 3 days after birth, when diagnosis of the cause of hypoglycemia may be made by interpreting the critical sample drawn at a time of a spontaneous or fasting-induced glucose < 50 mg/dL. It is vital that the diagnosis of the cause of hypoglycemia and decisions about need for treatment be completed prior to discharge from the nursery in order to avoid the potential hazard of a hypoglycemic episode after the infant is at home.

In infants born IUGR, the diminished glycogen reserves may have a significant impact on glucose control in the first 24 hours of life, resulting in hypoglycemia greater than expected. However, there are data that also suggest that small for gestational age (SGA) infants have a transient state of hyperinsulinism persisting over the first days of life as they adjust to extrauterine life.[39] Premature infants may also not have accumulated adequate glycogen and will have immaturity of their enzyme systems also increasing the risk of hypoglycemia. Infants of diabetic mothers or infants with perinatal stress hyperinsulinism, or even those with genetic defects in insulin secretion or other conditions, will not have the sudden drop in insulin levels after birth, thus impairing glycogenolysis and lipolysis and increasing the risk of hypoglycemia. It is important for the clinician to recognize these at-risk neonates and neonates whose glucose control is out of the range of transitional hypoglycemia during this period and treat appropriately. In particular, it is important to recognize conditions in which insulin secretion is increased, as these infants will neither mobilize glycogen nor be able to oxidize fatty acids, thus putting their brains at risk of damage due to the combination of hypoglycemia and hypoketosis.

Management of Hypoglycemia in the First 24 Hours

Management of hypoglycemia in the first 24 hours of life is important from two points of view. First, the management plan should avoid treatment of infants who do not need treatment; second, it should include a management plan for those at risk of having a permanent hypoglycemic disorder that both identifies those neonates and threats them adequately. It is not currently recommended that all newborns have glucose concentration measured. However, if an infant has symptoms consistent with hypoglycemia—such as lethargy, apnea, or seizures, or is unwell or in the at-risk category (e.g., infants with siblings with known hypoglycemic disorders, preterm infants, LGA infants, SGA infants, infants of a diabetic mother, or infants who

had birth asphyxia)—the infant's glucose levels should be measured.

If the baby has symptoms of hypoglycemia or is unwell and the glucose is < 2.8 mmol/L (50 mg/dL), then IV glucose should be administered to keep the glucose > 3.3 mmol/L (60 mg/dL).[40] Typically doses of glucose would be a 200 mg/kg (2 mL/kg dextrose 10%) bolus dose followed by a starting glucose infusion rate (GIR) of 4 to 6 mg/kg/min (60 to 90 mL dextrose 10% /kg/day) and rising by GIR of 1.6 mg/kg/min (1 mL/kg dextrose 10%/hour) until glucose levels are > 3.3 mmol/L (60 mg/dL) are obtained. Glucose levels should be checked every 15 minutes and the glucose infusion rate increased until the desired range is achieved. If after two dose increases of 1.6 mg/kg/min (1 mL/kg dextrose 10%/hour) the glucose levels remain < 3.3 mmol/L (60 mg/dL), then an increase by 3.2 mg/kg/min (2 mL/kg dextrose 10%/hour) every 15 minutes is recommended.

For asymptomatic infants at < 2 hours of life, intravenous (IV) glucose should be given if glucose levels are < 1.7 mmol/L (30 mg/dL) and the goal is to raise the glucose to > 3.3 mmol/L (60 mg/dL). For asymptomatic infants at 2 to 24 hours of life, IV glucose should be given if glucose levels are < 2.2 mmol/L (40 mg/dL) and the goal is to raise the glucose to > 3.3 mmol/L (60 mg/dL). If newborns continue to have asymptomatic glucose concentrations < 50 mg/dL after 24-48 hours consideration should be given to providing IV dextrose with the goal of maintaining glucose > 3.9 mmol/L (70 mg/dL).

There is no evidence-based medicine to show us how to differentiate neonates who failed the preceding protocols because of a more severe transition from neonates with a more serious and potentially harmful cause of hypoglycemia. Both the American Academy of Pediatrics guidelines[41] and the Canadian Pediatric Society guidelines[40] recommend further testing to identify neonates who may needing longer-term treatment and to identify when glucose metabolism has returned to normal, but they do not recommend how and when this should be done.

We strongly recommend that neonates requiring intravenous glucose to maintain the blood glucose levels > 3.3 mmol/L (60 mg/dL) at any time, or those having glucose levels < 2.8 mmol/L (50 mg/dL) past 48 hours of age, should have the cause of their abnormality in glucose metabolism identified prior to discharge. This means a critical sample should be drawn during a spontaneous episode of hypoglycemia, whether on intravenous glucose or not, and if a spontaneous event does not occur, a simple fast should be done after the neonate has been weaned from intravenous fluids and is feeding. Those who require IV glucose at any time for the treatment of hypoglycemia should skip 1 to 2 feeds and fast a total of 6 to 8 hours. If plasma glucose levels remain > 3.6 mmol/L (65 mg/dL), one can conclude the infant is unlikely to have a hypoglycemic disorder. For those with glucose levels < 2.8 mmol/L (50 mg/dL) after 48 hours of life and not requiring IV glucose, the recommendation is to skip one feeding and have the infant fast for 6 hours. If the infant maintains the glucose > 3.3 mmol/L (60 mg/dL), it is unlikely that the infant has a hypoglycemic disorder. One should note that there is no evidence that proves these protocols will identify 100% of infants, so caution should be made if clinical circumstances warrant further thought. Finally, it is important to emphasize to anxious parents the consequences of missing a diagnosis of a hypoglycemic disorder. In our experience most parents are willing to lengthen the hospital stay by 6 to 9 hours to ensure the long-term safety of their child.

HORMONAL AND METABOLIC SYSTEMS OF FASTING ADAPTATION

Hypoglycemia in neonates, infants, and children is essentially always a problem with fasting adaptation. Postprandial hypoglycemia is exceedingly rare and is limited to a few unusual situations, such as postprandial hypoglycemia after Nissen fundoplication and gastric tube feedings, hereditary fructose intolerance, or the protein-induced hypoglycemia seen in some forms of congential hyperinsulinism. Therefore, a consideration of the major hormonal and metabolic pathways that maintain fuel homeostasis during fasting provides an important framework for understanding the causes, diagnosis, and treatment of different forms of hypoglycemia.

Three metabolic systems regulate the physiologic response to fasting: (1) hepatic glycogenolysis, (2) hepatic gluconeogenesis, and (3) hepatic ketogenesis. The key enzymatic steps in these pathways are shown in Figure 6-1. These metabolic systems are coordinated by the endocrine system, consisting of suppression of insulin (the most important endocrine response to fasting, as insulin suppresses all three metabolic systems) balanced by the secretion of glucagon, epinephrine, cortisol, and growth hormone. Table 6-2 summarizes the counter-balancing effects of these counter-regulatory hormones on key metabolic pathways. There is a hierarchic redundancy in the interaction of these counter-regulatory hormones that provides a margin of safety ("fail-safe mechanism") if only one counter-regulatory hormone is impaired. Epinephrine and glucagon are quick acting, each signaling its effects by activation of cyclic AMP. Deficiencies of glucagon, as occurs in long-standing type 1 diabetes mellitus, can be largely compensated for by an intact autonomic nervous system with appropriate α- and β-adrenergic, and cholinergic effects. Conversely, autonomic failure can be largely compensated for if glucagon secretion remains intact.[1]

Hepatic glycogenolysis is sufficient to meet energy requirements for only a few hours. Beyond that time, glucose must be produced by hepatic gluconeogenesis from precursors such as amino acids, glycerol, and lactate recycled from glycolysis. The major source of gluconeogenic precursors is muscle protein. Although the pool of muscle protein is large, it is required for body function and thus in contrast to stores of glycogen and fat there are no "reserves" of protein to draw on during fasting. To spare the use of essential protein during extended fasting, glucose consumption must be suppressed by switching on the mobilization and oxidation of fatty acids from adipose triglyceride stores, producing ketone bodies and glycerol. The ketone bodies inhibit phosphofructokinase,

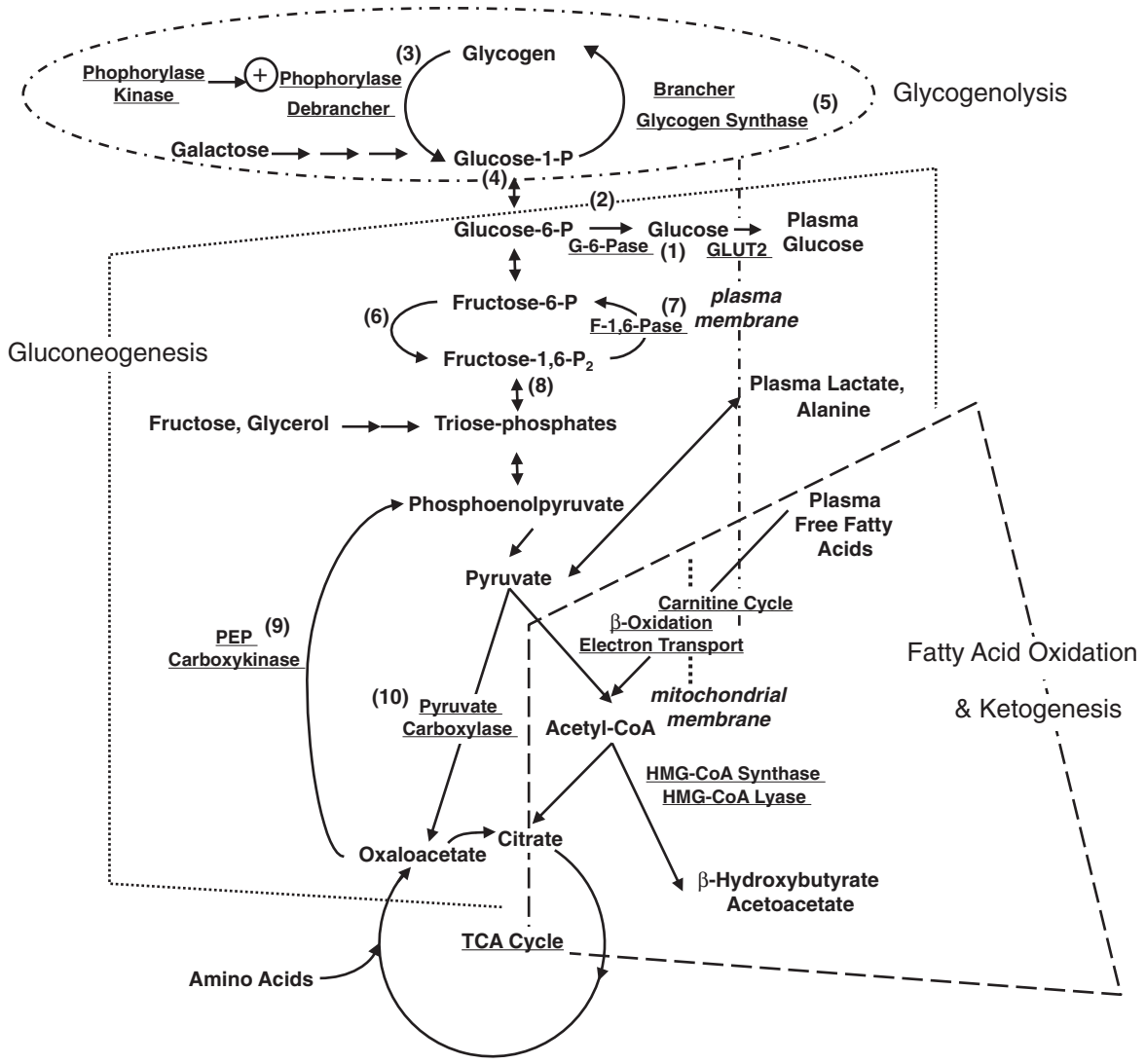


FIGURE 6-1 ■ Key metabolic pathways of intermediary metabolism. Disruption of the elements of these pathways may be pathogenetic in the development of hypoglycemia. Not shown is the hormonal control of these pathways. Indicated are (1) glucose 6-phosphatase, (2) glucokinase, (3) phosphorylase, (4) phosphoglucomutase, (5) glycogen synthetase, (6) phosphofructokinase, (7) fructose 1,6-diphosphatase, (8) fructose 1,6-diphosphate aldolase, (9) phosphoenolpyruvate carboxykinase, and (10) pyruvate carboxylase. (From Pagliara AS, Karl IE, Haymond M, Kipnis DM [1973]. Hypoglycemia in infancy and childhood. J Pediatr 82:365-379 and 82:558-577.)

TABLE 6-2 **Hormonal Regulation of Fasting Metabolic Systems**

Counter-Regulatory Hormone	Glycogenolysis	Gluconeogenesis	Lipolysis	Ketogenesis
Insulin	Inhibits	Inhibits	Inhibits	Inhibits
Glucagon	Stimulates	Stimulates		Stimulates
Cortisol		Stimulates		
Growth hormone			Stimulates	
Epinephrine	Stimulates		Stimulates	Stimulates

hexokinase, and inactivate pyruvate dehydrogenase, thus reducing glucose utilization in the liver and preserving glucose for those cells dependent of glucose alone.

The essential function of fasting adaptation is to maintain fuel supply to the brain. Glucose homeostasis is very limited in neonates and infants compared to adults, in part because of their smaller reserves of liver glycogen and muscle protein but also because of their relatively larger rates of glucose consumption due to their larger brain-to-body-mass ratio. For example, the fuel stores of a 10-kg infant are only 15% of those of an adult. However, the caloric needs are 60% of those of an adult and

glucose turnover rates per kilogram are two- to threefold greater. As shown in Figure 6-2, early in fasting, glucose is the primary brain fuel and accounts for over 90% of total body oxygen consumption. Glucose is provided chiefly from hepatic glycogenolysis, supplemented by hepatic gluconeogenesis utilizing amino acids released by muscle protein turnover. After 8 to 12 hours in normal infants (24 to 36 hours in adults), glucose production declines, because the supply of liver glycogen is limited and the rate of gluconeogenesis from amino acids remains constant. At this time, a transition to fat as the major fuel for the body begins, with accelerated adipose tissue lipolysis and increased fatty acid oxidation in muscle and ketogenesis in liver. Lipolysis also generates glycerol, which is an important gluconeogenic substrate once fasting adaptation is fully active. The brain cannot utilize fatty acids directly, because they do not pass the blood-brain barrier. However, the brain can substitute glucose consumption with the ketones acetoacetate and β-hydroxybutyrate, which are released by the liver as the end product of hepatic fatty acid oxidation. In late stages of fasting adaptation, fatty acid oxidation and ketone utilization account for 90% of total oxygen consumption.

The operation of the metabolic and endocrine systems of fasting adaptation is evidenced by the changes in circulating levels of metabolic fuels and hormones during a fast. As shown in Figure 6-3, in young infants a 24-hour fast is accompanied by a gradual fall in plasma glucose levels as hepatic glycogen stores are depleted, a progressive fall in concentrations of gluconeogenic substrate (e.g., lactate, alanine) as they are used for hepatic gluconeogenesis, a brisk rise in free fatty acids as lipolysis is activated, and a dramatic rise in β-hydroxybutyrate (the major ketone) as hepatic ketogenesis is turned on.[1,42]

By the time that plasma glucose concentration has fallen to < 2.8 mmol/L (50 mg/dL), all the metabolic systems and the hormonal responses described earlier will have fully engaged. A "critical sample" drawn at this time will therefore enable an evaluation of these systems and detect abnormalities of fasting adaptation. As an example, when insulin concentration is above the lower limit of detection according to the assay at the time when plasma glucose concentration is 2.8 mmol/L (50 mg/dL) or less, there is a hyperinsulinemic state reflecting failure of the mechanisms that normally result in suppression of insulin secretion during fasting or hypoglycemia.[43-45] Thus, a snapshot of the integrity of the metabolic and endocrine fasting systems can easily be obtained by measuring the plasma levels of the major fuels and hormones at the end of a fast, when the blood glucose approaches hypoglycemic levels ("critical samples") (Table 6-3; also see also Box 6-2, presented later in the chapter). The use of these critical samples in diagnosing the cause of hypoglycemia is discussed later.

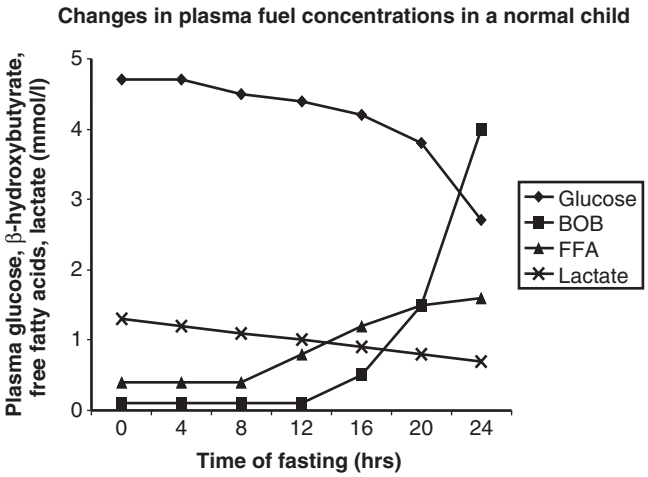


FIGURE 6-3 ■ In infants, a 24-hour fast is accompanied by a gradual fall in plasma glucose levels as hepatic glycogen stores are depleted, a progressive fall in concentrations of gluconeogenic substrate (e.g., lactate, alanine) as they are used for hepatic gluconeogenesis, a brisk rise in free fatty acids (FFA) as lipolysis is activated, and a dramatic rise in βhydroxybutyrate (BOB) (the major ketone) as hepatic ketogenesis is turned on.

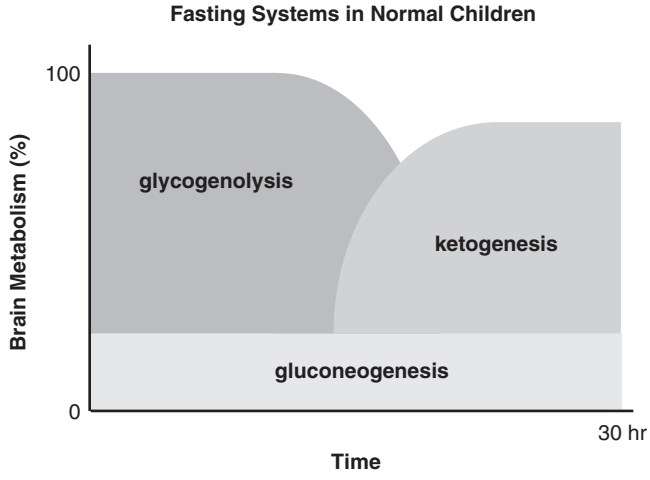


FIGURE 6-2 ■ Contribution of major fasting systems to brain metabolism over time in a typical normal infant. Note that glycogen stores are depleted by 8 to 12 hours and that ketogenesis becomes the major source of brain substrate by 24 hours.

DEFINITION OF HYPOGLYCEMIA IN NEONATES AND INFANTS

The classic definition of symptomatic hypoglycemia is "Whipple's triad"—symptoms, signs or both consistent with hypoglycemia, a low plasma glucose concentration, and resolution of symptoms/signs after the plasma glucose concentration is raised. These three criteria were originally used for diagnosing insulinomas in adults. However, in neonates and infants, who cannot dependably communicate their symptoms and in whom clinical signs of hypoglycemia are not specific, it may not be possible to satisfy Whipple's triad. In these cases, recognition of hypoglycemia may require confirmation by repeated measurements of plasma glucose concentrations on multiple occasions or by formal provocative testing.

Beyond the first 48 hours of life, normal plasma glucose concentration in neonates and infants are not different than in older children and adults. Thus, plasma glucose concentrations in the postabsorptive state range

TABLE 6-3 **Differential Diagnosis of Hypoglycemia in Neonates and Infants**

		Plasma Fuels at End of Fast (mmol/L)				*Plasma Hormones at End of Fast*				
DISORDER	LENGTH OF FAST (hour)	GLUCOSE	LACTATE	FREE FATTY ACIDS	β-HYDROXY-BUTYRATE	INSULIN (μU/mL)	CORTISOL (mg/dL)	GROWTH HORMONE (ng/mL)	GLYCEMIC RESPONSE TO GLUCAGON (mg/dL)	PHYSICAL EXAMINATION
Normal Infants	24-36	2.8	0.7-1.5	1.5-2.5	2-4	< 2	> 20	>10	< 30	
Endocrine System										
Hyperinsulinism	Varies	2.8	N	< 1.5	< 2	> 1	N	N	> 30	LGA
Cortisol deficiency	10-16	2.8	N	N	N	N	Low	Low	N	
GH deficiency	10-16	2.8	N	N	N	N	N	Low	N	Short stature
Panhypopituitarism	10-16	2.8	N	N	N	N	Low	N	N	Short stature, midline facial malformation, optic hypoplasia, micropenis
Epinephrine deficiency (beta-blocker)	10-16	2.8	N	<1.5	<2	N	N	N	N	
Glycogenolysis										
Debrancher deficiency (GSD3)	4-8	2.8	N	N	N	N	N	N	N	Hepatomegaly 4+
Phosphorylase deficiency (GSD6)	10-16	2.8	N	N	N	N	N	N	N	Hepatomegaly 2+
Phosphorylase kinase deficiency (GSD9)	10-16	2.8	N	N	N	N	N	N	N	Hepatomegaly 2+
Glycogen synthase deficiency (GSD0)	6-12	2.8	N	N	N	N	N	N	N	Hepatomegaly 1+
Gluconeogenesis										
Glucose 6-phosphatase deficiency (GSD1a and 1b)	2-4	2.8	4-8+	N	< 2	N	N	N	(lactate)	Hepatomegaly 4+
Fructose 1,6-diphosphatase deficiency	8-12	2.8	4-8+	N	N	N	N	N	N	Hepatomegaly 1+
Pyruvate carboxylase deficiency	8-12	2.8	4-8+	N	N	N	N	N	N	
Lipolysis										
Congenital lipodystrophy, familial dysautonomia, beta-blockers	10-16	2.8	N	< 1.5	< 2	N	N	N	N	
Fatty Acid Oxidation	10-16	2.8	N	> 2.5	< 1.5	N	N	N	N	Hepatomegaly 1+
Carnitine transporter, CPT-1, Translocase, CPT-2, VLCAD, MCAD, SCAD, LCHAD, MADD, HMG-CoA synthase, HMG-CoA lyase deficiency										

For definitions of abbreviations, see Table 6-5 footnote.
GH, growth hormone; GSD, glycogen storage disease.

between 3.9 and 5.6 mmol/L (70 and 100 mg/dL), with a mean of 4.4 to 4.7 mmol/L (80 to 85 mg/dL). A plasma glucose level of 2.8 mmol/L (50 mg/dL) is conventionally used as an end point for provocative diagnostic tests for hypoglycemia. This value is low enough to strongly stimulate the endocrine and metabolic defenses against hypoglycemia for identifying the mechanism responsible for hypoglycemia. Falling glucose levels elicit a typical sequence of responses: plasma insulin levels begin to decrease when plasma glucose falls to the range of 4.4 to 4.7 mmol/L (80 to 85 mg/dL) and insulin secretion is generally "switched off" at glucose concentrations below 2.5 to 3 mmol/L (45 to 54 mg/dL); glucagon secretion increases when plasma glucose levels are in the range of 3.6 to 3.9 mmol/L (65 to 70 mg/dL); epinephrine, cortisol, and growth hormone responses are activated in the range of 3.6 to 3.9 mmol/L (65 to 70 mg/dL). As the plasma glucose falls below 3.3 mmol/L (59 mg/dL), auditory and visual reaction time is prolonged and cognitive function begins to decline as the plasma glucose concentration declines below 2.5 to 3.5 mmol/L (45 to 63 mg/dL), with some of this variability dependent of the testing employed.[13,46]

In some disorders, such as defects in ketogenesis, signs and symptoms may begin to appear during fasting at plasma glucose levels of 3.3 mmol/L (60 mg/dL). On the other hand, some patients (those with glucose 6-phosphatase deficiency) may have few symptoms of neuroglycopenia at plasma glucose levels as low as 1.1 to 1.7 mmol/L (20 to 30 mg/dL) because their high plasma levels of lactate provide an alternative substrate for the brain. Plasma glucose levels between 2.8 and 3.9 mmol/L (50 and 70 mg/dL) should be regarded as suboptimal and below the goal for therapy for hypoglycemia.

A number of potential artifacts can interfere with measuring glucose levels in neonates and infants (Box 6-1). Whole-blood glucose concentrations are 10% to 15% lower than plasma glucose levels because erythrocytes have a higher concentration of protein (hemoglobin) versus plasma with its higher water content, and hence higher dissolved glucose concentrations. The difference may be greater in neonates with higher hematocrits. Blood samples that are not processed promptly can have erroneously low glucose levels, owing to glycolysis by red and white blood cells. At room temperature, the decline of whole-blood glucose can be 5 to 7 mg/dL/hr. The use of inhibitors, such as fluoride, in collection tubes avoids this problem.

Hospital bedside glucose meters and similar home glucose meters are less precise than clinical laboratory methods and can be expected to have an error range of 10% to 15%. These methods are also prone to errors, such as outdated strips or inadequate sampling—most of which result in falsely low glucose values. For this reason, bedside monitors can be used for screening purposes—but any glucose value below 3.3 mmol/L (60 mg/dL) should be verified in the clinical laboratory. Falsely low (or high) glucose values may occur with samples drawn from indwelling lines without adequate flushing of the saline (or glucose) infusate.

For infants beyond the transitional period, treatment should be initiated promptly for plasma glucose values below 2.8 mmol/L (50 mg/dL). Plasma glucose values below 3.3 mmol/L (60 mg/dL) should be rechecked, and treatment considered if values are confirmed to be below 3.3 mmol/L (60 mg/dL). Symptomatic infants and neonates should be treated with intravenous dextrose (0.2 g/kg bolus, followed by infusion at 5 to 10 mg/kg/min). These rates approximate the normal hepatic glucose production rates in neonates and young infants (Figure 6-4). Asymptomatic neonates with plasma glucose levels below 2.8 mmol/L (50 mg/dL) may be treated with oral glucose, but only if there is good reason to believe the problem is a transient one that will not recur. This essentially only applies to otherwise normal neonates during the first 12 to 24 hours after birth who have delayed feedings, such as with initiation of breastfeeding. *Beyond the first day of life, all neonates with verified plasma glucose concentration less than 2.8 mmol/L (50 mg/dL) should be suspected of having a hypoglycemic disorder.*

BOX 6-1 Factors Affecting Measurement of Blood Glucose Concentration

- Whole-blood versus plasma glucose concentration (plasma is 10% to 15% higher)
- Duration between sample collection and sample measurement
- Presence or absence of glycolytic inhibitors in collection tubes
- Sample collection from indwelling lines without adequate flushing

Compiled from Sacks DB (1994). Carbohydrates. In Burtis CA, Ashwood ER (eds.), *Tietz textbook of clinical chemistry*, 2nd ed. Philadelphia: WB Saunders.

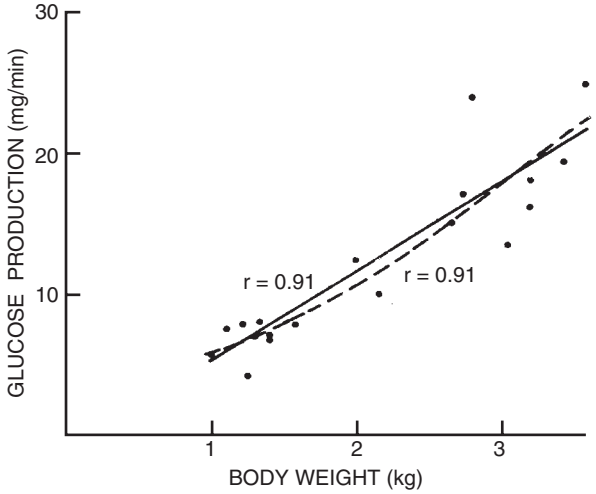


FIGURE 6-4 ■ Glucose production versus body weight determined in 19 newborns with the use of stable isotopic techniques. These studies provide support for the calculated rates of glucose administration required to correct hypoglycemia. (From Bier DM, Leake RD, Haymond MW, et al. [1977]. Measurement of "true" glucose production rates in infancy and childhood with 6,6-dideuteroglucose. Diabetes 26:1016.)

CLINICAL SYMPTOMS AND SIGNS ASSOCIATED WITH HYPOGLYCEMIA

The clinical features of hypoglycemia in infants may be associated with both neurogenic and neuroglycopenic components (Table 6-4). Symptoms are often subtle and not specific, therefore a high index of clinical suspicion must be maintained. Any alteration in clinical status in a newborn that suggests a change in neurologic behavior, fall in temperature, change in feeding pattern, or presence of tremors must be considered a possible initial presentation of a hypoglycemic episode. A seizure must always be considered a possible manifestation of hypoglycemia.

DIAGNOSTIC APPROACH

Treatment of hypoglycemia in neonates and infants requires making a specific diagnosis of the underlying cause. The diagnosis should be based on the combination of data obtained from the history, physical examination, laboratory findings, and, especially, the hormonal and fuel responses at the time of fasting hypoglycemia. A logical approach to diagnosis and treatment must be to analyze a hypoglycemic event as a maladaptation to fasting, and thus the most important information required for diagnosis comes from tests on the blood and urine specimens obtained at a time of hypoglycemia (also known as the critical samples).

Important facts from the history include the duration of fasting that provoked hypoglycemia. Onset within a few hours of a meal would be consistent with hyperinsulinism or glucose 6-phosphatase deficiency, whereas onset after 10 to 12 hours would be consistent with a defect in fatty acid oxidation. Pituitary deficiency with growth hormone or adrenocorticotropic hormone (ACTH)-cortisol deficiency might be suspected by the presence of midline facial malformations, microphthalmia, or microphallus (follicle-stimulating hormone [FSH]/luteinizing hormone [LH] deficiency in utero). Growth failure is also a prominent feature of glucose 6-phosphatase deficiency or debrancher deficiency glycogen storage disease after the first 3 months of life. Both of these disorders are associated with massive hepatomegaly. Abnormal results of liver function tests (transaminases) and hyperammonemia, with or without elevated creatine kinase level, would suggest a possible fatty acid oxidation disorder (see Table 6-3).

Figure 6-5 outlines an algorithm for diagnosis of different forms of hypoglycemia based on readily available laboratory tests on the "critical" blood and urine samples. The first discriminant is a measure of acidemia at the time of hypoglycemia using the serum bicarbonate. If the acidemia is caused by elevations of the ketoacids (β-hydroxybutyrate and acetoacetate), possibilities include a normal child fasted for too long (ketotic hypoglycemia), a defect in glycogenolysis (glycogen storage disease type 3), or counter-regulatory hormone deficiency (hypopituitarism). If the acidemia is caused by an elevation of lactic acid, a block of gluconeogenesis should be suspected (glucose 6-phosphatase or fructose 1,6-diphosphatase deficiency or ethanol ingestion).

If there is no acidemia (i.e., absence of the normal elevation of ketones) but free fatty acid levels are high, a defect in fatty acid oxidation and ketogenesis should be suspected (MCAD deficiency). If ketones are not appropriately increased but the free fatty acid concentrations are also suppressed, hyperinsulinism should be suspected. In the neonatal period, the features of hyperinsulinism can be mimicked by congenital pituitary deficiency. Further tests can then be planned using the initial critical specimens to confirm the suspected diagnosis. These may include physiologic tests (such as the glucagon stimulation test at a time of hypoglycemia to confirm hyperinsulinism) or specialized laboratory tests (such as a plasma acyl-carnitine profile) to identify a defect in mitochondrial β-oxidation.

TABLE 6-4 **Symptoms of Hypoglycemia in Infancy**

Neurogenic	Neuroglycopenic
SYMPTOMS DUE IN PART TO ACTIVATION OF AUTONOMIC NERVOUS SYSTEM (ADRENERGIC OR CHOLINERGIC)	SYMPTOMS DUE TO DECREASED CEREBRAL GLUCOSE AND OXYGEN USE
Shakiness, trembling, jitterness	Lethargy
Tachycardia	Irritability
Hunger	Poor feeding
Pallor	Seizures
Hypothermia	Cyanosis
	Tachypnea
	Apneic episodes
	Weak/High-pitched cry
	Floppiness
	Eye-rolling
	Lip smacking
	Twitching, convulsions

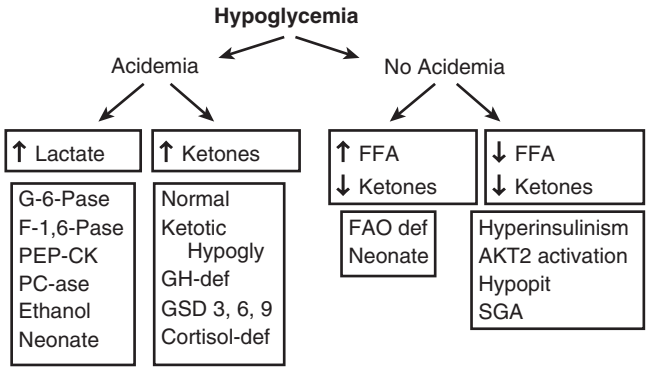


FIGURE 6-5 ■ Algorithm for diagnosis of hypoglycemia based on "critical" blood tests obtained during a period of hypoglycemia. FFA, free fatty acids; FAO: fatty acid oxidation; GSD, glycogen storage disorder; SGA, small for gestational age. (Modified from Stanley CA, Baker L [1978]. Hypoglycemia. In Kaye R, Oski FA, Barness LA (eds.), *Core textbook of pediatrics*. Philadelphia: JB Lippincott, 280-305.)

In some cases, a formal fasting test may be necessary to diagnose the cause of hypoglycemia. The goal of this test is to reproduce the setting in which hypoglycemia occurs in order to identify the underlying cause. The fasting test should be considered a method of testing a hypothesis that has already been developed, based on available clinical and laboratory data about the cause of the hypoglycemia. Thus, the test can be modified with additions or deletions to the basic protocol. This is important, because challenging an infant with fasting is not without risk—particularly if a genetic defect in fatty acid oxidation or adrenal insufficiency is present. Therefore, fasting or other diagnostic challenges should be done only in the hospital under carefully controlled settings with an experienced physician and nursing staff readily available.

Infants younger than 1 year are usually fasted for up to 24 hours, whereas in older children the maximum fast may be up to 36 hours. The fast is terminated when the plasma glucose falls below 2.8 mmol/L (50 mg/dL), but it may be ended sooner if plasma β-hydroxybutyrate rises to greater than 2.5 mmol/L or if there are any adverse signs or symptoms. Periodic blood samples are obtained for analysis of major fuels and hormones and for appropriate ancillary tests (e.g., serum total carnitine, acylcarnitine profile, liver transaminases, creatine phosphokinase, or urinary organic acids). If hyperinsulinism is suspected, the fasting test may be ended with glucagon (1 mg given intravenously) to evaluate the glycemic response.

Special note should be made that the most frequent cause of hypoglycemia in neonates, infants, and children (hyperinsulinism) often cannot be diagnosed based solely on the plasma insulin concentration. With very sensitive assays, serum insulin concentrations will be less than 1 to 2 μU/mL at times of hypoglycemia (i.e., below the sensitivity of most insulin assays). Therefore, the diagnosis must often be made based on evidence of inappropriate insulin effects: hypoketonemia, hypo-free fatty acidemia, and a positive glycemic response to glucagon.

CLASSIFICATION OF CAUSES OF PERSISTENT HYPOGLYCEMIA IN THE NEONATE AND INFANT (Box 6-2)

Disorders of Insulin Excess or Actions

Suppression of insulin secretion is critical for maintaining euglycemia during fasting through the activation of hepatic glycogenolysis, gluconeogenesis, and fatty acid oxidation. Indeed, failure to suppress insulin secretion or actions is the most common cause of persistent hypoglycemia in neonates and children. Whether this is the result of a genetic defect in factors involved in the insulin secretory pathway or in the insulin signaling cascade, the clinical and biochemical hallmarks in the neonate are: (1) increased birth weight because of the growth-promoting effects of insulin in utero, (2) increased glucose utilization, (3) suppression of lipolysis and ketogenesis (low free fatty acids and ketones at the time of hypoglycemia), and (4) inappropriate glycemic response to glucagon at time of hypoglycemia (an increase of > 30 mg/dL) (Box 6-3). These features are critical for making the diagnosis, but it is also essential to determine the underlying cause because the treatment and prognosis may vary among the different disorders. The potential risk of brain damage in an infant suffering from hyperinsulinism is high, placing this diagnosis in a critical position in the diagnostic evaluation of neonatal hypoglycemia.

BOX 6-2 Classification of Causes of Persistent Hypoglycemia in the Neonate and Infant

A. Disorders of Insulin Excess or Actions
  1. Hyperinsulinemic Hypoglycemia
    a. Perinatal Stress-Induced Hyperinsulinism
    b. Monogenic Hyperinsulinism
      K_{ATP} Hyperinsulinism
        Focal
        Diffuse
      GDH Hyperinsulinism
      SCHAD Hyperinsulinism
      GCK Hyperinsulinism
      HNF4Alpha Hyperinsulinism (HNF1Alpha)
      UCP2 Hyperinsulinism
    c. Beckwith-Wiedemann Syndrome
    d. Hyperinsulinemic Hypoglycemia after Fundoplication
  2. AKT2
B. Defects of Counter-Regulatory Response
  1. Hypopituitarism
  2. Congenital ACTH Deficiency (TBX19)
C. Defects in Glycogenolysis and Gluconeogenesis
  GSD Type 1
D. Defects in Fatty Acid Oxidation
  MCAD
E. Defects of Glucose Transporters
  GLUT1 Deficiency
  GLUT2 Deficiency

BOX 6-3 Criteria for Diagnosing Hyperinsulinism Based on "Critical" Samples (Drawn at a Time of Fasting Hypoglycemia: Plasma Glucose < 50 mg/dL)

1. Hyperinsulinemia (plasma insulin > 2 μU/mL)*
2. Hypofattyacidemia (plasma FFA < 1.5 mmol/L)
3. Hypoketonemia (plasma BOB < 2 mmol/L)
4. Inappropriate glycemic response to glucagon, 1 mg IV (delta glucose > 30 mg/dL)

*Depends on sensitivity of insulin assay.
BOB, β-hydroxybutyrate; FFA, free fatty acids.

Hyperinsulinemic Hypoglycemia

Transient Hyperinsulinism Resulting from Maternal Factors

Transient hyperinsulinism is a well-recognized complication in neonates after a pregnancy complicated by gestational diabetes. At birth, infants born to these mothers may be large and plethoric—and their body stores of glycogen, protein, and fat are replete. The classic clinical description of the effect of hyperinsulinism relates to the infant of the diabetic mother[47]:

> *These infants are remarkable not only because like fetal versions of Shadrack, Meshack, and Abednego, they emerge at least alive from within the fiery metabolic furnace of diabetes mellitus, but because they resemble one another so closely they might well be related. They are plump, sleek, liberally coated with vernix caseosa, full-faced, and plethoric. The umbilical cord and placenta share in the gigantism. During their first 24 or more extrauterine hours, they lie on their backs, bloated and flushed, their legs flexed and abducted, their lightly closed hands on either side of the head, the abdomen prominent and their respiration sighing. They convey a distinct impression of having had such a surfeit of both food and fluid pressed upon them by an insistent hostess that they desire only peace so that they may recover from their excesses. On the second day their resentment of the slightest noise improves the analogy when their trembling anxiety seems to speak of intrauterine indiscretions of which we know nothing.*

The hypoglycemia in infants of diabetic mothers is related chiefly to hyperinsulinemia and in part to diminished glucagon secretion. Hypertrophy and hyperplasia of their islets have been documented, as has their brisk, biphasic, and typically adult insulin response to glucose. This insulin response is absent in normal infants. Infants born to diabetic mothers also have a subnormal surge in plasma glucagon immediately after birth, subnormal glucagon secretion in response to stimuli, and (initially) excessive sympathetic activity that may lead to adrenomedullary exhaustion because urinary excretion of epinephrine is diminished. Thus, despite their abundance of tissue stores of available substrate, the normal plasma hormonal pattern of low insulin, high glucagon, and catecholamines is reversed. Their endogenous glucose production is inhibited and glucose utilization is increased compared with that in normal infants, thus predisposing them to hypoglycemia.

Mothers whose diabetes has been well controlled during pregnancy in general have near-normal-sized infants who are less likely to develop neonatal hypoglycemia and other complications formerly considered typical of maternal diabetes. Nevertheless, treatment of infants born to mothers with diabetes commonly requires provision of intravenous glucose for a few days until the hyperinsulinemia abates. For these infants, glucose should be provided at a rate of 5 to 10 mg/kg/min. However, the appropriate dosage for each patient should be individually adjusted.

During labor and delivery, maternal hyperglycemia should be avoided because it may result in fetal hyperglycemia—which predisposes to rebound hypoglycemia when the glucose supply is interrupted at birth. Other maternal factors that can result in transient neonatal hyperinsulinism include oral hypoglycemics (such as sulfonylureas) or other medications (terbutaline or propranolol).

By definition, transient hyperinsulinism as a cause of neonatal hypoglycemia in an infant of a diabetic mother should abate in 1 or 2 days. If the condition persists, prolonged or congenital hyperinsulinism must become a prominent consideration, and the index of suspicion must remain high until it is ruled out.

Prolonged Neonatal Hyperinsulinism: Perinatal Stress-Induced Hyperinsulinism

As shown in Figure 6-6, the risk of postnatal hypoglycemia is increased in neonates who are small for gestational age. There is increasing evidence that prolonged hypoglycemia in some neonates exposed to perinatal stress such as birth asphyxia, maternal toxemia, prematurity, or intrauterine growth retardation or other peripartum stress is due to hyperinsulinism.[48-50] The estimated incidence of prolonged neonatal hyperinsulinism is 1:12,000 live births.[50]

The clinical presentation of perinatal stress-induced hyperinsulinism is characterized by high glucose utilization, and the response to fasting hypoglycemia shows an elevated plasma insulin level (although it may be normal), low β-hydroxybutyrate and free fatty acid levels, and inappropriate glycemic response to glucagon at the time of hypoglycemia. Unlike the transient hyperinsulinism seen in the infant of the diabetic mother, perinatal stress-induced hyperinsulinism may persist for several days to several weeks. In a series of neonates with perinatal stress-induced hyperinsulinism, the median age of resolution was 6 months.[50] The mechanism responsible for the dysregulated insulin secretion is not known. Acute insulin responses (AIRs) show that in general the patterns of insulin response to secretagogues (calcium, tolbutamide, glucose, and leucine) in infants with prolonged neonatal hyperinsulinism resembled those of normal controls.[50]

Infants with prolonged neonatal hyperinsulinism usually respond very well to medical therapy with diazoxide.[48-50] Previously, it was common practice to use pharmacologic doses of glucocorticoids to treat such

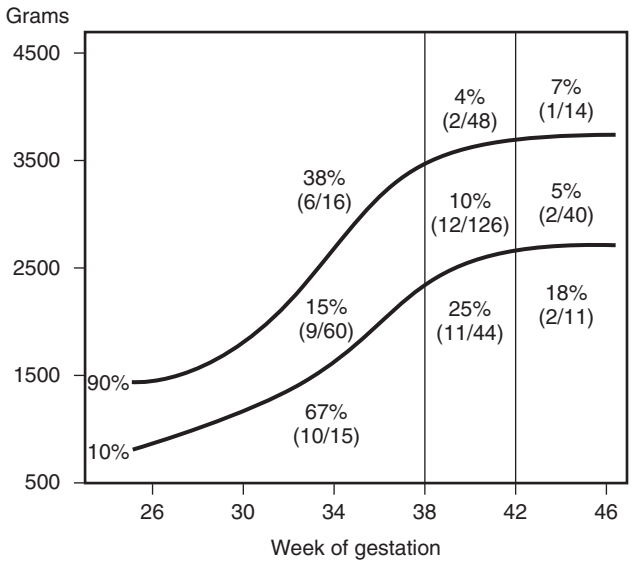


FIGURE 6-6 ■ Incidence of plasma glucose level being less than 30 mg/dL before first feeding at 3 to 6 hours of age in newborns, classified by birth weight and gestational age. (From Lubchenko LO, Bard H [1971]. Incidence of hypoglycemia in newborn infants by birth weight and gestational age. Pediatrics 47:831.)

neonates with persistent hypoglycemia. However, the use of glucocorticoids as nonspecific therapy for neonatal hypoglycemia is not recommended because they are not only ineffective but also can suppress the hypothalamic-pituitary-adrenal axis.

Hyperinsulinism in Beckwith-Wiedemann Syndrome

Beckwith-Wiedemann syndrome (BWS) is a heterogeneous disorder that results from various genetic and epigenetic anomalies in the imprinted 11p15.5 region. This region can be divided into two distinct imprinted domains separated by a nonimprinted region. The distal domain 1 contains the imprinted genes insulin-like growth factor 2 (*IGF2*), which is growth promoting and paternally expressed, and *H19*, a tumor suppressor expressed from the maternal allele. In the proximal domain 2, the imprinted region contains two genes that have been implicated in BWS, *KCNQ1OT1* (paternally expressed untranslated transcript) and *CDKN1C* (maternally expressed, encodes a negative regulator of cell proliferation). Molecular alterations affecting domains 1 and 2 associated with BWS include paternal uniparental disomy involving both imprinted gene clusters (20% of cases) and paternal duplications of 11p15 (approximately 1% of cases).[51]

The classical features of BWS include macrosomia, macroglossia, visceromegaly, abdominal wall defects, ear creases/pits, body asymmetry, and an increased risk for embryonal tumor development. Hypoglycemia due to hyperinsulinism is present in up to 50% of cases.[52] The mechanism responsible for hyperinsulinism in BWS has not been elucidated but may involve dysregulated insulin secretion due to loss of KATP channel function.[53] The pancreatic histology in BWS is characterized by hyperplasia of all pancreatic structures including ducts, acini, and islets with increased proliferation of endocrine cells.[54] However, is not known whether the hyperinsulinism in BWS is due to the increased β-cell mass, dysregulated insulin secretion, or both.

The hyperinsulinism in BWS may be mild and transient, although in some cases it can be severe and persistent.[52] Some BWS infants respond to diazoxide, whereas others require pancreatectomy to control the hypoglycemia. Because the hyperinsulinism will resolve within the first year of life in most cases, pancreatectomy should be reserved for those cases in which the hypoglycemia cannot be controlled with diazoxide, octreotide, or continuous enteral supplementation with dextrose or feedings.

Monogenic Hyperinsulinism

Congenital hyperinsulinism, also known as persistent hyperinsulinemic hypoglycemia of infancy, represents a group of clinically and genetically heterogeneous disorders characterized by dysregulated insulin secretion and resulting in severe and persistent hypoglycemia. First described in 1954 by MacQuarrie[55] as "idiopathic hypoglycemia of infancy," congenital hyperinsulinism is the most common cause of persistent hypoglycemia in children. Worldwide, the incidence of congenital hyperinsulinism is estimated at 1 in 50,000 live births with higher incidence of up to 1 in 2500 in areas of high consanguinity.[45]

To understand the pathophysiology of congenital hyperinsulinism, knowledge of the major pathways regulating insulin release by pancreatic β-cells is critical (outlined in Figure 6-7). Glucose-stimulated insulin secretion involves glucose uptake through glucose transporters (GLUT1, GLUT2, and GLUT3 are expressed in the islets[56]) and phosphorylation by glucokinase (GK), leading to glucose oxidation and an increased ATP/ADP ratio that results in inhibition of a plasma membrane ATP-dependent potassium (K_{ATP}) channel. The β-cell K_{ATP} channel is a hetero-octameric complex consisting of two subunits: a K^+-selective pore-forming subunit (Kir6.2) and a regulatory subunit (SUR-1). Four Kir6.2 subunits form the central pore, coupled to four SUR-1 subunits. The K_{ATP} channel is inhibited (closed) by sulfonylurea drugs (used therapeutically to stimulate insulin secretion in type 2 diabetes) and activated (opened) by diazoxide (the main medical treatment for congenital hyperinsulinism). In the unstimulated state, the β-cell ATP-sensitive potassium channels are open—keeping a resting membrane potential of approximately –65 mV. Following the uptake and metabolism of glucose, an increase in the intracellular ATP/ADP ratio results in closure of ATP-sensitive potassium channels, depolarization of the cell membrane, and subsequent opening of voltage-dependent Ca^{2+} channels. The resulting increase in cytosolic Ca^{2+} concentration triggers the release of stored insulin granules. Stimulation of insulin secretion by amino acids occurs through an allosteric activation of glutamate dehydrogenase (GDH) by leucine, which results in increased oxidation of glutamate—leading to an increased ATP/ADP ratio, inhibition of K_{ATP}-channel activity, and membrane depolarization.

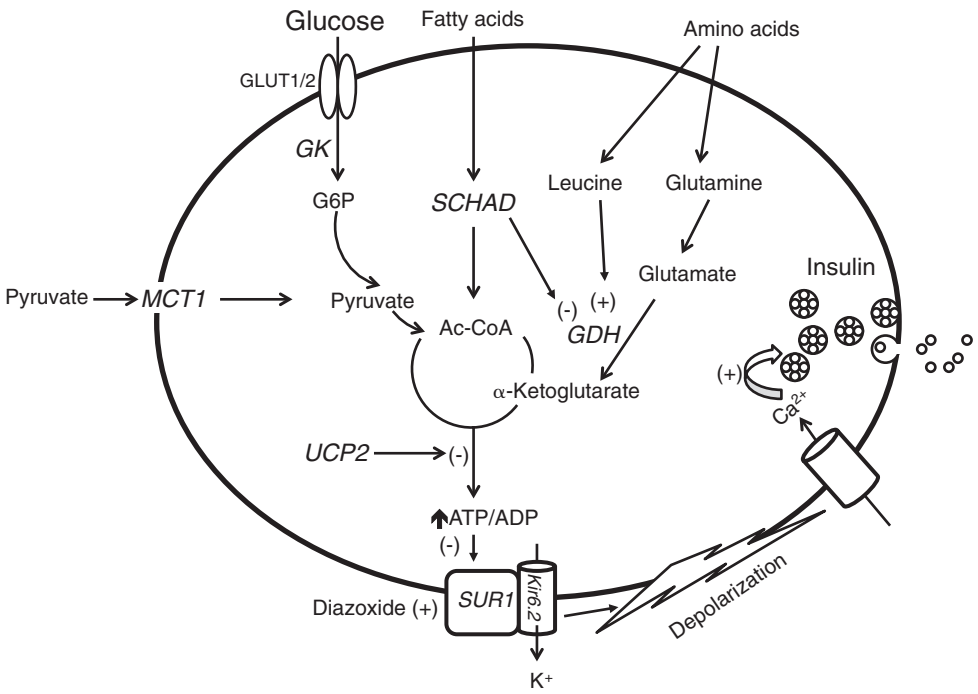


FIGURE 6-7 ■ Current model of mechanisms of insulin secretion by the beta cell of the pancreas. Glucose transported into the beta cell by the insulin-independent glucose transporter GLUT2/GLUT1 undergoes phosphorylation by glucokinase and is then metabolized, resulting in an increase in the adenosine triphosphate/adenosine diphosphate (ATP/ADP) ratio. The increase in the ATP/ADP ratio closes the K_{ATP} channel and initiates the cascade of events characterized by increase in intracellular potassium concentration, membrane depolarization, calcium influx, and release of insulin from storage granules. Leucine stimulates insulin secretion by allosterically activating glutamate dehydrogenase (GDH) and by increasing the oxidation of glutamate, thereby increasing the ATP/ADP ratio and closure of the K_{ATP} channel. Diazoxide inhibits insulin secretion by stimulating the K_{ATP} channel. The gene defects known to cause hyperinsulinism are shown in italics. Six are inactivating mutations: SUR1 (sulfonylurea receptor), Kir6.2 (potassium channel), SCHAD (short-chain 3-OH acyl-CoA dehydrogenase), UCP2 (uncoupling protein 2), HNF4a (hepatic nuclear transcription factor 4alpha), and HNF1a (hepatic nuclear transcription factor 1alpha). Three are activating mutations: GK (glucokinase), GDH (glutamate dehydrogenase), and MCT1 (monocarboxylate transporter 1). (−), inhibition; (+), stimulation.

Mutations in genes encoding nine different proteins involved in different steps of this pathway have been associated with congenital hyperinsulinism: the sulfonylurea receptor 1 (SUR-1, a member of the superfamily of ATP-binding cassette proteins), encoded by *ABCC8*[57]; Kir6.2, encoded by *KCNJ11*[58]; glucokinase (GK), encoded by *GCK*[59]; glutamate dehydrogenase (GDH), encoded by *GLUD1*[60]; the mitochondrial enzyme short-chain 3-hydroxyacyl-CoA dehydrogenase (SCHAD), encoded by *HADH*[61]; monocarboxyl transporter-1 (MCT-1), encoded by *SLC16A1*[62]; uncoupling protein-2 (UCP2), encoded by *Ucp2*[63]; hepatocyte nuclear factor 4 alpha (HNF-4 alpha), encoded by *HNF4A*[64]; and hepatocyte nuclear factor 1 alpha (HNF-1 alpha), encoded by *HNF1A*.[65] In this chapter we review the forms more likely to present in the neonatal period. For a discussion of MCT-1 hyperinsulinism, see Chapter 6.

Treatment in neonates with suspected congenital hyperinsulinism should be initiated promptly, as their risk of brain damage is high because their brain is deprived not only of glucose but also alternative fuels (ketones). Brain injury due to hypoglycemia is particularly likely in K_{ATP}HI due to the severity of the hypoglycemia. In the Children's Hospital of Philadelphia experience, the prevalence of developmental delay in patients with congenital hyperinsulinism is approximately 30%.[66] Patients with K_{ATP}HI requiring surgical therapy have a higher incidence of neurodevelopmental problems than diazoxide-responsive patients.[66] A homogenous cohort of children with the known Ashkenazi mutations who were treated without surgery showed good neurologic outcome by school age, based on parental reports, although earlier developmental deficits were present.[67] A higher rate (44%) of long-term neurodevelopmental retardation was reported in a large series of children (49 medically treated, 65 treated surgically).[68] In another series of 90 patients, severe mental retardation was found in 8%, with milder disability in another 18%. Psychomotor delays were more common in patients with neonatal onset than in those with onset of hypoglycemia later in infancy presumably reflecting more severe hypoglycemia.[69] In general, the high risk of brain damage appears to be due to delays in diagnosis and treatment rather than a consequence of the K_{ATP} channel defect and, thus, potentially preventable.

The goal of therapy is to restore plasma glucose to the normal range (> 70 mg/dL) and to maintain it there. Intravenous glucose at high rates, up to five times the normal glucose requirement, is usually required and central lines may be needed for administration of highly concentrated dextrose. The first line of therapy for hyperinsulinism is diazoxide, an agent that acts as a K_{ATP}-channel opener that

requires functional channels to be present at the cell surface to have an effect; thus, most patients with K_{ATP}HI do not respond to diazoxide. In contrast, patients with GDH-HI, GK-HI, SCHAD-HI, and those with perinatal stress-induced hyperinsulinism do respond to diazoxide. The usual dose of diazoxide is 5 to15 mg/kg/day, given orally once or twice a day. The major adverse event with diazoxide in neonates is fluid retention. Concomitant use of a diuretic (chlorothiazide or furosemide) should be considered, especially in infants receiving intravenous fluids. The half-life of diazoxide is 24 to 36 hours in adults; limited data indicate that in children the half-life is 9.5 to 24 hours.[70] The response to diazoxide should thus be evaluated after at least 5 days of therapy. Successful response should be demonstrated by showing maintenance of plasma glucose above 3.3 mmol/L (70 mg/dL) after fasting. The duration of the fast should be determined on a case-by-case basis, based in part on the age of the child.

The second line of medical therapy for infants unresponsive to diazoxide is octreotide. This long-acting somatostatin analog inhibits insulin secretion by inducing hyperpolarization of β-cells, direct inhibition of voltage-dependent calcium channels, and more distal events in the insulin secretory pathway. Octreotide is administered subcutaneously every 6 to 8 hours at 5 to 20 μg/kg/day, or as a continuous infusion. The initial response to octreotide is good in most infants with hyperinsulinism, but tachyphylaxis develops after a few doses, rendering therapy inadequate for long-term use in most cases. The use of octreotide has been associated with the occurrence of necrotizing enterocolitis in neonates and should be used with caution in this population.[71] Treatment with a long-acting release octreotide has been shown to be effective in older children.[72] Glucagon, given as a continuous intravenous infusion (1 mg/day), can help maintain euglycemia in infants waiting for surgery, but its long-term use as a subcutaneous infusion[73] is limited by crystallization of the glucagon and clogging in the tubing.[74]

Mutation analysis to establish the genetic cause of the hyperinsulinism is particularly helpful in diazoxide-unresponsive cases, as it helps to distinguish those with diffuse pancreatic involvement who may require extensive pancreatic resection from children in whom focal hyperinsulinism is likely, thereby permitting curative resection of the focal lesion. Thus, once the diagnosis is established and initial therapy is initiated, samples for the child and the child's parents should be obtained for DNA molecular analysis. Figure 6-8 depicts the diagnostic and treatment approach for children with hyperinsulinism. A cardiac evaluation should also be considered in children with hyperinsulinism, as a large proportion of the cases have cardiac structural abnormalities, more commonly ventricular hypertrophy.[75]

K_{ATP} Hyperinsulinism. Inactivating mutations of the K_{ATP} channels are responsible for the most common and most severe form of congenital hyperinsulinism. According to the severity of the molecular defect and the phenotype, K_{ATP}HI can be classified in three subtypes:

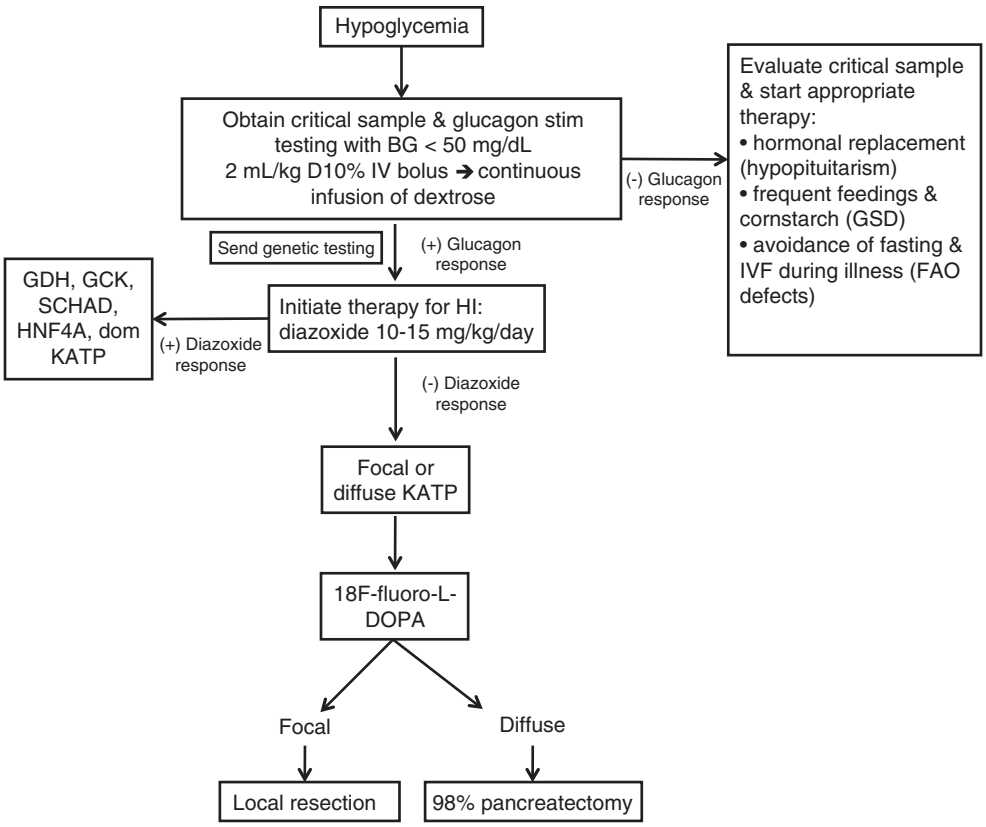


FIGURE 6-8 ■ Diagnostic and treatment approach in cases of hyperinsulinism.

(1) recessive, diazoxide-unresponsive; (2) dominant, diazoxide-unresponsive; and (3) dominant, diazoxide-responsive. Recessive mutations interfere with protein expression or channel trafficking, resulting essentially in a complete absence of channels on the plasma membrane, and therefore diazoxide is not effective. Recessive mutations are the most common defects identified in children with congenital hyperinsulinism. Dominant mutations allow normal trafficking of the channels to the plasma membrane but impair channel activity either completely or partially, resulting in a spectrum of phenotypes that go from severe, diazoxide-unresponsive to mild, diazoxide-responsive. To date, a total of 146 mutations in *ABCC8* (119 recessive and 27 dominant) and 22 mutations in *KCNJ11* (18 recessive and 4 dominant) have been reported.[76]

The clinical presentation of K_{ATP}HI then depends on the severity of the mutation effect. More commonly, these children are large for gestational age and have severe neonatal hypoglycemia that does not respond to diazoxide, except for the cases with a dominant diazoxide-responsive subtype in which birth weight is normal and the clinical presentation tends to occur later in life.[77]

The pathophysiology of K_{ATP}HI has been illustrated by functional evaluation of islet function in vivo and in vitro. The absence of functional K_{ATP} channels results in an uncoupling between plasma glucose and insulin secretion or β-cell "glucose blindness." Thus, insulin secretion is not turned off as plasma glucose decreases and fails to increase in response to a rapid raise in plasma glucose (Figure 6-9).[78-80] The former defect dominates the clinical manifestations in early infancy, whereas the latter may play a role in later development of glucose intolerance and, possibly, diabetes. In marked contrast with the failure to respond to changing concentrations of plasma glucose, these β-cells are hyperresponsive to stimulation with amino acids,[81] which results in protein-induced hypoglycemia.[82]

Other clinical features of infants with K_{ATP}HI include extremely high glucose requirements, frequently four to five times higher than normal to control plasma glucose (although in some cases the requirement may be normal), feeding aversion, and gastroesophageal reflux, likely consequences of force feeding. Treatment options for the diazoxide-unresponsive cases are limited. These infants often require pancreatectomy within the first few weeks after birth to manage the hypoglycemia. On the other hand, diazoxide is very effective for controlling hypoglycemia in cases with dominant diazoxide-responsive K_{ATP}HI. Octreotide, a long-acting somatostatin analog, is a second line medical therapy for infants unresponsive to diazoxide, although the Food and Drug Administration (FDA) has not approved it for this indication, and, as discussed earlier, because of concerns with a possible association with necrotizing enterocolitis, its use should be carefully considered.[71] A nonsurgical approach in diazoxide-unresponsive hyperinsulinism consisting of octreotide in combination with continuous or frequent enteral feedings has been proposed.[74] This approach is challenging for home management of these children, it requires feedings as frequently as every 2 to 3 hours during the day and continuously at night, frequent glucose testing, and may lead to loss of oral feeding skills.

There are two distinctive histologic forms of KATP-hyperinsulinism, focal and diffuse hyperinsulinism.

Focal K_{ATP}-Hyperinsulinism (Focal Adenomatosis). Approximately 40% to 60% of the cases of K_{ATP}HI (which require surgery) have focal disease.[83] Focal lesions arise by a "two-hit" mechanism of focal loss of heterozygosity for the maternal 11p15 region, leading to a somatic reduction to homozygosity (or hemizygosity) of a paternally inherited mutation of the *ABCC8* or *KCNJ11*

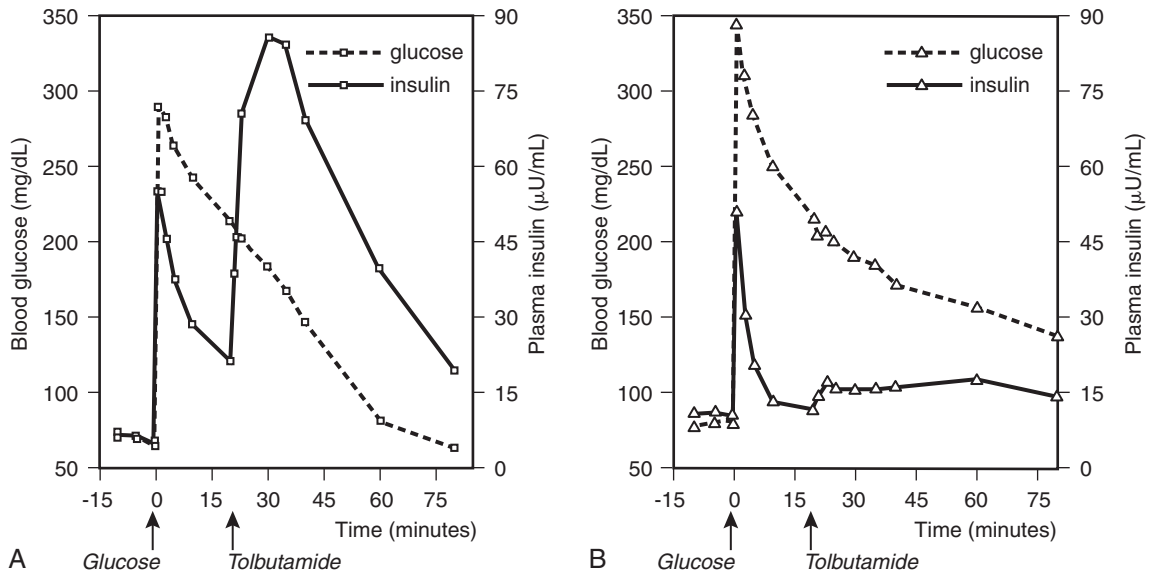


FIGURE 6-9 ■ AIR to glucose and tolbutamide in children with diffuse K_{ATP} hyperinsulinism (mean 11- and 13-minute increments). **A**, Normal adult control. **B**, Patient with diffuse K_{ATP} hyperinsulinism. (From Grimberg A, Ferry RJ, Kelly A, et al. [2000]. Dysregulation of insulin secretion in children with congenital hyperinsulinism due to sulfonylurea receptor mutations. Diabetes 50:322.)

gene. The 11p15 region, which carries the *ABCC8* and *KCNJ11* genes, contains several imprinted tumor suppressor genes (*H19* and *CDKN1C*) that are only expressed on the maternal chromosome, whereas IGF-II is expressed on the paternal allele. Loss of these growth-suppressing genes, while the paternal growth-promoting gene is active, may play an important permissive role in the clonal expansion of the cells expressing the mutated channel.[84] The majority of mutations causing the focal lesions involve the *ABCC8* gene.

Diffuse Hyperinsulinism. In diffuse hyperinsulinism, all β-cells in the pancreas are affected. It results from the inheritance of two recessive mutations in ABCC8 or KCNJ11 or a dominant mutation in these genes.

Clinically, the focal and diffuse forms of hyperinsulinism are identical in terms of the presentation and the lack of response to diazoxide. Histologically, they are distinct.[85] Diffuse hyperinsulinism is characterized by the presence of abnormally large islet cell nuclei distributed throughout the pancreas. In contrast, the pancreatic histology in focal hyperinsulinism is characterized by a lesion formed by the confluence of hyperplastic islets occupying more than 40% of the cross-sectional area of pancreatic lobules. In contrast to true adenomas, the focal adenomatous hyperplasia includes exocrine acinar cells intermixed within the lesion. The morphology of islets away from the focal lesion is normal.[86] The ability to interpret these histologic characteristics requires specialized training and is only available at centers with dedicated multidisciplinary teams to evaluate and treat hyperinsulinism.

Focal K_{ATP}HI is potentially curable by surgery, whereas diffuse K_{ATP}HI is not. Therefore, efforts to diagnose and localize focal lesions in infants with diazoxide-unresponsive hyperinsulinism before surgery are critical. Conventional imaging techniques, such as computed tomography (CT) or magnetic resonance imaging (MRI), are unable to detect focal lesions. Interventional radiology studies, such as transhepatic portal venous insulin sampling[87] and selective pancreatic arterial calcium stimulation,[88] have only modest success, are technically difficult, and are highly invasive. The gold standard technique to localize the focal lesions is positron emission tomography (PET) scanning with flurorine-18 L-3, 4-dihydroxyphenylalanine (^{18}F-fluoro-L-DOPA).[89-91] Pancreatic β-cells take up L-DOPA,[92] and DOPA decarboxylase is active in pancreatic islet cells.[93] In children with focal hyperinsulinism, there is localized accumulation of ^{18}F-fluoro-L-DOPA. Co-registration of PET and CT images allows the anatomic localization of the lesion (Figure 6-10; see also e-Figure 6-1 on the accompanying website for a full-color example).

Glutamate Dehydrogenase-Hyperinsulinism: The Hyperinsulinism Hyperammonemia Syndrome (HI/HA). Congenital hyperinsulinism due to gain-of-function mutations of *GLUD1* (encoding glutamate dehydrogenase, GDH) is the second most common form of genetic hyperinsulinism and the most common form that responds to treatment with diazoxide.[60,94-97] A total of 23 different disease-causing mutations have been identified. In approximately 70% of the cases, the mutations are de novo.[98] Among the 30% of familial cases, a clear autosomal dominant pattern of inheritance is evident.

The clinical presentation is characterized by hypoglycemia due to hyperinsulinism together with a characteristically persistent but asymptomatic elevation of plasma ammonia. Hypoglycemia is usually recognized by around 4 to 12 months of life and birth size is normal. Plasma ammonia levels in HI/HA are elevated three to five times above the normal range to approximately 60 to 150 μmol/L. Ammonia levels are quite constant, and in contrast to the urea cycle enzyme defects, do not increase with protein feeding. The hyperammonemia does not appear to cause symptoms and does not require treatment.

GDH is a mitochondrial matrix enzyme that is a key regulator of amino acid and ammonia metabolism in pancreatic β-cells, liver, kidney, and brain. As shown in Figure 6-7, GDH functions in the beta cell pathway of leucine-stimulated insulin secretion. Leucine is an allosteric activator of the enzyme, causing increased oxidation of glutamate to α-ketoglutarate and increased ATP production—which results in insulin release. The HI/HA mutations affect either the inhibitory GTP-binding site or the antenna loop of the enzyme, which communicates between adjacent subunits, impairing the inhibitory allosteric effect of GTP on GDH enzyme activity, thus leading to excessive insulin release.

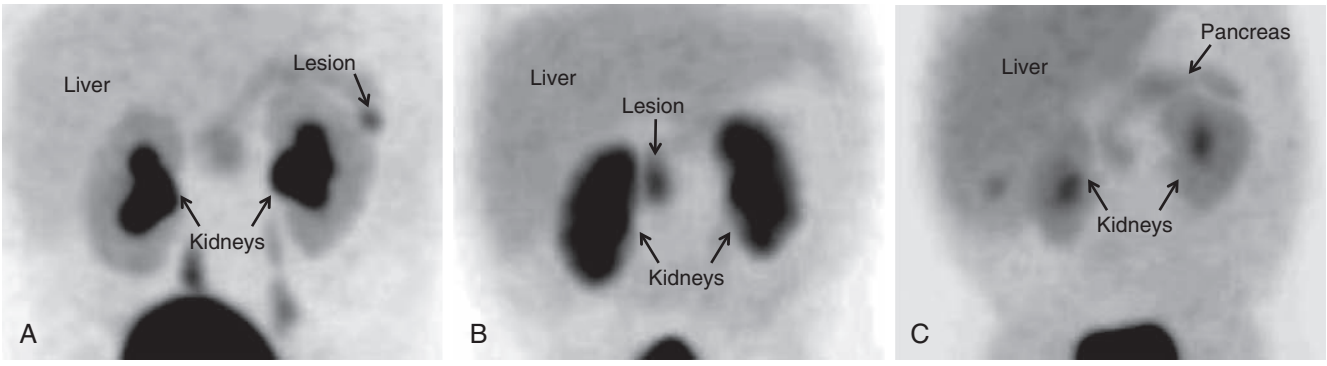


FIGURE 6-10 ■ ^{18}Fluoro-L-DOPA PET scan images showing L-DOPA uptake in the liver, kidneys, and pancreas. Note increased uptake in the tail (**A**) and head (**B**) of the pancreas demonstrating focal lesions. In contrast, diffuse hyperinsulinism uptake throughout the pancreas is uniform (**C**). *A full-color image of L-DOPA uptake can be viewed online at ExpertConsult.*

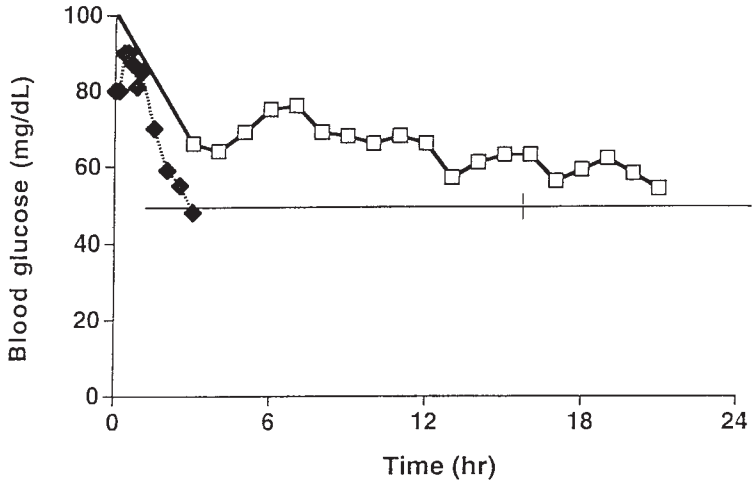


FIGURE 6-11 ■ Blood glucose responses to fasting (open squares) and protein feeding (solid diamonds) in a 16-year-old girl with the hyperinsulinism/hyperammonemia syndrome caused by a dominantly expressed R269H regulatory mutation of glutamate dehydrogenase. (From Hsu BY, Kelly A, Thornton PS, et al. [2001]. Protein-sensitive and fasting hypoglycemia in children with the hyperinsulinism/hyperammonemia syndrome. J Pediatr 138:383.)

The clinical phenotype of HI/HA is dominated by the effects of the activating mutations in the pancreatic β-cells. Isolated islets from transgenic mice expressing a mutated human GDH exhibit normal glucose-stimulated insulin secretion but enhanced leucine- and amino-acid–stimulated insulin secretion.[99] In children with HI/HA there is a dramatic increase in insulin following an intravenous bolus of leucine, but in contrast to children with K_{ATP}HI, they do not respond to calcium stimulation.[100] The hypoglycemia in children with HI/HA is provoked by fasting and by protein-rich meals. The fasting hypoglycemia may be relatively mild. Children may be able to fast for 8 to 12 hours before becoming hypoglycemic. However, these patients have dramatic protein-sensitive hypoglycemia—becoming severely hypoglycemic within 30 to 90 minutes of ingesting a protein meal[101] (Figure 6-11). Diazoxide therapy, 5 to 10 mg/kg/day, is usually effective in controlling both fasting and protein-induced hypoglycemia in HI/HA. Carbohydrate preloading may be helpful in avoiding the latter.[98]

Initially, the hyperammonemia was presumed to be due to the effects of the mutated enzyme in the liver; however, an alternative mechanism involving activation of renal GDH seems to be more likely.[102] The hyperammonemia is persistent and not associated to the classics signs of ammonia toxicity. It occurs in both the fed and fasted states and is unaffected by plasma glucose concentrations or protein intake.

GDH is also highly expressed in the brain, particularly astrocytes, but the consequences of increased GDH enzyme activity in the brain are less clear. Different groups have reported a propensity for seizures (atypical absence seizures, generalized tonic-clonic seizures, and focal motor seizures), learning disabilities, mental retardation, and behavior disorders in children with HI/HA.[103-105] The underlying mechanism responsible for the central nervous system manifestations is not known. It does not appear to be related to the hypoglycemia or the hyperammonemia, and it may be a direct effect of the activation of GDH.

SCHAD-Hyperinsulinism. A less common form of congenital hyperinsulinism also involving loss of regulation of GDH activity is due to inactivating mutations in *HADH* (the gene encoding the mitochondrial short-chain L-3-hydroxyacyl-CoA dehydrogenase [SCHAD]).[61,106,107] SCHAD-HI is an autosomal recessive disorder characterized by fasting hypoglycemia due to inappropriate insulin regulation. The cause of dysregulated insulin secretion was elucidated with the discovery that in the β cell, SCHAD plays an inhibitory role in GDH activity.[108] Thus, the insulin dysregulation observed in SCHAD deficiency is due to loss of this inhibition, explaining the clinical similarities between GDH-HI and SCHAD-HI.

In contrast to all other defects in fatty acid oxidation, children with SCHAD-HI have no signs of hepatic dysfunction or cardiomyopathy, or of affects on skeletal muscle, features usually recognized in children with fatty acid oxidation disorders. The clinical presentation of SCHAD-HI is heterogeneous, ranging from late onset of mild hypoglycemia to severe early onset of hypoglycemia in the neonatal period. In addition to fasting, hypoglycemia children with SCHAD-HI exhibit protein-induced hypoglycemia, similar to the children with GDH-HI.[109] The biochemical hallmarks, in addition to markers of increased insulin action, are increased levels of plasma 3-hydroxybutyryl-carnitine and increased levels of 3-hydroxyglutarate in urine, findings consistent with reduced SCHAD enzyme activity. In contrast to GDH-HI, children with SCHAD-HI do not have elevated ammonia, probably because of the lower expression of SCHAD in other tissues where GDH is expressed. Affected children are responsive to medical therapy with diazoxide.

Glucokinase-Hyperinsulinism. A less frequent form of congenital hyperinsulinism is due to activating mutations in *GCK* (encoding glucokinase-GK). Glucokinase is a hexokinase that serves as the glucose sensor in pancreatic β-cells[110] (see Figure 6-7). In contrast to other hexokinases, GK has a much lower affinity for its substrate with half-maximal activity ($S_{0.5}$) at a glucose

concentration of 7.6 mM. The properties of GK and the positive cooperative action of glucose on enzyme activity with a curve that is steep in the range of 5 mM glucose make GK well suited to tightly control plasma glucose in the normal physiologic range of 70 to 100 mg/dL.[111] In GK-HI, activating mutations result in increased affinity of glucokinase for glucose and insulin secretion at lower glucose concentrations. A total of 15 mutations have been associated with GK-HI[111]; some cases are sporadic, and some are dominantly inherited. All of the mutations have reduced glucose $S_{0.5}$ ranging from 1.1 to 4.5 mM.

The clinical presentation of GK-HI is characterized by large for gestational age birth weight, reflecting the growth-promoting effects of increased fetal insulin secretion. Hypoglycemia may present in the neonatal period but often is not recognized until later in infancy or childhood. The severity of the phenotype is variable with some mutations having a mild phenotype, with fasting hypoglycemia responsive to diazoxide, whereas others seem to lower the glucose threshold further and may be more difficult to treat. Based on the greater experience at the Children's Hospital of Philadelphia, diazoxide is uniformly unable to normalize blood glucose, although most children do not have frequent severe episodes of hypoglycemia. Treatment with octreotide has been tried in some children with GK-HI, with poor effect in our experience. Although some cases have undergone pancreatectomy, most children have continued to have hypoglycemia and required additional medical management after surgery. In some cases, it is necessary to provide continuous support with overnight feedings or glucose to prevent hypoglycemia.

From the few cases that have undergone a pancreatectomy, pancreatic histology has been reported to have little or no abnormal features.[112-114] Others have reported nuclear enlargement and increased islet size and β-cell proliferation.[115,116]

Uncoupling Protein-2 Hyperinsulinism. Loss-of-function mutations in the mitochondrial uncoupling protein UCP2 have been reported to cause congenital hyperinsulinism. UCP2 is a negative regulator of insulin secretion[117] that regulates insulin secretion by decreasing the ATP/ADP ratio in β-cells or modulating the production of reactive oxygen species.[118] The cases of UCP2 hyperinsulinism described to date were dominant and responsive to diazoxide.[63]

Hepatocyte Nuclear Factors and Hyperinsulinism: HNF1Alpha and HNF4Alpha-Hyperinsulinism. Hepatocyte nuclear factors 1 alpha and 4 alpha are transcription factors expressed in hepatocytes, pancreatic β-cells, intestinal epithelial cells, and renal tubular cells. In β-cells and hepatocytes, they form a feed-forward loop, such that the haploinsufficiency of either causes decreased expression of the other.[119] Both HNF1α and HNF4α play an important role on pancreatic β-cell function and heterozygous mutations in the genes encoding them: *HNF1A* and *HNF4A*, respectively, are known to cause familial monogenic diabetes (MODY 3 and MODY1). These autosomal dominant forms of diabetes are characterized by progressive impairment in glucose-stimulated insulin secretion leading to frank hyperglycemia usually manifesting before the age of 25.[120]

It has been demonstrated that heterozygous mutations in the *HNF4A* gene not only cause reduced insulin secretion leading to diabetes in early adolescence and young adulthood, but also lead to increased insulin secretion during fetal, neonatal, and childhood periods.[64,121-123] The consequences of the increased insulin secretion range from fetal macrosomia to persistent hyperinsulinism. The hyperinsulinism in neonates with *HNF4A* mutations in some cases is transient, but in some cases it persists into childhood. The hypoglycemia is well controlled with diazoxide.[124] The phenotype in children with *HNF4A* mutations may extend beyond the pancreatic β-cells, and an association with renal Fanconi tubulopathy and hepatomegaly with increased glycogen stores have been reported.[65]

HNF4 alpha hyperinsulinism represents 5% of all cases of diazoxide-responsive hyperinsulinism.[123] The precise mechanism by which mutation in *HNF4A* leads to hyperinsulinism is currently unknown, but it is likely that the defects in HNF4 alpha alter different gene expression patterns during early and later in life.

This dual phenotype of hyperinsulinism early in life and diabetes later is not limited to mutations in *HNF4A*, because dominant mutations in *HNF1A* have also been reported to present in the neonatal period with hyperinsulinemic hypoglycemia.[65] The phenotype of HNF1A hyperinsulinism is similar to that described in HNF4A mutation carriers: evidence of insulin excess in utero and early onset hyperinsulinemic hypoglycemia that resolves with age.

Hypoglycemia Due to Activating Mutations in AKT2

Recessive gain-of-function mutations in *AKT2*, a key component of the insulin signaling cascade, cause a phenotype similar to hyperinsulinemic hypoglycemia but without detectable plasma insulin levels despite classical signs of increased insulin action (suppressed free fatty acids and ketones).[125] The phenotype in these children is characterized by severe, persistent hypoglycemia unresponsive to medical therapy and requiring continuous intragastric feedings. These children also had overgrowth with body asymmetry.

Acquired Postprandial Hyperinsulinemic Hypoglycemia after Fundoplication

The Nissen fundoplication and other modified fundoplication procedures, frequently performed for the treatment of gastroesophageal reflux in neonates and children, have been associated with late dumping syndrome, particularly postprandial hyperinsulinemic hypoglycemia. In a study in neonates it was estimated that approximately 24% of those undergoing fundoplication developed postprandial hypoglycemia after the surgery.[126] Classically, dumping syndrome is characterized by early symptoms or "early dumping," thought to be due to the fluid shifts provoked by the osmotic load in

the small bowel,[127] and "late dumping" or postprandial hypoglycemia.[128] In our experience, dumping syndrome in children is characterized by severe postprandial hypoglycemia without the significant gastrointestinal symptoms observed in adults.[129] Very frequently, children with postprandial hyperinsulinemic hypoglycemia after fundoplication experience seizures, lethargy, and other symptoms of hypoglycemia for several months before the diagnosis is made. In the neonate particularly, the hypoglycemia may not be recognized unless these infants are screened with postfeeding blood glucose monitoring after the surgery.[126]

The pathophysiology of hyperinsulinemic hypoglycemia after fundoplication is poorly understood. Studies of the motor and sensory function of the stomach after fundoplication have shown significantly reduced postprandial gastric relaxation and significantly accelerated gastric emptying.[130] The rapid emptying of a meal into the small intestine results in rapid absorption of glucose into the bloodstream, with early hyperglycemia followed by an exaggerated insulin surge and subsequent hypoglycemia.[129] Increased secretion of the potent insulinotropic hormone, glucagon-like peptide-1 (GLP-1), after a meal may be partly responsible for the postprandial hyperinsulinemia.[131]

The diagnosis of hyperinsulinemic hypoglycemia in neonates and children after fundoplication is established based on the glucose and insulin profile after a mixed meal tolerance test or an oral glucose tolerance test.[131] The typical response is characterized by hyperglycemia that can reach up to > 11.1 mmol/L (200 mg/dL) within the first hour after the meal, followed by hypoglycemia at 90 to 120 minutes. Plasma insulin reach a peak that is > 200 μU/mL approximately 30 minutes after the meal. A variety of therapies have been used with varying success to treat dumping syndrome, including uncooked cornstarch,[132] pectin,[133] octreotide,[134] acarbose,[129] and dietary manipulations.[135] Many of the patients suffering from postprandial hyperinsulinemic hypoglycemia continue to have severe symptoms despite these interventions and require a regimen of continuous enteral feedings to avoid hypoglycemia but continue to be at high risk of hypoglycemic events if feedings are abruptly stopped. In most cases, the postprandial hyperinsulinemic hypoglycemia improves with time, especially after solids have been introduced to the diet.

Defects in Counter-Regulatory Response

Hypoglycemia associated with endocrine deficiency is usually caused by glucocorticoid or growth hormone deficiency, more commonly the combination of both. In children with panhypopituitarism, isolated growth hormone deficiency, or a combination of ACTH deficiency and growth hormone deficiency, the incidence of hypoglycemia is as high as 20%. In the newborn period, hypoglycemia may be the presenting feature of hypopituitarism. In males, a microphallus may provide a clue to a coexistent deficiency of gonadotropin[136,137] (Figure 6-12). Newborns with hypopituitarism may also have liver dysfunction resembling cholestatic liver disease, and some have midline malformations such as the syndrome of septo-optic dysplasia.[138]

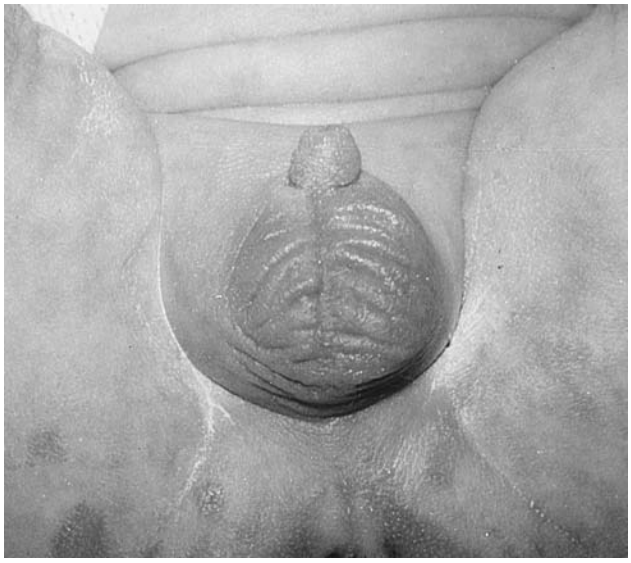


FIGURE 6-12 ■ Micropenis and undescended testes in an infant with congenital hypopituitarism. The infant was hypoglycemic at 12 hours of age (glucose, 24 mg/dL). At 72 hours of age, he was jaundiced—and a liver biopsy demonstrated neonatal hepatitis. His endocrine evaluation was positive for hypothyroidism, low cortisol level, undetectable growth hormone level, and an elevated prolactin level (confirming hypothalamic hypopituitarism).

Although older infants and children with pituitary deficiency present with ketotic hypoglycemia, in the neonatal period the hypoglycemia may mimic hyperinsulinism. However, their hypoglycemia is not responsive to diazoxide and only remits with replacement of deficient hormones (including thyroxine, as well as growth hormone and cortisol). When adrenal disease is severe, as in congenital adrenal hyperplasia, adrenal hemorrhage, or congenital absence or hypoplasia of the adrenals,[139,140] disturbances in serum electrolytes with hyponatremia and hyperkalemia or ambiguous genitalia may provide diagnostic clues.

Abnormalities of the ACTH receptor or adrenal hypoplasia may be phenotypically difficult to distinguish from cortisol deficiency of other causes, with the exception of the marked elevations of serum ACTH concentration noted if the ACTH receptor or adrenal gland is malfunctioning.[141] However, all states with ACTH elevations may be clinically suspected by virtue of the associated hyperpigmentation (see Chapter 13). These cases of isolated adrenal insufficiency very rarely cause hypoglycemia in neonates, whereas in older infants and children, treated congenital adrenal hyperplasia and isolated ACTH deficiency may cause profound stress-induced hypoglycemia. Congenital ACTH deficiency due to mutation in *TPIT*, which encodes TBX19, a transcription factor that is required for the expression of the proopiomelanocortin (POMC) gene and for terminal differentiation of the pituitary corticotroph lineage, is the exception, presenting in the neonatal period with severe and persistent hypoglycemia until hormonal replacement is established.[142] It is important to have in mind that a low growth hormone and cortisol level at the time of hypoglycemia is not

diagnostic of a true deficiency[143] and provocative testing may be needed to establish the diagnosis.

The mechanism of hypoglycemia in growth hormone deficiency may be the result of decreased lipolysis. The mechanism of hypoglycemia with cortisol deficiency may be reduced liver glycogen reserves plus diminished gluconeogenesis, owing to a failure to supply endogenous gluconeogenic substrate in the form of amino acids from muscle proteolysis. Investigation of a child with hypoglycemia therefore requires exclusion of ACTH, cortisol, or growth hormone deficiency—and if diagnosed appropriate replacement with cortisol or growth hormone.

Although glucagon deficiency[144,145] had been described, the authors have retracted their conclusions and now believe these patients had SCHAD hyperinsulinism; thus, this disorder is exceedingly rare and may be nonexistent. Epinephrine deficiency is also rare but must be a consideration in familial dysautonomia or in children treated with beta-blockers.

Defects in Glycogenolysis and Gluconeogenesis

Most of the disorders of glycogenolysis and gluconeogenesis that result in hypoglycemia are usually diagnosed in childhood and will be discussed in Chapter 6. The exception is glucose 6-phosphatase deficiency, which impairs the release of glucose from glycogenolysis and gluconeogenesis and presents in the neonatal period, although in some cases it is diagnosed later.

Glucose 6-Phosphatase Deficiency (GSD Type 1)

Glycogen storage disease type 1 is an autosomal recessive disorder caused by defects in the glucose 6-phosphatase (G6Pase) complex, which catalyzes the terminal steps of both hepatic gluconeogenesis and glycogenolysis, the hydrolysis of glucose 6-phosphate to glucose and inorganic phosphate. Deficiency of G6Pase activity in liver, kidney, and intestine results in the accumulation of glycogen in these organs, fasting hypoglycemia as a result of inadequate glucose production, and other secondary biochemical abnormalities including hyperlactacidemia, hyperuricemia, and hyperlipidemia.

Glucose 6-phosphatase is a multicomponent complex consisting of a catalytic unit, G6Pase, localized on the luminal side of the endoplasmic reticulum, and a G6P-specific bidirectional translocase (G6PT) that allow the entry of glucose 6-phosphate to the catalytic unit.[146] The gene for the catalytic unit has been cloned and located on chromosome 17,[147] whereas the gene for the glucose 6-phosphate translocase is located on chromosome 11.[148] Mutations in the catalytic unit cause GSD type 1a,[149] whereas mutations in G6PT cause GSD type 1b.[148] The phenotype for these two subtypes is identical, except that in type 1b, in addition to the liver phenotype, there are recurrent bacterial infections and inflammatory bowel disease associated with neutropenia and neutrophil dysfunction.[150] Although two additional types have been reported (GSD type 1c and GSD type 1d), there is insufficient available evidence to support the existence of these defects.

The estimated incidence of GSD type 1 is 1 in 100,000 live births, with GSD type 1a representing approximately 80% of cases. Clinically, GSD type 1 may present in the neonatal period with severe hypoglycemia occurring 2 to 2½ hours after a meal and tachypnea secondary to respiratory compensation for the metabolic acidemia. However, because lactate and ketones may provide adequate brain substrate to protect central nervous system function (and because in early infancy regular feedings are consistently provided) the diagnosis may be delayed for months until massive hepatomegaly brings the infant to medical attention, although the hepatomegaly may be missed because the liver is soft. After infancy, affected patients may be seen walking with a waddling gait secondary to their prominent abdomen and muscle weakness. Other consistent features are hyperuricemia, hypophosphatemia, a bleeding diathesis secondary to impairment of platelet adhesiveness, and growth retardation.

Hypoglycemia may occur anytime these children are exposed to even brief periods of fasting. They are completely dependent on the provision of glucose from exogenous sources, with the exception of the small amount of free glucose—which is released as part of the process of debranching glycogen. Because less than 10% of glycogen consists of branch points, this mechanism provides little protection against hypoglycemia during fasting. Hypoglycemia in the setting of suppressed insulin and increased glucagon promotes glycogenolysis, but the absence of G6Pase commits the glucose-1-phosphate produced by phosphorylase to glycolytic catabolism resulting in increased lactate production (see Figure 6-1). Another consequence of the impaired activity of G6Pase is the shunting of 6P through the pentose phosphate pathway, to yield ribose-6-phosphate, which ultimately yields uric acid, resulting in hyperuricemia. Hypertriglyceridemia results from increased triglyceride formation as a major route of disposal of pyruvate from lactate and amino acids when glucose yield is blocked in G6Pase deficiency.[151] Massive accumulation of fat in the liver is responsible for the massive hepatomegaly characteristic of GSD type 1.

Renal disease is a frequent complication of GSD type 1 (with an estimated prevalence of 30%).[152] Manifestations include proximal renal tubular dysfunction (Fanconi-like syndrome), distal tubular acidification defect, and hypercalciuria. There is an inverse relationship between age and citrate excretion, and the combination of low citrate excretion and hypercalciuria predispose these children to nephrocalcinosis and nephrolithiasis.[153] Citrate supplementation may prevent or ameliorate these complications.[154] The widespread prevalence and serious prognosis of kidney involvement is manifested by severe glomerular hyperfiltration and microalbuminuria over time, systemic arterial hypertension, and consequently renal failure.[155-157] The early implementation of treatment with angiotensin-converting enzyme inhibitors has been shown to delay the progression of renal damage.[152] The pathologic findings include focal segmental glomerulosclerosis with interstitial fibrosis. The etiology of the renal involvement is unclear, but it correlates negatively with metabolic control. It has been proposed that the dyslipidemia contributes to the kidney injury.[158]

In addition to the characteristic hepatomegaly, the liver undergoes adenomatous changes. Adenomas are usually first observed in the second and third decades of life but may appear before puberty.[157,159] The adenomas may undergo malignant degeneration or hemorrhage and are frequently associated with chronic iron resistant anemia.[160] Other complications of GSD 1 include osteopenia and growth retardation.

The diagnosis of GSD type 1 is based on the clinical and biochemical characteristics: hypoglycemia after a short period of fasting, hepatomegaly, lactic academia, and elevation of uric acid and triglycerides. Glucagon stimulation 2 to 4 hours after a meal containing carbohydrates results in increased lactate concentration, but plasma glucose does not rise.[160] The diagnosis can be confirmed by mutation analysis.

The goal of treatment of children with glucose 6-phosphate deficiency is to completely eliminate hypoglycemia and suppress secondary metabolic decompensation. Continuous nasogastric or intragastric feedings during the night have been demonstrated to either reduce or eliminate the metabolic and clinical findings through complete avoidance of hypoglycemia.[161] However, this approach places children at risk of severe hypoglycemia if feedings are abruptly stopped. A safer approach is the introduction of oral uncooked cornstarch, which helps prolonged the fasting tolerance[162,163] and can be used in older infants, usually older than 6 months of age. The doses of cornstarch used are approximately 1.6 g/kg per dose every 4 hours in infants and 1.7 to 2.5 g/kg per dose every 6 hours in older patients. A typical regimen consists of daytime feedings every 3 to 4 hours that are calculated to provide adequate carbohydrate calories to avoid the need for hepatic glucose output.[160] Most of these calories consist of carbohydrates, primarily providing pure glucose as an energy source and avoiding disaccharides containing fructose or galactose. At night, the regimen consists of an intragastric infusion of glucose with or without protein designed to infuse at rates of about 125% calculated hepatic glucose output[151] for normal young infants. For older children, a regimen of uncooked cornstarch can be implemented at nighttime.

Meticulous dietary control of blood glucose levels can lead to a significant clinical and metabolic improvement and prevention of complications. Adjunctive therapies should include careful monitoring of the uric acid level and treatment with allopurinol if the uric acid level is elevated. With increasing awareness of the renal tubular dysfunction, treatment of the hyperfiltration state with an angiotensin-converting enzyme inhibitor should be initiated promptly. Treatment with granulocyte-macrophage colony-stimulating factor to augment neutrophil production has been shown to ameliorate mouth ulcers and the enteritis in type 1b.[164]

Disorders of Fatty Acid Oxidation: Medium-Chain Acyl-Coenzyme A Dehydrogenase Deficiency (MCAD)

The inborn errors of metabolism associated with deficiencies of fatty acid oxidation (Figure 6-13) are inherited in an autosomal-recessive fashion. All of these disorders may be provoked by fasting and exhibit life-threatening events characterized by varying degrees of hypoglycemia associated with a relative deficiency in the generation of ketone bodies.[165] Disorders of fatty acid oxidation may be divided according to the site of the defect: defects of fatty acid and carnitine transport, β-oxidation defects, electron transport chain defects, and defects of ketone body synthesis and utilization. The presentation is usually beyond the neonatal period, but because of the severe consequences of the manifestations, newborn screening for the most common defect, MCAD deficiency, is now offered in all U.S. states and many other countries. In the United States, the screening also detects cases of long-chain L-3-hydroxyacyl-CoA dehydrogenase (LCHAD), carnitine transport defect, and very long-chain acyl-CoA dehydrogenase (VLCAD). We review the diagnosis, clinical characteristics, and treatment of MCAD deficiency in this chapter; other fatty acid oxidation defects are reviewed in Chapter 6.

MCAD deficiency is the most frequent defect on fatty acid oxidation. Neonatal screening in Pennsylvania has shown an incidence of 1 in 15,700 newborns.[166] MCAD (encoded by *ACADM*) is responsible for the initial dehydrogenation of acyl-CoAs with a chain length between 4 and 12 carbon atoms. Thus, defects in MCAD enzyme activity leads to accumulation of medium-chain fatty acids (between 6 and 10 carbons) that can be detected by mass spectrometry. The most prominent metabolite in MCAD deficiency is octanoylcarnitine (C8). Other metabolites presenting as abnormally elevated in MCAD deficiency include hexanoylcarnitine (C6), decanoylcarnitine (C10), and decenoylcarnitine (C10:1).

Children with MCAD deficiency may appear normal at birth, although symptoms may manifest before results from newborn screening are available.[167] The clinical presentation (if not detected by newborn screening) occurs between 3 and 24 months of life precipitated in response to prolonged fasting (at time of weaning from nighttime feedings) or during an intercurrent illnesses. The resulting metabolic stress may rapidly progress to coma and death. In children in which the diagnosis has not been previously established, the rate of death with the first episode is as high as 18%.[168]

Although there is significant heterogeneity in the presentation of MCAD, the most frequent clinical presentation is one of intermittent hypoketotic hypoglycemia with little or no acidemia, elevation of serum urea, ammonia, and uric acid, liver function abnormalities, and hepatic steatosis. The risk of severe complications and death is very high unless appropriate treatment to reverse the catabolic state is implemented. The diagnosis of these cases may be confused with Reye syndrome. Although hypoglycemia may be a prominent late feature with MCAD, the phenotype of a defect of a fatty acid oxidation disorder may not manifest if a fasting state is avoided. A high index of suspicion for fatty acid oxidation defects is important because appropriate therapy may result in an interruption and prevention of these potentially life-threatening episodes. Affected patients have also been misdiagnosed with idiopathic sudden infant death syndrome.[169] Decreased plasma carnitine levels and

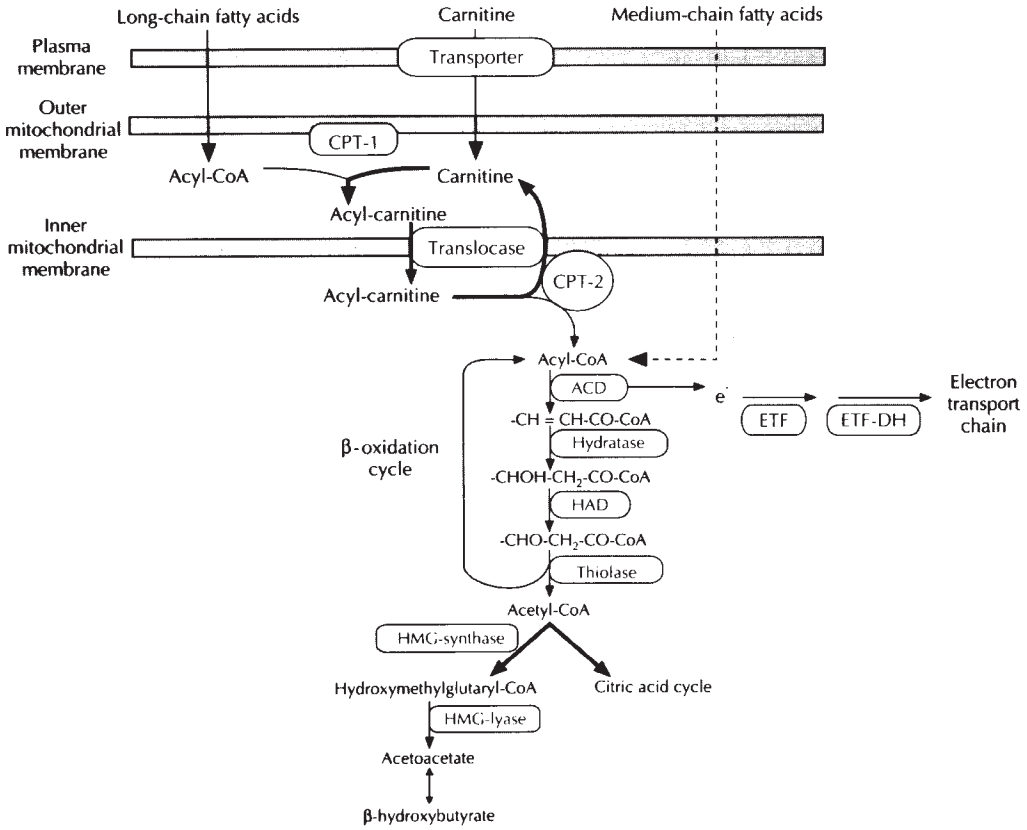


FIGURE 6-13 ■ The pathways of mitochondrial fatty acid oxidation and ketone body synthesis. ACD, acyl-CoA dehydrogenase; CPT-1 and CPT-2, carnitine palmitoyltransferase I and II; ETF 5, electron-transferring flavoprotein; ETF-DH, electron-transferring flavoprotein dehydrogenase; HAD, hydroxyacyl-CoA dehydrogenase. (From Stanley CA, Hale DE [1994]. Genetic disorders of mitochondrial fatty acid oxidation. Curr Opin Pediatr 6:476.)

an increase in the ratio of esterified to free carnitine is a frequently associated laboratory finding.

Evaluation of suspected errors in fatty acid oxidation should first include a determination of the profile of plasma acylcarnitines by mass spectrometry and measurement of plasma total, esterified, and free carnitine. Most, but not all, of the fatty acid oxidation disorders are associated with specific abnormalities of plasma acylcarnitines—octanoylcarnitine in cases with MCAD deficiency (Table 6-5). Determinations of urinary organic acids with assessment of the presence or absence of dicarboxylic aciduria are also very useful. Patients whose disorder cannot be identified by these tests may require further evaluations, including assays of fatty acid oxidation and specific enzyme assays in cultured skin fibroblasts or lymphoblasts. Since the early 1990s, the use of tandem mass espectometry has made newborn screening possible for most fatty acid oxidation disorders based on the acylcarnitine profile in blood spots. Presymptomatic identification of these individuals can prevent catastrophic events such as sudden death. Direct DNA mutational analysis can be performed for many of these defects, which is particularly useful in MCAD in which most cases are due to a single common mutations (Lys304Gly).[170]

The primary treatment of disorders of fatty acid oxidation is a devoted avoidance of fasting. For infants younger than 1 year old, 6 to 8 hours of fasting may be sufficient to precipitate an episode. On the other hand, as children become older they appear to be able to withstand periods of fasting of as long as 10 to 12 hours without decompensation. A high index of suspicion and rapid institution of intravenous glucose will often reverse an evolving episode. The presence of hypoglycemia is usually an event that occurs late in the evolution of an episode of metabolic decompensation. High-fat diets should be avoided, although normal amounts of dietary fats do not appear to be toxic. An adjunct approach may involve the use of cornstarch (as used for the treatment of type 1 glycogen storage disease) in doses of 1.5 to 2 g/kg.[171]

Defects of Glucose Transporters

GLUT1 Deficiency

Glucose transporter type 1 (GLUT1) deficiency syndrome is an autosomal dominant inborn error of glucose transport characterized by seizures, developmental delay, spasticity, acquired microcephaly, and ataxia. The biochemical hallmark is the finding of hypoglycorrhachia (low cerebrospinal fluid glucose concentration) despite normal plasma glucose concentrations.

GLUT1, encoded by *SLC2A1*, is the fundamental vehicle that facilitates glucose entry into the brain. Diagnosis of GLUT1 deficiency is based on the finding of low

TABLE 6-5 **Fatty Acid Oxidation Disorders with Distinguishing Metabolic Markers**

Disorder	Plasma Acylcarnitines	Urinary Acylglycines	Urinary Organic Acids
VLCAD	Tetradecenoyl-		
MCAD	Octanoyl	Hexanoyl-Suberyl-Phenylpropionyl-	
SCAD	Butyryl-	Butyryl-	Ethylmalonic
LCHAD	3-Hydroxy-palmitoyl-3-Hydroxy-oleoyl-3-Hydroxy-linoleoyl-		3-Hydroxydicarboxylic
DER	Dodecadienoyl-		
ETF and ETF-DH	Butyryl-Isovaleryl-Glutaryl-	Isovaleryl-	Ethylmalonic
		Hexanoyl-	Glutaric
			Isovaleric
HMG-CoA lyase	Methylglutaryl-		3-Hydroxy-3-methyl-glutaric

DER, 2,4-dienoyl-coenzyme A reductase; ETF, electron-transferring flavoprotein; ETF-DH, ETF dehydrogenase; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; LCHAD, long-chain 3-hydroxyacyl-coenzyme A dehydrogenase; MCAD, medium-chain acyl-coenzyme A dehydrogenase; SCAD, short-chain acyl-coenzyme A dehydrogenase; VLCAD, very-long-chain acyl-coenzyme A dehydrogenase.
From Stanley CA (1990). Disorders of fatty acid oxidation. In Fernandes J, Bremer E, Saudubray J-M (eds.), Inborn metabolic diseases: diagnosis and treatment. *New York: Springer-Verlag, 394-410.*

cerebrospinal fluid glucose, in the absence of hypoglycemia, and identification of *SLC2A1* mutation (located on chromosome 1). The classic phenotype is a severe form of early onset epileptic encephalopathy in approximately 90% of cases (classic form). A nonepileptic form represents 10% of cases, which includes a broad phenotypic spectrum that may present with paroxysmal nonepileptic manifestations that have been reported to include intermittent ataxia, choreoathetosis, dystonia, and alternating hemiplegia.[172]

Treatment efforts have been based on providing alternative brain fuel sources by a ketogenic diet.[11,173] The ketogenic diet effectively controls the seizures and other paroxysmal activities, but it is has less effect on the cognitive function.

GLUT2 Deficiency

Originally described by Fanconi and Bickel as a syndrome characterized by hypoglycemia and ketonuria in the fasting state and hyperglycemia in the postabsorptive state,[174] Fanconi-Bickel syndrome is due to recessive mutations of the GLUT2 plasma membrane transporter for glucose (encoded by *SLC2A2*).[175] The defect on GLUT2 results in hepatorenal glycogen accumulation, proximal renal tubular dysfunction, and impaired utilization of glucose and galactose.[176] GLUT2 is expressed in hepatocyte, pancreatic β-cells, and the basolateral membranes of intestinal and renal tubular epithelial cells.[177] The clinical manifestations reflect impairment of glucose release from liver and of glucose reabsorption from renal tubular cells. Galactose clearance and conversion to glucose is delayed.

Patients with GLUT2 deficiency usually present at an age of 3 to 10 months. The typical clinical signs are hepatomegaly due to glycogen accumulation, a severe Fanconi-type renal tubulopathy with disproportionately severe glucosuria, glucose and galactose intolerance, hypophosphatemic rickets, and severely stunted growth.[178] These patients may present with a combination of fasting hypoglycemia and postprandial hyperglycemia. The hypoglycemia during fasting is explained by altered glucose transport out of the liver, resulting in an increased intracellular glucose concentration that may inhibit glycogen degradation, leading to glycogen storage and hepatomegaly. The hypoglycemia is exacerbated by renal loss of glucose due to a transport defect for glucose and galactose across the basolateral membranes of the tubular cells. Hyperglycemia (and hypergalactosemia) in the fed state is explained by decreased monosaccharide uptake by the liver and enhanced by an inappropriately low insulin secretion due to impaired glucose-sensing by the pancreatic β-cells.[175]

The therapeutic goal for patients with GLUT2 deficiency is to ameliorate the consequences of renal tubulopathy by replacing water, electrolytes, and alkali—and by providing phosphate and vitamin D supplementation. In terms of diet, an adequate caloric intake is recommended to compensate for renal and intestinal glucose loss—given as frequent feedings containing slowly absorbed carbohydrates (such as cornstarch) to avoid fasting hypoglycemia.

TREATMENT

A rational therapeutic approach to the treatment of hypoglycemia relies on a systematic diagnostic evaluation. The key to effective treatment is diagnosis specific. The maintenance of euglycemia is critical to the preservation of central nervous system function. Even in the presence of an uncertain diagnosis, every effort must be used to maintain euglycemia until a diagnosis is made or hypoglycemia resolves. Intravenous glucose infusion remains the mainstay of emergency therapy, particularly for the child with severe hypoglycemia. It is important to follow

an initial glucose bolus with a continuous glucose infusion to prevent further episodes of hypoglycemia until specific therapies are established.

The therapeutic goal is to maintain plasma glucose > 3.3 mmol/L (70 mg/dL). Ideally, treatment should maintain normoglycemia on a normal feeding schedule for the patient's age. It is advisable to periodically reassess the efficacy of the treatment for any form of hypoglycemia by a formal fasting study on treatment.

CONCLUSIONS

Since the early reports of neonatal hypoglycemia in the 1950s by McQuarrie[55] and Cornblath and colleagues,[179] our understanding of the pathogeneses and treatment of hypoglycemia in neonates and infants has progressed significantly. The ongoing identification of new molecular causes of congenital hyperinsulinism represents significant new discoveries. The development of noninvasive techniques (such as the ^{18}F-fluoro-L-DOPA PET scan) that can accurately identify patients with focal hyperinsulinism and the potential for cure with surgical removal of the lesion are promising advances in the treatment of these children. These and future discoveries should result in continued improvement in outcome for hypoglycemic patients.

REFERENCES

1. Cryer, P. E. (2003). Glucose homeostasis and hypoglycemia. In H. L. P. Kronenberg, S. Melmed, K. Polonsky (Eds.), *Williams textbook of endocrinology* (11th ed.). Philadelphia: Elsevier.
2. Nehlig, A. (1997). Cerebral energy metabolism, glucose transport and blood flow: changes with maturation and adaptation to hypoglycaemia. Diabetes Metab, *23*, 18–29.
3. Devaskar, S. U., & Mueckler, M. M. (1992). The mammalian glucose transporters. *Pediatr Res*, *31*, 1–13.
4. Scheepers, A., Joost, H. G., & Schurmann, A. (2004). The glucose transporter families SGLT and GLUT: molecular basis of normal and aberrant function. *JPEN J Parenter Enteral Nutr*, *28*, 364–371.
5. Kahn, B. B. (1992). Facilitative glucose transporters: regulatory mechanisms and dysregulation in diabetes. *J Clin Invest*, *89*, 1367–1374.
6. Pardridge, W. M., Boado, R. J., & Farrell, C. R. (1990). Brain-type glucose transporter (GLUT-1) is selectively localized to the blood-brain barrier: studies with quantitative western blotting and in situ hybridization. *J Biol Chem*, *265*, 18035–18040.
7. Kang, L., Routh, V. H., Kuzhikandathil, E. V., et al. (2004). Physiological and molecular characteristics of rat hypothalamic ventromedial nucleus glucosensing neurons. *Diabetes*, *53*, 549–859.
8. James, D. E., Strube, M., & Mueckler, M. (1989). Molecular cloning and characterization of an insulin-regulatable glucose transporter. *Nature*, *338*, 83–87.
9. Birnbaum, M. J. (1989). Identification of a novel gene encoding an insulin-responsive glucose transporter protein. *Cell*, *57*, 305–315.
10. Settergren, G., Lindblad, B. S., & Persson, B. (1980). Cerebral blood flow and exchange of oxygen, glucose ketone bodies, lactate, pyruvate and amino acids in anesthetized children. *Acta Paediatr Scand*, *69*, 457–465.
11. De Vivo, D. C., Trifiletti, R. R., Jacobson, R. I., et al. (1991). Defective glucose transport across the blood-brain barrier as a cause of persistent hypoglycorrhachia, seizures, and developmental delay. *N Engl J Med*, *325*, 703–709.
12. Fishman, R. A. (1991). The glucose-transporter protein and glucopenic brain injury. *N Engl J Med*, *325*, 731–732.
13. Gruetter, R., Ugurbil, K., & Seaquist, E. R. (1998). Steady-state cerebral glucose concentrations and transport in the human brain. *J Neurochem*, *70*, 397–408.
14. Choi, I. Y., Lee, S. P., Kim, S. G., & Gruetter, R. (2001). In vivo measurements of brain glucose transport using the reversible Michaelis-Menten model and simultaneous measurements of cerebral blood flow changes during hypoglycemia. *J Cereb Blood Flow Metab*, *21*, 653–663.
15. Gerich, J. E. (1998). Lilly lecture 1988. Glucose counterregulation and its impact on diabetes mellitus. *Diabetes*, *37*, 1608–1617.
16. Cryer, P. E., & Gerich, J. E. (1985). Glucose counterregulation, hypoglycemia, and intensive insulin therapy in diabetes mellitus. *N Engl J Med*, *313*, 232–241.
17. Lubchenco, L. O., & Bard, H. (1971). Incidence of hypoglycemia in newborn infants classified by birth weight and gestational age. *Pediatrics*, *47*, 831–838.
18. Greenham, R. (1989). Brain damage by neonatal hypoglycaemia. *Lancet*, *2*, 446–447.
19. Lucas, A., Morley, R., & Cole, T. J. (1988). Adverse neurodevelopmental outcome of moderate neonatal hypoglycaemia. *BMJ*, *297*, 1304–1308.
20. Morriss, F. H., Jr., Makowski, E. L., Meschia, G., & Battaglia, F. C. (1974). The glucose/oxygen quotient of the term human fetus. *Biol Neonate*, *25*, 44–52.
21. Marconi, A. M., Cetin, I., Davoli, E., et al (1993). An evaluation of fetal glucogenesis in intrauterine growth-retarded pregnancies. *Metabolism*, *42*, 860–864.
22. Bloch, C. A., Sperling, M. A. (1988). Sources and disposition of fetal glucose: studies in the fetal lamb. *Am J Perinatol*, *5*, 344–352.
23. Townsend, S. F., Rudolph, C. D., & Rudolph, A. M. (1991). Cortisol induces perinatal hepatic gluconeogenesis in the lamb. *J Dev Physiol*, *16*, 71–79.
24. Kalhan, S. C., Parimi, P., Van Beek, R., et al (2001). Estimation of gluconeogenesis in newborn infants. *Am J Physiol Endocrinol Metab*, *281*, E991–E997.
25. Fukumoto, H., Seino, S., Imura, H., Seino, Y., & Bell, G. I. (1988). Characterization and expression of human HepG2/erythrocyte glucose-transporter gene. *Diabetes*, *37*, 657–661.
26. Gao, L., Lv, C., Xu, C., et al (2012). Differential regulation of glucose transporters mediated by CRH receptor type 1 and type 2 in human placental trophoblasts. *Endocrinology*, *153*, 1464–1471.
27. Bier, D. M., Leake, R. D., Haymond, M. W., et al (1977). Measurement of "true" glucose production rates in infancy and childhood with 6,6-dideuteroglucose. *Diabetes*, *26*, 1016–1023.
28. Marconi, A. M., Paolini, C., Buscaglia, M., et al. (1996). The impact of gestational age and fetal growth on the maternal-fetal glucose concentration difference. *Obstet Gynecol*, *87*, 937–942.
29. Whaley, W. H., Zuspan, F. P., & Nelson, G. H. (1966). Correlation between maternal and fetal plasma levels of glucose and free fatty acids. *Am J Obstet Gynecol*, *94*, 419–421.
30. Hauguel, S., Desmaizieres, V., & Challier, J. C. (1986). Glucose uptake, utilization, and transfer by the human placenta as functions of maternal glucose concentration. *Pediatr Res*, *20*, 269–273.
31. Menon, R. K., Cohen, R. M., Sperling, M. A., et al. (1990). Transplacental passage of insulin in pregnant women with insulin-dependent diabetes mellitus: its role in fetal macrosomia. *N Engl J Med*, *323*, 309–315.
32. Sperling, M. A., Ganguli, S., Leslie, N., & Landt, K. (1984). Fetal-perinatal catecholamine secretion: role in perinatal glucose homeostasis. *Am J Physiol*, *247*, E69–E74.
33. Sperling, M. A., DeLamater, P. V., Phelps, D., et al. (1974). Spontaneous and amino acid-stimulated glucagon secretion in the immediate postnatal period: relation to glucose and insulin. *J Clin Invest*, *53*, 1159–1166.
34. Granner, D., Andreone, T., Sasaki, K., & Beale, E. (1983). Inhibition of transcription of the phosphoenolpyruvate carboxykinase gene by insulin. *Nature*, *305*, 549–551.
35. Stanley, C. A., Anday, E. K., Baker, L., & Delivoria-Papadopolous, M. (1979). Metabolic fuel and hormone responses to fasting in newborn infants. *Pediatrics*, *64*, 613–619.
36. Bougneres, P. F., Lemmel, C., Ferre, P., & Bier, D. M. (1986). Ketone body transport in the human neonate and infant. *J Clin Invest*, *77*, 42–48.
37. Patel, D., & Kalhan, S. (1992). Glycerol metabolism and triglyceride-fatty acid cycling in the human newborn: effect of maternal diabetes and intrauterine growth retardation. *Pediatr Res*, *31*, 52–58.

38. Alkalay, A. L., Sarnat, H. B., Flores-Sarnat, L., et al. (2006). Population meta-analysis of low plasma glucose thresholds in full-term normal newborns. *Am J Perinatol*, *23*, 115–119.
39. Hawdon, J. M., Weddell, A., Aynsley-Green, A., & Ward Platt, M. P. (1993). Hormonal and metabolic response to hypoglycaemia in small for gestational age infants: *Arch Dis Child*, *68*, 269–273.
40. Fetus and Newborn Committee, Screening guidelines for newborns at risk for low blood glucose. (2004). *Paediatr Child Health*, *9*, 723–740.
41. Adamkin, D. H. (2011). Postnatal glucose homeostasis in late-preterm and term infants. *Pediatrics*, *127*, 575–579.
42. Chaussain, J. L., Georges, P., Olive, G., & Job, J. C. (1974). Glycemic response to 24-hour fast in normal children and children with ketotic hypoglycemia: II. Hormonal and metabolic changes. *J Pediatr*, *85*, 776–781.
43. McGarry, J. D. (1979). Lilly Lecture 1978. New perspectives in the regulation of ketogenesis. *Diabetes*, *28*, 517–523.
44. Hirsch, H. J., Loo, S. W., & Gabbay, K. H. (1977). The development and regulation of the endocrine pancreas. *J Pediatr*, *91*, 518–520.
45. De Leon, D. D., & Stanley, C. A. (2007). Mechanisms of disease: advances in diagnosis and treatment of hyperinsulinism in neonates. *Nat Clin Pract Endocrinol Metab*, *3*, 57–68.
46. Blackman, J. D., Towle, V. L., Lewis, G. F., et al. (1990). Hypoglycemic thresholds for cognitive dysfunction in humans. *Diabetes*, *39*, 828–835.
47. Farquhar, J. W. (1959). The child of the diabetic woman. *Arch Dis Child*, *34*, 76–96.
48. Collins, J. E., & Leonard, J. V. (1984). Hyperinsulinism in asphyxiated and small-for-dates infants with hypoglycaemia. *Lancet*, *2*, 311–313.
49. Collins, J. E., Leonard, J. V., Teale, D., et al. (1990). Hyperinsulinaemic hypoglycaemia in small for dates babies. *Arch Dis Child*, *65*, 1118–1120.
50. Hoe, F. M., Thornton, P. S., Wanner, L. A., et al. (2006). Clinical features and insulin regulation in infants with a syndrome of prolonged neonatal hyperinsulinism. *J Pediatr*, *148*, 207–212.
51. Choufani, S., Shuman, C., & Weksberg, R. (2010). Beckwith-Wiedemann syndrome. *Am J Med Genet C Semin Med Genet*, *154C*, 343–354.
52. DeBaun, M. R., King, A. A., & White, N. (2000). Hypoglycemia in Beckwith-Wiedemann syndrome. *Semin Perinatol*, *24*, 164–171.
53. Hussain, K., Cosgrove, K. E., Shepherd, R. M., et al. (2005). Hyperinsulinemic hypoglycemia in Beckwith-Wiedemann syndrome due to defects in the function of pancreatic beta-cell adenosine triphosphate-sensitive potassium channels. *J Clin Endocrinol Metab*, *90*, 4376–4382.
54. Stefan, Y., Bordi, C., Grasso, S., & Orci, L. (1985). Beckwith-Wiedemann syndrome: a quantitative, immunohistochemical study of pancreatic islet cell populations. *Diabetologia*, *28*, 914–919.
55. McQuarry I (1954). Idiopathic spontaneously occurring hypoglycemia in infants; clinical significance of problem and treatment. *AMA Am J Dis Child*, *87*, 399–428.
56. McCulloch, L. J., van de Bunt, M., Braun, M., et al. (2011). GLUT2 (SLC2A2) is not the principal glucose transporter in human pancreatic β-cells: implications for understanding genetic association signals at this locus. *Mol Genet Metab*, *104*, 648–653.
57. Thomas, P. M., Cote, G. J., Wohllk, N., et al. (1995). Mutations in the sulfonylurea receptor gene in familial persistent hyperinsulinemic hypoglycemia of infancy. *Science*, *268*, 426–429.
58. Thomas, P., Ye, Y., & Lightner, E. (1996). Mutation of the pancreatic islet inward rectifier Kir6.2 also leads to familial persistent hyperinsulinemic hypoglycemia of infancy. *Hum Mol Genet*, *5*, 1809–1012.
59. Glaser, B., Kesavan, P., Heyman, M., et al. (1998). Familial hyperinsulinism caused by an activating glucokinase mutation. *N Engl J Med*, *338*, 226–230.
60. Stanley, C. A., Lieu, Y. K., Hsu, B. Y., et al. (1998). Hyperinsulinism and hyperammonemia in infants with regulatory mutations of the glutamate dehydrogenase gene. *N Engl J Med*, *338*, 1352–1357.
61. Clayton, P. T., Eaton, S., Aynsley-Green, A., et al. (2001). Hyperinsulinism in short-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency reveals the importance of beta-oxidation in insulin secretion. *J Clin Invest*, *108*, 457–465.
62. Otonkoski, T., Jiao, H., Kaminen-Ahola, N., et al. (2007). Physical exercise-induced hypoglycemia caused by failed silencing of monocarboxylate transporter 1 in pancreatic β-cells. *Am J Hum Genet*, *81*, 467–474.
63. Gonzalez-Barroso, M. M., Giurgea, I., Bouillaud, F., et al. (2008). Mutations in UCP2 in congenital hyperinsulinism reveal a role for regulation of insulin secretion. *PLoS One*, *3*, e3850.
64. Pearson, E. R., Boj, S. F., Steele, A. M., et al. (2007). Macrosomia and hyperinsulinaemic hypoglycaemia in patients with heterozygous mutations in the HNF4A gene. *PLoS Med*, *4*, e118.
65. Stanescu, D. E., Hughes, N., Kaplan, B., et al. (2012). Novel presentations of congenital hyperinsulinism due to mutations in the MODY genes: HNF1A and HNF4A. *J Clin Endocrinol Metab*, *97*, E2026–E2030.
66. Steinkrauss, L., Lipman, T. H., Hendell, C. D., et al. (2005). Effects of hypoglycemia on developmental outcome in children with congenital hyperinsulinism. *J Pediatr Nurs*, *20*, 109–118.
67. Mazor-Aronovitch, K., Gillis, D., Lobel, D., et al. (2007). Long-term neurodevelopmental outcome in conservatively treated congenital hyperinsulinism. *Eur J Endocrinol*, *157*, 491–497.
68. Meissner, T., Wendel, U., Burgard, P., et al. (2003). Long-term follow-up of 114 patients with congenital hyperinsulinism. *Eur J Endocrinol*, *149*, 43–51.
69. Menni, F., de Lonlay, P., Sevin, C., et al. (2001). Neurologic outcomes of 90 neonates and infants with persistent hyperinsulinemic hypoglycemia. *Pediatrics*, *107*, 476–479.
70. Dayton, P. G., Pruitt, A. W., Faraj, B. A., & Israili, Z. H. (1975). Metabolism and disposition of diazoxide: a mini-review. *Drug Metab Dispos*, *3*, 226–229.
71. Laje, P., Halaby, L., Adzick, N. S., & Stanley, C. A. (2010). Necrotizing enterocolitis in neonates receiving octreotide for the management of congenital hyperinsulinism. *Pediatr Diabetes*, *11*, 142–147.
72. Le Quan Sang KH, Arnoux JB, Mamoune A, et al. (2012). Successful treatment of congenital hyperinsulinism with long-acting release octreotide. *Eur J Endocrinol 166*(2):333-9.
73. Mohnike, K., Blankenstein, O., Pfuetzner, A., et al. (2008). Long-term non-surgical therapy of severe persistent congenital hyperinsulinism with glucagon. *Horm Res*, *70*, 59–64.
74. Mazor-Aronovitch, K., Landau, H., & Gillis, D. (2009). Surgical versus non-surgical treatment of congenital hyperinsulinism. *Pediatr Endocrinol Rev*, *6*, 424–430.
75. Banerjee, I., Avatapalle, B., Petkar, A., et al. (2012). The association of cardiac ventricular hypertrophy with congenital hyperinsulinism. *Eur J Endocrinol*, *167*, 619–624.
76. De Leon, D. D., & Stanley, C. A. (2012). Pathophysiology of diffuse ATP-sensitive potassium channel hyperinsulinism. In D. D. De Leon, & C. A. Stanley (Eds.), *Monogenic hyperinsulinemic hypoglycemia disorders* (pp. 18–29). Basel: Karger.
77. Macmullen, C. M., Zhou, Q., Snider, K. E., et al. (2011). Diazoxide-unresponsive congenital hyperinsulinism in children with dominant mutations of the beta-cell sulfonylurea receptor SUR1. *Diabetes*, *60*, 1797–1804.
78. Grimberg, A., Ferry, R. J., Jr., Kelly, A., et al. (2001). Dysregulation of insulin secretion in children with congenital hyperinsulinism due to sulfonylurea receptor mutations. *Diabetes*, *50*, 322–328.
79. Shiota, C., Larsson, O., Shelton, K. D., et al. (2002). Sulfonylurea receptor type 1 knock-out mice have intact feeding-stimulated insulin secretion despite marked impairment in their response to glucose. *J Biol Chem*, *277*, 37176–37183.
80. Henquin, J. C., Nenquin, M., Sempoux, C., et al. (2011). In vitro insulin secretion by pancreatic tissue from infants with diazoxide-resistant congenital hyperinsulinism deviates from model predictions. *J Clin Invest*, *121*, 3932–3942.
81. Li, C., Buettger, C., Kwagh, J., et al. (2004). A signaling role of glutamine in insulin secretion. *J Biol Chem*, *279*, 13393–13401.
82. Fourtner, S. H., Stanley, C. A., & Kelly, A. (2006). Protein-sensitive hypoglycemia without leucine sensitivity in hyperinsulinism caused by K(ATP) channel mutations. *J Pediatr*, *149*, 47–52.
83. de Lonlay-Debeney, P., Poggi-Travert, F., Fournet, J. C., et al. (1999). Clinical features of 52 neonates with hyperinsulinism. *N Engl J Med*, *340*, 1169–1175.
84. de Lonlay, P., Fournet, J. C., Rahier, J., et al. (1997). Somatic deletion of the imprinted 11p15 region in sporadic persistent hyperinsulinemic hypoglycemia of infancy is specific of focal adenomatous hyperplasia and endorses partial pancreatectomy. *J Clin Invest*, *100*, 802–807.

85. Lord K, Dzata E, Snider KE, et al (2013). Clinical presentation and management of children with diffuse and focal hyperinsulinism: a review of 223 cases. *J Clin Endocrinol Metab* Sep 20 [Epub ahead of print].
86. Suchi, M., MacMullen, C., Thornton, P. S., et al. (2003). Histopathology of congenital hyperinsulinism: retrospective study with genotype correlations. *Pediatr Dev Pathol*, *6*, 322–333.
87. Dubois, J., Brunelle, F., Touati, G., et al. (1995). Hyperinsulinism in children: diagnostic value of pancreatic venous sampling correlated with clinical, pathological and surgical outcome in 25 cases. *Pediatr Radiol*, *25*, 512–516.
88. Stanley, C. A., Thornton, P.S., Ganguly, A., et al. (2004). Preoperative evaluation of infants with focal or diffuse congenital hyperinsulinism by intravenous acute insulin response tests and selective pancreatic arterial calcium stimulation. *J Clin Endocrinol Metab*, *89*, 288–296.
89. Ribeiro, M. J., De Lonlay, P., Delzescaux, T., et al. (2005). Characterization of hyperinsulinism in infancy assessed with PET and 18F-fluoro-L-DOPA. *J Nucl Med*, *46*, 560–566.
90. Otonkoski, T., Nanto-Salonen, K., Seppanen, M., et al. (2006). Noninvasive diagnosis of focal hyperinsulinism of infancy with [18F]-DOPA positron emission tomography. *Diabetes*, *55*, 13–18.
91. Hardy, O. T., Hernandez-Pampaloni, M., Saffer, J. R., et al. (2007). Diagnosis and localization of focal congenital hyperinsulinism by 18F-fluorodopa PET scan. *J Pediatr*, *150*, 140–145.
92. Ericson, L. E., Hakanson, R., & Lundquist, I. (1977). Accumulation of dopamine in mouse pancreatic β-cells following injection of L-DOPA: localization to secretory granules and inhibition of insulin secretion. *Diabetologia*, *13*, 117–124.
93. Borelli, M. I., Villar, M. J., Orezzoli, A., & Gagliardino, J. J. (1997). Presence of DOPA decarboxylase and its localisation in adult rat pancreatic islet cells. *Diabetes Metab*, *23*, 161–163.
94. Miki, Y., Taki, T., Ohura, T., et al. (2000). Novel missense mutations in the glutamate dehydrogenase gene in the congenital hyperinsulinism-hyperammonemia syndrome. *J Pediatr*, *136*, 69–72.
95. MacMullen, C., Fang, J., Hsu, B. Y., et al. (2001). Hyperinsulinism/hyperammonemia syndrome in children with regulatory mutations in the inhibitory guanosine triphosphate-binding domain of glutamate dehydrogenase. *J Clin Endocrinol Metab*, *86*, 1782–1787.
96. Huijmans, J. G., Duran, M., de Klerk, J. B., et al. (2000). Functional hyperactivity of hepatic glutamate dehydrogenase as a cause of the hyperinsulinism/hyperammonemia syndrome: effect of treatment. *Pediatrics*, *106*, 596–600.
97. Yorifuji, T., Muroi, J., Uematsu, A., et al. (1999). Hyperinsulinism-hyperammonemia syndrome caused by mutant glutamate dehydrogenase accompanied by novel enzyme kinetics. *Hum Genet*, *104*, 476–479.
98. Kelly, A., Palladino, A., & Stanley, C. A. (2012). Congenital hyperinsulinism due to activating mutations of glutamate dehydrogenase: the hyperinsulinism/hyperammonemia syndrome. In D. D. De Leon, & C. A. Stanley (Eds.), *Monogenic hyperinsulinemic hypoglycemia disorders* (pp. 100–111). Basel: Karger.
99. Li, C., Matter, A., Kelly, A., et al. (2006). Effects of a GTP-insensitive mutation of glutamate dehydrogenase on insulin secretion in transgenic mice. *J Biol Chem*, *281*, 15064–15072.
100. Kelly, A., Ng, D., Ferry, R. J. Jr., et al. (2001). Acute insulin responses to leucine in children with the hyperinsulinism/hyperammonemia syndrome. *J Clin Endocrinol Metab*, *86*, 3724–3728.
101. Hsu, B. Y., Kelly, A., Thornton, P. S., et al. (2001). Protein-sensitive and fasting hypoglycemia in children with the hyperinsulinism/hyperammonemia syndrome. *J Pediatr*, *138*, 383–389.
102. Treberg, J. R., Clow, K. A., Greene, K. A., et al. (2010). Systemic activation of glutamate dehydrogenase increases renal ammoniagenesis: implications for the hyperinsulinism/hyperammonemia syndrome. *Am J Physiol Endocrinol Metab*, *298*, E1219–E1225.
103. Bahi-Buisson, N., Roze, E., Dionisi, C., et al. (2008). Neurological aspects of hyperinsulinism-hyperammonaemia syndrome. *Dev Med Child Neurol*, *50*, 945–949.
104. Raizen, D. M., Brooks-Kayal, A., Steinkrauss, L., et al. (2005). Central nervous system hyperexcitability associated with glutamate dehydrogenase gain of function mutations. *J Pediatr*, *146*, 388–394.
105. Kapoor, R. R., Flanagan, S. E., Fulton, P., et al. (2009). Hyperinsulinism-hyperammonaemia syndrome: novel mutations in the GLUD1 gene and genotype-phenotype correlations. *Eur J Endocrinol*, *161*, 731–735.
106. Molven, A., Matre, G. E., Duran, M., et al. (2004). Familial hyperinsulinemic hypoglycemia caused by a defect in the SCHAD enzyme of mitochondrial fatty acid oxidation. *Diabetes*, *53*, 221–227.
107. Hussain, K., Clayton, P. T., Krywawych, S., et al. (2005). Hyperinsulinism of infancy associated with a novel splice site mutation in the SCHAD gene. *J Pediatr*, *146*, 706–708.
108. Li, C., Chen, P., Palladino, A., et al. (2010). Mechanism of hyperinsulinism in short-chain 3-hydroxyacyl-CoA dehydrogenase deficiency involves activation of glutamate dehydrogenase. *J Biol Chem*, *285*, 31806–31818.
109. Kapoor, R. R., James, C., Flanagan, S. E., et al. (2009). 3-Hydroxyacyl-coenzyme A dehydrogenase deficiency and hyperinsulinemic hypoglycemia: characterization of a novel mutation and severe dietary protein sensitivity. *J Clin Endocrinol Metab*, *94*, 2221–2225.
110. Matschinsky, F. M. (2002). Regulation of pancreatic beta-cell glucokinase: from basics to therapeutics. *Diabetes*, *51*(suppl 3), S394–S404.
111. Sayed, S., Matschinsky, F. M., & Stanley, C. A. (2012). Hyperinsulinism due to activating mutations of glucokinase. In C. A. Stanley, D. D. De Leon (Eds.), *Monogenic hyperinsulinism hypoglycemia disorders* (pp. 146–157). Basel: Karger.
112. Gloyn, A. L., Noordam, K., Willemsen, M. A., et al. (2003). Insights into the biochemical and genetic basis of glucokinase activation from naturally occurring hypoglycemia mutations. *Diabetes*, *52*, 2433–440.
113. Wabitsch, M., Lahr, G., Van de Bunt, M., et al. (2007). Heterogeneity in disease severity in a family with a novel G68V GCK activating mutation causing persistent hyperinsulinaemic hypoglycaemia of infancy. *Diabet Med*, *24*, 1393–1399.
114. Sayed, S., Langdon, D. R., Odili, S., et al. (2009). Extremes of clinical and enzymatic phenotypes in children with hyperinsulinism caused by glucokinase activating mutations. *Diabetes*, *58*, 1419–1427.
115. Kassem, S., Bhandari, S., Rodriguez-Bada, P., et al. (2010). Large islets, beta-cell proliferation, and a glucokinase mutation. *N Engl J Med*, *362*, 1348–1350.
116. Cuesta-Munoz, A. L., Huopio, H., Otonkoski, T., et al. (2004). Severe persistent hyperinsulinemic hypoglycemia due to a de novo glucokinase mutation. *Diabetes*, *53*, 2164–2168.
117. Zhang, C. Y., Baffy, G., Perret, P., et al. (2001). Uncoupling protein-2 negatively regulates insulin secretion and is a major link between obesity, beta cell dysfunction, and type 2 diabetes. *Cell*, *105*, 745–755.
118. Gonzalez-Barroso, M. M., de Lonlay, P., & Ricquier, D. (2012). Hyperinsulinism due to mutations of uncoupling protein 2. In C. A. Stanley, D. D. De Leon (Eds.), *Monogenic hyperinsulinemic hypoglycemia disorders* (pp. 158–171). Basel: Karger.
119. Ferrer, J. (2002). A genetic switch in pancreatic β-cells: implications for differentiation and haploinsufficiency. *Diabetes*, *51*, 2355–2362.
120. Fajans, S. S., Bell, G. I., & Polonsky, K. S. (2001). Molecular mechanisms and clinical pathophysiology of maturity-onset diabetes of the young. *N Engl J Med*, *345*, 971–980.
121. Fajans, S. S., & Bell, G. I. (2007). Macrosomia and neonatal hypoglycaemia in RW pedigree subjects with a mutation (Q268X) in the gene encoding hepatocyte nuclear factor 4alpha (HNF4A). *Diabetologia*, *50*, 2600–2601.
122. Kapoor, R. R., Locke, J., Colclough, K., et al. (2008). Persistent hyperinsulinemic hypoglycemia and maturity-onset diabetes of the young due to heterozygous HNF4A mutations. *Diabetes*, *57*, 1659–1663.
123. Flanagan, S. E., Kapoor, R. R., Mali, G., et al. (2010). Diazoxide-responsive hyperinsulinemic hypoglycemia caused by HNF4A gene mutations. *Eur J Endocrinol*, *162*, 987–992.
124. Kapoor, R. R., James, C. T., & Hussain, K. (2012). HNF4A and hyperinsulinemic hypoglycemia. In C. A. Stanley, D. D. De Leon (Eds.), *Monogenic hyperinsulinemic hypoglycemia disorders* (pp. 182–190). Basel: Karger.
125. Hussain, K., Challis, B., Rocha, N., et al. (2011). An activating mutation of AKT2 and human hypoglycemia. *Science*, *334*, 474.
126. Calabria, A. C., Gallagher, P. R., Simmons, R., et al. (2011). Postoperative surveillance and detection of postprandial hypoglycemia after fundoplasty in children. *J Pediatr*, *159*, 597–601 e1.
127. Ralphs, D. N., Thomson, J. P., Haynes, S., et al. (1978). The relationship between the rate of gastric emptying and the dumping syndrome. *Br J Surg*, *65*, 637–641.

128. Rivkees, S., & Crawford, J. (1987). Hypoglycemia pathogenesis in children with dumping syndrome. *Pediatrics*, *80*, 937–942.
129. Ng, D. D., Ferry, R. J. J., Kelly, A., et al. (2001). Acarbose treatment of postprandial hypoglycemia in children after Nissen fundoplication. *J Pediatr*, *139*, 877–879.
130. Vu, M. K., Straathof, J. W. A., v d Schaar, P. J., et al. (1999). Motor and sensory function of the proximal stomach in reflux disease and after laparoscopic Nissen fundoplication. *Am J Gastroenterol*, *94*, 1481–1489.
131. Palladino, A. A., Sayed, S., Levitt Katz, L. E., et al. (2009). Increased glucagon-like peptide-1 secretion and postprandial hypoglycemia in children after Nissen fundoplication. *J Clin Endocrinol Metab*, *94*, 39–44.
132. Borovoy, J., Furuta, L., & Nurko, S. (1998). Benefit of uncooked cornstarch in the management of children with dumping syndrome fed exclusively by gastrostomy. *Am J Gastroenterol*, *93*, 814–818.
133. Samuk, I., Afriat, R., Horne, T., et al. (1996). Dumping syndrome following Nissen fundoplication, diagnosis, and treatment. *J Pediatr Gastroenterol Nutr*, *23*, 235–240.
134. Lehnert, H., Beyer, J., Weber, P., et al. (1990). Treatment of severe reactive hypoglycemia with a somatostatin analogue (SMS 201-995). *Arch Intern Med*, *150*, 2401–2402.
135. Khoshoo, V., Reifen, R. M., Gold, B. D., et al. (1991). Nutritional manipulation in the management of dumping syndrome. *Arch Dis*, *66*, 1447–1448.
136. Salisbury, D. M., Leonard, J. V., Dezateux, C. A., & Savage M. O. (1984). Micropenis: an important early sign of congenital hypopituitarism. *Br Med J* (Clin Res Ed), *288*, 621–622.
137. Lovinger, R. D., Kaplan, S. L., & Grumbach, M. M. (1975). Congenital hypopituitarism associated with neonatal hypoglycemia and microphallus: four cases secondary to hypothalamic hormone deficiencies. *J Pediatr*, *87*, 1171–1181.
138. Kaufman, F. R., Costin, G., Thomas, D. W., et al. (1984). Neonatal cholestasis and hypopituitarism. *Arch Dis Child*, *59*, 787–789.
139. Renier, W. O., Nabben, F. A., Hustinx, T. W., et al. (1983). Congenital adrenal hypoplasia, progressive muscular dystrophy, and severe mental retardation, in association with glycerol kinase deficiency, in male sibs. *Clin Genet*, *24*, 243–251.
140. Toyofuku, T., Takashima, S., Takeshita, K., & Nagafuji, H. (1986). Progressive muscular dystrophy with congenital adrenal hypoplasia: an unusual autopsy case. *Brain Dev*, *8*, 285–289.
141. Clark, A. J., & Weber, A. (1994). Molecular insights into inherited ACTH resistance syndromes. *Trends Endocrinol Metab*, *5*, 209–214.
142. Atasay, B., Aycan, Z., Evliyaoglu, O., et al. (2004). Congenital early onset isolated adrenocorticotropin deficiency associated with a TPIT gene mutation. *J Pediatr Endocrinol Metab*, *17*, 1017–1020.
143. Kelly, A., Tang, R., Becker, S., & Stanley, C. A. (2008). Poor specificity of low growth hormone and cortisol levels during fasting hypoglycemia for the diagnoses of growth hormone deficiency and adrenal insufficiency. *Pediatrics*, *122*, e522–e528.
144. Vidnes, J., & Oyasaeter, S. (1977). Glucagon deficiency causing severe neonatal hypoglycemia in a patient with normal insulin secretion. *Pediatr Res*, *11*, 943–949.
145. Kollee, L. A., Monnens, L. A., Cecjka, V., & Wilms, R. M. (1978). Persistent neonatal hypoglycaemia due to glucagon deficiency. *Arch Dis Child*, *53*, 422–4224.
146. Foster, J. D., & Nordlie, R. C. (2002). The biochemistry and molecular biology of the glucose-6-phosphatase system. *Exp Biol Med* (Maywood), *227*, 601–608.
147. Lei, K. J., Pan, C. J., Shelly, L. L., et al. (1994). Identification of mutations in the gene for glucose-6-phosphatase, the enzyme deficient in glycogen storage disease type 1a. *J Clin Invest*, *93*, 1994–1999.
148. Gerin, I., Veiga-da-Cunha, M., Achouri, Y., et al. (1997). Sequence of a putative glucose 6-phosphate translocase, mutated in glycogen storage disease type Ib. *FEBS Lett*, *419*, 235–238.
149. Lei, K. J., Shelly, L. L., Pan, C. J., et al. (1993). Mutations in the glucose-6-phosphatase gene that cause glycogen storage disease type 1a. *Science*, *262*, 580–583.
150. Visser, G., Rake, J. P., Fernandes, J., et al. (2000). Neutropenia, neutrophil dysfunction, and inflammatory bowel disease in glycogen storage disease type Ib: results of the European Study on Glycogen Storage Disease type I. *J Pediatr*, *137*, 187–191.
151. Stanley, C. A., Mills, J. L., & Baker, L. (1981). Intragastric feeding in type I glycogen storage disease: factors affecting the control of lactic acidemia. *Pediatr Res*, *15*, 1504–158.
152. Melis, D., Parenti, G., Gatti, R., et al. (2005). Efficacy of ACE-inhibitor therapy on renal disease in glycogen storage disease type 1: a multicentre retrospective study. *Clin Endocrinol* (Oxf), *63*, 19–25.
153. Restaino, I., Kaplan, B. S., Stanley, C., & Baker, L. (1993). Nephrolithiasis, hypocitraturia, and a distal renal tubular acidification defect in type 1 glycogen storage disease. *J Pediatr*, *122*, 392–396.
154. Weinstein, D. A., Somers, M. J., & Wolfsdorf, J. I. (2001). Decreased urinary citrate excretion in type 1a glycogen storage disease. *J Pediatr*, *138*, 378–382.
155. Chen, Y. T., Coleman, R. A., Scheinman, J. I., et al. (1988). Renal disease in type I glycogen storage disease. *N Engl J Med*, *318*, 7–11.
156. Chen, Y. T., Scheinman, J. I., Park, H. K., et al. (1990). Amelioration of proximal renal tubular dysfunction in type I glycogen storage disease with dietary therapy. *N Engl J Med*, *323*, 590–593.
157. Rake, J. P., Visser, G., Labrune, P., et al. (2002). Glycogen storage disease type I: diagnosis, management, clinical course and outcome. Results of the European Study on Glycogen Storage Disease Type I (ESGSD I). *Eur J Pediatr*, *161*(suppl 1), S20–S34.
158. Moses, S. W. (2002). Historical highlights and unsolved problems in glycogen storage disease type 1. *Eur J Pediatr*, *161*(suppl 1), S2–S9.
159. Bianchi, L. (1993). Glycogen storage disease I and hepatocellular tumours. *Eur J Pediatr*, *152*(suppl 1), S63–S70.
160. Wolfsdorf, J. I., & Weinstein, D. A. (2003). Glycogen storage diseases. *Rev Endocr Metab Disord*, *4*, 95–102.
161. Greene, H. L., Slonim, A. E., O'Neill, J. A., Jr., & Burr, I. M. (1976). Continuous nocturnal intragastric feeding for management of type 1 glycogen-storage disease. *N Engl J Med*, *294*, 423–425.
162. Chen, Y. T., Cornblath, M., & Sidbury, J. B. (1984). Cornstarch therapy in type I glycogen-storage disease. *N Engl J Med*, *310*, 171–175.
163. Wolfsdorf,, J. I., Keller R. J., Landy, H., & Crigler J. F., Jr. (1990). Glucose therapy for glycogenosis type 1 in infants: comparison of intermittent uncooked cornstarch and continuous overnight glucose feedings. *J Pediatr*, *117*, 384–391.
164. Roe, T. F., Coates, T. D., Thomas, D. W., et al. (1992). Brief report: treatment of chronic inflammatory bowel disease in glycogen storage disease type Ib with colony-stimulating factors. *N Engl J Med*, *326*, 1666–1669.
165. Hale, D. E., & Bennett, M. J. (1992). Fatty acid oxidation disorders: a new class of metabolic diseases. *J Pediatr*, *121*, 1–11.
166. Chace, D. H., Kalas, T. A., & Naylor, E. W. (2002). The application of tandem mass spectrometry to neonatal screening for inherited disorders of intermediary metabolism. *Annu Rev Genomics Hum Genet*, *3*, 17–45.
167. Ensenauer, R., Winters, J. L., Parton, P. A., et al. (2005). Genotypic differences of MCAD deficiency in the Asian population: novel genotype and clinical symptoms preceding newborn screening notification. *Genet Med*, *7*, 339–343.
168. Iafolla, A. K., Thompson, R. J. Jr., & Roe, C. R. (1994). Medium-chain acyl-coenzyme A dehydrogenase deficiency: clinical course in 120 affected children. *J Pediatr*, *124*, 409–415.
169. Ding, J. H., Roe, C. R., Iafolla A. K., & Chen, Y. T. (1991). Medium-chain acyl-coenzyme A dehydrogenase deficiency and sudden infant death. *N Engl J Med*, *325*, 61–62.
170. Rhead, W. J. (2006). Newborn screening for medium-chain acyl-CoA dehydrogenase deficiency: a global perspective. *J Inherit Metab Dis*, *29*, 370–377.
171. Matern, D., & Rinaldo, P. (2000, April 20). Medium-chain acyl-coenzyme a dehydrogenase deficiency. In R. A. Pagon, T. D. Bird, C. R. Dolan, et al. (Eds.). Retrieved from GeneReviews, University of Washington, Seattle, Dec. 7, 2012: http://www.ncbi.nlm.nih.gov/books/NBK1424/
172. Leen, W. G., Klepper, J., Verbeek, M. M., et al. (2010). Glucose transporter-1 deficiency syndrome: the expanding clinical and genetic spectrum of a treatable disorder. *Brain*, *133*, 655–670.
173. Klepper, J., & Voit, T. (2002). Facilitated glucose transporter protein type 1 (GLUT1) deficiency syndrome: impaired glucose transport into brain—a review. *Eur J Pediatr*, *161*, 295–304.

174. Santer, R., Schneppenheim, R., Suter, D., et al. (1998). Fanconi-Bickel syndrome: the original patient and his natural history, historical steps leading to the primary defect, and a review of the literature. *Eur J Pediatr*, *157*, 783–797.
175. Santer, R., Schneppenheim, R., Dombrowski, A., et al. (1997). Mutations in GLUT2, the gene for the liver-type glucose transporter, in patients with Fanconi-Bickel syndrome. *Nat Genet*, *17*, 324–326.
176. Manz, F., Bickel, H., Brodehl, J., et al. (1987). Fanconi-Bickel syndrome. *Pediatr Nephrol*, *1*, 509–518.
177. Mueckler, M., Kruse, M., Strube, M., et al. (1994). A mutation in the Glut2 glucose transporter gene of a diabetic patient abolishes transport activity. *J Biol Chem*, *269*, 17765–17767.
178. Santer, R., Steinmann, B., & Schaub, J. (2002). Fanconi-Bickel syndrome: a congenital defect of facilitative glucose transport. *Curr Mol Med*, *2*, 213–227.
179. Cornblath, M., Odell, G. B., & Levin, E. Y. (1995). Symptomatic neonatal hypoglycemia associated with toxemia of pregnancy. *J Pediatr*, *55*, 545–562.

QUESTIONS

1. An infant girl was admitted to a children's hospital on day 3 of life following an apneic episode at home for which emergency medical services (EMS) was dispatched after a 911 call. EMS recorded a glucose of 24 mg/dL (meter) and treated it with a bolus of 10% glucose followed by transfer to the children's hospital. History revealed an induced delivery at 34W gestation because of U/S confirmed IUGR presumed secondary to "placental insufficiency." The mother admitted to smoking half a pack per day during pregnancy but denied use of any other medication or drugs; a screening OGTT was normal. At birth, the baby weighed 2.6 kg, had Apgar of 9/9, and was found to have symmetric growth retardation. Despite feeding and intravenous glucose at 15 mg/kg/min, hypoglycemia persisted. A "critical sample" on day 5 of life was performed when the glucose was noted to be 33 mg/dL via meter and confirmed via laboratory glucose. In this "critical sample," insulin was 62 μU/mL, cortisol 32 μg/dL, growth hormone 35 ng/mL; βOHB 0.2 mmol/L. What should the net step be?
   a. Genetic mutational analysis with emphasis on KCNJ11, ABCC8.
   b. Liver biopsy to rule out GSD-1.
   c. Start diazoxide 10 mg/kg/D in divided doses.
   d. Refer to an institution for PET scan with ^{18}F-L DOPA immediately preceding scheduled surgery for pancreatectomy.

Answer: c

2. A 17-month-old female was admitted to a children's hospital for evaluation of a seizure. Three weeks before the seizure, she had a "spell" and was taken to a local emergency department where metered blood glucose was 17 mg/dL and a formal laboratory glucose was 30 mg/dL. In that sample, insulin concentration was < 2 μU/mL, GH 11.1 ng/dl, and cortisol 32 μg/dL. Because of the low insulin, a diagnosis of "ketotic hypoglycemia" was made and the infant discharged to her home. Past history revealed normal gestation, delivery and birth weight, normal developmental milestones, and normal weight gain and growth. While awaiting the results of an EEG and MRI of the brain, it was noted that the fasting blood glucose was 60 mg/dL and 1 hour post prandial glucose was 44 mg/dL. A "controlled fast" was begun after the child had her usual evening meal. Two hours after this meal, a metered blood glucose was 36 mg/dL and lab glucose was 37 mg/dL. Other results included the following:
   β-OHB of 0.27 mmol/L
   Lactate 1.8 mM/L
   Insulin 9 μU/ML
   NH_3 93 μM/L

Given the presentation beginning at approximately 17 months, what is the most likely diagnosis?
   a. Idiopathic ketotic hypoglycemia of infancy
   b. Hyperinsulinism due to an activating mutation in the insulin receptor
   c. Medium-chain acyl-CoA dehydrogenase deficiency
   d. Hyperinsulinism due to an activating mutation in GLUD1

Answer: d

What are the correct next steps for this condition?
   a. Frequent feedings, especially milk to avoid fasting hypoglycemia
   b. Diazoxide 10 mg/kg/day in divided doses
   c. Supplemental carnitine in all feedings
   d. Confirmation of the diagnosis by mutation analysis of glutamate dehydrogenase
   e. a and c
   f. b and d

Answer: f

3. A 4 month-old boy is referred to endocrine clinic for poor growth. He was the product of a full-term, normal pregnancy complicated by gestational diabetes treated with diet. A plasma glucose concentration at 2 hours of age was 40 mg/dL. He was formula-fed and a plasma glucose concentration at discharge on day of life 2 was 50 mg/dL. Over the past 2 months, his mother has noted that he has been increasingly fussy and sweaty if not fed every 2 hours. Despite the frequent feedings, his length and weight have decreased from the 50 and 80 percentiles, respectively, at birth to the 10 and 25 percentiles at 4 months of age. On physical examination, he seems irritable and is breathing rapidly; the liver is enlarged to 8 cm below the costal margin; the phallus is 3.2 cm in length and the testes are descended 2 mL in volume bilaterally. Lab tests show plasma glucose 25 mg/dL; normal sodium, potassium, and chloride, HCO_3; 11 mEq/L; calcium 10 mg/dL; phosphate 4.5 mg/dL; growth hormone 2 ng/mL, cortisol 8 μg/dL, insulin 3 μU/mL; and urine ketones small.

Which of the following is the most likely diagnosis?
   a. Hyperinsulinism associated with recessive mutations of short-chain 3-hydroxy-acyl-CoA dehydrogenase
   b. Hypopituitarism associated with recessive mutation of PIT1
   c. Recessive deficiency of the GLUT2 glucose transporter
   d. Mosaic paternal isodisomy for 11p
   e. Recessive deficiency of glucose 6-phosphatase (G6PC)

Answer: e

The diagnosis is best confirmed by which of the following results?
   a. Following injection of glucagon 1 mg IV, the plasma glucose rises from 35 to 90 mg/dL after 30 minutes.
   b. Following injection of glucagon 1 mg IV, the blood lactate rises from 3.5 to 7 mM/L after 15 minutes.
   c. Following ingestion of an oral protein supplement, 1 gm/kg, the plasma glucose falls from 75 to 45 mg/dL after 20 minutes.
   d. Following ingestion of oral glucose, 1.75 gm/kg, the plasma glucose rises to 250 mg/dL at 1 hour and then falls to 35 mg/dL at 1.5 hour.

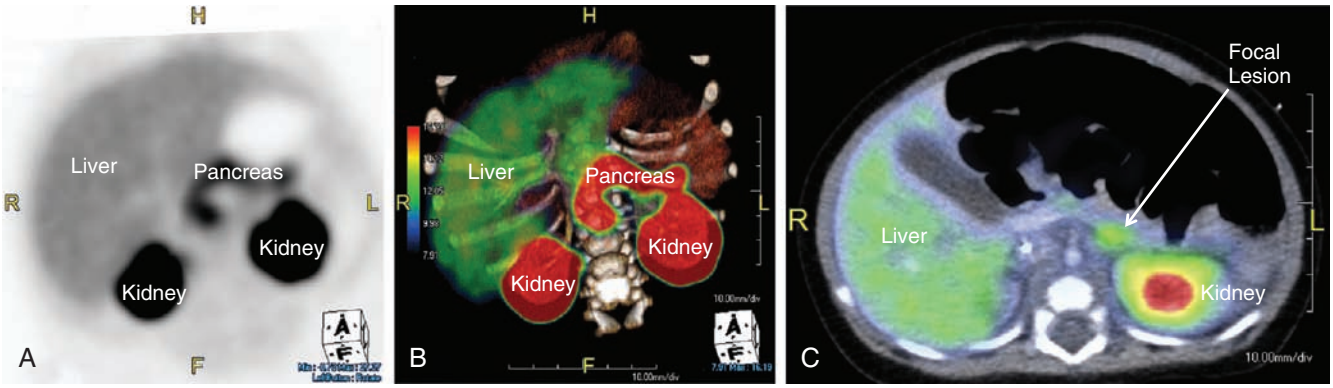
e. Following injection of calcium 200 mg/kg IV, the plasma insulin rises from 10 up to 45 μU/mL after 3 minutes.

Answer: b

What would be the most appropriate treatment for this condition?

a. A high-protein, low–glycemic index diet
b. Frequent feedings using a normal milk-based formula
c. Frequent feedings using a non–lactose-containing formula, with the addition of raw cornstarch after age 1 to 2 years old
d. Oral diazoxide plus a low-leucine diet
e. Growth hormone, 0.3 mg/kg/week, divided into twice daily injections

Answer: c



e-FIGURE 6-1 ■ A, ^{18}F-fluoro-L-DOPA PET scan image of a diffuse case of hyperinsulinism showing L-DOPA uptake in the liver, in the kidneys, and throughout the pancreas with equal intensity. **B,** Fused 3D image of a contrast-enhanced CT scan fused with an ^{18}F-fluoro-L-DOPA PET scan for the same case as in (**A**). **C,** An axial image of a contrast-enhanced CT scan fused with an ^{18}F-fluoro-L-DOPA PET scan showing a focus of increased uptake in the posterior body of the pancreas. Note the normal tracer uptake in the liver and left kidney. (Images courtesy of Dr. Lisa States.)

CHAPTER 7

Disorders of the Thyroid in the Newborn and Infant

Guy Van Vliet, MD • Johnny Deladoëy, MD, PhD

CHAPTER OUTLINE

INTRODUCTION

The management of the fetus, newborn, and infant with disorders of thyroid function has benefited greatly from our ability, since the 1970s, to measure hormones in minute amounts of biologic fluids. Specifically, biochemical screening of newborns for congenital hypothyroidism (CH) is now routine throughout the industrialized world and has resulted in the disappearance of the overt intellectual disability that affected 8% to 28% of patients with this condition in the prescreening era.[1] More recently, the molecular basis of several monogenic disorders of thyroid development and function has been deciphered. However, most cases of abnormal thyroid gland development in humans, the most common cause of CH, are unexplained and constitute one of the remaining mysteries of thyroid physiopathology.[2] On the other hand, progress in ultrasound techniques has led to an increasing number of goiters being identified in the fetus in utero, which raises the possibility of prenatal treatment via the mother's amniotic fluid in highly selected cases.[3] This chapter reviews thyroid disorders in the fetus, newborn, and infant from a developmental, molecular, and clinical perspective.

EMBRYOLOGY, PHYSIOLOGY, AND PHYSIOPATHOLOGY

Development of the Thyrotropic Axis

As for all organs, anatomic development of the hypothalamic-pituitary-thyroid system occurs during the first trimester of gestation. The human embryonic forebrain and hypothalamus begin to differentiate by 3 weeks of gestation under the influence of a series of homeodomain proteins or transcription factors. Immunoreactive thyrotropin-releasing hormone (TRH) becomes detectable in human embryonic hypothalami by 8 to 9 weeks of postconceptional age and is also produced by the fetal gut and pancreas.[4,5]

Anatomically, the pituitary gland develops from two ectodermal anlagen: a neural component from the floor of the primitive forebrain and Rathke's pouch from the primitive oral cavity. The latter is visible by 5 weeks, evolving to a morphologically mature pituitary gland by 14 to 15 weeks. The pituitary-portal blood vessels are present by this time and mature further through 30 to 35 weeks. A wide spectrum of congenital malformations collectively named "midline defects," including holoprosencephaly

and septo-optic dysplasia, may be associated with central hypothyroidism and other anterior pituitary deficiencies.[6] The molecular mechanisms underlying these malformations have been identified in some cases.[7] Within the pituitary itself, PROP-1 and PIT-1 are terminal factors in the differentiation cascade of pituitary cells and PIT-1 or PROP-1 deficiency results in profound defects in growth hormone, prolactin, and thyroid-stimulating hormone (TSH) secretion as well as age-dependent pituitary hypoplasia.[8-10]

The human thyroid gland develops from a median anlage derived from the primitive pharyngeal floor and from paired lateral anlagen from the fourth pharyngeal pouches. The long-held belief that the lateral anlagen were the only source of calcitonin-producing cells has been challenged by the observation that sublingual thyroids that are derived exclusively from the median anlage contain calcitonin mRNA and protein.[11] Conversely, thyroid follicular cells can differentiate within the lateral anlagen, as illustrated by histologic observations[12] as well as by patients in whom the only thyroid tissue is a lateral ectopy.[13] Both the median and lateral structures are visible by day 16 to 17 of gestation; by 50 days they have fused and the thyroid gland has migrated to its definitive location in the anterior neck. The thyroglossal duct, from the foramen cecum to the final location of the thyroid, may persist and is constituted of degenerated thyroid follicular cells. Within the thyroid gland, iodine concentration, TSH receptors, thyroglobulin, and thyroid peroxidase mRNA and protein can be demonstrated by 70 days.[14]

Thyroid embryogenesis is dependent on the expression of a programmed sequence of homeobox and transcription factors, including thyroid transcription factors-1 and -2 (TTF-1 or TiTF-1, also known as NK2 homeobox 1 -NKX2-1- and TTF-2, also known as Forkhead box E1 -FOXE-1) and paired box gene 8 (PAX8).[15] Embryonic stem cells in which NKX2-1 and PAX 8 are overexpressed form fully differentiated thyroid cells and will form the typical follicular architecture under the influence of TSH.[16] In keeping with this concept, mice with a naturally occurring mutation inactivating the TSH receptor have a disorganized follicular architecture,[17] and this is also probably the case in humans with similar mutations in which a high serum thyroglobulin in spite of profound thyroid hypoplasia suggests leaky follicles.[18] In newborn mice, biallelic inactivation of Nkx2.1 results in absence of both pituitary and thyroid glands, with complete absence of both thyroid follicular cells and of calcitonin-producing C cells,[19] whereas that of PAX8 results in a small thyroid gland composed almost exclusively of C cells.[20] FOXE-1 null mouse embryos have either an absent thyroid or an ectopic sublingual gland, but all newborn pups have athyreosis in addition to cleft palate.[21] Mutations in the homologous genes, however, account for at most 2% of cases of thyroid dysgenesis in humans.[22]

Placental Transfer of Iodine, T4, TRH, Antithyroid Drugs, and Immunoglobulins

Iodine is an essential component of thyroid hormones. In this chapter, the term *iodine* is used to designate both iodine itself (I^2) and iodide (I^-). The human placenta expresses the sodium–iodine symporter throughout gestation,[23] which explains why the mother's iodine status is reflected in the fetus (Figure 7-1) . If the mother's iodine intake is suboptimal, the fetal thyroid cannot constitute appropriate stores of iodine and fetal hypothyroidism may ensue. Worldwide, inadequate maternal iodine intake leading to irreversible consequences, known as endemic cretinism, remains a major public health problem. Prevention of this condition by supplying the mother with adequate iodine is one of the best and most important examples of considering the fetus as a patient and of treating this patient through its mother.[24,25]

In contrast to iodine, thyroxine (T4) was for a long time thought not to cross the placenta in substantial amounts.[26] However, T4 is detectable in human embryonic tissues before the onset of fetal thyroid function and must, therefore, be of maternal origin.[27] Later in gestation, the transfer of T4 from mother to fetus must continue, because the concentration in cord blood from neonates with complete absence of thyroid function is 30% to 50% of that of normal neonates.[28] More recently, it was shown that normal neonates born to a mother who has chronically higher T4 concentrations due to a mutation inactivating the thyroid hormone receptor have lower birth weight and TSH concentrations at newborn screening than those born to normal mothers.[29] Taken together, these data indicate that maternal T4 must cross the placenta in physiologically relevant amounts throughout gestation.

That this transplacental transfer of T4 from mother to fetus might be of clinical relevance was suggested by the severe developmental delay observed in an infant with central hypothyroidism caused by a maternally inherited

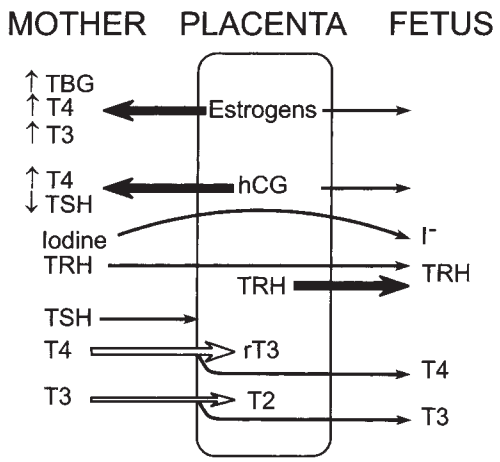


FIGURE 7-1 ■ The placental role in thyroid metabolism during human pregnancy. The placenta produces estrogens and a human chorionic gonadotropin (hCG), which increase maternal TBG levels and stimulate maternal thyroid hormone production, respectively. Both activities tend to increase maternal T4 and T3 concentrations and inhibit maternal TSH secretion. Iodide and TRH readily cross the placenta. In addition, the placenta synthesizes TRH. The placenta is impermeable to TSH and only partially permeable to T4 and T3. Placental type 3 iodothyronine monodeiodinase enzymes degrade T4 to reverse T3 and T3 to 3,3′ T2. The placenta is also permeable to the thiourea drugs used to treat maternal Graves disease.

heterozygous mutation inactivating PIT1, and whose equally hypothyroid mother had stopped thyroxine treatment at midpregnancy.[30] At the population level, children born to mothers who have low T4 concentrations during pregnancy have been reported to have lower IQ than children born to mothers with normal circulating concentrations of thyroid hormones.[31-33] However, in a randomized trial, developmental outcome was similar in the offspring of T4- and placebo-treated women who had either elevated TSH concentrations (> 97.5 percentile) or lower circulating T4 concentrations (< 2.5 percentile) when treatment was begun toward the end of the first trimester.[34] Moreover, in two recent case series of women with severe hypothyroidism diagnosed during pregnancy but corrected by the third trimester, the intellectual outcome of the offspring was normal.[35,36] Thus, universal screening for thyroid dysfunction during pregnancy remains questionable at this time. On the other hand, 85% of women who are already receiving T4 therapy require an increase in dose of about 30% to 50% during pregnancy because of the estradiol-induced increase in serum thyroxine-binding globulin (TBG).[37]

The transplacental transfer of T4 is not always sufficient to prevent the development of fetal goiter if the fetus has severe thyroid dyshormonogenesis. Fetal goiters may be big enough to interfere with the flow of amniotic fluid into the airways, causing progressive hydramnios and eventual lung hypoplasia. In such occurrences, levothyroxine can be injected into the amniotic fluid, which the fetus then swallows, leading to a decrease in the size of the fetal thyroid and in the degree of hydramnios.[38] The injection of thyroxine into the umbilical vein, which carries an even higher risk of triggering premature labor or fetal loss than amniocentesis, should be restricted to fetuses with a goiter that continues to increase in spite of repeated intra-amniotic injections. Invasive and potentially risky procedures should not be undertaken to protect the brain of affected fetuses: indeed, the fetal brain is, to a large extent, protected from the deleterious effect of hypothyroidism through upregulation of brain type 2 deiodinase, which converts the prohormone T4 into its biologically active derivative, T3.[39] This likely accounts for the observation that even in CH with delayed bone maturation at diagnosis (indicating a prenatal onset), the intellectual outcome is within normal limits if continuous and adequate treatment is instituted shortly after birth.[40] Thus, the in utero treatment of fetal hypothyroidism should only be considered in exceptional circumstances, such as for goiter causing progressive hydramnios. Although the identification of a goiter by prenatal ultrasound may be increasing,[41] it remains rare and even direct examination at birth often fails to detect goiters that are obvious on scintigraphy. Goiters can also be observed in fetuses borne by women with Graves disease if they are overtreated with antithyroid drugs, which readily cross the placenta. Reducing the dose of the antithyroid medication given to the mother should decrease the size of the fetal thyroid in such circumstances[42] (Figure 7-2).

Although the placenta produces a pro-TRH molecule, TRH concentrations in the maternal circulation are very low and thus have little effect on fetal thyroid function. However, TRH, with its tripeptide structure (the smallest of the hypothalamic hypophysiotropic peptides), crosses the placenta readily and, when injected to the mother, increases thyroid hormone concentrations in the fetus. Because thyroid hormones stimulate fetal lung maturation, maternal TRH treatment to decrease neonatal respiratory distress syndrome has been attempted, but without successful outcome.[43]

Because immunoglobulins of the IgG type cross the placenta, transient fetal/neonatal hyperthyroidism from transplacental transfer of TSH-receptor activating antibodies can occur in women with past or present Graves disease. On the other hand, when pregnant women are overtreated with antithyroid drugs, which also cross the placenta readily, their fetus may develop goitrous hypothyroidism, as noted previously. However, in the Québec database for neonatal thyroid screening, only one case out of 30,000 births is attributable to this mechanism (unpublished observations). Lastly, transient neonatal hypothyroidism from materno-fetal transfer of TSH-receptor–blocking antibodies can also occur, but only accounts for 2% of cases of neonatal hypothyroidism identified by screening[44]; hence, for babies born to women with Hashimoto thyroiditis, a specific screening strategy is not required. However, thyroid function tests are often ordered clinically in newborns whose mother has a history of thyroid disease and, if abnormal, require special consideration of optimal approach to therapy. For example, hypothyroidism at birth caused by treatment of maternal Graves disease with antithyroid medication may only require observation, in the expectation that the effects of the drugs will dissipate over a few days; hyperthyroidism may follow, albeit exceptionally. For hypothyroidism or hyperthyroidism resulting respectively from maternal TSH receptor blocking or stimulating antibodies, treatment will be required as these effects may last several months.[45]

Maturation of Thyroid Hormone Synthesis and Secretion

Maturation of thyroid function in the fetus reflects changes at the level of the hypothalamus, pituitary, and thyroid. Serum TRH concentrations are relatively high in the human fetus, because it is produced at both hypothalamic and extrahypothalamic sites and because of the TRH-degrading activity of fetal blood is low.[46] Fetal serum TSH increases from a low concentration at 18 to 20 weeks to a peak value of approximately 7 to 10 mU/L at term. After delivery, in response to exposure to the cold extrauterine environment, there is an acute release of TSH with mean serum concentrations peaking at 30 minutes at approximately 70 mU/L. The subsequent increase in serum T4 concentration immediately after birth is TSH dependent.

Only free thyroid hormones enter cells, and hormones bound to serum TBG and other transport proteins are not available to tissues. In addition, T4 is a prohormone and it is T3 that is biologically active to exert intracellular effects, so that deiodination of T4 is essential for tissue euthyroidism. Both serum transport

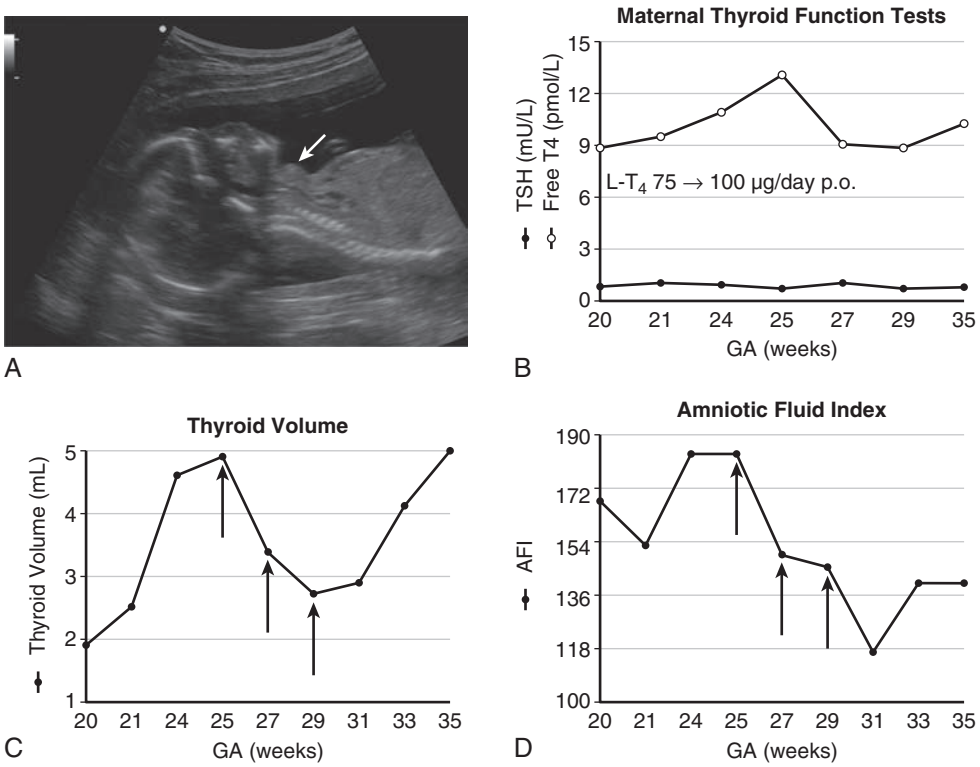


FIGURE 7-2 ■ **A,** Fetal goiter discovered at a routine ultrasound at 19 weeks (white arrow). The mother was euthyroid and had no autoantibodies. Cordocentesis showed a serum TSH of 90 mU/L. **B,** initial management was through oral treatment of the mother with increasing doses of levothyroxine; this resulted in increased maternal free T4 concentrations, but presumably not enough to cross the placenta in sufficient amounts to prevent the progression of the fetal goiter (panel C) and the development of hydramnios (panel D). **C,** Effect of 3 intra-amniotic injections of 200 μg of levothyroxine (black arrows) on fetal thyroid size. **D,** Effect of three intra-amniotic injections of 200 μg of levothyroxine (black arrows) on amniotic fluid index. At birth, cord serum TSH was 224 mU/L and thyroglobulin was low at 3.55 μg/L; thyroglobulin deficiency was confirmed by molecular genetic analyses. Treatment with 50 μg of levothyroxine orally from day 1 has allowed this child, now age 4 years, to have normal intellectual development. (Adapted and updated from Stoppa-Vaucher S, Francoeur D, Grignon A, et al. [2010]. Non-immune goiter and hypothyroidism in a 19-week fetus: a plea for conservative treatment. J Pediatr 156:1026-1029.)

proteins and intracellular deiodination change during development. As previously mentioned, the fetal thyroid gland is capable of iodine concentration and iodothyronine synthesis as early as 70 days of gestation, a reflection of a sharp increase in the expression of the sodium-iodine symporter and of the appearance of a follicular architecture.[47] Starting at 18 to 20 weeks, total T4 and TBG concentrations in fetal serum increase steadily until the final weeks of pregnancy.

The study of free T4 concentrations in fetal/neonatal blood has been hampered by the relative inadequacy of the commercially available immunoassay systems for measurements in these samples.[48] The fetal serum T3 concentration remains low until 30 weeks due to two factors: first, the low activities of type 1 iodothyronine monodeiodinase result in relatively low rates of T4 to T3 conversion in fetal tissues; second, active type 3 monodeiodinase in placenta and selected fetal tissues degrades T3 to T2. After 30 weeks, serum T3 increases slowly until birth. This prenatal increase in serum T3 is due to progressive maturation of liver type 1 deiodinase activity increasing hepatic conversion of T4 to T3, and to decreased placental T3 degradation. Postnatally, T3 and T4 serum concentrations increase two- to sixfold within the first few hours, peaking on the second day of life. These levels then gradually decline to levels characteristic of infancy over the first 4 to 5 weeks of life (Figures 7-3 and 7-4).

In the human, the fetal thyroid gland grows and its production increases under the influence of the increasing serum TSH level during the second half of gestation, as illustrated by the severely atrophic and hypofunctional glands observed in newborns with mutations that inactivate either the β-subunit of TSH[49] or the TSH receptor.[50] On the other hand, the maturation of the negative feedback control system appears to occur earlier than previously thought, as an elevated TSH in serum obtained at cordocentesis can be seen in fetuses with primary hypothyroidism as early as 18 weeks.[51]

Thyroid function in the premature infant (before 30 to 32 weeks) is characterized by low circulating concentrations of T4 and free T4, a normal or low concentration of TSH, and a normal or prolonged TSH response to TRH, suggesting a degree of relative hypothalamic (tertiary) hypothyroidism. In summary, fetal thyroid hormone secretion results from increasing hypothalamic TRH secretion that stimulates the secretion of TSH from the pituitary and increased thyroid follicular cell sensitivity to TSH. In turn, this process is regulated by increasing pituitary sensitivity to inhibition by thyroid

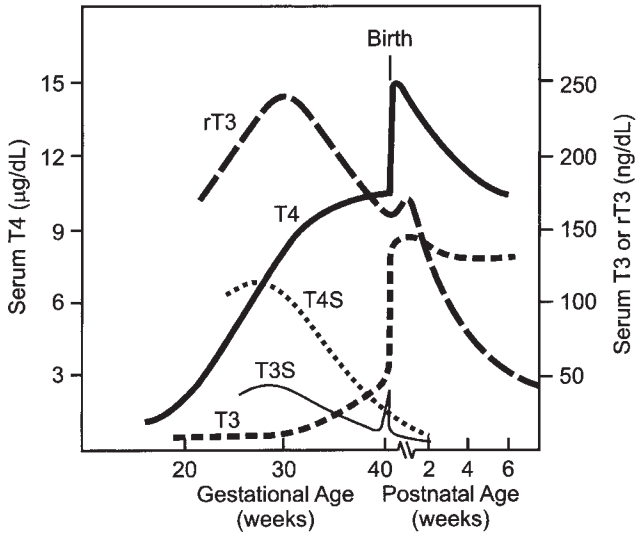


FIGURE 7-3 ■ The pattern of change in fetal and neonatal thyroid function parameters during pregnancy and extrauterine adaptation. Fetal serum thyroxine (T4) concentrations begin to increase at midgestation and increase progressively thereafter to term. This increase is due largely to the increase in thyroxine-binding globulin concentration, but free T4 concentrations (not shown) also increase progressively between 20 and 40 weeks. T4 in the fetus is metabolized predominantly to inactive reverse triiodothyronine (rT3) and sulfated analogs (T4S, T3S). Monodeiodination of T4 to active triiodothyronine (T3) increases at about 30 weeks to levels approximating 50 ng/dL at term. The TSH surge (not shown), which peaks at 25 to 30 minutes after extrauterine exposure, stimulates thyroidal T4 and T3 secretion. Neonatal T4 and T3 peak at 2 to 3 days. Serum T3 concentrations remain at higher postnatal levels because of the increased T4-to-T3 conversion mediated by increased type 1 iodothyronine deiodinase activity in newborn tissues.

hormone of TSH release. The marked cold-stimulated TRH-TSH surge at birth is associated with a marked increase in T4 secretion and free T4 concentration with a new equilibrium reached by 1 to 2 months. During infancy and childhood, there is a progressive decrease in T4 secretion rate (based on a μg/kg/day) correlating with a decreasing metabolic rate.[52]

Maturation of Thyroid Hormone Metabolism and Transport

The thyroid gland is the sole source of T4. Most of the circulating T3 after birth is derived from conversion of T4 to T3 via monodeiodination in peripheral tissues. Deiodination of the iodothyronines is the major route of metabolism, and monodeiodination may occur either at the outer (phenolic) ring or the inner (tyrosyl) ring of the iodothyronine molecule. Outer ring monodeiodination of T4 produces T3, the form of thyroid hormone with greatest affinity for the thyroid nuclear receptor. Inner ring monodeiodination of T4 produces reverse T3 (rT3), an inactive metabolite. In adults, between 70% and 90% of circulating T3 is derived from peripheral conversion of T4, and 10% to 30% from direct glandular secretion. Nearly all circulating rT3 derives from peripheral conversion, with only 2% to 3% coming directly from the thyroid gland. T3 and rT3 are progressively metabolized to diiodo, monoiodo, and noniodinated forms of thyroxine, none of which possess biologic activity.

Two types of outer ring iodothyronine monodeiodinases have been described.[53] Type 1 deiodinase (predominantly expressed in liver, kidney, and thyroid) is a high-Km enzyme inhibited by propylthiouracil and stimulated by thyroid hormone. Type 2 deiodinase (predominantly located in brain, pituitary, placenta, skeletal muscle, heart, thyroid, and brown adipose tissue) is a low-Km enzyme insensitive to propylthiouracil and inhibited by thyroid hormone. Types 1 and 2 deiodinases contribute to circulating T3 production, whereas type 2 acts to increase local tissue levels of T3 as well. An inner ring deiodinase (type 3 deiodinase) has been characterized in most fetal tissues, including placenta. This enzyme system catalyzes the conversion of T4 to rT3 and T3 to diiodothyronine. All three deiodinase enzymes are selenoproteins (Figure 7-5).

Deiodination is developmentally and thyroid-state regulated. In the human fetal brain, type 2 deiodinase activity in the cortex increases between 13 and 20 weeks gestation and by about 50% over the last third of gestation. There is a general inverse correlation of type 2 and type 3 activities. Both of these deiodinase species are thyroid hormone responsive.

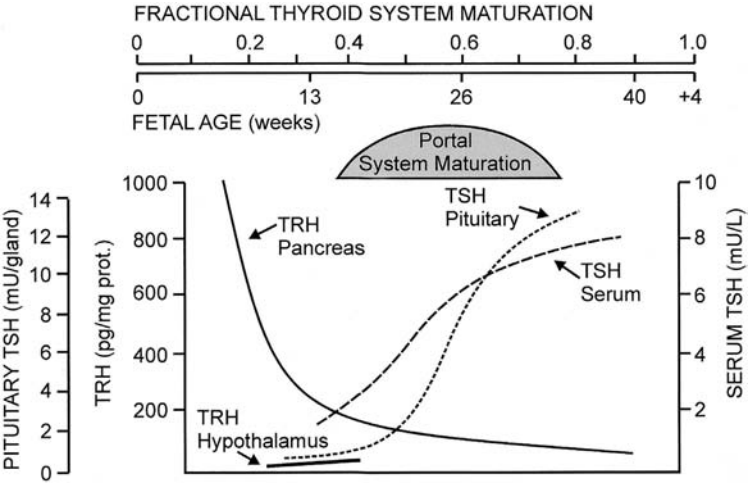


FIGURE 7-4 ■ Changes in fetal TRH and TSH levels in pancreas, hypothalamus, serum, and pituitary during human gestation. Hypothalamic TRH concentrations increase progressively after midgestation, but the pattern of change has not been documented in the human fetus. (Reproduced with permission from Fisher DA, Polk DH [1994]. Development of the fetal thyroid system. In Thorburn GD, Harding R [eds.], *Textbook of fetal physiology*. Oxford, UK: Oxford University Press, 359-368.)

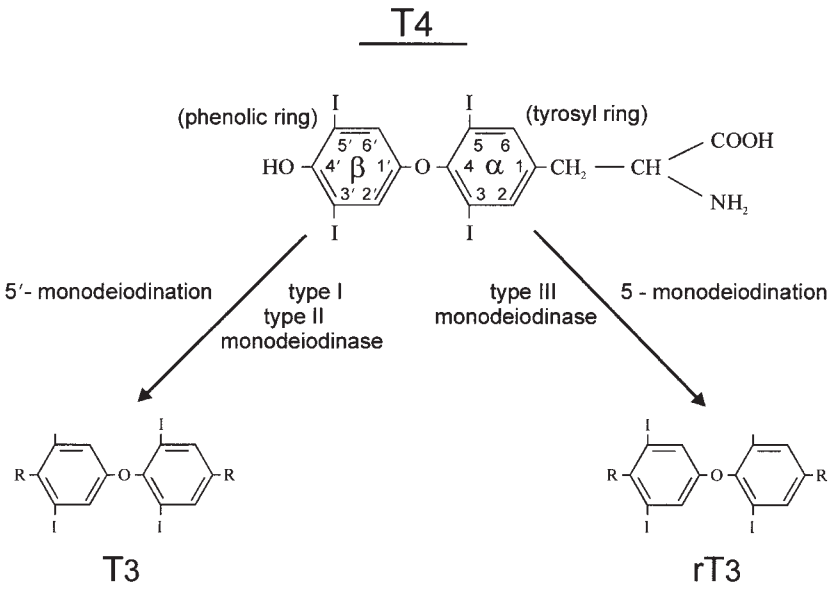


FIGURE 7-5 ■ The deiodination of thyroxine by types 1, 2, and 3 iodothyronine monodeiodinase enzymes. The type 1 enzyme is also capable of inner ring monodeiodination, particularly of the sulfated conjugates (not shown).

Fetal thyroid hormone metabolism is characterized by a predominance of type 3 enzyme activity (particularly in liver, kidney, and placenta), accounting for the increased circulating concentrations of rT3 observed in the fetus. However, the persistence of high circulating rT3 concentrations for several weeks in the newborn indicate that type 3 deiodinase activity expressed in nonplacental tissues is important. The mixture of type 2 and type 3 deiodinase activities in the placenta provides for the conversion of T4 to T3 and of T4 and T3 to rT3 and T2, respectively.

Sulfated iodothyronines are the major thyroid hormone metabolites circulating in the fetus.[54] Sulfokinase enzymes are present early in fetal life, and sulfation of the phenolic hydroxyl group of the iodothyronine molecule may be a normal prerequisite step for monodeiodination. The sulfated iodothyronines are preferred substrates for the type 1 deiodinase, and concentrations are high in fetal serum in part because of low type 1 deiodinase activity. However, increased production of sulfated metabolites is also involved. There is evidence that T3S has biologic activity (i.e., suppresses TSH in vivo), suggesting that it can be de-sulfated by one or more tissue sulfatase enzymes. The low production rates and low levels of T3 metabolites and the high ratio of inactive to active metabolites suggest that fetal thyroid hormone metabolism is largely oriented to inactivating T4, presumably to avoid tissue thermogenesis and to potentiate the anabolic state of the rapidly growing fetus. This is mediated by early activation of type 3 monodeiodinase, inactivation of type 1 monodeiodinase, and augmented iodothyronine sulfation.

The developmental expression of type 2 deiodinase in brain and other tissues provides for local T3 supply to specific tissues (particularly in the event of T4 deficiency) and helps guarantee provision of T3 during gestation, when brain development is thyroid hormone dependent.[39]

Extrauterine Thyroid Adaptation

At the time of parturition, the neonate must rapidly convert from the fetal state of predominant thyroid hormone inactivation to a state of relative thyroidal hyperactivity. During the first hours after birth, there is an acute increase in circulating T4 and T3 levels. This is due to the abrupt increase in hypothalamic TRH stimulating increased pituitary TSH secretion and, in turn, thyroid hormone secretion. The cold-stimulated TRH-TSH surge is short lived and mean TSH concentrations decrease progressively to normal infant levels by 3 to 5 days, but the serum-free T4 level remains elevated for several weeks.[55]

Serum T3 levels increase in response to the TSH surge, because of stimulation of thyroidal T3 secretion and of increased hepatic type 1 deiodinase activity. Placental separation decreases T3 deiodination (to inactive T2), contributing to the early postnatal increase in serum T3 concentration. The type 2 deiodinase activity in brown adipose tissue increases during the last weeks of gestation to potentiate catecholamine-stimulated brown adipose tissue thermogenesis, thereby contributing to the maintenance of the body temperature of the neonate.[53]

Thyroid Hormone Actions

Evidence suggests that all thyroid-sensitive cell populations express iodothyronine membrane transporters. These belong to several families of integrin, organic anion, amino acid, and monocarboxylate solute carriers. The importance of these transporters is highlighted by the role of mutations inactivating human monocarboxylate transporter 8 (MCT8) in an X-linked syndrome of severe psychomotor retardation (previously called the Allan-Herndon-Dudley syndrome) combined with mild abnormalities of thyroid function, characterized by high T3, low T4, and normal or high TSH.[56,57] MCT8 is thought to play a role in the entry of T3 into neurons,

after deiodination of T4 to T3 in neighboring astrocytes. In addition, MCT8 is involved in the transfer of T3 across the blood-brain barrier. Lastly, the abnormalities in thyroid hormone levels and TSH are also due to the effect of MCT8 on deiodination.[58]

Thyroid hormone effects are mediated predominantly via nuclear thyroid hormone protein receptors (TR), which act as DNA-binding transcription factors regulating gene transcription. Two mammalian genes code for TR, TRα and TRβ, and alternative mRNA splicing lead to the production of four major thyroid hormone-binding transcripts: TRα1, TRα2, TRβ1, and TRβ2. The TRs exist as monomers, homodimers, and heterodimers with other nuclear receptor family members such as the retinoid X receptors. TRα1 is the predominant subtype in the bone, gastrointestinal tract, heart, and brain. TRβ1 is expressed in liver, kidney, heart, lung, brain, cochlea, and pituitary. TRβ2 is expressed in pituitary gland, retina, and cochlea. The receptors function redundantly, as indicated by knockout studies in mice, but predominant effects of one or another TR have been described.

In humans, the specific roles of TRα and TRβ are illustrated by the phenotypes observed in patients with inactivating mutations in the corresponding genes. The syndrome of thyroid hormone resistance initially described in 1967 was later found to be generally due to mutations inactivating TRβ that occur either *de novo* or are inherited in an autosomal dominant fashion; however, in some patients with thyroid hormone resistance, TRβ is normal and the molecular defect remains elusive.[59] Mutations in TRα have been described, occurring de novo in one patient[60] and transmitted from father to daughter in one pedigree.[61]

Thyroid hormone-programmed development of fetal tissues requires the interaction of local tissue monodeiodinase 1 and 2, TRs, thyroid receptor coactivators, and thyroid-responsive genes. In most responsive tissues, the timing of maturation events is controlled by the TRs acting as a molecular switch. In the absence of T3, the unliganded receptor recruits corepressors, thereby repressing gene transcription. Local tissue maturation events are stimulated by the coincident availability of T3, liganded T3 receptor, T3-mediated receptor exchange of corepressors with coactivators, and activation of responsive gene transcription.[62]

In the human fetus, low levels of TR binding have been detected in brain tissue at 10 weeks gestational age—and liver, heart, and lung TR binding is observed at 16 to 18 weeks. TR levels in human fetal cerebral cortex and cerebellum increase markedly during the second and third trimesters.[63] Information is limited regarding the timing of the appearance of thyroid hormone tissue effects in the human fetus.

The birth length of the athyroid human neonate is within normal limits: the linear growth of the human fetus is programmed independently of thyroid hormones by a complex interplay of genetic, nutritional, and hormonal factors, as well by the mechanical uterine constraint.[64] However, 50% to 60% of athyroid newborns manifest delayed epiphyseal maturation and have large fontanelles.[65] At birth, severe CH may be suspected in the presence of a large anterior fontanelle, a persistent posterior fontanelle at term with diastasis of the parietal bones, macroglossia and umbilical hernia; delayed meconium emission, feeding difficulties, and prolonged jaundice may be noted soon thereafter.[66] However, the classic clinical manifestations of CH appear progressively during the early months of life. These include soft-tissue myxedema, a slow linear growth, and retarded central nervous system (CNS) development.[64] The normal IQ in most athyroid infants treated early thanks to newborn screening[1] seems attributable to the low but significant levels of maternal thyroxine derived from placental transfer, shown in humans,[28] and the upregulation of type 2 monodeiodinase in fetal brain tissue in the face of low fetal serum thyroxine concentrations, shown in rats.[39]

Postnatal thermogenesis is mediated via the brown adipose tissue prominent in subscapular and perirenal areas in the mammalian fetus and neonate. Heat production in brown adipose tissue is stimulated by catecholamines via β-adrenergic receptors and is thyroid hormone dependent. The uncoupling protein thermogenin unique to brown adipose tissue is located on the inner mitochondrial membrane and uncouples phosphorylation by dissipating the proton gradient created by the mitochondrial respiratory chain. The type 2 monodeiodinase in brown adipose tissue mediates local T4 to T3 conversion. Full thermogenin expression in brown adipose tissue requires both catecholamine and T3 stimulation.[67] Brown adipose tissue matures progressively in the fetus but remains thermoneutral until stimulated by catecholamines in the perinatal period. Brown adipose tissue thermogenesis is immature in small premature infants, and brown adipose tissue mass decreases in the neonatal period in full-term infants as the capacity for nonshivering thermogenesis develops in other tissues.[68] Uncoupling protein-2 is found in many tissues, but it does not appear to be regulated by β-adrenergic agonists or thyroid hormone. Uncoupling protein-3 is expressed in muscle and white adipose tissue as well as brown adipose tissue. Muscle uncoupling protein-3 is regulated by β3-adrenergic stimulation and thyroid hormone and presumably contributes to nonshivering thermogenesis in humans. mRNA levels for uncoupling protein-3 are also regulated by dexamethasone, leptin, and starvation, but the regulation differs in brown adipose tissue and muscle. Starvation increases muscle and decreases brown adipose tissue uncoupling protein-3, suggesting that muscle serves a larger role in thermoregulation during starvation.[69]

The critical role of thyroid hormones in CNS maturation has long been recognized. Nervous system development involves neurogenesis, gliogenesis, neural cell migration, neuronal differentiation, dendritic and axonal growth, synaptogenesis, myelination, and neurotransmitter synthesis. Thyroid hormones have been shown to stimulate a number of developmentally regulated nervous tissue genes, but the role of these factors in the CNS developmental program remains undefined. Available evidence suggests that deficiency or excess of thyroid hormones alters the timing or synchronization of the CNS developmental program, presumably by initiating critical gene actions or other genetic CNS maturation events.[70]

There is increasing evidence for a critical period for thyroid-dependent brain maturation in utero. Early maternal hypothyroxinemia in the rat alters histogenesis and cerebral cortical architecture of the progeny.[71] As noted earlier, maternal hypothyroxinemia in humans has been reported to be associated with IQ reduction in offspring,[31-33] but association does not mean causation as shown by the negative results of the randomized controlled trial of T4 treatment.[34] In contrast, iodine supplementation in pregnant women in geographic areas of severe iodine deficiency improves developmental outcome of the offspring if given before the third trimester of gestation.[24] The reasons for the discrepancy between the effect of iodine in iodine-deficient women and the lack of effect of T4 in hypothyroid women are unknown. Studies of the dose and timing of thyroid hormone therapy in infants with CH suggest a second critical period of thyroid hormone action during the very early neonatal period,[72] but the period of CNS thyroid hormone dependency extends further, to at least 2 years of age.[73]

CONGENITAL HYPOTHYROIDISM

Newborn Screening

CH screening tests are usually carried out in dried blood spot samples collected via skin puncture, although a few programs use cord serum.[74-76] The historical controversy between the proponents of using total T4[77] or TSH[78] as the primary test was related in part to the greater precision of T4 measurements around the cutoff value, but this is no longer an issue. Because primary hypothyroidism is at least 10-fold more common than central hypothyroidism and because only 19% of cases of central hypothyroidism have T4 below the cutoff,[6] primary TSH screening makes more sense. About half of the states in the United States and the Netherlands still use a primary T4 strategy, but, in effect, all are screening for elevated serum TSH concentrations as the secondary screening test. In the Dutch program, for instance, TSH is measured in almost half of the population: all those with a T4 below 0.8 SD *and* all infants born with a weight $\leq$ 2.5 kg or a gestational age of $\leq$ 36 weeks.[79] Conversely, the Québec program uses T4 to sort cases with borderline TSH elevation (15 to 30 mU/L) as shown on the algorithm in Figure 7-6.

With the trend toward earlier discharge of newborns in the 1990s, the minimal age at which TSH screening should be performed was assessed: in the first 24 hours, 9% of the population had a TSH above 15 mU/L (a reflection of the neonatal surge), but this percentage dropped precipitously to only 0.2% to 0.3% from days 2 to 5. Thus, any sample collected after 24 hours is acceptable.[80]

A more recent controversy relates to the threshold value for a significant TSH elevation, with cutoffs varying from 6 to 20 mU/L of whole blood.[81] Predictably, the lower the cutoff, the greater the number of children labeled as having CH. However, the additional cases identified have mostly functional disorders whose impact on cognition and behavior, if left untreated, is unknown.[82]

False-negative results are very rare and most are due to errors in specimen handling, testing and data analysis, or reporting of results. Some jurisdictions obtain screening samples in addition to the initial one in low-birth-weight infants, based on observations of a delayed TSH rise in some.[83,84] The mechanisms for such delayed rises are unknown, but the widespread use of dopamine infusions, a potent inhibitor of TSH secretion, in the first few days of life may play a role.[85] An update on a subset of

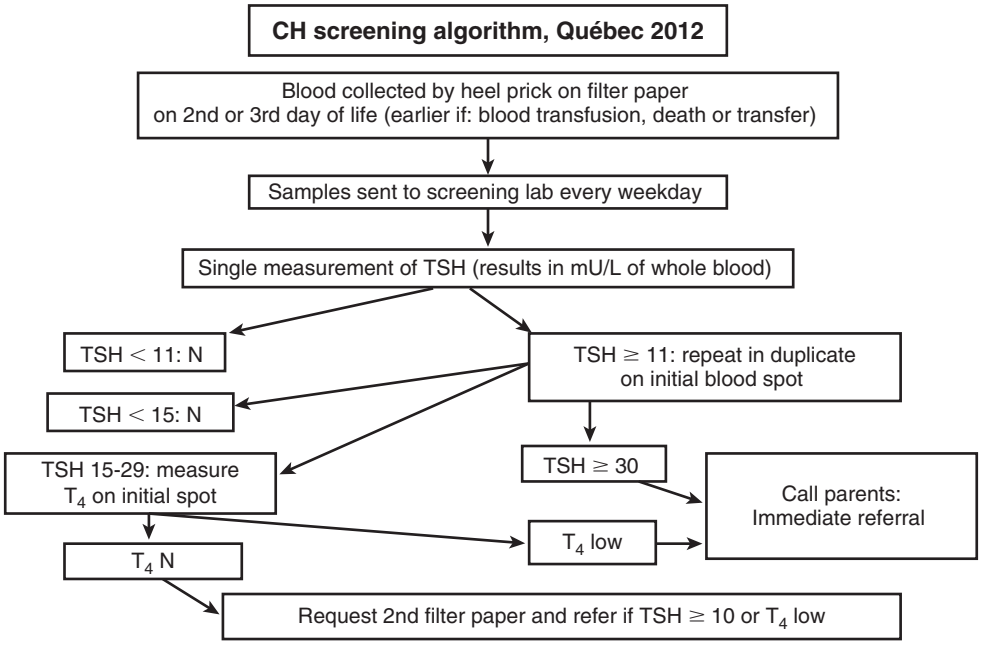


FIGURE 7-6 ■ Algorithm presently used in Québec for screening newborns for hypothyroidism.

low-birth-weight newborns with a delayed TSH rise has shown that the problem was transient, with no evidence of benefit from treatment.[86] Thus, the repeat screening policy in low-birth-weight newborns remains debatable.[87] By contrast, the case for a repeat screening policy in same-sex twins risk is stronger, as fetal blood mixing may lead to a falsely negative screening TSH in the affected member of a monozygotic twin pair.[88]

As is obvious from the previous discussion, estimating the prevalence of CH depends on the ascertainment method. Before population-based biochemical screening of neonates, it was 1 in 6700 births.[89] Over the almost four decades of the screening era, it has increased progressively from 1 in 4000 to 1 in 2500 with our current screening algorithm [82] or even 1 in 1500 in some jurisdictions.[90,91] Geographical and temporal variations are due almost exclusively to differences or changes in screening algorithms.[92] In keeping with this concept, permanent CH due to thyroid dysgenesis documented by radionuclide imaging is not increasing, whereas the prevalence of dyshormonogenesis, a group of autosomal recessive conditions, is predictably higher in inbred populations[93] or in those with a founder effect.[94] Interestingly, the introduction of folic acid fortification, which has resulted in a marked decrease in the prevalence of neural tube defects[95] and of some heart malformations,[96] has not affected the prevalence of CH due to thyroid dysgenesis in the same jurisdiction.[82] Universal newborn screening has also provided estimates of the relative birth prevalence of various congenital thyroid disorders at the population level (Table 7-1).

TABLE 7-1 **Estimated Birth Prevalence of Various Types of Permanent Thyroid Disorders in Iodine-Sufficient Populations, Based on Universal Newborn Screening with Either TSH or T4**

Type of Permanent Congenital Disorder	Estimated Birth Prevalence
Thyroid dysgenesis	
—Ectopy	1:5,000
—Athyreosis	1:15,000
Thyroid dyshormonogenesis	1:30,000
Hypothalamic-pituitary hypothyroidism	1:21,000
Thyroid hormone resistance	1:40,000

From Deladoey J, Ruel J, Giguere Y, Van Vliet G (2011). Is the incidence of congenital hypothyroidism really increasing? A 20-year retrospective population-based study in Quebec. J Clin Endocrinol Metab 96:2422-2429; Kempers MJ, Lanting CI, van Heijst AF, et al. (2006). Neonatal screening for congenital hypothyroidism based on thyroxine, thyrotropin, and thyroxine-binding globulin measurement: potentials and pitfalls. J Clin Endocrinol Metab 91:3370-3376; Lafranchi SH, Snyder DB, Sesser DE, et al. (2003). Follow-up of newborns with elevated screening T4 concentrations. J Pediatr 143:296-301; Tajima T, Jo W, Fujikura K, et al. (2009). Elevated free thyroxine levels detected by a neonatal screening system. Pediatr Res 66: 312-316.

Thyroid Dysgenesis

Dysgenesis is defined as abnormal organogenesis, a process that occurs during the embryonic period. The most common developmental defect, currently accounting for about half of CH cases,[82] is a defect in the migration of the median anlage, resulting in an ectopic (sub)lingual thyroid. These ectopic thyroids are round structures, lacking lateral lobes, and are the only thyroid tissue present in affected individuals. They can only be unequivocally visualized by radionuclide scanning.[97] In almost 10% of cases, there is a dumbbell appearance, with two adjacent areas of ectopic tissue.[98] Histologically, ectopic thyroids are fully differentiated with a normal follicular architecture. Thus, the associated hypothyroidism, which is variable in severity, is likely due to a smaller number of cells, due to the absence of lateral lobes, and to a limitation to TSH-induced cell growth.[99] The second category of thyroid dysgenesis, athyreosis, is heterogeneous. Indeed, about half of newborns with CH and undetectable technetium uptake on imaging have a detectable serum thyroglobulin.[100] We have proposed to call this situation "apparent athyreosis." Apparent athyreosis can be permanent in patients with a severely hypoplastic and hypofunctional orthotopic thyroid due to mutations that completely inactivate the TSH receptor.[50] The term *transient athyreosis* has been used to describe the absent uptake that can be observed in babies with CH due to the transplacental transfer of TSH receptor-blocking antibodies.[101]

Thyroid dysgenesis is usually sporadic but familial cases occur more often than by chance alone.[102] On the other hand, monozygotic twins are almost always discordant.[88] To reconcile these seemingly discrepant findings, a two-hit model combining germ-line susceptibility and early postzygotic mutations has been proposed,[22] but its validation requires access to ectopic thyroids, which generally do not need to be removed. The limited data available seem to exclude somatic mutations in TTF-1, TTF-2, and PAX-8[99] and suggest the implication of the Wnt-beta catenin pathway in thyroid migration.[103]

Also inconsistent with simple Mendelian genetics is the observation that thyroid dysgenesis is about twofold more prevalent in female than in male infants. In most series, the skewing of the sex ratio is more pronounced in ectopy than in athyreosis.[104] Thyroid dysgenesis is less prevalent in blacks, who are more genetically diverse than Caucasians, and this supports an oligogenic first-hit susceptibility.[105] In some of the rare multiplex families, mutations in TTF-1, TTF-2, or PAX8 can be found but these genes have been excluded by linkage analysis in other multiplex families.[106] TTF-2 mutations appear to be the least common, having been found in the homozygous state in only three consanguineous pedigrees in which probands present with true athyreosis and cleft palate, sometimes associated with kinky hair, choanal atresia, and a bifid epiglottis.[107] TTF-1 heterozygous mutations, de novo or dominantly inherited, present with a spectrum of anomalies, the "brain-lung-thyroid syndrome," in which CH is usually mild and associated with normal thyroid gland morphology[108,109] but may rarely be severe with apparent athyreosis.[110] Heterozygous

mutations in PAX8 can occur de novo or be inherited in an autosomal dominant fashion.[111] They have been associated with hypothyroidism of greatly variable severity and age of onset, even within the same pedigree, with either apparent athyreosis or orthotopic thyroid hypoplasia. In spite of the ubiquitous expression of PAX8, the phenotype is generally limited to hypothyroidism.[112] Lastly, mutations of another transcription factor (GL1S3) have been described as the cause of a rare syndrome of CH with apparent athyreosis or normal thyroid anatomy, neonatal diabetes, congenital glaucoma and deafness, liver, kidney, and pancreas abnormalities.[113,114] Thus, all transcription factor mutations in humans have been found to cause true athyreosis (TTF2), apparent athyreosis, or orthotopic thyroid hypoplasia (TTF1, PAX8, GLIS3) (Table 7-2) but thyroid ectopy, the most common form of thyroid dysgenesis, remains unexplained.

Newborns with trisomy 21, as a group, have a slight skewing of their T4 distribution toward low values and of TSH toward higher values.[115] However, these shifts are very subtle so that TSH rarely exceeds screening cutoffs. In fact, not a single case of CH was found in a newborn with trisomy 21 over 20 years in Québec (1.6 million births), so that previous statements that the prevalence of CH is markedly increased in trisomy 21 are clearly unfounded.[82]

An increased prevalence of extrathyroid abnormalities has consistently been found in several large series; specifically, defects in heart septation are observed in about 5% of dysgenetic CH cohorts.[104,116,117] The molecular mechanisms underlying this association are unknown. These septal defects are usually mild and close spontaneously so that, beyond a careful clinical examination, they do not need to be searched for by other tests.

Overt CH due to defects in TSH binding/action resulting in severe thyroid hypoplasia at birth[18] represents a developmental disorder (growth being part of development), and we therefore think it is more appropriate to discuss this entity under dysgenesis rather than under dyshormonogenesis. Severe TSH resistance was postulated in 1968[118] and the molecular characterization of the TSH receptor, a member of the family of G-protein–coupled receptors,[119] has led to the identification of homozygote or compound heterozygote loss-of-function mutations of the TSH receptor gene in several patients with this phenotype.[18,50,120] However, the phenotype resulting from biallelic TSH receptor inactivating mutations is generally milder.[121,122] When only one allele bears a mutation inactivating the TSH receptor, carriers are typically normal as expected in traditional autosomal recessive transmission, although some may have asymptomatic hyperthyrotropinemia.[123] Likewise, TSH resistance from defects in the Gs subunits, as seen in pseudohypoparathyroidism, is typically mild (although it may be picked up on neonatal screening[124]), and these patients usually maintain normal serum T4 concentrations.

Thyroid Dyshormonogenesis

General Features

The thyroid gland can be envisioned as an iodine pump coupled to an assembly line destined to return iodine to the bloodstream in a hormonally active form.[125] The major substrates for thyroid hormone synthesis are iodine and tyrosine. Tyrosine is not rate limiting even in individuals with phenylketonuria, in whom tyrosine becomes an essential amino acid. Iodine, by contrast, is a trace element that can be rate limiting in thyroid hormone synthesis. The process of thyroid hormone biosynthesis is initiated by TSH binding to the follicular cell TSH receptor and cAMP activation.[126]

Processes stimulated by cAMP include cell membrane iodine transport, thyroglobulin synthesis, oxidation and organification of trapped iodine, activation of colloid endocytosis and intracellular phagolysosome formation, hydrolysis of thyroglobulin to release the iodotyrosines (monoiodotyrosine [MIT] and diiodotyrosine [DIT]) and iodothyronine (T4 and T3) residues, deiodination of MIT and DIT by a dehalogenase leading to intracellular iodine recycling, and release of T4 and T3 into the circulation.[127] Significant amounts of thyroglobulin

TABLE 7-2 **Thyroid and Extrathyroid Phenotypes Associated with TSHR and Transcription Factor Mutations and Mode of Inheritance**

Thyroid Phenotype	Other Features	Gene	Transmission
From apparent athyreosis to normally appearing gland	None	*TSHR*	AR
From apparent athyreosis to normal gland, usually mild ↑ TSH	RDS Developmental delay/hypotonia Ataxia/choreoathetosis	*TTF-1*	De novo or AD
True athyreosis	Cleft palate Choanal atresia Kinky hair Bifid epiglottis	*TTF-2*	AR
From apparent athyreosis to normally appearing gland	Cysts within thyroid remnants	*PAX-8*	AD or de novo
From apparent athyreosis to normally appearing gland	Congenital glaucoma Deafness Liver, kidney, and pancreas abnormalities	*GLIS3*	AR

RDS, respiratory distress syndrome; TTF, thyroid transcription factor; AR, autosomal recessive; AD, autosomal dominant.

also escape from the gland, predominantly via the thyroid lymphatic system. These events are summarized in Figure 7-7. Infants with CH due to dyshormonogenesis leading to goiter constitute some 10% to 15% of newborns with CH.

The causes of thyroid dyshormonogenesis include decreased iodine trapping, defective organification of trapped iodine, abnormalities of thyroglobulin structure, and deficiency of iodotyrosine deiodination and recycling. With few exceptions, these disorders are transmitted as autosomal recessive traits.[128] Except for the familial incidence and tendency for affected individuals to develop goiter, the clinical manifestations of CH due to a biochemical defect are similar to those in infants with thyroid dysgenesis. Thyroid enlargement may be manifest at birth or even before, but in many patients development of the goiter is delayed. From surveys of the specific molecular defects, it appears that mutations in thyroperoxidase are the most common cause of thyroid dyshormonogenesis.[129] However, identifying the specific disorder in an individual patient is not required as it does not affect management: a risk of 25% in siblings of affected subjects can be given empirically, treatment is lifelong except in DuOX2 deficiency (discussed later), and surveillance for the development of multinodular goiter, a frequent occurrence, is required.

Sodium-Iodine Symporter Defects

The transport of iodine across the thyroid follicular cell membrane from plasma to cytosol is the first step in thyroid hormone biosynthesis. Under normal circumstances, the thyroid cell membrane iodine pump generates a thyroid/serum (T/S ratio) concentration gradient in excess of 20 to 30. This gradient can reach several hundredfold when the thyroid gland is stimulated by a low-iodine diet, by TSH, by thyroid-stimulating immunoglobulins in Graves disease, or by drugs that impair the efficiency of hormone synthesis. Other tissues (such as the salivary glands, gastric mucosa, mammary glands, ciliary body, choroid plexus, and placenta) are also capable of concentrating iodine against a gradient. However, these tissues are not capable of organifying inorganic iodine.

The discovery of the gene encoding the sodium-iodine symporter (NIS; called *SLC5A5* and which has been mapped to chromosome 19) has allowed detecting the presence of mutations in 31 patients with a defect in iodine transport as of 2006. The patients present most frequently with neonatal or infantile hypothyroidism (which may rarely be transient) and the diagnosis is based on the presence of a goiter on clinical examination or ultrasound imaging contrasting with limited or absent uptake on scintiscanning. The timing of onset of hypothyroidism tends to correlate with the genotype-specific residual NIS activity and iodine intake. Neonatal onset is likely with mutations associated with lower radioactive iodine uptake. Importantly, infantile onset after normal neonatal TSH screening has been reported, sometimes with neurodevelopmental sequelae.[130]

Pendred Syndrome

Pendred syndrome, defined as congenital bilateral neurosensory deafness and goiter, is transmitted as an autosomal recessive trait. By some estimates, it is the cause of 10% of cases of congenital deafness.[131] The etiology of the deafness remains controversial but it is characteristically associated with dilated semicircular canals on CT scanning of the temporal bone (an abnormality named "Mondini's cochlea"). The thyroid phenotype is usually mild, appears to depend on nutritional iodine intake, and is seldom identified by neonatal TSH screening.[132,133] Over time, affected subjects may develop a goiter and subtle hypothyroidism.

One century after its clinical description, the gene responsible for this syndrome was discovered. This gene, *SLC26A4*, maps to chromosome 7, and the predicted

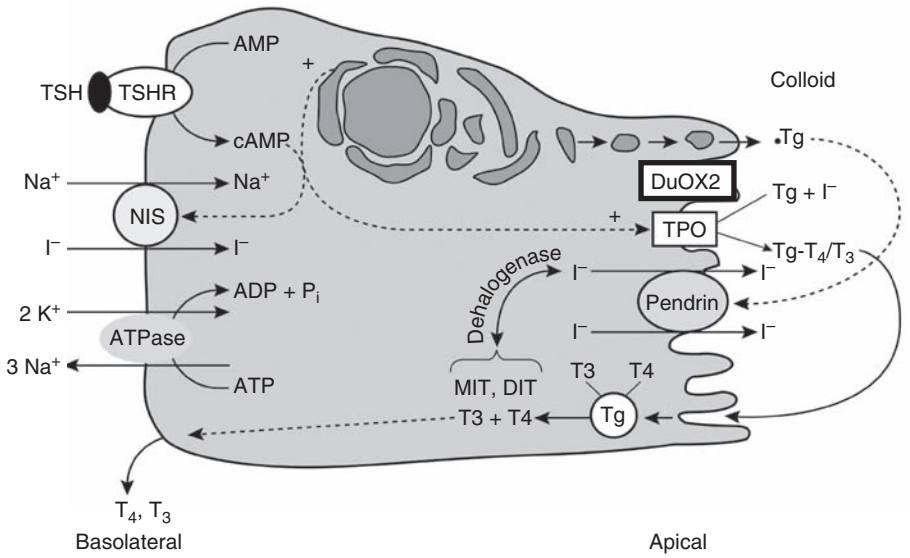


FIGURE 7-7 ■ Schematic representation of a thyroid follicular cell indicating all the important steps in thyroid hormone biosynthesis.

protein, named pendrin, is a multifunctional anion exchanger.[131] It is mainly expressed in the thyroid, the inner ear, and the kidney. In the thyroid, pendrin localizes to the apical membrane of thyrocytes, where it may be involved in mediating iodide efflux. In the inner ear, pendrin is important in the maintenance of a normal anion transport and of the endocochlear potential. Molecular studies in affected families from different geographic areas have shown independent point mutations and no large deletions.[134]

Thyroperoxidase Defects

Normally, iodide concentrated by the thyroid follicular cell is rapidly oxidized and bound in organic form. Organification of iodide involves two processes: oxidation of iodide and iodination of thyroglobulin-bound tyrosine. Trapped iodide is oxidized to an active intermediate, followed by iodination of thyroglobulin-bound tyrosyl residues to form the iodotyrosines MIT and DIT. Two DIT residues are "coupled" to form T4, while MIT and DIT couple to form T3. Tyrosyl iodination and coupling are catalyzed by a thyroid peroxidase enzyme system located at the apical membrane of the thyroid follicular cell in association with thyroid (NADPH) oxidases. Thyroid peroxidase (TPO) is a membrane-bound heme protein that requires peroxide and an acceptor, which in the normal thyroid gland is thyroglobulin but can be albumin or other proteins or peptides. The clinical presentation of thyroperoxidase deficiency is permanent goitrous CH with high uptake on scintiscanning and a high serum thyroglobulin. Historically, complete iodide organification defects were identified by a rapid and profound discharge of the thyroidal radioiodine trapped after administration of perchlorate (the "perchlorate discharge test").[127] This test is not specific for TPO deficiency (it can also be positive in Pendred syndrome and in DuOX2 deficiency, as discussed later) and clinical grade perchlorate is not available in all jurisdictions. Detecting mutations in the TPO gene will establish the diagnosis if this is deemed important, such as in atypical pedigrees due to uniparental disomy[135] or other mechanisms.[128]

The thyroid peroxidase gene is located on chromosome 2 and encodes a glycoprotein that is located at the apical membrane of the thyroid follicular cell. Since the 1990s, a variety of mutations have been described, including homozygous and compound heterozygous missense mutations, frame-shift mutations, base pair duplications, and single-nucleotide substitutions,[136,137] but no systematic genotype-phenotype correlations have been established. In about 17% of patients with a TPO deficiency phenotype, only one TPO mutations is found and the TPO deficiency may arise from the monoallelic expression of mutant TPO in the thyroid.[138]

Defects in H_2O_2 Generation

Since the first description of mutations inactivating DuOX2,[139] one of the enzymes generating H_2O_2 at the apical membrane, DuOX2 mutations have been found with increasing frequency.[127] It was initially suggested that monoallelic mutations led to transient CH while mutations on both alleles led to permanent CH. It is now clear that even patients with biallelic mutations can have a transient phenotype[140] and that their phenotypic variability, even within a family, is wide. It has been speculated that iodine intake is a possible disease modifier[141] but genetic variants in DuOX1, the other H_2O_2-generating enzyme in the thyroid (and also located on chromosome 15), may also play a role in the variable phenotype of DuOX2 deficiency.[140] By contrast, in a patient with a phenotype reminiscent of DuOX2 deficiency, the DuOX2 gene was normal but the gene encoding the "DuOX2 maturation factor" (that is required for translocation to the plasma membrane of functional DuOX2[142]) was mutated.[143] Other clinical observations show redundancy between the two DuOX enzymes and their respective maturation factors.[127]

Thyroglobulin Defects

Thyroglobulin is an essential substrate for organification and is the major protein component of the colloid in the thyroid follicular lumen. It is an iodinated glycoprotein with a molecular weight approximating 650,000 daltons. It consists of two monomeric chains, each with 67 tyrosine residues. About one third of the tyrosine residues are spatially oriented so that they are susceptible to iodination. The thyroglobulin gene is located on chromosome 8 and encodes a 300-kilodalton monomer molecule.[144]

Genetic defects can lead to thyroglobulin deficiency or structural/functional abnormalities of the protein. Goiter and hypothyroidism are usually manifest at birth or even before, and a low or undetectable serum thyroglobulin in this context points to thyroglobulin mutations. Interestingly, five of the cases of nonautoimmune fetal goiters in whom the gene defect was searched for have had thyroglobulin defects,[42,145-147] whereas a thyroperoxidase defect has been found in only one.[148] To date, 52 mutations in thyroglobulin have been described, leading to various defects including defective transport with carbohydrate-deficient thyroglobulin sequestered in Golgi or cytoplasmic membranes, thyroglobulin with deficient tyrosine residues or tyrosine residues buried within the molecule and not available for iodination, and sialic-acid–deficient thyroglobulin (due to a sialyltransferase deficiency) containing MIT and DIT but manifesting defective coupling.[149]

Dehalogenase Defects

Iodotyrosine deiodinase recycles the iodine contained in MIT and DIT as they are released from thyroglobulin within the thyroid follicular cell. Failure to deiodinate MIT and DIT leads to their being leaking out of the thyroid and being excreted in the urine. Iodotyrosine deiodinases are present in both thyroid cells and peripheral tissues, and abnormalities involving both systems have been described.

A clinical phenotype was originally described in the 1950s, combining hypothyroidism; goiters with early, rapid thyroid radioiodine uptake and rapid spontaneous discharge; and high serum and urine concentrations of MIT and DIT. The cloning of the gene encoding

iodothyronine dehalogenase (DEHAL1, IYD, located on chromosome 6) has led to the identification of mutations of the DEHAL1 gene in five consanguineous pedigrees. The variability of the phenotype between siblings carrying the same mutation in the homozygous state and in time of onset of hypothyroidism between pedigrees may be due to variation in iodine intake but appears to also involve yet unidentified modifier genes.[150-152] The development of the phenotype between ages 9 and 15 years in one patient with a single mutated allele is unexplained, but monoallelic expression in the thyroid was ruled out.[151] Importantly, some patients had intellectual disability as a consequence of early-onset, but not congenital, hypothyroidism.[150] This emphasizes that hypothyroidism should be looked for in any infant with suggestive signs, even if TSH was normal at neonatal screening.

Abnormal Thyroid Hormone Metabolism

Selenium Incorporation Defects

As discussed previously, iodothyronines are metabolized via three iodothyronine monodeiodinases (types 1, 2, and 3). To date, no defects have been identified in the genes encoding the enzymes themselves. All are selenoproteins requiring the incorporation of selenocysteine (sec) during biosynthesis. Incorporation of selenocysteine into the iodothyronine monodeiodinase enzymes requires several components: a selenocysteine insertion sequence (SECIS) element, a SECIS-binding protein (SECISBP2), and a selenoprotein-specific elongation factor (EFSec) and its transfer RNA $tRNA^{sec}$.

Homozygous or compound heterozygous mutations in the SECISBP2 gene on chromosome 9 have been reported in four probands (including one who had had a high TSH at neonatal screening, but no follow-up because of normal T4), ascertained for transient delay in growth, and found to have elevated T4, reverse T3 and TSH, and decreased T3.[153-155] A broader and more severe phenotype including myopathy[156] azoospermia and photosensitivity,[157] in addition to abnormal thyroid function tests, has also been described, consistent with the ubiquitous functions of selenoproteins other than thyroid deiodinases. All subjects with biallelic inactivation of SECISBP2 have had low serum selenium levels, which is therefore a helpful diagnostic clue to detect this disorder.

Thyroid Hormone Resistance

Receptor Defects

Patients with thyroid hormone resistance classically present with increased circulating levels of T4 and T3 with a (inappropriately nonsuppressed) normal or increased serum TSH concentration. In the newborn, TSH may be sufficiently high to be picked up at screening.[158] The prevalence of this condition estimated from two population-based studies of infants with a high T4 on the neonatal blood spot is 1 in 40,000 births.[159,160] Resistance to thyroid hormone has been classified into three phenotypes (generalized, pituitary, and peripheral), but this classification is somewhat artificial because patients and even siblings with the same mutation in TRβ can have different phenotypes.

Inheritance is generally autosomal dominant but 15% to 20% of cases appear sporadically. Many patients are asymptomatic or demonstrate nonspecific symptoms. Deafness is observed in 20%, and a syndrome of attention deficit hyperactivity has been documented in half of affected patients. Hypothyroid features include growth retardation, delayed bone maturation, and intellectual impairment. Some children exhibit features of thyrotoxicosis, including failure to thrive, accelerated growth, and hyperkinetic behavior.

Thyroid hormone action is mediated via nuclear thyroid hormone receptor proteins with zinc-finger DNA-binding regions and thyroid hormone-binding domains. The latter have a 10:1 relative binding affinity for T3 rather than for T4. The receptors act as DNA-transactivating factors to stimulate or suppress responsive genes. Two genes coding for the TR proteins have been described: an alpha gene on chromosome 17 and a beta gene on chromosome 3. To date, the most common molecular defect in all cases studied has involved the TRβ1 gene on chromosome 3. In about 80% of subjects with features of resistance to thyroid hormone, specific TRβ gene mutations can be demonstrated. More than 120 different defects have been described, mostly involving single-amino-acid deletions or substitutions in the hormone-binding domain at the carboxy terminal end of the receptor molecule. A few in-frame deletions and frame-shift insertions have been described. Only very recently have the first mutations in TRα been described, and the markedly retarded bone maturation and severe constipation found in these three patients is consistent with the known tissue distribution of this receptor isoform.[60,61]

TRs (like other steroid hormone superfamily receptors) bind to DNA response elements as monomers, homodimers, or heterodimers—and heterodimerization can involve another TR, including the TRα2 receptors, or other transactivation factors. Affected members of most families studied have one normal and one abnormal TRβ allele. The abnormal TRβ with minimal or reduced T3 binding fails to mediate T3-regulated transcription and may block the action of the normal allele. This dominant negative effect is presumably mediated by binding of the defective allele with normal TRβ and producing an inactive homodimer.[59]

Treatment of thyroid hormone resistance remains a challenge, especially in severely affected individuals, such as the rare homozygotes.[161] Symptomatic treatments with beta-blockers or attempts to override or bypass the hormone resistance with high-dose thyroxine or TRIAC, a thyroid hormone analog, have been reported.[161,162] Anti-thyroid drugs result in increasing TSH and goiter size,[163] with the theoretical risk of developing a tumor of the pituitary or the thyroid in the long term. In the majority of patients with only biochemical abnormalities and mild clinical manifestations, the dictum *primum non nocere* applies.[164]

Membrane Transporter Defects

Among the proteins involved in carrier-mediated energy-dependent transport of thyroid hormones for which

there is in vitro evidence, only the monocarboxylate transporter MCT-8 (encoded by the SLC16A2 gene on the X chromosome) has now been shown to be involved in human disease.[56,57]

Interestingly, Allan-Herndon-Dudley syndrome, an X-linked mental retardation syndrome described in 1944,[165] was also found to be due to MCT8 mutations. It is not surprising that the neurologic phenotype was recognized decades earlier, as it is much more severe than the abnormalities of thyroid function. It develops from infancy or childhood with hypotonia, poor head control, involuntary athetoid and dystonic movements, hyperreflexia, nystagmus, and severe mental retardation. Thyroid function test reveal elevated serum T3, low serum T4, free T4, and normal or slightly elevated TSH without signs of hypothyroidism.

The gene defects have included deletions and missense mutations. The MCT-8 gene in mice is expressed in brain (choroid plexus, cerebrum, hippocampus, amygdala, striatum, and hypothalamus), liver, kidney, and pituitary and thyroid tissues. In brain tissues, MCT-8 transporters seem localized to neuronal rather than to glial cells. Much of the brain damage due to decreased neuronal T4 content occurs in utero, and the phenotype resembles that of severe endemic cretinism. Accordingly, women hemizygous for MCT8 mutations should be counseled regarding pregnancy, not only about genetic risk but also because they may be prone to gestational hypothyroxinemia.[166] In affected children, the thyroid hormone analog DITPA, which has been shown in mice not to require MCT8 to enter tissues,[167] could be an effective alternative.[168]

Consumptive Hypothyroidism

This entity, which typically develops in infants, may be considered under thyroid hormone resistance, because it is characterized by the need for extremely high amounts of thyroxine to maintain euthyroidism. This is because both endogenous and endogenous thyroxine is inactivated, most often within hemangiomas that express high levels of deiodinase 3.[169] Hemangiomas are not typically present at birth but grow rapidly during the first few months of life. Accordingly, hypothyroidism is generally not congenital but develops during early infancy. Large cutaneous hemangiomas are easy to diagnose but consumptive hypothyroidism was the clue that led to diagnosing a liver hemangiomas in one infant.[170] An elevated reverse T3 concentration in serum may also be suggestive.[171] Thyroid scintigraphy shows a gland of normal size but with increased uptake.[170] It is unclear whether the series reported by Ayling and colleagues represents a different entity, because immunohistochemical studies of deiodinase 3 were not performed.[172] Aside from high-dose T4, treatment with T3 has also been reported.[169,171] With disappearance of the hemangiomas, either spontaneously or through medical or surgical treatment, euthyroidism is restored. The exact prevalence of this entity is unknown but it is likely low: in a retrospective review of 1555 patients with hemangiomas, 92 had thyroid function tests but only 3 had overt hypothyroidism.[169]

Hypothalamo-Pituitary Hypothyroidism

Congenital central hypothyroidism is uncommon, and isolated TRH/TSH deficiency is exceedingly rare. Multiple pituitary hormone deficiencies are estimated to be present in about 1 in 20,000 to 30,000 newborns.[173] However, these patients usually do not come to medical attention because of thyroid hormone deficiency. Rather, hypoglycemia reveals growth hormone or adrenocorticotropic hormone (ACTH)/cortisol deficiency or micropenis and cryptorchidism reveal gonadotrophin deficiency. Although hypothyroidism may reduce bile flow,[174] the major factor leading to jaundice in primary CH is immature glucuronoconjugation[175] and the hyperbilirubinemia is mostly unconjugated. By contrast, in congenital ACTH or cortisol deficiency, it is *conjugated* because cortisol is necessary for neonatal choleresis.[176,177] Congenital hypopituitarism is most often associated with the "classical triad" (ectopic posterior pituitary, interrupted stalk, and small anterior pituitary) on magnetic resonance imaging.[178] This malformation occurs almost always sporadically and its cause is generally unknown.

TRH Defects

No mutations of TRH itself have been described to date in humans. By contrast, homozygosity and compound heterozygosity for loss-of-function mutations of the TRH receptor gene, located on chromosome 8, have been described. The phenotype of the probands was mild, with ascertainment at 9 and 11 years because of short stature and delayed bone maturation. The clue to the diagnosis was a complete absence of response of both TSH and prolactin (which were measurable at baseline) to exogenous TRH. In the first proband, a low T4 had been measured on the neonatal blood spot but IQ was similar to that of unaffected siblings.[179] In the other family, an affected sister had breastfed her two children, showing that the TRH receptor is not required for lactation.[180]

Isolated TSH Deficiency

Severe congenital hypothyroidism due to isolated TSH deficiency was first described in consanguineous Japanese pedigrees.[181] Cloning of the gene encoding the TSHβ subunit gene, located on chromosome 1, quickly led to the identification of homozygous mutations in the Japanese patients,[182] but also in patients with similar phenotypes from different parts of the world.[183] Typically, serum TSH is undetectable or low and the response to TRH is blunted but, in contrast to what is observed in patients with TRH receptor mutations, prolactin increases after TRH. Serum TSH may be slightly elevated with some radioimmunoassays.[49] There may be no isotope uptake on scintigraphy but the thyroid becomes visible after exogenous TSH stimulation.[49] The most prevalent mutation involves a 1–base pair deletion from codon 105 and has been identified in different populations, which may indicate common ancestry[184] or a mutational hot spot.[183]

Transient Neonatal Hypothyroidism, Hyperthyrotropinemia, and Hypothyroxinemia

A blood-spot TSH above the screening cutoff but with spontaneous normalization in serum taken at the time of diagnostic evaluation could be described as "false positive" of CH screening or as transient neonatal hyperthyrotropinemia. Its prevalence obviously depends on screening cutoffs. In Québec, it occurs in approximately 1 in 18,000 births and is generally ascribed to transplacental passage of either antithyroid drugs or TSH receptor-blocking antibodies. In both of these situations, overt hypothyroidism has also been reported but is transient, lasting a few days in the former and a few months (and therefore justifying treatment, until TSH receptor antibody levels are cleared) in the latter.

Transient CH can also result from iodine deficiency or excess. In severe iodine deficiency, up to 10% of newborns have severe primary CH[185] associated with the increased thyroid hormone requirements in the neonatal period.[52] In borderline iodine deficiency, transient CH may be provoked by a superimposed acute iodine overload (the Wolff-Chaikoff effect), especially in premature newborns.[186] On the other hand, iodine in quantities more than 10-fold the upper limit of the recommended intake, from nutritional supplements taken throughout pregnancy, can also cause transient CH.[187] Amiodarone, an iodine-rich anti-arrhythmic drug, can also lead to hypothyroidism when given either to the pregnant woman[188] or to the newborn or infant.[189] The use of povidone-iodine disinfection of the skin or of the mediastinum in infants undergoing heart surgery may also induce hypothyroidism,[190] although this is not common in North America.[191]

Asymptomatic hyperthyrotropinemia is a relatively common disorder at all ages and, when detected at newborn screening, may be permanent and reflect either a large ectopic thyroid,[82] thyroid hemiagenesis,[192] heterozygous inactivating mutations in the TSH receptor,[123] or dominant TSH resistance of unknown origin.[193]

Transient isolated hypothyroxinemia is a very common finding in premature neonates and is correlated with poorer outcome in terms of mortality, morbidity, and developmental outcome. However, the results of a long-term randomized placebo-controlled trial do not suggest causation.[194,195] Pending the results of an ongoing trial comparing placebo, thyroxine, and iodine,[196] hypothyroxinemia of prematurity should be viewed as a state of physiologic adaptation that does not need to be screened for or treated routinely. The same likely applies to the nonthyroidal illness syndrome (low free T4, T3, and TSH), which is sometimes observed in severely ill infants[197] who, upon recovery, may have transient hyperthyrotropinemia.

Evaluation of Newborns with Positive Screening Results

A positive screening report for CH is only the beginning of a process that should lead to confirmation of the diagnosis, establishment of etiology, optimal treatment, and documentation of outcome.

Family history should focus on consanguinity, which increases the likelihood of dyshormonogenesis,[94] and on the existence of even distant relatives with CH.[102] Given the very high prevalence of thyroid disorders with onset in later life, a family history of these disorders is usually irrelevant. However, a maternal history of thyroid disease of documented autoimmune etiology[198] or of consumption of iodine-rich nutritional supplements[187] is relevant. Personal history should include length of gestation and birth weight, and the mother should be asked if she has noticed the baby is hypotonic or is feeding poorly. Physical examination may reveal large fontanelles, persistent jaundice, macroglossia, dry and mottled skin, and an umbilical hernia.[66,199] However, only 1% to 4% of newborns are under clinical suspicion of CH when the results of biochemical screening become available.[76,200] Even with the infant's neck hyperextended, experienced clinicians often fail to detect a goiter that will only be evidenced on imaging. Physical examination should also include a search for dysmorphic features[201] and for a heart murmur suggesting a defect in heart septation.[104,116,117]

At a minimum, the diagnosis of CH should be confirmed biochemically with a serum TSH and free T4. Indeed, a low free T4 has been associated in several studies with a higher risk of neurocognitive sequelae.[202,203] When a thyroid ectopy or a goiter is documented on nuclear medicine scanning, no other tests are required for clinical management. In cases with undetectable radioisotope uptake, serum thyroglobulin will allow distinguishing true athyreosis (a permanent condition) from apparent athyreosis (which may be transient if due to antibodies to the TSH receptor[100,101]).

The role of nuclear medicine in establishing the etiology of CH cannot be overemphasized.[204] In 50% of the cases, it will reveal a sublingual thyroid ectopy,[82] a permanent condition that is almost always sporadic. In 15%, there will be no detectable uptake and, if serum thyroglobulin is also undetectable, the condition is also sporadic and will be permanent. In about 10%, it will reveal a goiter reflecting dyshormonogenesis, in which case the hypothyroidism will almost always be permanent and the recurrence risk in siblings is one in four. Thus, a clear etiology can be established on the day of diagnosis, and this is an important decision that can offer help for physicians and families (Figure 7-8).

We favor the use of sodium pertechnetate, which is available daily in all nuclear medicine services and allows imaging in 20 minutes,[205] in contrast to iodine scintigraphy, which requires several hours. Care should be taken to feed the baby after the injection of the tracer, to avoid tracer accumulation in the salivary glands[205] and to ensure that the baby will sleep while under the camera. Although thyroid scintigraphy can be postponed until reevaluation of the diagnosis at 3 years of age, it is much easier to perform in a sleepy 10-day-old. We do not advise the routine use of ultrasound scanning, which is prone to movement artifacts, does not unequivocally allow the identification of a sublingual ectopy, and may constitute a diagnostic trap.[97] By contrast, demonstrating absence of the knee epiphyses

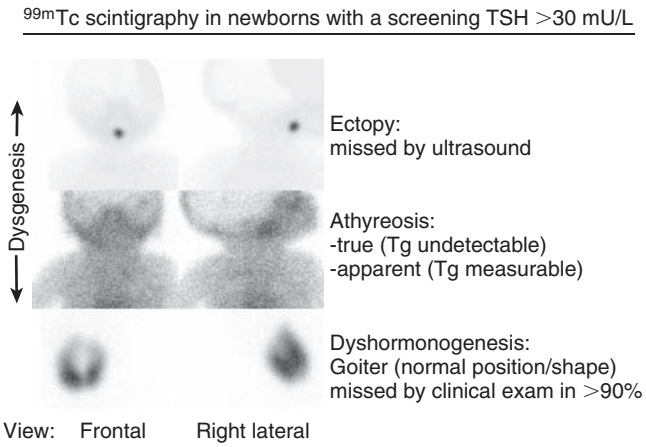


FIGURE 7-8 ■ The three most commonly seen etiologies of congenital hypothyroidism as evidenced by nuclear medicine scintigraphy using sodium pertechnetate.

on an antero-posterior x-ray, which indicates hypothyroidism of prenatal onset, has prognostic value for neurocognitive development.[206,207]

Although it has been repeatedly and rightly stated that delay in obtaining imaging should not delay treatment, it has been our experience that nuclear medicine services can be motivated to carry out a sodium pertechnetate scan with a 24-hour notice. In severe cases, it can even be performed after a few days of treatment,[208] as long as serum TSH is still above 30 mU/L. Treating an asymptomatic newborn on the basis of TSH alone may give the parents the impression that a number, rather than a patient, is being treated. This may account for the observation that about 40% of patients labeled as having CH by neonatal screening laboratories in the United States are no longer treated after 4 years of age,[209] without the recommended documentation of outcome of such thyroxine withdrawal.[210]

Treatment of Congenital Hypothyroidism

The following discussion applies to infants with definite CH from ectopy, athyreosis, or goiter and not to those with a normal gland in situ whose hypothyroidism is usually milder but who represent about half of contemporary CH cohorts.[82] Treatment should be instituted as soon as the diagnosis is confirmed. The goal of newborn CH screening is the institution of early adequate thyroid hormone replacement therapy. Most of the brain cell thyroid hormone is derived from local T4-to-T3 conversion. Thus, the preferred thyroid hormone preparation for treatment of infants with CH is thyroxine, and the addition of T3 offers no advantage.[211]

There is only one randomized controlled trial of different starting doses of thyroxine in CH, the results of which support the practice of giving 50 μg/day of thyroxine to infants weighing 3 to 4 kg. Indeed, not only does this dose most rapidly normalize serum TSH,[212] it is also associated with the best neurodevelopmental outcome at 5 years.[213] This starting dose leads to a mean serum fT4 concentration of 56.3 pmol/L (4.37ng/dL) after 2 weeks of treatment; although this figure seems high, it should be pointed out that the mean serum fT4 in normal children at 1 to 4 days of age is 48.1 pmol/L (3.73 ng/dL).[214] Later during infancy, there appears to be a resetting of the feedback threshold for T4 suppression of TSH release in CH. This resetting may occur in utero and its mechanisms remain obscure.[215] Accordingly, serum fT4 is often high but T3 is generally normal and TSH remains measurable with no clinical evidence of hyperthyroidism.[40] Unless serum TSH is persistently suppressed, the thyroxine dose does not need to be decreased: although there is evidence that overtreatment may be associated with greater distractibility at school age,[216] there is also evidence that undertreatment is associated with school delay.[217] The frequency at which infants are seen and thyroid function tests obtained varies greatly between jurisdictions but should be no less than 3 months in the first year and 6 months in older infants.

Growth in length and weight of infants with CH are normal if they are treated early and adequately. Head circumference is, on average, 0.8 SD above the mean[218] and in some infants this relative macrocrania leads to unnecessary imaging: it is a reflection of an immaturity of the skeleton and not of the size of the brain or cerebral ventricles.[219] Bone maturation is normal at 3 years of age, showing complete catch-up and no undue advancement, again suggesting that hyperthyroxinemia during treatment does not indicate iatrogenic hyperthyroidism.[40]

Although neurocognitive development and behavior is, on average, within normal limits in CH regardless of severity,[40] a few children still have clinically significant learning difficulties.[202,203] Their exact number and whether their difficulties are due to disease- or treatment-related factors remain controversial. Importantly, the known influence of the family's socioeconomic status on child development interacts with the biologic effect of CH: there is a steeper IQ decline with decreasing social class in CH than in controls.[203] Clinicians should therefore be particularly alert to developmental delay in children with severe CH from an economically disadvantaged family.

CONGENITAL HYPERTHYROIDISM

Graves Disease

Symptomatic Graves disease in a fetus or neonate is very uncommon: in the United States, a population-based study based on follow-up of newborns with a high total T4 on the neonatal screening specimen yielded an estimate of at most 1 in 80,000 births.[159] The incidence may be higher in Japan[160] or if only premature neonates are considered.[220] On the other hand, new-onset hyperthyroidism is diagnosed in about 1 in 500 to 1 in 1000 women of childbearing age,[221] to which women with established or even cured Graves disease (who may still produce thyroid-stimulating immunoglobulins [TSIs]) should be added. It must therefore be concluded that not all fetuses borne by women with positive TSIs develop overt hyperthyroidism. Although exceptional, fetal/neonatal hyperthyroidism is nevertheless a serious condition that should not be missed. However, earlier mortality estimates, as high as 25%, likely no longer apply to the present, when

awareness of the diagnosis is higher and supportive therapy more sophisticated. No death occurred in the seven infants reported by Smith and colleagues (and in our own unpublished experience), in spite of confounding factors such as prematurity[220] and of delayed diagnosis in some.[222] On the other hand, in utero death from fetal hyperthyroidism still occurs, especially when referral to a highly specialized center is delayed.[223]

Ideally, all women with Graves disease should have serum TSIs measured in early pregnancy. This applies to those currently treated for hyperthyroidism as well as to those who are now euthyroid, spontaneously or on replacement after ablative therapy.[3] Indeed, a high TSI titer in maternal serum makes hyperthyroidism more likely in the fetus or newborn. Unfortunately, this information is often not available at first evaluation and clinicians are confronted at first with the classical signs heralding fetal hyperthyroidism, such as tachycardia and intrauterine growth retardation. In the hands of highly skilled radiologists, a fetal goiter may be the first sign but whether this will be generalizable remains unproven.[223]

Once fetal hyperthyroidism is suspected, it can generally be managed through the mother by initiating or increasing antithyroid drug treatment. Occasionally, the hyper- or hypothyroid nature of a goiter in a fetus cannot be made on the basis of maternal TSI concentrations and antithyroid drug dose, in which case the ultrasound characteristics of the goiter may be helpful,[224] although this is highly operator dependent. In exceptional cases, cordocentesis is necessary to determine fetal thyroid function and to guide management, but it should be kept in mind that this procedure carries a 1% to 2% risk of fetal loss.[225]

Traditionally, the mainstay of treatment of hyperthyroidism during pregnancy has been the administration of propylthiouracil (PTU) because of the risk of methimazole (MMZ)-induced embryopathy.[226] However, because of the greater liver toxicity of PTU,[227] it has been suggested that MMZ should be preferred after organogenesis is complete, but the practicality of switching from one drug to another has been questioned[228] and data suggest PTU may be teratogenic as well.[229] Whatever drug is used, maternal antithyroid treatment should normalize fetal heart rate within 2 weeks. The antithyroid drug dose can usually be decreased progressively.

Graves disease in the newborn is manifested by irritability, flushing, tachycardia, hypertension, poor weight gain, thyroid enlargement, and exophthalmos. Thrombocytopenia, hepatosplenomegaly, jaundice, hypoprothrombinemia, and cardiac failure may occur. The diagnosis is confirmed by an undetectable serum TSH, high levels of serum T4, free T4, and T3. By contrast, on blood drawn on the second day of life in normal children, free T4 is high but TSH is not suppressed. In some neonates, the onset of symptoms and signs may be delayed as long as 8 to 9 days, if they were born to a mother treated with antithyroid drugs, and also reflecting the switch from an inactivating to an activating conversion of T4 to active T3 by liver and other tissues after birth. Neonatal Graves disease resolves spontaneously as maternal TSIs are cleared from the infant's circulation. The usual clinical course of neonatal Graves disease extends from 3 to 12 weeks.

Newborns with symptomatic hyperthyroidism should be admitted and their heart rate monitored. Treatment includes propranolol (1 to 2 mg/kg/day divided into four doses) and methimazole in a dose of 0.5 to 1 mg per kilogram daily in divided doses at 8-hour intervals. Iodine is also often used, because it rapidly inhibits hormone release: Lugol solution (5% iodine and 10% potassium iodide, 126 mg of iodine per milliliter) is given in a dose of one drop (about 8 mg) three times daily. A therapeutic response should be observed within 24 to 36 hours. If a satisfactory response is not observed, the dose of antithyroid drug and iodine can be increased by 50%. Glucocorticoids in high doses diminish T4 to T3 conversion and may therefore be helpful. Radiographic contrast agents have also been used,[230] and exchange transfusions will also be effective.[231] In severe cases, sedatives and digitalization may be necessary.

Nonautoimmune Hyperthyroidism

Mutations that result in increased constitutive activity of the TSH receptor give rise to hyperthyroidism. Premature delivery and low birth weight are consistent features in these infants (as in unaffected children of mothers with thyroid hormone resistance[29]), suggesting that an increase in thyroid hormone levels in the fetus independently stunts growth and shortens gestation.[232] Hyperthyroidism is generally associated with a goiter, although this is not always present at diagnosis. Acceleration of bone maturation occurs very early and rapid linear growth, relative thinness, and microcephaly are observed.

Mutations activating the TSH receptor may occur de novo in the germline. The first cases described had very severe hyperthyroidism,[233,234] but subsequent cases have not all been as severe.[235,236] They can also be transmitted in an autosomal dominant pattern,[237] in which case they tend to lead to a less severe phenotype with variable age of onset. Although generally detected at later ages, some cases are recognized in the neonatal period or during infancy.[238] Lastly, mutations activating the TSHR may occur at the somatic level, leading to the development of a hyperfunctioning nodule and to hyperthyroidism. This is a common disease in adults, but can occur before birth[239] or in infancy.[240]

In contrast to neonatal Graves disease, nonautoimmune hyperthyroidism is life-long and thyroidectomy is the only curative approach and should be preferred in infants with a hyperfunctioning nodule. In those with a germline mutation and, consequently, a diffuse goiter, surgery is also often required.

DISORDERS OF THYROID HORMONE TRANSPORT

Several genetic abnormalities of the iodothyronine-binding serum proteins have been described and all are manifest at birth. These include complete TBG deficiency, partial TBG deficiency, TBG excess, transthyretin (TTR) (prealbumin) variants, and familial dysalbuminemic hyperthyroxinemia (FDH). Total T4 is low or high, but free T4 is normal and the patients are therefore euthyroid.

Nowadays, these abnormalities are only picked up by screening programs that measure total T4, in which an elevated total T4 is at least 10-fold more likely due to TBG excess than to Graves disease.[159] In institutions where total serum T4 is still measured at later ages, clinicians should be aware of abnormalities in TBG, which are common, and that total T4 should always be evaluated together with the serum TSH. Free T4, instead of total T4, is measured by an ever-increasing number of clinical laboratories but, depending on the assay method, free T4 can also be spuriously elevated in familial dysalbuminemic hyperthyroxinemia. Here again, considering the clinical context and the serum TSH will avoid an erroneous diagnosis of hyperthyroidism.[241]

REFERENCES

1. Grosse, S. D., & Van Vliet, G. (2011). Prevention of intellectual disability through screening for congenital hypothyroidism: how much and at what level? *Arch Dis Child*, *96*, 374–349.
2. Vassart, G., & Dumont, J. E. (2005). Thyroid dysgenesis: multigenic or epigenetic...or both? *Endocrinology*, *146*, 5035–5037.
3. Van Vliet, G., Polak, M., & Ritzen, E. M. (2008). Treating fetal thyroid and adrenal disorders through the mother. *Nat Clin Pract Endocrinol Metab*, *4*, 675–682.
4. Winters, A. J., Eskay, R. L., & Porter, J. C. (1974). Concentration and distribution of TRH and LRH in the human fetal brain. *J Clin Endocrinol Metab*, *39*, 960–963.
5. Morley, J. E. (1979). Extrahypothalamic thyrotropin releasing hormone (TRH): its distribution and its functions. *Life Sci 29*, *25*, 1539–1550.
6. Nebesio, T. D., McKenna, M. P., Nabhan, Z. M., & Eugster, E. A. (2010). Newborn screening results in children with central hypothyroidism. *J Pediatr*, *156*, 990–993.
7. McCabe, M. J., Alatzoglou, K. S., & Dattani, M. T. (2011). Septo-optic dysplasia and other midline defects: the role of transcription factors: HESX1 and beyond. *Best Pract Res Clin Endocrinol Metab*, *25*, 115–124.
8. Ward, L., Chavez, M., Huot, C., et al. (1998). Severe congenital hypopituitarism with low prolactin levels and age-dependent anterior pituitary hypoplasia: a clue to a PIT-1 mutation. *J Pediatr*, *132*, 1036–1038.
9. Deladoey, J., Fluck, C., Buyukgebiz, A., et al. (1999). "Hot spot" in the PROP1 gene responsible for combined pituitary hormone deficiency. *J Clin Endocrinol Metab*, *84*, 1645–1650.
10. Pfaffle, R., & Klammt, J. (2011). Pituitary transcription factors in the aetiology of combined pituitary hormone deficiency. *Best Pract Res Clin Endocrinol Metab*, *25*, 43–60.
11. Vandernoot, I., Sartelet, H., Abu-Khudir, R., et al. (2012). Evidence for calcitonin-producing cells in human lingual thyroids. *J Clin Endocrinol Metab*, *97*, 951–956.
12. Williams, E. D., Toyn, C. E., & Harach, H. R. (1989). The ultimobranchial gland and congenital thyroid abnormalities in man. *J Pathol*, *159*, 135–141.
13. Kumar, R., Gupta, R., Bal, C. S., et al. (2004). Thyrotoxicosis in a patient with submandibular thyroid. *Thyroid*, *10*, 363–365.
14. De Felice, M., & Di Lauro, R. (2004). Thyroid development and its disorders: genetics and molecular mechanisms. *Endocr Rev*, *25*, 722–746.
15. De Felice, M., & Di Lauro, R. (2011). Minireview: intrinsic and extrinsic factors in thyroid gland development: an update. *Endocrinology*, *152*, 2948–2956.
16. Antonica, F., Kasprzyk, D. F., Opitz, R., et al.(2012). Generation of functional thyroid from embryonic stem cells. *Nature*, *491*, 66–71.
17. Stein, S. A., Shanklin, D. R., Krulich, L., et al.(1989). Evaluation and characterization of the hyt/hyt hypothyroid mouse. II. Abnormalities of TSH and the thyroid gland. *Neuroendocrinology*, *49*, 509–519.
18. Abramowicz, M. J., Duprez, L., Parma, J., et al.(1997). Familial congenital hypothyroidism due to inactivating mutation of the thyrotropin receptor causing profound hypoplasia of the thyroid gland. *J Clin Invest*, *99*, 3018–3024.
19. Kimura, S., Hara, Y., Pineau, T., et al.(1996). The T/ebp null mouse: thyroid-specific enhancer-binding protein is essential for the organogenesis of the thyroid, lung, ventral forebrain, and pituitary. *Genes Dev*, *10*, 60–69.
20. Mansouri, A., Chowdhury, K., & Gruss, P. (1998). Follicular cells of the thyroid gland require Pax8 gene function. *Nat Genet*, *19*, 87–90.
21. De Felice, M., Ovitt, C., Biffali, E., Rodriguez-Mallon A., et al.(1998). A mouse model for hereditary thyroid dysgenesis and cleft palate. *Nat Genet*, *19*, 395–398.
22. Deladoey, J., Vassart, G., & Van Vliet, G. (2007). Possible non-mendelian mechanisms of thyroid dysgenesis. *Endocr Dev*, *10*, 29–42.
23. Di Cosmo, C., Fanelli, G., Tonacchera, M., et al.(2006). The sodium-iodide symporter expression in placental tissue at different gestational age: an immunohistochemical study. *Clin Endocrinol* (Oxf), *65*, 544–548.
24. Cao, X. Y., Jiang, X. M., Dou, Z. H., et al.(1994). Timing of vulnerability of the brain to iodine deficiency in endemic cretinism. *N Engl J Med*, *331*, 1739–1744.
25. Glinoer, D. (2007). The importance of iodine nutrition during pregnancy. *Public Health Nutr*, *10*, 1542–1546.
26. Fisher, D. A., Lehman, H., & Lackey, C. (1964). Placental transport of thyroxine. *J Clin Endocrinol Metab*, *24*, 393–400.
27. Calvo, R. M., Jauniaux, E., Gulbis, B., et al.(2002). Fetal tissues are exposed to biologically relevant free thyroxine concentrations during early phases of development. *J Clin Endocrinol Metab*, *87*, 1768–1777.
28. Vulsma, T., Gons, M. H., & de Vijlder, J. J. (1989). Maternal-fetal transfer of thyroxine in congenital hypothyroidism due to a total organification defect of thyroid agenesis. *N Eng J Med*, *321*, 13–16.
29. Anselmo, J., Cao, D., Karrison, T., et al.(2004). Fetal loss associated with excess thyroid hormone exposure. *JAMA*, *292*, 691–695.
30. de Zegher, F., Pernasetti, F., Vanhole, C., et al.(1995). The prenatal role of thyroid hormone evidenced by fetomaternal Pit-1 deficiency. *J Clin Endocrinol Metab*, *80*, 3127–3130.
31. Man, E. B., Brown, J. F., & Serunian, S. A. (1991). Maternal hypothyroxinemia: psychoneurological deficits of progeny. *Ann Clin Lab Sci*, *21*, 227–239.
32. Haddow, J. E., Palomaki, G. E., Allan, W. C., et al.(1999). Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. *N Engl J Med*, *341*, 549–555.
33. Pop, V. J., Kuijpens, J. L., vanBaar, A. L., et al.(1999). Low maternal free thyroxine concentrations during early pregnancy are associated with impaired psychomotor development in infancy. *Clin Endocrinol*, *50*, 155.
34. Lazarus, J. H., Bestwick, J. P., Channon, S., et al.(2012). Antenatal thyroid screening and childhood cognitive function. *N Engl J Med*, *366*, 493–501.
35. Momotani, N., Iwama, S., & Momotani, K. (2012). Neurodevelopment in children born to hypothyroid mothers restored to normal thyroxine (t4) concentration by late pregnancy in Japan: no apparent influence of maternal t4 deficiency. *J Clin Endocrinol Metab*, *97*, 1104–1108.
36. Downing, S. D., Halpern, L., Carswell, J., & Brown, R. S. (2012). Severe early maternal hypothyroidism corrected prior to the third trimester associated with normal cognitive outcome in the offspring. *Thyroid*, *22*, 625-630.
37. Alexander, E. K., Marqusee, E., Lawrence, J., et al.(2004). Timing and magnitude of increases in levothyroxine requirements during pregnancy in women with hypothyroidism. *N Engl J Med*, *351*, 241–249.
38. Stoppa-Vaucher, S., Francoeur, D., Grignon, A., et al.(2010). Non-immune goiter and hypothyroidism in a 19-week fetus: a plea for conservative treatment. *J Pediatr*, *156*, 1026–1029.
39. Calvo, R., Obregon, M. J., Ruiz de Ona, C., et al.(1990). Congenital hypothyroidism, as studied in rats: crucial role of maternal thyroxine but not of 3,5,3'-triiodothyronine in the protection of the fetal brain. *J Clin Invest*, *8*, 889–899.
40. Simoneau-Roy, J., Marti, S., Deal, C., et al.(2004). Cognition and behavior at school entry in children with congenital hypothyroidism treated early with high-dose levothyroxine. *J Pediatr*, *144*, 747–752.
41. Stewart, C. J., Constantatos, S., Joolay, Y., & Muller, L. (2012). In utero treatment of fetal goitrous hypothyroidism in a euthyroid mother: a case report. *J Clin Ultrasound*, *40*, 603–606.

42. Stoppa-Vaucher, S., Van Vliet, G., & Deladoey, J. (2011). Discovery of a fetal goiter on prenatal ultrasound in women treated for Graves' disease: first, do no harm. *Thyroid*, *21*, 931.
43. Ballard, R. A., Ballard, P. L., Cnaan, A., et al.(1990). Antenatal thyrotropin-releasing hormone to prevent lung disease in preterm infants. North American Thyrotropin-Releasing Hormone Study Group. *N Engl J Med*, *338*, 493–498.
44. Brown, R. S., Bellisario, R. L., Botero, D., et al.(1996). Incidence of transient congenital hypothyroidism due to maternal thyrotropin receptor-blocking antibodies in over one million babies. *J Clin Endocrinol Metab*, *81*, 1147–1151.
45. Zakarija, M., McKenzie, J. M., & Hoffman, W. H. (1986). Prediction and therapy of intrauterine and late-onset neonatal hyperthyroidism. *J Clin Endocrinol Metab*, *62*, 368–371.
46. Aratan-Spire, S., & Czernichow, P. (1980). Thyrotropin-releasing hormone-degrading activity of neonatal human plasma. *J Clin Endocrinol Metab*, *50*, 88–92.
47. Szinnai, G., Lacroix, L., Carre, A., et al.(2007). Sodium/iodide symporter (NIS) gene expression is the limiting step for the onset of thyroid function in the human fetus. *J Clin Endocrinol Metab*, *92*, 70–76.
48. Deming, D. D., Rabin, C. W., & Hopper, A. O. (2007).Direct equilibrium dialysis compared with two non-dialysis free T4 methods in premature infants. *J Pediatr*, *151*, 404–408.
49. Heinrichs, C., Parma, J., Scherberg, N. H., et al.(2000). Congenital central isolated hypothyroidism caused by a homozygous mutation in the TSH-beta subunit gene. *Thyroid*, *10*, 387–391.
50. Gagne, N., Parma, J., Deal, C., et al.(1998). Apparent congenital athyreosis contrasting with normal plasma thyroglobulin levels and associated with inactivating mutations in the thyrotropin receptor gene: are athyreosis and ectopic thyroid distinct entities? *J Clin Endocrinol Metab*, *83*, 1771–1775.
51. Ribault, V., Castanet, M., & Bertrand, A. M. (2009). Experience with intraamniotic thyroxine treatment in nonimmune fetal goitrous hypothyroidism in 12 cases. *J Clin Endocrinol Metab*, *94*, 3731–3739.
52. Delange, F. (1993). Biochemistry and hysiology. In J. Bertrand, R. Rappaport, & P. C. Sizonenko (Eds.), *Pediatric endocrinology: physiology, pathophysiology, and clinical aspects*, (2nd ed.) (pp. 242–251). Baltimore: Williams & Wilkins.
53. Bianco, A. C. (2001). Minireview: cracking the metabolic code for thyroid hormone signaling. *Endocrinology*, *152*, 3306–3311.
54. Stanley, E. L., Hume, R., Visser, T. J., & Coughtrie, M. W. (2001). Differential expression of sulfotransferase enzymes involved in thyroid hormone metabolism during human placental development. *J Clin Endocrinol Metab*, *86*, 5944–5955.
55. Djemli, A., Van Vliet, G., Belgoudi, J., et al.(2004). Reference intervals for free thyroxine, total triiodothyronine, thyrotropin and thyroglobulin for Quebec newborns, children and teenagers. *Clin Biochem*, *37*, 328–330.
56. Dumitrescu, A. M., Liao, X. H., Best, T. B., et al.(2004). A novel syndrome combining thyroid and neurological abnormalities is associated with mutations in a monocarboxylate transporter gene. *Am J Hum Genet*, *74*, 168–175.
57. Friesema, E. C., Grueters, A., Biebermann, H., et al.(2004). Association between mutations in a thyroid hormone transporter and severe X-linked psychomotor retardation. *Lancet*, *364*, 1435–1437.
58. Visser, W. E., Friesema, E. C., & Visser, T. J. (2011). Minireview: thyroid hormone transporters: the knowns and the unknowns. *Mol Endocrinol*, *25*, 1–14.
59. Refetoff, S., Weiss, R. E., & Usala, S. J. (1993). The syndromes of resistance to thyroid hormone. *Endocr Rev*, *14*, 348–399.
60. Bochukova, E., Schoenmakers, N., Agostini, M., et al.(2012). A mutation in the thyroid hormone receptor alpha gene. *N Engl J Med*, *366*, 243–249.
61. van Mullem, A., van Heerebeek, R., Chrysis, D., et al.(2012). Clinical phenotype and mutant TRalpha1. *N Engl J Med*, *366*, 1451–1453.
62. Flamant, F., & Gauthier, K. (2013). Thyroid hormone receptors: the challenge of elucidating isotype-specific functions and cell-specific response. *Biochim Biophys Acta*, *1830*, 3900–3907.
63. Chan, S., & Kilby, M. D. (2000). Thyroid hormone and central nervous system development. *J Endocrinol*, *165*, 1–8.
64. Andersen, H. J. (1961). Studies of hypothyroidism in children. *Acta Paediatr Scand*, *50*(suppl 125), 1–150.
65. Smith, D. W., & Popich, G. (1972). Large fontanels in congenital hypothyroidism: a potential clue toward earlier recognition. *J Pediatr*, *80*, 753–756.
66. Lenz, A. M., & Root, A. W. (2008). Congenital hypothyroidism: a forgotten clinical diagnosis? *J Pediatr Endocrinol Metab*, *21*, 623–624.
67. Silva, J. E. (2011). Physiological importance and control of non-shivering facultative thermogenesis. *Front Biosci* (Schol Ed), *3*, 352–371.
68. Tews, D., & Wabitsch, M. (2011). Renaissance of brown adipose tissue. *Horm Res Paediatr*, *75*, 231–239.
69. Sluse, F. E. (2012). Uncoupling proteins: molecular, functional, regulatory, physiological and pathological aspects. *Adv Exp Med Biol*, *942*, 137–156.
70. Morreale de Escobar, G., Obregon, M. J., & Escobar del Rey, F. (2004). Role of thyroid hormone during early brain development. *Eur J Endocrinol*, *151*(suppl 3), U25–U37.
71. Lavado-Autric, R., Auso, E., Garcia-Velasco, J. V., et al.(2003). Early maternal hypothyroxinemia alters histogenesis and cerebral cortex cytoarchitecture of the progeny. *J Clin Invest*, *111*, 1073–1082.
72. Bongers-Schokking, J. J., & de Muinck Keizer-Schrama, S. M. (2005). Influence of timing and dose of thyroid hormone replacement on mental, psychomotor, and behavioral development in children with congenital hypothyroidism. *J Pediatr*, *147*, 768–774.
73. Joergensen, J. V., Oerbeck, B., Jebsen, P., et al.(2005). Severe hypothyroidism due to atrophic thyroiditis from second year of life influenced developmental outcome. *Acta Paediatr*, *94*, 1049–1054.
74. Virtanen, M., Maenpaa, J., Pikkarainen, J., et al.(1989). Aetiology of congenital hypothyroidism in Finland. *Acta Paediatr Scand*,*78*, 67–73.
75. Alvarez, M. A., Guell, R., Gonzalez, J., et al.(1992). Neuropsychological development during the first two years of life in children with congenital hypothyroidism screened at birth: the Cuban experience. *Screening*, *1*, 167–173.
76. Van Vliet, G., & Czernichow, P. (2004). Screening for neonatal endocrinopathies: rationale, methods and results. *Semin Neonatol*, *9*, 75–85.
77. Dussault, J. H., & Laberge, C. (1973). [Thyroxine (T4) determination by radioimmunological method in dried blood eluate: new diagnostic method of neonatal hypothyroidism?]. *Union Med Can*, *102*, 2062–2064.
78. Delange, F., Camus, M., Winkler, M., et al.(1977). Serum thyrotrophin determination on day 5 of life as screening procedure for congenital hypothyroidism. *Arch Dis Child*, *52*, 89–96.
79. Kempers, M. J., Lanting, C. I., van Heijst, A. F., et al.(2006). Neonatal screening for congenital hypothyroidism based on thyroxine, thyrotropin, and thyroxine-binding globulin measurement: potentials and pitfalls. *J Clin Endocrinol Metab*, *91*, 3370–3376.
80. Dussault, J. H., Grenier, A., Morissette, J., & Mitchell, M. (1995). Preliminary report on filter paper TSH levels in the first 24h of life and the following days in a program screening for congenital hypothyroidism. In K. A. Pass, & H. L. Levy (Eds.), *Early hospital discharge: impact on newborn screening.* (pp. 267–269). Atlanta: Council of Regional Networks for Genetics Services, Emory University School of Medicine.
81. Korada, S. M., Pearce, M., Ward Platt, M. P., et al.(2010). Difficulties in selecting an appropriate neonatal thyroid stimulating hormone (TSH) screening threshold. *Arch Dis Child*, *95*, 169–173.
82. Deladoey, J., Ruel, J., Giguere, Y., & Van Vliet, G. (2011). Is the incidence of congenital hypothyroidism really increasing? A 20-year retrospective population-based study in Quebec. *J Clin Endocrinol Metab*, *96*, 2422–2429.
83. Larson, C., Hermos, R., Delaney, A., et al.(2003). Risk factors associated with delayed thyrotropin elevations in congenital hypothyroidism. *J Pediatr*, *143*, 587–591.
84. Bijarnia, S., Wilcken, B., & Wiley, V. C. (2001). Newborn screening for congenital hypothyroidism in very-low-birth-weight babies: the need for a second test. *J Inherit Metab Dis*, *34*, 827–833.
85. Filippi, L., Pezzati, M., Cecchi, A., & Poggi, C. (2006). Dopamine infusion: a possible cause of undiagnosed congenital hypothyroidism in preterm infants. *Pediatr Crit Care Med*, *7*, 249–251.
86. Woo, H. C., Lizarda, A., Tucker, R., et al.(2011). Congenital hypothyroidism with a delayed thyroid-stimulating hormone elevation in very premature infants: incidence and growth and developmental outcomes. *J Pediatr*, *158*, 538–542.

87. Vincent, M. A., Rodd, C., Dussault, J. H., & Van Vliet, G. (2002). Very low birth weight newborns do not need repeat screening for congenital hypothyroidism. *J Pediatr*, *140*, 311–334.
88. Perry, R., Heinrichs, C., Bourdoux, P., et al.(2002). Discordance of monozygotic twins for thyroid dysgenesis: implications for screening and for molecular pathophysiology. *J Clin Endocrinol Metab*, *87*, 4072–4077.
89. Alm, J., Larsson, A., & Zetterstrom, R. (1978). Congenital hypothyroidism in Sweden: incidence and age at diagnosis. *Acta Paediatr Scand*, *67*, 1–3.
90. Corbetta, C., Weber, G., Cortinovis, F., et al. (2009). A 7-year experience with low blood TSH cutoff levels for neonatal screening reveals an unsuspected frequency of congenital hypothyroidism (CH). *Clin Endocrinol* (Oxf), *71*, 739–745.
91. Mengreli, C., Kanaka-Gantenbein, C., Girginoudis, P., et al. (2010). Screening for congenital hypothyroidism: the significance of threshold limit in false-negative results. *J Clin Endocrinol Metab*, *95*, 4283–4290.
92. Olney, R. S., Grosse, S. D., & Vogt, R. F., Jr. (2010). Prevalence of congenital hypothyroidism—current trends and future directions: workshop summary. *Pediatrics*, *125*(suppl 2), S31–S36.
93. Majeed-Saidan, M. A., Joyce, B., Khan, M., & Hamam, H. D. (1993). Congenital hypothyroidism: the Riyadh Military Hospital experience. *Clin Endocrinol* (Oxf), *38*, 191–195.
94. Albert, B. B., Cutfield, W. S., Webster, D., et al. (2012). Etiology of increasing incidence of congenital hypothyroidism in New Zealand from 1993-2010. *J Clin Endocrinol Metab*, *97*, 3155–3160.
95. De Wals, P., Tairou, F., Van Allen, M. I., et al. (2007). Reduction in neural-tube defects after folic acid fortification in Canada. *N Engl J Med*, *357*, 135–142.
96. Ionescu-Ittu, R., Marelli, A. J., Mackie, A. S., & Pilote, L. (2009). Prevalence of severe congenital heart disease after folic acid fortification of grain products: time trend analysis in Quebec, Canada. *BMJ*, *338*, B1673.
97. Jones, J. H., Attaie, M., Maroo, S., et al. (2010). Heterogeneous tissue in the thyroid fossa on ultrasound in infants with proven thyroid ectopia on isotope scan: a diagnostic trap. *Pediatr Radiol*, *40*, 725–731.
98. Wildi-Runge, S., Stoppa-Vaucher, S., Lambert, R., et al. (2012). A high prevalence of dual thyroid ectopy in congenital hypothyroidism: evidence for insufficient signaling gradients during embryonic thyroid migration or for the polyclonal nature of the thyroid gland? *J Clin Endocrinol Metab*, *97*, E978–E981.
99. Stoppa-Vaucher, S., Lapointe, A., Turpin, S., et al. (2010). Ectopic thyroid gland causing dysphonia: imaging and molecular studies. *J Clin Endocrinol Metab*, *95*, 4509–4510.
100. Djemli, A., Fillion, M., Belgoudi, J., et al. (2004). Twenty years later: a reevaluation of the contribution of plasma thyroglobulin to the diagnosis of thyroid dysgenesis in infants with congenital hypothyroidism. *Clin Biochem*, *37*, 818–822.
101. Connors, M. H., & Styne, D. M. (1986). Transient neonatal "athyreosis" resulting from thyrotropin-binding inhibitory immunoglobulins. *Pediatrics*, *78*, 287–290.
102. Castanet, M., Lyonnet, S., Bonaiti-Pellie, C., et al. (2000). Familial forms of thyroid dysgenesis among infants with congenital hypothyroidism. *N Engl J Med*, *343*, 441–442.
103. Abu-Khudir, R., Paquette, J., Lefort, A., et al. (2010). Transcriptome, methylome and genomic variations analysis of ectopic thyroid glands. *PLoS One*, *5*, e13420.
104. Devos, H., Rodd, C., Gagne, N., et al. (1999). A search for the possible molecular mechanisms of thyroid dysgenesis: sex ratios and associated malformations. *J Clin Endocrinol Metab*, *84*, 2502–2506.
105. Stoppa-Vaucher, S., Van Vliet, G., & Deladoey, J. (2011). Variation by ethnicity in the prevalence of congenital hypothyroidism due to thyroid dysgenesis. *Thyroid*, *21*, 13–18.
106. Castanet, M., Sura-Trueba, S., Chauty, A., et al. (2005). Linkage and mutational analysis of familial thyroid dysgenesis demonstrate genetic heterogeneity implicating novel genes. *Eur J Hum Genet*, *13*, 232–239.
107. Castanet, M., & Polak, M. (2010). Spectrum of human Foxe1/TTF2 mutations. *Horm Res Paediatr*, *73*, 423–429.
108. Maquet, E., Costagliola, S., Parma, J., et al. (2009). Lethal respiratory failure and mild primary hypothyroidism in a term girl with a de novo heterozygous mutation in the TITF1/NKX2.1 gene. *J Clin Endocrinol Metab*, *94*, 197–203.
109. Carre, A., Szinnai, G., Castanet, M., et al. (2009). Five new TTF1/NKX2.1 mutations in brain-lung-thyroid syndrome: rescue by PAX8 synergism in one case. *Hum Mol Genet*, *18*, 2266–2276.
110. Krude, H., Schutz, B., Biebermann, H., et al. (2002). Choreoathetosis, hypothyroidism, and pulmonary alterations due to human NKX2-1 haploinsufficiency. *J Clin Invest*, *109*, 475–480.
111. Macchia, P. E., Lapi, P., Krude, H., et al. (1998). PAX8 mutations associated with congenital hypothyroidism caused by thyroid dysgenesis. *Nat Genet*, *19*, 83–86.
112. Al Taji, E., Biebermann, H., Limanova, Z., et al. (2007). Screening for mutations in transcription factors in a Czech cohort of 170 patients with congenital and early-onset hypothyroidism: identification of a novel PAX8 mutation in dominantly inherited early-onset non-autoimmune hypothyroidism. *Eur J Endocrinol*, *156*, 521–529.
113. Senee, V., Chelala, C., Duchatelet, S., et al. (2006). Mutations in GLIS3 are responsible for a rare syndrome with neonatal diabetes mellitus and congenital hypothyroidism. *Nat Genet*, *38*, 682–687.
114. Dimitri, P., Warner, J. T., Minton, J. A., et al. (2011). Novel GLIS3 mutations demonstrate an extended multisystem phenotype. *Eur J Endocrinol*, *164*, 437–443.
115. van Trotsenburg, A. S., Vulsma, T., Van Santen, H. M., et al. (2003). Lower neonatal screening thyroxine concentrations in down syndrome newborns. *J Clin Endocrinol Metab*, *88*, 1512–1515.
116. Castanet, M., Polak, M., Bonaiti-Pellie, C., et al. (2011). Nineteen years of national screening for congenital hypothyroidism: familial cases with thyroid dysgenesis suggest the involvement of genetic factors. *J Clin Endocrinol Metab*, *86*, 2009–2014.
117. Olivieri, A., Stazi, M. A., Mastroiacovo, P., et al. (2002). A population-based study on the frequency of additional congenital malformations in infants with congenital hypothyroidism: data from the Italian registry for congenital hypothyroidism (1991-1998). *J Clin Endocrinol Metab*, *87*, 557–562.
118. Stanbury, J. B., Rocmans, P., Buhler, U. K., & Ochi, Y. (1968). Congenital hypothyroidism with impaired thyroid response to thyrotropin. *N Engl J Med*, *279*, 1132–1136.
119. Parmentier, M., Libert, F., Maenhaut, C., et al. (1989). Molecular cloning of the thyrotropin receptor. *Science*, *246*, 1620–1602.
120. Biebermann, H., Schoneberg, T., Krude, H., et al. (1997). Mutations of the human thyrotropin receptor gene causing thyroid hypoplasia and persistent congenital hypothyroidism. *J Clin Endocrinol Metab*, *82*, 3471–3480.
121. Sunthornthepvarakul, T., Gottschalk, M. E., Hayashi, Y., & Refetoff, S. (1995). Brief report: resistance to thyrotropin caused by mutations in the thyrotropin-receptor gene. *N Engl J Med*, *332*, 155–160.
122. de Roux, N., Misrahi, M., Brauner, R., et al. (1996). Four families with loss of function mutations of the thyrotropin receptor. *J Clin Endocrinol Metab*, *81*, 4229–4235.
123. Calebiro, D., Gelmini, G., Cordella, D., et al. (2012). Frequent TSH receptor genetic alterations with variable signaling impairment in a large series of children with nonautoimmune isolated hyperthyrotropinemia. *J Clin Endocrinol Metab*, *97*, E156–E160.
124. Yokoro, S., Matsuo, M., Ohtsuka, T., & Ohzeki, T. (1990). Hyperthyrotropinemia in a neonate with normal thyroid hormone levels: the earliest diagnostic clue for pseudohypoparathyroidism. *Biol Neonate*, *58*, 69–72.
125. Polak, M., & Van Vliet, G. (2008). Disorders of the thyroid gland. In K. Sarafoglou, G. F. Hoffmann, & K. S. Roth (Eds.), *Pediatric endocrinology and inborn errors of metabolism.* New York: McGraw-Hill, 355–382.
126. Vassart, G., & Dumont, J. E. (1992). The thyrotropin receptor and the regulation of thyrocyte function and growth. *Endocr Rev*, *13*, 596–611.
127. Grasberger, H., & Refetoff, S. (2011). Genetic causes of congenital hypothyroidism due to dyshormonogenesis. *Curr Opin Pediatr*, *23*, 421–428.
128. Deladoey, J., Pfarr, N., Vuissoz, J. M., et al. (2008). Pseudodominant inheritance of goitrous congenital hypothyroidism caused by TPO mutations: molecular and in silico studies. *J Clin Endocrinol Metab*, *93*, 627–633.
129. Avbelj, M., Tahirovic, H., Debeljak, M., et al. (2007). High prevalence of thyroid peroxidase gene mutations in patients with thyroid dyshormonogenesis. *Eur J Endocrinol*, *156*, 511–519.

130. Szinnai, G., Kosugi, S., Derrien, C., et al. (2006). Extending the clinical heterogeneity of iodide transport defect (ITD): a novel mutation R124H of the sodium/iodide symporter (NIS) gene and review of genotype-phenotype correlations in ITD. *J Clin Endocrinol Metab*, *91*, 1199–1204.
131. Everett, L. A., Glaser, B., Beck, J. C., et al. (1997). Pendred syndrome is caused by mutations in a putative sulphate transporter gene (PDS). *Nat Genet*, *17*, 411–422.
132. Gaudino, R., Garel, C., Czernichow, P., & Leger, J. (2005). Proportion of various types of thyroid disorders among newborns with congenital hypothyroidism and normally located gland: a regional cohort study. *Clin Endocrinol* (Oxf), *62*, 444–448.
133. Banghova, K., Al, T. E., Cinek, O., et al. (2008). Pendred syndrome among patients with congenital hypothyroidism detected by neonatal screening: identification of two novel PDS/SLC26A4 mutations. *Eur J Pediatr*, *167*, 777–783.
134. Bizhanova, A., & Kopp, P. (2010). Genetics and phenomics of Pendred syndrome. *Mol Cell Endocrinol*, *322*, 83–90.
135. Bakker, B., Bikker, H., & Hennekam, R. C., (2001). Maternal isodisomy for chromosome 2p causing severe congenital hypothyroidism. *J Clin Endocrinol Metab*, *86*, 1164–1168.
136. Abramowicz, M. J., Targovnik, H. M., Varela, V., et al. (1992). Identification of a mutation in the coding sequence of the human thyroid peroxidase gene causing congenital goiter. *J Clin Invest*, *90*, 1200–1204.
137. Belforte, F. S., Miras, M. B., Olcese, M. C., et al. (2012). Congenital goitrous hypothyroidism: mutation analysis in the thyroid peroxidase gene. *Clin Endocrinol* (Oxf), *76*, 568–576.
138. Fugazzola, L., Cerutti, N., Mannavola, D., et al. (2003). Monoallelic expression of mutant thyroid peroxidase allele causing total iodide organification defect. *J Clin Endocrinol Metab*, *88*, 3264–3271.
139. Moreno, J. C., Bikker, H., Kempers, M. J., et al. (2002). Inactivating mutations in the gene for thyroid oxidase 2 (THOX2) and congenital hypothyroidism. *N Engl J Med*, *347*, 95–102.
140. Hoste, C., Rigutto, S., Van Vliet, G., et al. (2010). Compound heterozygosity for a novel hemizygous missense mutation and a partial deletion affecting the catalytic core of the H2O2-generating enzyme DUOX2 associated with transient congenital hypothyroidism. *Hum Mutat*, *31*, E1304–E1319.
141. Vigone, M. C., Fugazzola, L., Zamproni, I., et al. (2005). Persistent mild hypothyroidism associated with novel sequence variants of the DUOX2 gene in two siblings. *Hum Mutat*, *26*, 395.
142. Grasberger, H., & Refetoff, S. (2006). Identification of the maturation factor for dual oxidase: evolution of an eukaryotic operon equivalent . *J Biol Chem*, *281*, 18269–18272.
143. Zamproni, I., Grasberger, H., Cortinovis, F., et al. (2008). Biallelic inactivation of the dual oxidase maturation factor 2 (DUOXA2) gene as a novel cause of congenital hypothyroidism. *J Clin Endocrinol Metab*, *93*, 605–610.
144. Vassart, G., Bacolla, A., Brocas, H., et al. (1985). Structure, expression and regulation of the thyroglobulin gene. *Mol Cell Endocrinol*, *40*, 89–97.
145. Caron, P., Moya, C. M., Malet, D., et al. (2003). Compound heterozygous mutations in the thyroglobulin gene (1143delC and 6725G—>A [R2223H]) resulting in fetal goitrous hypothyroidism. *J Clin Endocrinol Metab*, *88*, 3546–3553.
146. Reynolds, B. C., Simpson, J. H., Macara, L., et al. (2006). Goitrous congenital hypothyroidism in a twin pregnancy causing respiratory obstruction at birth: implications for management. *Acta Paediatr*, *95*, 1345–1348.
147. Citterio, C. E., Coutant, R., Rouleau, S., et al. (2011). A new compound heterozygous for c.886C>T/c.2206C>T [p.R277X/p.Q717X] mutations in the thyroglobulin gene as a cause of foetal goitrous hypothyroidism. *Clin Endocrinol* (Oxf), *74*, 533–535.
148. Borgel, K., Pohlenz, J., Holzgreve, W., & Bramswig, J. H. (2005). Intrauterine therapy of goitrous hypothyroidism in a boy with a new compound heterozygous mutation (Y453D and C800R) in the thyroid peroxidase gene: a long-term follow-up. *Am J Obstet Gynecol*, *193*, 857–858.
149. Targovnik, H. M., Citterio, C. E., & Rivolta, C. M. (2011). Thyroglobulin gene mutations in congenital hypothyroidism. *Horm Res Paediatr*, *75*, 11–321.
150. Moreno, J. C., Klootwijk, W., van, T. H., Pinto, G., et al. (2008). Mutations in the iodotyrosine deiodinase gene and hypothyroidism. *N Engl J Med*, *358*, 1811–1818.
151. Afink, G., Kulik, W., Overmars, H., et al. (2008). Molecular characterization of iodotyrosine dehalogenase deficiency in patients with hypothyroidism. *J Clin Endocrinol Metab*, *93*, 4894–4901.
152. Burniat, A., Pirson, I., Vilain, C., et al. (2012). Iodotyrosine deiodinase defect identified via genome-wide approach. *J Clin Endocrinol Metab*, *97*, E1276–E1283.
153. Dumitrescu, A. M., Liao, X. H., Abdullah, M. S., et al. (2005). Mutations in SECISBP2 result in abnormal thyroid hormone metabolism. *Nat Genet*, *37*, 1247–1252.
154. Di Cosmo, C., McLellan, N., Liao, X. H., et al. (2009). Clinical and molecular characterization of a novel selenocysteine insertion sequence-binding protein 2 (SBP2) gene mutation (R128X). *J Clin Endocrinol Metab*, *94*, 4003–4009.
155. Hamajima, T., Mushimoto, Y., Kobayashi, H., et al. (2012). Novel compound heterozygous mutations in the SBP2 gene: characteristic clinical manifestations and the implications of GH and triiodothyronine in longitudinal bone growth and maturation. *Eur J Endocrinol*, *166*, 757–764.
156. Azevedo, M. F., Barra, G. B., Naves, L. A., et al. (2010). Selenoprotein-related disease in a young girl caused by nonsense mutations in the SBP2 gene. *J Clin Endocrinol Metab*, *95*, 4066–4071.
157. Schoenmakers, E., Agostini, M., Mitchell, C., et al. (2010). Mutations in the selenocysteine insertion sequence-binding protein 2 gene lead to a multisystem selenoprotein deficiency disorder in humans. *J Clin Invest*, *120*, 4220–4235.
158. Weiss, R. E., Balzano, S., Scherberg, N. H., & Refetoff, S. (1990). Neonatal detection of generalized resistance to thyroid hormone. *JAMA*, *264*, 2245–2250.
159. Lafranchi, S. H., Snyder, D. B., Sesser, D. E., et al. (2003). Follow-up of newborns with elevated screening T4 concentrations. *J Pediatr*, *143*, 296–301.
160. Tajima, T., Jo, W., Fujikura, K., et al. (2009). Elevated free thyroxine levels detected by a neonatal screening system. *Pediatr Res*, *66*, 312–316.
161. Ferrara, A. M., Onigata, K., Ercan, O., et al. (2012). Homozygous thyroid hormone receptor beta-gene mutations in resistance to thyroid hormone: three new cases and review of the literature. *J Clin Endocrinol Metab*, *97*, 1328–1336.
162. Anzai, R., Adachi, M., Sho, N., et al. (2012). Long-term 3,5,3'-triiodothyroacetic acid therapy in a child with hyperthyroidism caused by thyroid hormone resistance: pharmacological study and therapeutic recommendations. *Thyroid*, *22*, 1061-1075.
163. Tsai, S. L., & Ahmet, A. (2012). Treatment with methimazole in a 3-year-old male with thyroid hormone resistance. *Horm Res Paediatr*, *77*, 402–406.
164. Weiss, R. E., & Refetoff, S. (1999). Treatment of resistance to thyroid hormone—primum non nocere. *J Clin Endocrinol Metab*, *84*, 401–404.
165. Stevenson, R. E., Goodman, H. O., Schwartz C. E., et al. (1990). Allan-Herndon syndrome. I. Clinical studies. *Am J Hum Genet*, *47*, 446–453.
166. Ramos, H. E., Morandini, M., Carre, A., et al. (2011). Pregnancy in women heterozygous for MCT8 mutations: risk of maternal hypothyroxinemia and fetal care. *Eur J Endocrinol*, *164*, 309–314.
167. Di Cosmo, C., Liao, X. H., Dumitrescu, A. M., et al. (2009). A thyroid hormone analog with reduced dependence on the monocarboxylate transporter 8 for tissue transport. *Endocrinology*, *150*, 4450–4458.
168. Verge, C. F., Konrad, D., Cohen, M., et al. (2012). Diiodothyropropionic acid (DITPA) in the treatment of MCT8 deficiency. *J Clin Endocrinol Metab*, *97*, 4515–4523.
169. Huang, S. A., Tu, H. M., Harney, J. W., et al. (2000). Severe hypothyroidism caused by type 3 iodothyronine deiodinase in infantile hemangiomas. *N Engl J Med*, *343*, 185–189.
170. Konrad, D., Ellis, G., & Perlman, K. (2003). Spontaneous regression of severe acquired infantile hypothyroidism associated with multiple liver hemangiomas. *Pediatrics*, *112*, 1424–1426.
171. Peters, C., Langham, S., Mullis, P. E., & Dattani, M. T. (2010). Use of combined liothyronine and thyroxine therapy for consumptive hypothyroidism associated with hepatic haemangiomas in infancy. *Horm Res Paediatr*, *74*, 149–152.

172. Ayling, R. M., Davenport, M., Hadzic, N., et al. (2001). Hepatic hemangioendothelioma associated with production of humoral thyrotropin-like factor. *J Pediatr*, *138*, 932–935.
173. Schweizer, R., Blumenstock, G., Mangelsdorf, K., et al. (2010). Prevalence and incidence of endocrine disorders in children: results of a survey in Baden-Wuerttemberg and Bavaria (EndoPrIn BB) 2000-2001. *Klin Padiatr*, *222*, 67–72.
174. Kurtoglu, S., Coban, D., Akin, M. A., et al. (2009). Neonatal sludge: a finding of congenital hypothyroidism. *J Clin Res Pediatr Endocrinol*, *1*, 197–200.
175. Labrune, P., Myara, A., Huguet, P., et al. (1992). Bilirubin uridine diphosphate glucuronosyltransferase hepatic activity in jaundice associated with congenital hypothyroidism. *J Pediatr Gastroenterol Nutr*, *14*, 79–82.
176. Leblanc, A., Odievre, M., Hadchouel, M., et al. (1981). Neonatal cholestasis and hypoglycemia: possible role of cortisol deficiency. *J Pediatr*, *99*, 577–580.
177. Braslavsky, D., Keselman, A., Galoppo, M., et al. (2011). Neonatal cholestasis in congenital pituitary hormone deficiency and isolated hypocortisolism: characterization of liver dysfunction and follow-up. *Arq Bras Endocrinol Metabol*, *55*, 622–627.
178. Di Iorgi, N., Allegri, A. E., Napoli, F., et al. (2012). The use of neuroimaging for assessing disorders of pituitary development. *Clin Endocrinol* (Oxf), *76*, 161–176.
179. Collu, R., Tang, J., Castagne, J., et al. (1997). A novel mechanism for isolated central hypothyroidism: inactivating mutations in the thyrotropin-releasing hormone receptor gene. *J Clin Endocrinol Metab*, *82*, 1561–1565.
180. Bonomi, M., Busnelli, M., Beck-Peccoz, P., et al. (2009). A family with complete resistance to thyrotropin-releasing hormone. *N Engl J Med*, *360*, 731–734.
181. Miyai, K., Azukizawa, M., & Kumahara, Y. (1971). Familial isolated thyrotropin deficiency with cretinism. *N Engl J Med*, *285*, 1043–1048.
182. Hayashizaki, Y., Hiraoka, Y., Tatsumi, K., et al. (1990). Deoxyribonucleic acid analyses of five families with familial inherited thyroid stimulating hormone deficiency. *J Clin Endocrinol Metab*, *71*, 792–796.
183. Deladoey, J., Vuissoz, J. M., Domene, H. M., et al. (2003). Congenital secondary hypothyroidism due to a mutation C105Vfs114X thyrotropin-beta mutation: genetic study of five unrelated families from Switzerland and Argentina. *Thyroid*, *13*, 553–559.
184. Brumm, H., Pfeufer, A., Biebermann, H., et al. (2002). Congenital central hypothyroidism due to homozygous thyrotropin beta 313 Delta T mutation is caused by a Founder effect. *J Clin Endocrinol Metab*, *87*, 4811–4816.
185. Thilly, C. H., Delange, F., Lagasse, R., et al. (1978). Fetal hypothyroidism and maternal thyroid status in severe endemic goiter. *J Clin Endocrinol Metab*, *47*, 354–360.
186. Delange, F., Dalhem, A., Bourdoux, P., et al. (1984). Increased risk of primary hypothyroidism in preterm infants. *J Pediatr*, *105*, 462–469.
187. Connelly, K. J., Boston, B. A., Pearce, E. N., et al. (2012). Congenital hypothyroidism caused by excess prenatal maternal iodine ingestion. *J Pediatr*, *161*, 760–762.
188. De Wolf, D., De Schepper, J., Verhaaren, H., et al. (1988). Congenital hypothyroid goiter and amiodarone. *Acta Paediatr Scand*, *77*, 616–618.
189. Trudel, K., Sanatani, S., & Panagiotopoulos, C. (2011). Severe amiodarone-induced hypothyroidism in an infant. *Pediatr Crit Care Med*, *12*, E43–E45.
190. Linder, N., Sela, B., German, B., et al. (1997). Iodine and hypothyroidism in neonates with congenital heart disease. *Arch Dis Child Fetal Neonatal Ed*, *77*, F239–F240.
191. Brown, R. S., Bloomfield, S., Bednarek, F. J., et al. (1997). Routine skin cleansing with povidone-iodine is not a common cause of transient neonatal hypothyroidism in North America: a prospective controlled study. *Thyroid*, *7*, 395–400.
192. Castanet, M., Leenhardt, L., Leger, J., et al. (2005). Thyroid hemiagenesis is a rare variant of thyroid dysgenesis with a familial component but without Pax8 mutations in a cohort of 22 cases. *Pediatr Res*, *57*, 908–913.
193. Grasberger, H., Mimouni-Bloch, A., Vantyghem, M. C., et al. (2005). Autosomal dominant resistance to thyrotropin as a distinct entity in five multigenerational kindreds: clinical characterization and exclusion of candidate loci. *J Clin Endocrinol Metab*, *90*, 4025–4034.
194. van Wassenaer, A. G., Kok, J. H., de Vijlder, J. J., et al. (1997). Effects of thyroxine supplementation on neurologic development in infants born at less than 30 weeks' gestation. *N Eng J Med*, *336*, 21–26.
195. van Wassenaer, A. G., Westera, J., Houtzager, B. A., & Kok, J. H. (2005). Ten-year follow-up of children born at <30 weeks' gestational age supplemented with thyroxine in the neonatal period in a randomized, controlled trial. *Pediatrics*, *116*, E613–E618.
196. La Gamma, E. F., van Wassenaer, A. G., Ares, S., et al. (2009). Phase 1 trial of 4 thyroid hormone regimens for transient hypothyroxinemia in neonates of < 28 weeks' gestation. *Pediatrics*, *124*, E258–E268.
197. Marks, S. D. (2009). Nonthyroidal illness syndrome in children. *Endocrine*, *36*, 355–367.
198. Pacaud, D., Huot, C., Gattereau, A., et al. (1995). Outcome in three siblings with antibody-mediated transient congenital hypothyroidism. *J Pediatr*, *127*, 275–277.
199. Van Vliet, G., Larroque, B., Bubuteishvili, L., et al. (2003). Sex-specific impact of congenital hypothyroidism due to thyroid dysgenesis on skeletal maturation in term newborns. *J Clin Endocrinol Metab*, *88*, 2009–2013.
200. New England Congenital Hypothyroidism Collaborative (1981). Effects of neonatal screening for hypothyroidism: prevention of mental retardation by treatment before clinical manifestations. *Lancet*, *2*, 1095–1098.
201. Van Vliet, G. (2007). Congenital hypothyroidism: look at the whole patient! *Horm Res*, *68*, 276–277.
202. Kempers, M. J., van der Sluijs, V., Nijhuis-van der Sanden, R. W., et al. (2007). Neonatal screening for congenital hypothyroidism in the Netherlands: cognitive and motor outcome at 10 years of age. *J Clin Endocrinol Metab*, *92*, 919–924.
203. Dimitropoulos, A., Molinari, L., Etter, K., et al. (2009). Children with congenital hypothyroidism: long-term intellectual outcome after early high-dose treatment. *Pediatr Res*, *65*, 242–248.
204. Schoen, E. J., Clapp, W., To, T. T., & Fireman, B. H. (2004). The key role of newborn thyroid scintigraphy with isotopic iodide (123I) in defining and managing congenital hypothyroidism. *Pediatrics*, *114*, E683–E688.
205. Verelst, J., Chanoine, J. P., Delange, F., et al. (1991). Radionuclide imaging in primary permanent congenital hypothyroidism. *Clin Nucl Med*, *16*, 652–655.
206. Glorieux, J., Desjardins, M., Letarte, J., et al. (1988). Useful parameters to predict the eventual mental outcome of hypothyroid children. *Pediatr Res*, *24*, 6–8.
207. Wasniewska, M., De Luca, F., Cassio, A., et al. (2003). In congenital hypothyroidism bone maturation at birth may be a predictive factor of psychomotor development during the first year of life irrespective of other variables related to treatment. *Eur J Endocrinol*, *149*, 1–6.
208. Delange, F. (1997). Neonatal screening for congenital hypothyroidism: results and perspectives. *Horm Res*, *48*, 51–61.
209. Kemper, A. R., Ouyang, L., & Grosse, S. D. (2010). Discontinuation of thyroid hormone treatment among children in the United States with congenital hypothyroidism: findings from health insurance claims data. *BMC Pediatr*, *10*, 19.
210. Rose, S. R., Brown, R. S., Foley, T., et al. (2006). Update of newborn screening and therapy for congenital hypothyroidism. *Pediatrics*, *117*, 2290–2303.
211. Cassio, A., Cacciari, E., Cicognani, A., et al. (2003). Treatment for congenital hypothyroidism: thyroxine alone or thyroxine plus triiodothyronine? *Pediatrics*, *111*, 1055–1060.
212. Selva, K. A., Mandel, S. H., Rien, L., et al. (2002). Initial treatment dose of L-thyroxine in congenital hypothyroidism. *J Pediatr*, *141*, 786–792.
213. Selva, K. A., Harper, A., Downs, A., et al. (2005). Neurodevelopmental outcomes in congenital hypothyroidism: comparison of initial T4 dose and time to reach target T4 and TSH. *J Pediatr*, *147*, 775–780.
214. Nelson, J. C., Clark, S. J., Borut, D. L., et al. (1993). Age-related changes in serum free thyroxine during childhood and adolescence. *J Pediatr*, *123*, 899–905.

215. Fisher, D. A., Schoen, E. J., La Franchi, S., et al. (2000). The hypothalamic-pituitary-thyroid negative feedback control axis in children with treated congenital hypothyroidism. *J Clin Endocrinol Metab*, *85*, 2722–2727.
216. Alvarez, M., Iglesias, F. C., Rodriguez, S. A., et al. (2010). Episodes of overtreatment during the first six months in children with congenital hypothyroidism and their relationships with sustained attention and inhibitory control at school age. *Horm Res Paediatr*, *74*, 114–120.
217. Leger, J., Larroque, B., & Norton, J. (2001). Influence of severity of congenital hypothyroidism and adequacy of treatment on school achievement in young adolescents: a population-based cohort study. *Acta Paediatr*, *90*, 1249–1256.
218. Dubuis, J. M., Glorieux, J., Richer, F., et al. (1996). Outcome of severe congenital hypothyroidism: closing the developmental gap with early high dose levothyroxine treatment. *J Clin Endocrinol Metab*, *81*, 222–227.
219. Bucher, H., Prader, A., & Illig, R. (1985). Head circumference, height, bone age and weight in 103 children with congenital hypothyroidism before and during thyroid hormone replacement. *Helv Paediatr Acta*, *40*, 305–316.
220. Smith, C., Thomsett, M., Choong, C., et al. (2001). Congenital thyrotoxicosis in premature infants. *Clin Endocrinol* (Oxf), *54*, 371–376.
221. Abraham-Nordling, M., Bystrom, K., Torring, O., et al. (2011). Incidence of hyperthyroidism in Sweden. *Eur J Endocrinol*, *165*, 899–905.
222. Smith, C. M., Gavranich, J., Cotterill, A., & Rodda, C. P. (2000). Congenital neonatal thyrotoxicosis and previous maternal radioiodine therapy. *BMJ*, *320*, 1260–1261.
223. Luton, D., Le Gac, I., Vuillard, E., et al. (2005). Management of Graves' disease during pregnancy: the key role of fetal thyroid gland monitoring. *J Clin Endocrinol Metab*, *90*, 6093–6098.
224. Huel, C., Guibourdenche, J., Vuillard, E., et al. (2009). Use of ultrasound to distinguish between fetal hyperthyroidism and hypothyroidism on discovery of a goiter. *Ultrasound Obstet Gynecol*, *33*, 412–420.
225. Kilpatrick, S. (2003). Umbilical blood sampling in women with thyroid disease in pregnancy: Is it necessary? *Am J Obstet Gynecol*, *189*, 1–2.
226. Clementi, M., Di, G. E., Cassina, M., et al. (2010). Treatment of hyperthyroidism in pregnancy and birth defects. *J Clin Endocrinol Metab*, *95*, E337–E341.
227. Rivkees, S. A., & Szarfman, A. (2010). Dissimilar hepatotoxicity profiles of propylthiouracil and methimazole in children. *J Clin Endocrinol Metab*, *95*, 3260–3267.
228. Glinoer, D., & Cooper, D. S. (2012). The propylthiouracil dilemma. *Curr Opin Endocrinol Diabetes Obes*, *19*, 402–407.
229. Benavides, V. C., Mallela, M. K., Booth, C. J., et al. (2012). Propylthiouracil is teratogenic in murine embryos. *PLoS One*, *7*, e35213.
230. Earles, S. M., Gerrits, P. M., & Transue, D. J. (2004). Iopanoic acid in the management of neonatal Graves' disease. *J Perinatol*, *24*, 105–108.
231. Wit, J. M., Gerards, L. J., Vermeulen-Meiners, C., & Bruinse H. W. (1985). Neonatal thyrotoxicosis treated with exchange transfusion and Lugol's iodine. *Eur J Pediatr*, *143*, 317–319.
232. Vaidya, B., Campbell, V., Tripp, J. H., et al. (2004). Premature birth and low birth weight associated with nonautoimmune hyperthyroidism due to an activating thyrotropin receptor gene mutation. *Clin Endocrinol* (Oxf), *60*, 711–718.
233. Kopp, P., Van Sande, J., Parma, J., et al. (1995). Brief report: congenital hyperthyroidism caused by a mutation in the thyrotropin-receptor gene. *N Engl J Med*, *332*, 150–154.
234. de Roux, N., Polak, M., Couet, J., et al. (1996). A neomutation of the thyroid-stimulating hormone receptor in a severe neonatal hyperthyroidism. *J Clin Endocrinol Metab*, *81*, 2023–2026.
235. Aycan, Z., Agladioglu, S. Y., Ceylaner, S., et al. (2010). Sporadic nonautoimmune neonatal hyperthyroidism due to A623V germline mutation in the thyrotropin receptor gene. *J Clin Res Pediatr Endocrinol*, *2*, 168–172.
236. Agretti, P., De, M. G., Biagioni, M., et al. (2012). Sporadic congenital nonautoimmune hyperthyroidism caused by P639S mutation in thyrotropin receptor gene. *Eur J Pediatr*, *171*, 1133–1137.
237. Duprez, L., Parma, J., Van Sande, J., et al. (1994). Germline mutations in the thyrotropin receptor gene cause non-autoimmune autosomal dominant hyperthyroidism. *Nat Genet*, *7*, 396–401.
238. Pohlenz, J., Pfarr, N., Kruger, S., & Hesse, V. (2006). Subclinical hyperthyroidism due to a thyrotropin receptor (TSHR) gene mutation (S505R). *Acta Paediatr*, *95*, 1685–1687.
239. Kopp, P., Muirhead, S., Jourdain, N., et al. (1997). Congenital hyperthyroidism caused by a solitary toxic adenoma harboring a novel somatic mutation (serine281—>isoleucine) in the extracellular domain of the thyrotropin receptor. *J Clin Invest*, *10*, 1634–1639.
240. Kohn, B., Grasberger, H., Lam, L. L., et al. (2009). A somatic gain-of-function mutation in the thyrotropin receptor gene producing a toxic adenoma in an infant. *Thyroid*, *19*, 187–191.
241. Desai, P. R., Lipscomb, A. P., & Slater, J. (2004). Familial dysalbuminaemic hyperthyroxinaemia, a thyroid trap. *Arch Dis Child*, *89*, 1161–1162.

QUESTIONS

1. A newborn screens positive for congenital hypothyroidism with a TSH of 25 mU/L on whole blood. At 9 days of life, the diagnosis is confirmed with a serum TSH of 45 mU/L, a free T4 of 12 pmol/L, and a Tg of 54 μg/L. Thyroid scintigraphy shows no uptake. What might explain these findings? (Choose all that apply.)
   a. TSH too low to induce isotope uptake
   b. Transplacental passage of maternal TSH receptor-blocking antibodies
   c. Use of povidone-iodine disinfection during delivery
   d. Inactivating mutations of the TSH receptor
   e. True athyreosis

Answers: b, c, and d

2. Among these statements regarding congenital hypothyroidism, which are true? (Choose all that apply.)
   a. Thyroid dysgenesis is an autosomal recessive disease.
   b. TPO and Tg mutations are the most common causes of dyshormonogenesis.
   c. A delayed bone age is a good prognostic factor for cognitive development.
   d. A low T4 is a poor prognostic factor for cognitive development.
   e. The initial recommended dose of L-T4 is 5 μg/kg/day.
   f. A child with congenital hypothyroidism from thyroid dysgenesis has a slightly increased risk of also having congenital heart disease.

Answers: b, d, and f

3. A 6-month-old infant with athyreosis was previously well controlled with 50 μg of levothyroxine daily and comes to your clinic with the following serum values: TSH 70 mU/L, free T4 5.6 pmol/L. Which of the following may explain this condition? (Choose all that apply.)
   a. The thyroxine was given in the morning, now it is given in the evening.
   b. There is noncompliance with the medication.
   c. The infant took cow's milk the first 5 months and now takes soy milk.
   d. The infant has started taking elemental iron simultaneously with thyroxine.
   e. The infant now receives thyroxine in liquid form.

Answers: b, c, and d

4. What is the likelihood that a mother with Hashimoto's thyroiditis will have a hypothyroid newborn?
   a. 50%
   b. 10%
   c. 5%
   d. < 1%

Answer: d

5. For the treatment of a newborn with symptomatic hyperthyroidism, which should *not* be used?
   a. Propranolol
   b. Propylthiouracil
   c. Methimazole
   d. Lugol solution
   e. Glucocorticoids

Answer: b

CHAPTER 8

Disorders of Calcium and Phosphorus Homeostasis in the Newborn and Infant

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CHAPTER OUTLINE

Calcium (Ca), phosphorus (as phosphate [HPO_4^{2-}], as phosphorus does not exist in the free state in living tissues), and magnesium (Mg) are essential nutrients that are indispensable for the structural integrity of the body and for the function of each of its cells.[1,2] The genetic and physiologic mechanisms that regulate normal mineral homeostasis and bone development, composition, and strength from the prenatal period through adolescence are complex. Table 8-2 lists some of the many genes that direct these processes. Figures 8-1 and 8-2 schematically depict the factors that regulate serum concentrations of calcium and phosphate, respectively.

CALCIUM

Calcium is an irreplaceable component of the mineral portion of bone and is required for the function of each of the body's cell. Together, calcium and phosphate form the hydroxyapatite crystal—$Ca_{10}(PO_4)_{10}(OH)_2$—of bone; hydroxyapatite accounts for 65% of bone weight and provides its mechanical and weight bearing strength and also serves as a reservoir for calcium that may be quickly mobilized for homeostatic and functional purposes. Although 99% of total body calcium is present in the slowly exchangeable, deeply deposited skeletal crystal, it is the rapidly exchangeable 1% of body calcium in recently accumulated surface bone and in the vascular, extracellular, and intracellular (soft tissues) spaces, with which it is in equilibrium, that modulates gene expression, intercellular communication and intracellular signal transduction, neural transmission, cell-to-cell adhesion, clotting, striated, smooth and cardiac muscular contraction, cardiac rhythmicity, enzyme action, synthesis and secretion of endocrine and exocrine factors, fertilization, and cellular proliferation and apoptosis.[3,4] Approximately 50% of total serum calcium is bound to albumin and globulin; 5% is complexed or chelated to citrate, phosphate, lactate, bicarbonate, and sulfate; and 45% is present as biologically active and closely regulated ionized extracellular calcium (Ca^{2+}_e). Approximately 75% of the variability in total serum calcium concentrations is accounted for by genetic factors. Serum total and ionized calcium concentrations are related to levels of albumin, creatinine, parathyroid hormone (PTH), phosphate, and serum pH. For every 1 g/dL decline in the serum concentration of albumin below 4 g/dL, the total serum calcium level declines by 0.8 mg/dL. The measured

TABLE 8-1 **Abbreviations**

$1,25(OH)_2D_3$	1,25-Dihydroxyvitamin D_3 (calcitriol)
$24R,25(OH)_2D_3$	24,25-Dihydroxyvitamin D_3
$25OHD_3$	25-Hydroxyvitamin D_3 (calcidiol)
ADHR	Autosomal dominant hypophosphatemic rickets
AF	Activating function
AKT	see PKB
AMP	Adenosine monophosphate
APC	Adenomatous polyposis coli
ARHR	Autosomal recessive hypophosphatemic rickets
ASARM	Acidic serine aspartate-rich MEPE-associated motif
ATP	Adenosine triphosphate
ATPase	Adenosine triphosphatase
BAP	Bone alkaline phosphatase
BMAD	Bone mineral apparent density (~volumetric BMD)
BMC	Bone mineral content
BMD	Bone mineral density
BMP	Bone morphogenetic protein
BRU	Bone remodeling unit
BTT	Bone transmission time
Ca^{2+}_e	Calcium—ionized, extracellular
Ca^{2+}_i	Calcium—ionized, intracellular
CaMK	Ca^{2+}/calmodulin dependent protein kinase
CART	Cocaine amphetamine regulated transcript
CaSR	Calcium sensing receptor
Cl^-	Chloride
CNP	Natriuretic hormone type C
COMP	Cartilage oligomeric protein
CREB	Cyclic AMP-responsive element binding protein
CTX	Carboxyl-terminal cross-link telopeptide α1 chain of type I collagen (see ICTP)
DAG	1,2-Diacylglycerol
DBP	Vitamin D binding protein
DEXA	Dual energy x-ray absorptiometry
DMP	Dentin matrix protein
DNA	Deoxyribonucleic acid
DPD	Deoxypyridinoline
DRIP	Vitamin D interacting protein
ECF	Extracellular fluid
EGF	Epidermal growth factor
ER	Estrogen receptor
ERK	Extracellular signal-regulated kinase
FGF	Fibroblast growth factor
FGFR	Fibroblast growth factor receptor
FOS	FBJ osteosarcoma oncogene
FRP4	Frizzled related protein-4
FRS	FGF receptor substrate
GABA	Gamma (γ)-aminobutyric acid
GDNF	Glial cell line-derived neurotrophic factor
$G_{i\alpha}$	Alpha subunit of inhibitory GTP-binding protein
$G_{s\alpha}$	Alpha subunit of stimulatory GTP-binding protein
$G_{q\alpha}$	Alpha subunit of a stimulatory GTP-binding protein
GH	Growth hormone
GMP	Guanosine monophosphate
GPCR	G protein–coupled receptor
GRB2	GRB2-associated binding protein 2
GRK	G protein–coupled receptor kinase
GSK3	Glycogen synthase kinase 3
GTP	Guanosine triphosphate
H^+	Hydrogen ion (proton)
HNRPD	Heterogeneous nuclear ribnucleoprotein D
HRpQCT	High-resolution peripheral quantitative computed tomography
IGF	Insulin-like growth factor
ICTP	Carboxyl terminal cross-link telopeptide of collagen type I generated by matrix metalloproteinase (see CTX)
IGF	Insulin-like growth factor (somatomedin)
Ihh	Indian hedgehog
IL	Interleukin
IP_3	Inositol-1,4,5-trisphosphate
ITAM	Immunoreceptor tyrosine-based motif
K^+	Potassium
LRP	Low-density lipoprotein receptor-related protein
μFE	Microfinite element
MAPK	Mitogen activated protein kinase
MARRS	Membrane-associated rapid response steroid binding protein (see TBP-2)
M-CSF	Macrophage colony stimulating factor
MEPE	Matrix extracellular phosphoglycoprotein
Mg^{2+}	Magnesium
MITF	Microphthalmia-associated transcription factor
MMP	Matrix metalloproteinase
MRI	Magnetic resonance imaging
Na^+	Sodium
NADPH	Nicotinamide adenine dinucleotide phosphate
NFκB	Nuclear factor-κB
NFATC1	Nuclear factor of activated T cells, cytoplasmic, calcineurin-dependent 1
NHERF	Sodium-hydrogen exchanger regulatory factor
NPT2	Sodium/phosphate transporter 2
NTX	Amino terminal cross-link telopeptide of collagen type I
OMIM	Online Mendelian Inheritance in Man
OPG	Osteoprotegerin
OSCAR	Osteoclast-associated receptor
OSTM1	Osteopetrosis-associated transmembrane protein 1
P3H1	Prolyl 3-hydroxylase-1
PHEX	Phosphate-regulating gene with homologies to endopeptidases on the X chromosome
PICP	Procollagen type I carboxyl-terminal propeptide
PINP	Procollagen type I amino-terminal propeptide
PIIINP	Procollagen type III amino-terminal propeptide
PI3K	Phosphoinositide-3-kinase
PKA	Protein kinase A

TABLE 8-1 **Abbreviations—cont'd**

PKB	Protein kinase B (AKT)
PLC	Phospholipase C
PO_4	Phosphate (HPO_4^{2-})
PPARγ	Peroxisome proliferator activator receptor gamma
PTH	Parathyroid hormone
PTHrP	PTH-related protein
PTH1R	PTH/PTHrP-1 receptor (PTH/PTHrP-1R)
PYD	Pyridinoline
QCT	Quantitative computed tomography
QUS	Quantitative ultrasonography
RANK	Receptor activator of NFκB
RANKL	RANK-ligand
RDA	Recommended dietary allowance
RNA	Ribonucleic acid
RXR	Retinoid X receptor
SIBLING	Small integrin-binding ligand, N-glycosylated (proteins)
SOS	Speed of sound
SOX	SRY-Box
SPP1	Secreted phosphoprotein1 (osteopontin)
Src	SRC oncogene
SRC	Steroid receptor coactivator
STAT	Signal transduction and transcription
SYK	Spleen tyrosine kinase
TALH	Thick ascending loop of Henle
TBP-2	Thioredoxin binding protein-2 (see MARRS)
TCF/LEF	T cell factor/lymphoid enhancer binding factor
TGF	Transforming growth factor
TALH	Thick ascending loop of Henle
TIP	Tuberoinfundibular protein (hypothalamic)
TNF	Tumor necrosis factor
TNSALP	Tissue nonspecific alkaline phosphatase
TR	Thyroid (hormone) receptor
TRAF	TNF receptor associated factor
TRANCE	TNF-related activation induced cytokine
TRAP5b	Tartrate resistant acid phosphatase type 5b isoform
TREM2	Triggering receptor expressed on myeloid cells-2
TRP	Transient receptor potential (channel)
VDIR	Vitamin D–interacting repressor
VDR	Vitamin D receptor
VDRE	Vitamin D response element
VEGF	Vascular endothelial growth factor
WIF	WNT inhibitory factor
WINAC	WSTF including nucleosome assembly complex
WSTF	Williams syndrome transcription factor
XHR	X-linked hypophosphatemic rickets

A=Adenine C=Cytidine G=Guanine T=Thymine

TABLE 8-2 **Human Genes Involved in Mineral Homeostasis and Bone Metabolism**

Protein	Gene	Chromosome	OMIM*
Aggrecan 1	*AGC1*	15q26.1	155760
Alkaline phosphatase	*ALPL*	1p36.2	171760
Arrestin, beta, 2	*ARBB2*	17p13.2	107941
ATPase, $Ca(2^+)$-transporting, plasma membrane, 1	*ATP2B1*	12q21.33	108731
ATPase, $Ca(2^+)$-transporting, slow twitch	*ATP2A2*	12q24.11	108740
Axis inhibitor 1	*AXIN1*	16p13.3	603816
β1-Catenin	*CTNNB1*	3p22-p21.3	116806
Bone morphogenetic protein-2	*BMP2*	20p12	112261
Bone morphogenetic protein-4	*BMP4*	14q22-q23	112262
Bone morphogenetic protein-7	*BMP7*	20	112267
BMP receptor 1A	*BMPR1A*	10q22.3	601299
BMP receptor 2	*BMPR2*	2q33	600799
Bromodomain adjacent to zinc-finger domain, 1B (WSTF)	*BAZ1B*	7q11.23	605681
BSND gene (Barttin)	BSND	1p32.3	606412
C-type natriuretic protein	*NPPC*	2q24-qter	600296
Calbindin 3 (D9k)	*CABP3*	Xp22.2	302020
Calcitonin	*CALCA*	11p15.2	114130
Calcitonin receptor	*CALCR*	7q21.3	114131
Calcium release-activated calcium modulator 1	*CRACM1*	12q24	610277
Calcium sensing receptor	*CASR*	3q13.3-q21	601199
Calcium channel, L-type, subunit α_1	*CACNA1C*	12p13.3	114205
Calmodulin 1	*CALM1*	14q32.11	114180
Carbonic anhydrase II	*CA2*	8q21.2	611492
Cartilage-associated protein	*CRTAP*	3p22	605497
Cartilage oligomeric matrix protein	*COMP*	19p13.11	600310
β-Catenin	*CTNNB1*	3p22.1	116806

Continued

TABLE 8-2 **Human Genes Involved in Mineral Homeostasis and Bone Metabolism—cont'd**

Protein	Gene	Chromosome	OMIM*
Cathepsin K	*CTSK*	3q	603959
Chloride channel 5	*CLCN5*	Xp11.22	300008
Chloride channel 7	*CLCN7*	16p13.3	602727
Chloride channel, kidney, B	*CLCNKB*	1p36.13	602023
Claudin 2	*CLDN2*	Xq22.3-23	300520
Claudin 16 (Paracellin-1)	*CLDN16*	3q28	603959
Claudin 19	*CLDN19*	1p34.2	610036
Cocaine amphetamine regulated transcript	*CART*	5q13.2	602606
Collagen type I(α1)	*COL1A1*	17q21.31-q24	120150
Collagen type I(α2)	*COL1A2*	7q22.1	120160
Collagen type II(α1)	*COL2A1*	12q13.11-q13.2	120140
Collagen type III(α1)	*COL3A1*	2q31	120180
Collagen type IV(α1)	*COL4A1*	13q34	120130
Collagen type IX(α1)	*COL9A1*	6q13	120210
Collagen type X(α1)	*COL10A1*	6q21-q22.3	120110
Collagen type XI(α1)	*COL11A1*	1p21	120280
Cartilage oligomeric matrix protein	*COMP*	19p13.11	600310
Core binding factor, beta subunit	*CBFB*	16q22.1	121360
Cyclophilin B	*PIPB*	15	123841
Cytochrome P450, III A, 4	*CYP3A4*	7q22.1	124010
Cytochrome P450, subfamily IIR, polypeptide 1 (25-hydroxylase)	*CYP2R1*	11p15.2	608713
Cytochrome P450, subfamily XXVIIB, polypeptide 1 (25OHD-1α-hydroxylase)	*CYP27B1*	12q14.1	609506
Cytochrome P450, family 24, subfamily A, polypeptide 1 (25OHD-24-hydroxylase)	*CYP24A1*	20q13.2	126065
Dextrin matrix acidic phosphoprotein 1	*DMP1*	4q22.1	600980
Dikkopf	*DKK1*	10q11.2	605189
Disheveled 1	*DVL1*	1p36.33	601365
Distal-less 5	*DLX5*	7q22	600028
Ectonucleotide pyrophosphatase/phosphodiesterase 1	*ENPP1*	6p23.2	173335
Fibroblast growth factor-1 (acidic)	*FGF1*	5q31	131220
Fibroblast growth factor-2 (basic)	*FGF2*	4q25-q27	176943
Fibroblast growth factor-5	*FGF5*	4q21	165190
Fibroblast growth factor-7	*FGF7*	15q15-q21.1	148180
Fibroblast growth factor-18	*FGF18*	5q35.1	603726
Fibroblast growth factor-23	*FGF23*	12p13.3	605380
Fibroblast growth factor receptor-1	*FGFR1*	8p11.2-p11.1	136350
Fibroblast growth factor receptor-2	*FGFR2*	10q25.3-q26	176943
Fibroblast growth factor receptor 3	*FGFR3*	4p16.3	134934
Fibroblast growth factor receptor-4	*FGFR4*	5q356qter	134935
Frizzled receptor	*FZD1*	7q21	603408
Frizzled related protein-4	*FRP4*	7p14-p13	606570
UDP-N-acetyl-alpha-D-galactosamine: Polypeptide N-acetylgalactosaminyl- transferase 3	*GALNT3*	2q24.3	601756
Gamma-carboxyglutamic acid protein, bone (osteocalcin)	*BGLAP*	1q22	112260
Glial cells missing, Drosophila, homologue of, 2	*GCM2*	6p24.2	603716
Group specific component (DBP)	*GC*	4q12	139200
Guanine nucleotide binding protein, alpha stimulating	*GNAS1*	20q13.2	139320
Hairless	*HR*	8p21.1	602302
Heterogeneous nuclear ribonucleoprotein D	*HNRPD*	4q21.1-q21.2	601324
Indian hedgehog	*IHH*	2q35	600726
Inositol trisphosphate receptor	*ITPR1*	3p26-p25	147265
Integrin αv	*ITGAV*	2q31	193210
Integrin β3	*ITGB3*	17q21.32	173470
Insulin-like growth factor I	*IGF1*	12q22-q24.1	147440
IGF-I receptor	*IGF1R*	15q25-q26	147370

TABLE 8-2 **Human Genes Involved in Mineral Homeostasis and Bone Metabolism—cont'd**

Protein	Gene	Chromosome	OMIM*
Klotho	*KL*	13q13.1	604824
Leptin	*LEP*	7q31.3	164160
Low-density lipoprotein receptor-related protein 2 (megalin)	*LRP2*	2q31.1	600073
Low-density lipoprotein receptor-related protein 5	*LRP5*	11q13.4	603506
Low-density lipoprotein receptor-related protein 6	*LRP6*	12p13.3-p11.2	603507
Macrophage-colony stimulating factor	*CSF1*	1p13.3	120420
M-CSF receptor (c-Fms)	*CFS1R*	5q32	164770
Matrix extracellular phosphoglycoprotein	*MEPE*	4q21.1	605912
Matrix GLA-protein	*MGP*	12p13.1-p13.2	154870
Mediator complex subunit 4 (vitamin D interacting protein)	*MED4*	13q14.2	605718
Mitochondrial RNA-processing endoribonuclease	*RMRP*	9p21-p12	157660
Natriuretic peptide precursor C	*NPPC*	2q37.1	600296
Natriuretic peptide receptor B	*NPR2*	9p13.3	108961
Nuclear factor-κB, subunit 1	*NF*κB1	4q23-q24	164011
Nuclear factor-κB, inhibitor (IKB)	*NFKB1A*	14q13	164008
Nuclear factor of activated T cells, cytoplasmic, calcineurin-dependent 1	*NFATC1*	18q23	600489
Orai calcium release-activated calcium modulator 1	*ORAI1*	12q24.31	610277
Osteoclast-associated receptor	*OSCAR*	19q13.42	606862
Osteopetrosis-associated transmembrane protein 1	*OSTM1*	6q21	607649
Parathyroid hormone	*PTH*	11p15.2	168450
PTH related protein	*PTHLH*	12p11.22	168470
PTH 1 receptor	*PTH1R*	3p21.31	168468
PTH receptor 2	*PTHR2*	2q34	601469
Patched 1	*PTCH1*	9q22.3	601309
Peroxisome proliferator-activated receptor γ	*PPARG*	3p25	601487
Phosphate regulating gene with homologies to endopeptidases on the X chromosome	*PHEX*	Xp22.1	307800
Potassium channel, inwardly rectifying, subfamily 1, member 1	*KCNJ1*	11q24.3	600359
Potassium channel, inwardly rectifying, subfamily J, member 10	*KCNJ10*	1q23.2	602208
Potassium channel, voltage-gated, Shaker-related subfamily, member 1	*KCNA1*	12p13.32	176260
Pregnane X receptor	*NR112*	3q13-q21	603065
Prolyl 3-hydroxylase 1	*LEPRE1*	1p34	610339
Protein phosphatase 1, regulatory subunit 1B (Phospho1)	PPP1R1B	—	604399
Protein phosphatase 3, catalytic subunit, alpha isoform (calcineurin A)	*PPP3CA*	4q24	114105
Receptor activator of NF-κB (RANK)	*TNFRSF11A*	18q22.1	603499
RANK-ligand	*TNFSF11*	13q14	602642
Retinoid X receptor α	*RXRA*	9q34.3	180245
Runt-related transcription factor 2	*RUNX2*	6p21.1	600211
Sclerostin	*SOST*	17q21.31	605740
Secreted protein 1 (osteopontin)	*SPP1*	4q22.1	166490
Secreted protein, acidic, cysteine-rich (osteonectin)	*SPARC*	5q33.1	182120
Sirtuin 1	*SIRT1*	10	604479
Smoothened	*SMOH*	7q31-q32	601500
Sodium phosphate cotransporter solute carrier family 34, member 1 (NPT2a)	*SLC34A1*	5q35	182309
Sodium phosphate cotransporter solute carrier family 34, member 2 (NPT2b)	*SLC34A2*	4p15.2	604217
Sodium phosphate cotransporter solute carrier family 34, member 3 (NPT2c)	*SLC34A3*	9q34	609826
Solute carrier family 8 (sodium-calcium exchanger), member 1	*SLC8A1*	2p22.1	182305

Continued

TABLE 8-2 **Human Genes Involved in Mineral Homeostasis and Bone Metabolism—cont'd**

Protein	Gene	Chromosome	OMIM*
Solute carrier family 8 (sodium-calcium exchanger), member 2	*SLC8A2*	19q13.32	601901
Solute carrier family 9, member 3, regulator 1 (NHERF1)	*SLC9A3R1*	17q25.1	604990
Solute carrier family 12 (sodium/potassium/chloride transporter), member 1	*SLC12A1*	15q21.1	600839
Solute carrier family 12 (sodium/chloride transporter), member 3	*SLC12A3*	16q13	600968
SRY-box 9	*SOX9*	17q24.3	608160
Stromal interaction molecule 1	*STIM1*	11p15.4	605921
T-cell factor/ lymphoid enhancement factor	*LEF1*	4q23-q25	153245
T-cell immune regulator 1	*TCIRG1*	11q13.2	604592
Thioredoxin-interacting protein	*TXNIP*	1q21.1	606599
Transcription factor 3/VDR interacting repressor	*TCF3*	19p13.3	147141
Transcription factor Sp7 (osterix)	*SP7*	12q13.13	606633
Transmembrane protein 142A	*TMEM142A*		610277
Transient receptor potential cation channel, subfamily M, member 6	*TRPM6*	9q21.13	607009
Transient receptor potential cation channel, subfamily V, member 4	*TRPV4*	12q24.1	605427
Transient receptor potential cation channel, subfamily V, member 5	*TRPV5*	7q34	606679
Transient receptor potential cation channel, subfamily V, member 6	*TRPV6*	7q34	606680
Triggering receptor expressed on myeloid cells 2	*TREM2*	6p21.1	605086
Tumor necrosis factor ligand superfamily, member 11 (RANKL, OPGL)	*TNFSF11*	13q14.11	602642
Tumor necrosis factor receptor superfamily, member 11B (osteoprotegerin)	*TNFRSF11B*	8q24.12	602643
Tumor necrosis factor-associated factor 6	TRAF6	11p12	602355
Tuberoinfundular peptide 39	*TIP39*	19q13.33	608386
Vascular endothelial growth factor	*VEGF*	6p12	192240
Vitamin D binding protein	*GC*	4q13.3	139200
Vitamin D receptor	*VDR*	12q12-q14	601769
Voltage-gated calcium channel α_1	*CACNA1C*	12p13.3	114205
Wingless 1	*WNT1*	12q12-q13	164820
Wingless 9A	*WNT9A*	1q42	602863
WNT inhibitory factor 1	WIF1	12q14.3	605186

*Online Mendelian Inheritance in Man, www3.ncbi.nlm.nih.gov/htbin-post/Omim.

Ca^{2+}_e level is dependent on the serum pH (normal adult range 1.1 to 1.3 mmol/L at pH 7.4); increase in alkalinity (higher pH) raises calcium binding to albumin thus decreasing Ca^{2+}_e, whereas acidic changes (lower pH) decrease binding thereby increasing Ca^{2+}_e. The relationship between pH and Ca^{2+}_e is best described by an inversely S-shaped third-degree function.[5]

The serum concentration of Ca^{2+}_e is maintained within narrow limits by an integrated system involving the plasma membrane Ca^{2+}_e sensing receptor (CaSR), PTH and its receptor (PTH/PTH related protein [PTHrP]-1R), the thyroidal parafollicular C cell product calcitonin and its receptor, and the vitamin D hormone system acting on the intestinal tract, bone, and kidney (see Figure 8-1). With increase in serum Ca^{2+}_e concentration, the CaSR on the chief cell of the parathyroid gland is activated; the intracellular signal transduction pathway stimulated by the CaSR depresses PTH secretion instantly and decreases expression of *PTH*. Activation of the CaSR in the distal renal tubule decreases reabsorption of calcium filtered through the glomerulus and increases urinary calcium excretion. When the serum Ca^{2+}_e concentration falls, signaling through the CaSR also declines, thereby increasing PTH secretion and renal tubular reabsorption of filtered calcium, activating osteoclastic bone reabsorption, and somewhat later increasing synthesis of 1,25-dihydroxyvitamin D_3 (calcitriol) and intestinal absorption of ingested calcium.[1]

The intracellular concentration of cytosolic free calcium (Ca^{2+}_i) (approximately 100 nM) is 10,000-fold less than that in serum and extracellular fluid, a gradient maintained by exchange of Ca^{2+} across the cell's plasma membrane and across the membranes of intracellular structures. Within the cell, Ca^{2+}_i is primarily (99%) stored within the endoplasmic reticulum (sarcoplasmic reticulum in muscle cells) and mitochondria as well as

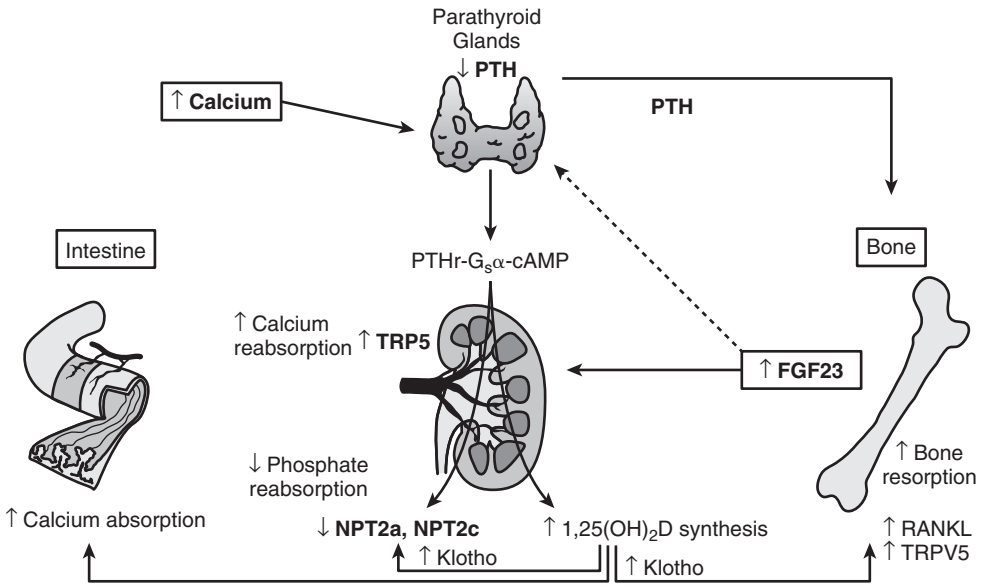


FIGURE 8-1 ■ Regulation of calcium homeostasis. Calcium (Ca^{2+}) is absorbed from the intestinal tract, kidney tubule, and bone in response to calcitriol [1,25$(OH)_2D_3$] and parathyroid hormone (PTH). Calcitonin inhibits resorption of calcium from bone. The Ca^{2+}-sensing receptor (CaSR) modulates Ca^{2+}-mediated activity of the parathyroid glands and the renal tubules. Both hypocalcemia and hypophosphatemia enhance renal tubular generation of calcitriol and absorption of intestinal phosphate. PTH inhibits renal tubular reabsorption of phosphate. Fibroblast growth factor-23 (FGF23), a phosphatonin secreted by osteoblasts and osteocytes, inhibits renal tubular reabsorption of phosphate and synthesis of calcitriol. (Please see Figure 8-2 and the text for further details.) (Reproduced from Levine, M. A. (2010). Investigation & management of hypocalcemia. ENDO 2010: Meet the Professor, The Endocrine Society, Bethesda, MD, with permission.)

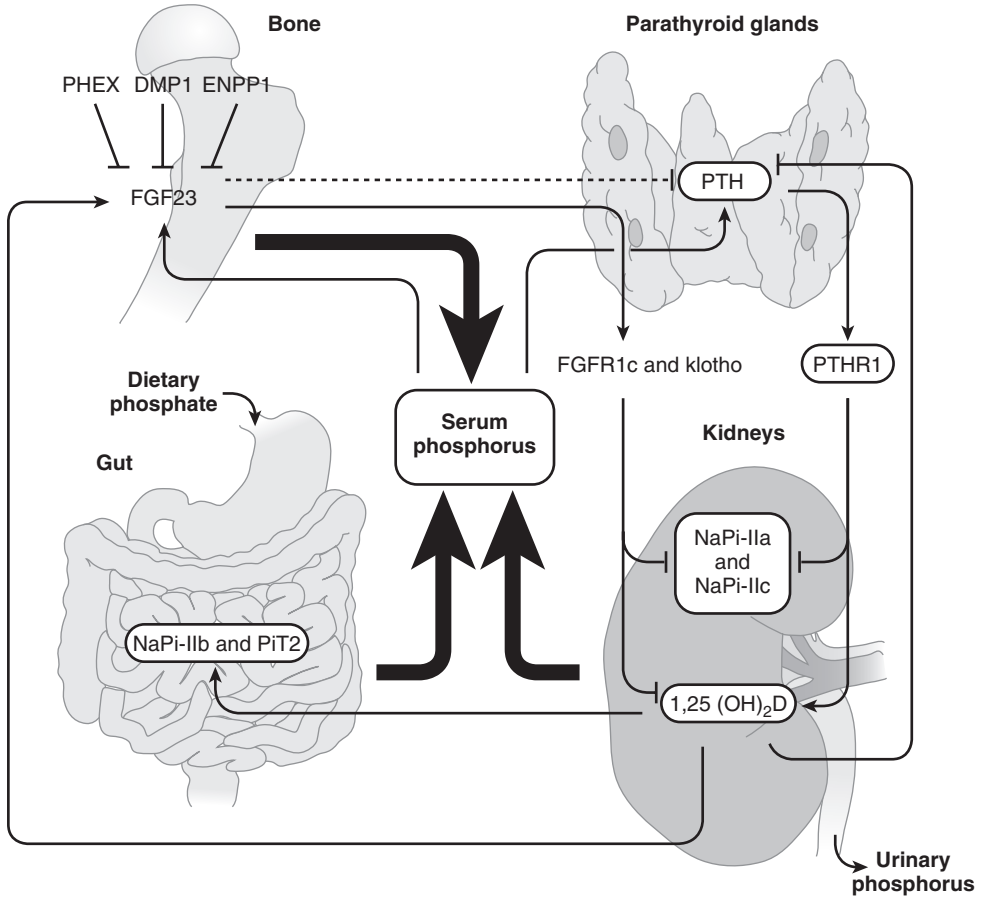


FIGURE 8-2 ■ Regulation of phosphate homeostasis. Serum concentrations of phosphate are depressed by parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23), both of which increase urinary excretion of this cation by decreasing renal tubular expression of phosphate cotransporter proteins. Synthesis of FGF23, a product of osteoblasts and osteocytes, is depressed by low serum phosphate levels and by PHEX (phosphate regulating gene with homologies to endopeptidases on the X chromosome), DMP1 (dentin matrix protein 1), and ENPP1 (ectonucleotide pyrophosphate-phosphodiesterase 1), all three of which proteins are also synthesized by bone cells. PTH increases and FGF23 depresses synthesis of calcitriol (1,25-dihydroxyvitamin D). (See text for details.) (Reproduced from Bergwitz, C., Collins, M. T., Kamath, R. S., & Rosenberg, A. E. (2011). Case 33-2011: a 56-year-old man with hypophosphatemia. *N Engl J Med, 365*, 1625–1635, with permission.)

within endosomes, lysosomes, the Golgi apparatus and secretory granules and bound to the inner plasma membrane from which sites it can be released by chemical signals—that is, inositol-1,4,5-trisphosphate (IP_3).[6] IP_3 acts on IP_3 receptors (encoded by *ITPR1*; see Table 8-2) located in the membrane of the endoplasmic reticulum to effect rapid egress of Ca^{2+} from storage, thereby quickly increasing $Ca^{2+}{}_i$ levels.[7] $Ca^{2+}{}_i$ serves as a second messenger and signal transducer that controls many cellular activities including gene transcription, cell division and growth, cell movement, and secretion of synthesized products. Ca^{2+} enters the cell through transmembrane protein pores such as voltage-gated and store-operated calcium channels. Voltage-gated Ca^{2+} channels are active in cells that are electrically excitable such as cardiac, skeletal, and smooth muscle cells, neurons, gastric mucosa, and pancreatic β cells and that open in response to depolarization of the plasma membrane permitting rapid influx of $Ca^{2+}{}_e$ into the cell cytoplasm leading to further depolarization of the membrane and activation of cell function.[8] They may be activated by high or low electrical voltage. The skeletal muscle high-voltage-activated calcium channel is composed of five subunits: α_1, α_2, β, γ, and δ. The voltage-sensing, pore-forming Ca^{2+}-binding α_1 subunit has four repeated domains, each with 6-transmembrane spanning regions (or helices) and intracytoplasmic amino and carboxyl terminals; transmembrane helix 4 serves as the voltage sensor; the α_1 subunit also has a sequence of amino acids between transmembrane helices 5 and 6 that is partially inserted into the membrane to serve as a selectivity filter.[8] The assembly, intracellular movement, interaction with other proteins, activation, and kinetic properties of the α_1 subunit are modified by the extracellular, glycosylated α_2 subunit, the β subunit (a cytoplasmic globular protein), a small membrane spanning δ subunit that is disulfide-linked to form dimeric $\alpha_2\delta$, and a second transmembrane subunit γ. The voltage-gated calcium channels are designated in accord with their cloned specific α_1 subunits and have been termed Cav 1.1, Cav 1.2, Cav 1.3, . . . Cav 3.3; formerly, they were designated in accord with the high- or low-voltage strength required for activation and for their sensitivity to specific inhibitors (e.g., L, N, P, Q, R, T subtypes). The high-voltage–dependent L type calcium channels (Cav1.1 through Cav 1.4) are present in skeletal, cardiac, and vascular smooth muscle cells; endocrine cells; neurons; and fibroblasts. L-type calcium channels are activated by the guanine triphosphate (GTP)-binding α_q subunit of G_q-proteins through stimulation of phospholipase C (PLC) leading to phosphorylation of the channel protein. L-type calcium channels modulate the growth and proliferation of fibroblasts and smooth muscle cells, the synthesis of extracellular matrix collagen proteins, and the activation of specific transcription factors.[9]

$Ca^{2+}{}_e$ also enters the cytosol through the plasma membrane store-operated Ca^{2+} channel, ORAI1 (also termed CRACM1 or TMEM142A [transmembrane protein 142A]).[7,10] Store-operated Ca^{2+} channels are active in cells that are electrically nonexcitable such as lymphocytes and other immune cells.[4] Nevertheless, both voltage-gated and store-operated Ca^{2+} channels often coexist in the same cell. Which type of Ca^{2+} channel is utilized in a specific cell is determined by the expression of *STIM1* (see Table 8-2) encoding a protein that transverses the membrane of the endoplasmic reticulum. When the Ca^{2+} content of the endoplasmic reticulum is depleted, STIM1 unfolds and bridges the cytosolic distance between the endoplasmic reticulum and the cell's plasma membrane where it binds to and opens the ORAI1 pore, thereby permitting Ca^{2+} influx into the cytoplasm and the restoration of Ca^{2+} stores within cytoplasmic organelles. At the same time that it activates the ORAI1 Ca^{2+} channel, STIM1 inhibits the voltage-gated Ca^{2+} channel.[4,11,12] Loss-of-function mutations in *ORAI1* have been identified in patients with severe combined immune deficiency and Kaposi sarcoma.[13,14]

In addition to traversing rapidly across the cell's plasma membrane through voltage-gated and store-operated channels, Ca^{2+} enters the cytosol, but at a markedly slower rate, through bifunctional membrane-associated IP_3 receptors that also serve as calcium channels.[7,15] Encoded by *ITPR1*, the tetrameric IP_3 receptor has six transmembrane domains and a pore-forming loop between the fifth and sixth transmembrane segments. Whereas many IP_3 receptor/channels are expressed in the membranes of the endoplasmic reticulum where IP_3 activation leads to depletion of Ca^{2+} stored in the endoplasmic reticulum thereby triggering store-operated calcium channel activation, only one to two such channels are expressed in the plasma membrane of each cell. Ca^{2+} is translocated not only through specific Ca^{2+} channels but also through paracellular transport channels[16]. $Ca^{2+}{}_i$ is extruded from cell cytoplasm by calcium and energy-dependent adenosine triphosphate (ATP)-driven calcium pumps and in exchange for sodium (Na^+) through H^+/ATPase and Na^+/Ca^{2+} exchangers.[3] The Ca^{2+}-ATPases are encoded by four genes; *ATP2B1* encodes a 1220-amino-acid plasma membrane protein with a cyclic AMP-dependent protein kinase domain and a carboxyl domain to which calbindin binds, which links hydrolysis of ATP with transport of cations across membranes. The Ca^{2+}-ATPases have the capacity to transport Ca^{2+} against high concentration gradients. The Na^+/Ca^{2+} exchangers are widely distributed plasma membrane Ca^{2+} export proteins.[1,3] A homologue of the human Na^+/Ca^{2+} exchanger is composed of 10 transmembrane domains consisting of two sets of five transmembrane helices in inverted reverse order with the amino and carboxyl termini within the cytoplasm.[17,18] Transmembrane helices 2, 3, 7, and 8 form an ion-binding pocket with three low-affinity Na^+ and one high-affinity Ca^{2+} binding sites symmetrically arranged in a diamond formation. Through conformational changes of the structure of the Na^+/Ca^{2+} exchanger, the Na^+ and Ca^{2+} binding sites are alternately exposed to either the intracellular or extracellular surfaces of the cell's plasma membrane. As Na^+ from the extracellular space occupies its three binding sites, the binding affinity for Ca^{2+} decreases, thereby releasing this cation into the cytosol and reversing the orientation of the Na^+/Ca^{2+} channel; inasmuch as cytosolic levels of Na^+ are low, these cations are then released into the cytosol restoring the affinity of the

exchanger for Ca^{2+}. The human cardiac Na^+/Ca^{2+} exchanger NCX1 (encoded by *SLC8A1*) has Na^+/Ca^{2+} binding configurations that are similar to those of the homologue.[17] The human skeletal muscle Na^+/Ca^{2+} exchanger NCX2 is encoded by *SLC8A2*. Within the cell, cytosolic Ca^{2+} is transported across the membrane and into the endoplasmic reticula of muscle cells through Ca^{2+}-ATPase channels encoded by *ATP2A2*; linkage of small ubiquitin-like modifier type 1 (sumoylation) to this Ca^{2+}-ATPase extends its half-life and increases its intrinsic activity.[19]

There are many intracellular calcium binding proteins (e.g., calcium binding proteins 1 and 4, calbindin, sorcin, calmodulin, and so forth). Calmodulin 1 (*CALM1*) is a widely distributed 149-amino-acid protein with an amino terminal lobe linked to a carboxyl terminal lobe that can assume more than 30 three-dimensional conformations.[8] Each lobe has two globular calcium binding domains consisting of two helix-loop-helix motifs connected by an α-helical linker; calcium binding exposes hydrophobic pockets that allow calmodulin to bind to and regulate the activity of target proteins.[20] When bound to a target protein, calmodulin can assume any one of an exceedingly large number of conformations. This versatility enables calmodulin to act as a calcium sensor for many different proteins subserving distinct processes within a single cell including voltage-gated calcium channels and calcium/calmodulin-dependent protein kinases.

In the gastrointestinal tract, calcium is absorbed by both active transcellular and passive paracellular processes.[21] During growth in younger subjects and when calcium intake and consequently intestinal calcium concentrations are low, active transcellular absorption of calcium predominates; when calcium intake is adequate or high, passive paracellular absorption of calcium is the major process by which calcium is absorbed.[21,22] Transcellular gastrointestinal absorption of calcium is an active and saturable process that is primarily stimulated by calcitriol by regulating the availability of transmembrane calcium "pumps" and channels on the luminal and basolateral surface membranes of duodenal enterocytes.[1] Calcitriol acting through the vitamin D receptor (VDR) increases duodenal expression of calcium transport protein-1 (epithelial calcium channel-2 encoded by *TRPV6*, transient receptor potential-vanilloid 6), a luminal calcium channel with six transmembrane domains and intracytoplasmic amino and carboxyl terminals and a pore region between the fifth and sixth transmembrane domains that forms a homotetramer or heterotetramer combined with TRPV5; TRPV5 and TRPV6 specifically permit Ca^{2+} permeation at physiologically levels of this cation.[22] Calcium is also actively absorbed in the intestinal tract by mechanisms that are independent of the VDR.[23] Thus, the expression of *TRPV6* can be increased by both estrogen and prolactin; prolactin can increase the transcellular absorption of calcium independently of calcitriol and the VDR.[24] After entering the enterocyte, Ca^{2+} diffuses across its interior within the cytosol or within a lysosomal vesical bound to calbindin D_{9k} (encoded by *CALB3*), a protein with two high-affinity calcium binding sites that transports calcium from the apical to the basolateral membrane of the enterocyte. After fusion of vesicle and basolateral plasma membrane, Ca^{2+} is extruded into the extracellular space (and circulation) through a basolateral Ca^{2+}-Mg^{2+}-dependent ATPase calcium channel (*ATPB21*), also termed plasma membrane Ca(2$^+$) ATPase, type 1 (PMCA1b). Experimental data in mice suggest that neither TRPV6 nor calbindin D_{9k} is essential for vitamin D–mediated intestinal absorption of calcium.[25,26] However, the applicability of these observations to human physiology is uncertain.[21] When calcium intake is adequate or high, the bulk of ingested calcium is passively absorbed through paracellular channels between enterocytes in the jejunum and ileum. Although calcitriol regulates synthesis of several tight junction proteins that may comprise the paracellular channels, including claudins-2 and -12, and cadherin-17, there is no known direct regulation of paracellular intestinal calcium absorption.[21,27]

Intestinal calcium absorption is influenced by vitamin D status, its food source (the bioavailability of calcium in cow milk formulas is 38%, that in human breast milk is 58%; leafy green vegetables are also a good source of dietary calcium), the form of the calcium salt in dietary supplements, and the presence in food of inhibitors of calcium absorption such as phytates, oxalates, or phosphates (e.g., cola beverages).[28] Intestinal calcium absorption is increased during adolescence, pregnancy, and lactation, and depressed in patients with nutritional or functional vitamin D deficiency, chronic renal disease, and hypoparathyroidism. The amount of calcium ingested influences the net amount of calcium absorbed; the lower the calcium intake, the greater is the efficiency of its absorption. In the adult when the dietary calcium intake is $<$ 200 mg/day, fecal calcium excretion exceeds intake; thus, active absorption of calcium cannot compensate for very low intake. As intake of dietary calcium increases from 200 to 1000 mg/day, active calcium absorption also increases but at a progressively decreasing rate. When dietary calcium exceeds 1000 mg/day, active calcium absorption remains relatively constant at 400 mg/day but passive absorption of calcium through paracellular channels continues to increase.[1] Thus, hypercalcemia may result from dietary calcium excess as in the milk-alkali syndrome. PTH indirectly increases intestinal calcium absorption by enhancing renal 25-hydroxyvitamin D-1α-hydroxylase activity and hence calcitriol synthesis. Growth hormone (GH) and estrogens also increase intestinal absorption of calcium; glucocorticoids and thyroid hormone inhibit this process. Calcium is excreted into the intestinal tract in the ileum and in pancreatic and biliary secretions.

Low calcium intake is associated with increased fracture rate in children and adolescents, and therefore, adequate dietary intake of calcium during infancy, childhood, and adolescence is necessary to attain a peak bone mass that may lessen the risk of fracture and the later development of osteopenia.[29] In an effort to ensure optimal mineralization of the developing skeleton, age-related dietary intakes of elemental calcium for infants, children, and adolescents have been recommended (Table 8-3).[30] Because calcium is an essential nutrient for bone mineralization, intake of calcium is optimal when skeletal calcium content is maximal for the age and gender of the subject.[2] In normally

TABLE 8-3 **Recommended Dietary Intakes of Calcium and Vitamin D in Infants, Children, and Adolescents**

Age (in years)	Calcium (mg/day)	Calcium (upper limit)	Vitamin D (IU/day)	Vitamin D (upper limit)	Minimum [25OHD] (ng/mL)
< 0.5	400		400	1000	20
0.5-1	600		400	1500	20
1-3	700	2500	600	2500	20
4-8	1000	2500	600	3000	20
9-13	1300	3000	600	4000	20
14-18	1300	3000	600	4000	20
Pregnant or lactating (14-18)	1300	3000	600	4000	20

Recommendations for calcium and vitamin D are the Recommended Dietary Allowance (RDA) intakes necessary for ≥97.5% of population. *Adapted from Ross, A. C., Manson, J. E., Abrams, S. A., et al. (2011). The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know.* J Clin Endocrinol Metab, 96, *53–58; Holick, M. F. (2011). Vitamin D: evolutionary, physiological and health perspectives.* Curr Drug Targets, 12, *4–18; Holick, M. F., Binkley, N. C., Bischoff-Ferrari, H. A., et al. (2011). Evaluation, treatment, and prevention of Vitamin D deficiency: an Endocrine Society Clinical Practice Guideline.* J Clin Endocrinol Metab, 96, *1911–1930.*

weighted adolescents, a calcium intake above 1600 mg/day results in maximal calcium retention (approximately 35%).[31] (Thus, the recommended dietary allowance [RDA] of calcium for adolescents of 1300 mg/day [see Table 8-3] may be underestimated.) Calcium retention and bone mass increase as the body mass index rises when calcium intake is sufficient. The retention of oral calcium depends not only on the level of nutrient intake but also on gender and race: white adolescent males retain more calcium than do white girls (37% versus 30%); black adolescent girls retain more calcium than do white girls (38% versus 30%). Besides dietary intake of calcium and vitamin D status, the most significant modifiable determinant of bone mineralization is weight-bearing physical activity (discussed later).[32]

Calcium is excreted primarily by the kidney. Ultrafiltrable serum calcium (representing both the ionized Ca^{2+}_e form and calcium that is complexed or chelated) crosses the renal glomerular membrane; 98% of filtered calcium is reabsorbed in the renal tubule. Paracellular reabsorption of most (93%) of filtered calcium occurs in the proximal renal tubule and thick ascending limb of Henle (TALH). The renal distal convoluted tubule, connecting tubule, and first part of the collecting duct actively reabsorb approximately 5% of filtered calcium transcellularly through TRPV5 calcium channels expressed on the apical membrane of renal tubular epithelial cells.[22] In the cytoplasm, calbindin D_{9k} then shuttles Ca^{2+}_e across the cell for extrusion by ATPB1 channels and the Na^+-Ca^{2+} exchanger type 1 (encoded by *SLC8A1*) on the basolateral membrane of the renal tubular cell. Calcitriol, PTH, low dietary calcium, estrogens and androgens, and acid/base balance increase expression of *TRPV5*. PTH also enhances posttranslational phosphorylation of TRPV5 and hence its movement to and insertion into the apical membrane of the renal tubular cell and inhibits its endocytosis, the paired effects thus resulting in increased numbers of TRPV5 channels on the apical surface of the renal tubular cell.[22] Klotho (encoded by *KL*), a cofactor/coreceptor essential for normal phosphate homeostasis (discussed later) that also has intrinsic beta glucuronidase activity, increases the retention and hence the activity of the TRPV5 calcium channel by removing oligosaccharide chains during its posttranslational processing.[33,34] Calmodulin, an intracellular Ca^{2+}-sensing protein that binds to and inhibits the action of TRPV5, modulates renal tubular reabsorption of Ca^{2+}, a process reversed by PTH-mediated phosphorylation of the TRPV5 calbindin-binding site.[35] Although calbindin D_{9k} is not expressed in the proximal renal tubule, cells lining the lumen of this segment have deep invaginations shortening the distance that reabsorbed Ca^{2+} must travel by diffusion between apical and basolateral membranes before extrusion.[21] In the TALH, calcium and magnesium are reabsorbed through voltage-driven paracellular channels (in part through paracellin-1, a tight junction protein channel that is instrumental in renal reabsorption of filtered calcium and magnesium [discussed later]). Cells of the TALH also express the CaSR on their basolateral membrane; when activated by peritubular Ca^{2+}, this G protein–coupled receptor (GPCR) decreases renal tubular reabsorption of calcium through the paracellular channels by inhibiting activity of the Na^+-K^+-$2Cl^-$ transporter and lowering lumen-positive voltage. Increased glomerular filtration or decreased renal tubular reabsorption increase renal excretion of calcium, phosphate, and magnesium. Urinary excretion of calcium is augmented by increased dietary intake, hypercalcemia of diverse pathophysiology (with the exception of that associated with familial hypocalcemic hypercalciuria [discussed later]), expansion of extracellular volume, metabolic acidosis, and loop diuretics (furosemide).[1] PTH and PTHrP increase renal tubular reabsorption of Ca^{2+}, whereas glucocorticoids, mineralocorticoids, and Ca^{2+} itself suppress Ca^{2+} reabsorption.

Calcium Sensing Receptor

Plasma Ca^{2+}_e concentrations are monitored by the CaSR, a 1078-amino-acid cell membrane GPCR encoded by *CASR* whose extracellular domain recognizes and binds Ca^{2+}, Mg^{2+}, gadolinium, aromatic amino acids, antibiotics, and other compounds.[36,37] Functionally, the CaSR is a homodimer linked by disulfide bonds between extracellular

domain cysteine residues 129 and 131 of each CaSR. Homodimerization of the CaSR takes place in the endoplasmic reticulum after core N-linked glycosylation; homodimeric CaSR is then packaged within the Golgi apparatus where it is further glycosylated and transported to the cell membrane.[38] The amino terminal extracellular domain of the CaSR is composed of 612 amino acids with five likely Ca^{2+} binding sites constructed in a Venus flytrap configuration; there are 250 amino acids in the transmembrane domain composed of seven transmembrane helices and three alternating extracellular and intracellular loops, and 216 amino acids in the carboxyl terminal intracellular domain.[37] Transmembrane helix 5 may also be necessary for receptor dimerization. The second extracellular loop contains sites (Asp758, Glu759, Glu767) that modulate the sensitivity of the CaSR to Ca^{2+}. Within the intracellular domain of the CaSR are three protein kinase C (PKC) phosphorylation sites; phosphorylation of Thr888 downregulates Ca^{2+}-mediated CASR intracellular signal transduction.[36] Because Ca^{2+}-CaSR interaction stimulates PKC signaling, function of the CaSR can thus be autoregulated.[37] Through its binding of Ca^{2+}_e, the CaSR finely regulates the extracellular concentration of this cation by modulating the secretion of PTH and the renal tubular reabsorption of filtered calcium. The CaSR is encoded by *CASR* and is a member of the C family of GPCRs with extremely long extracellular domains (500- to 600-amino-acid residues) and homologies with receptors that bind glutamate, γ-aminobutyric acid, and pheromones as well as with the taste receptors. The very long extracellular domain is heavily glycosylated, a posttranslational modification essential for efficient movement of the receptor to the cell surface. The CaSR is expressed on the plasma membrane of parathyroid chief cells, at the apical or basolateral membranes of most renal tubular cells—particularly the TALH and the collecting ducts, on the cell membranes of the parafollicular (C) cells of the thyroid, in cartilage and bone, lungs, adrenals, breast, intestines, skin, lens, placental cytotrophoblasts, and nervous tissue.[39] Expression of *CASR* is regulated in part by *GCM2*, encoding a transcription factor essential for formation of the parathyroid glands, and enhanced by calcitriol, high Ca^{2+}_e levels, and interleukin (IL)-1β.[40]

The serum Ca^{2+}_e concentration is related to polymorphic variants of the CaSR; 70% of individuals are homozygous for alanine at amino acid position 986 within the intracellular domain, 3% are homozygous for serine, and the remainder are heterozygous for the two amino acids. In heterozygous Arg986Ser and homozygous Ser986 subjects, Ca^{2+}_e levels are significantly higher than in those who are homozygous for Ala986.[41] Also, there are cellular Ca^{2+} sensors that are unrelated structurally to the CaSR as well as subtypes of the CaSR itself.[39] The CaSR also serves as a Mg^{2+}_e sensor and modulates renal tubular reabsorption of this cation decreasing its reabsorption when Mg^{2+}_e levels rise. By binding to the CaSR, aromatic L-amino acids appear to "sensitize" the receptor to a given level of Ca^{2+}_e (discussed later).[36]

Although in the kidney the CaSR is found in greatest abundance in the medullary and cortical TALH, it is also found in glomerular cells and other segments of the renal tubule. *CASR* is expressed on the apical brush-border membranes of cells in the proximal renal tubule, on basolateral cell membranes in cells in the TALH, and on the apical (luminal) surface of cells in the distal renal collecting ducts where it is exposed to intratubular Ca^{2+}.[37] Increasing peritubular concentrations of Ca^{2+}_e and Mg^{2+}_e inhibit renal tubular reabsorption of filtered Ca^{2+} and Mg^{2+}. In the kidney, binding of Ca^{2+}_e to the CaSR decreases not only transcellular transport of filtered Ca^{2+} but also its paracellular transport in the TALH. Inactivating mutations in *CASR* result in familial hypocalciuric hypercalcemia, whereas gain-of-function mutations in this gene are associated with autosomal dominant hypoparathyroidism (familial hypercalciuric hypocalcemia).[37] Stimulation of the CaSR suppresses PTH-mediated renal tubular Ca^{2+} and phosphate reabsorption.[37,42] In addition to effects on renal tubular cation transport, the Ca^{2+}/CaSR complex inhibits antidiuretic hormone–induced renal tubular permeability to water by decreasing the number of apical aquaporin-2 water channels in the inner medullary collecting ducts, thus leading to polyuria. Stimulation of the CaSR expressed in cells of the renal distal collecting ducts also increases local H^+-ATPase activity resulting in urine acidification.[42]

Acting through the CaSR, rising serum Ca^{2+}_e concentrations stimulate release of calcitonin from thyroid C cells. The CaSR is expressed throughout the intestinal tract, where it may modulate the changes in intestinal motility that accompany low (increased) and high (depressed) serum Ca^{2+}_e values.[36] Additionally, in gastric cells stimulation of CaSR activity results in release of gastrin into the circulation and increased intragastric acidity.[37] Expression of the CaSR in the brain suggests a mechanism whereby Ca^{2+} may influence neural function locally by modulating neurotransmitter and neuroreceptor function (such as the metabotropic glutamate receptor which also recognizes Ca^{2+}). Cell membrane CaSRs are present in (mouse, rat, bovine) articular and hypertrophic chondrocytes of the epiphyseal growth plate and to a lesser extent in proliferating and maturing chondrocytes.[43] Osteoblasts, osteocytes, and osteoclasts also express the CaSR, and agonist (Ca^{2+}, neomycin, gadolinium) activation of the CaSR stimulates intracellular signal transduction in these cells. Increase in CaSR activity in osteoblasts enhances their proliferation.[37] The CaSR may mediate recruitment of osteoblast precursor cells to sites of high Ca^{2+} levels, the residue of local osteoclast activity, one of the factors linking the bone remodeling processes of resorption and formation (discussed later).[39] In mice in which expression of bone-specific *Casr* has been eliminated, skeletal development is impaired with diffuse decrease in bone mineralization.[37] In vitro, Ca^{2+} inhibits bone reabsorptive activity of (rabbit) osteoclasts by causing the osteoclast to decrease secretion of acid and proteolytic enzymes and to withdraw from the site of bone resorption.[44]

Through its intracellular carboxyl terminal, the CaSR is linked to G proteins and their Gα subunits that couple the ligand-receptor message to several intracellular signal transduction pathways.[36,37,39] After binding of Ca^{2+} to the extracellular domain of the CaSR, $G_{\alpha q/11}$ dissociates from its βγ subunit complex and activates membrane bound

PLC-β1; in turn, this enzyme hydrolyzes membrane-bound phosphatidylinositol 4,5-bisphosphate (PIP_2) to diacylglycerol (DAG) and IP_3, the latter leading to release of Ca^{2+} from the endoplasmic reticulum and increased cytosolic concentrations of $Ca^{2+}{}_i$. Activation of the CaSR also stimulates activity of phospholipases A_2 and D, mitogen-activated protein kinases (MAPK), and protein kinases B (Akt) and C but inhibits that of adenylyl cyclase, the latter through stimulation of adenylate cyclase-inhibitory Gαi activity.[37] Increases in plasma $Ca^{2+}{}_e$ and rising cytosolic levels of $Ca^{2+}{}_i$ within the parathyroid gland chief cells suppress expression of *PTH* and increase degradation of PTH mRNA, thereby limiting synthesis and secretion of PTH; CaSR signaling also inhibits the proliferation of chief cells.[37] A decline in $Ca^{2+}{}_e$ leads to a decrease in CaSR activity, lower $Ca^{2+}{}_i$ values, and thence to increased PTH secretion, thus enabling the CaSR to exercise minute-to-minute control over the release of PTH and hence of the concentration of $Ca^{2+}{}_e$.

Calcimimetics are agonist drugs that activate the CaSR; calcilytics are antagonists of the CaSR.[45] As noted, the CaSR binds and responds to not only $Ca^{2+}{}_e$ but also $Mg^{2+}{}_e$, selected L-amino acids, and some antibiotics; the latter are designated type I calcimimetics. Synthetic compounds (e.g., phenylalkylamines) that bind to the second and third extracellular loops of the transmembrane domain of the CaSR and allosterically modulate (increase or decrease) the sensitivity of the CaSR to ambient $Ca^{2+}{}_e$ are designated type II calcimimetic agonists or calcilytic antagonists, respectively.[36,37] The most widely employed of the clinical calcimimetics is cinacalcet [N-[1-(R)-(-)-(1-naphthyl)ethyl] -3-[3-(trifluoromethyl)phenyl]-1-aminopropane hydrochloride]; this agent has been effective in decreasing secretion of PTH in patients with either primary or secondary hyperparathyroidism.[46] Calcilytics decrease the sensitivity of the CaSR to $Ca^{2+}{}_e$ and thus increase the secretion of PTH and depress renal tubular reabsorption of Ca^{2+}. Mutations in CASR may prevent its normal biosynthesis (class I), disturb movement of the CaSR protein to the plasma membrane (class II), interfere with binding of ligand to the CaSR (class III), impair activation of the CaSR (class IV), or disrupt receptor function by an unknown mechanism (class V).

PHOSPHATE

Eighty-five percent of body phosphate is deposited in bone as hydroxyapatite. Fourteen percent of phosphate is intracellular (in the cytosol or mitochondria in the form of inorganic phosphate esters or salts, cell and organelle membrane phospholipids, and phosphorylated metabolic intermediate compounds involved in energy metabolism and formation of ATP and signal transduction and in the cytosol and nucleus as an essential component of RNA and DNA) or in interstitial fluid or serum (approximately 1%). There it circulates as free orthophosphate anions HPO_4^{2-} and $H_2PO_4^-$ (55%), bound to proteins (10%), or complexed to calcium, magnesium or sodium (35%).[1,47] At pH 7.4, serum HPO_4^{2-} and $H_2PO_4^-$ are present in a molar ratio of 4:1; in alkalotic states the ratio increases, with acidosis it declines. (At pH 7.4, 1 mmol/L of orthophosphate = 1.8 mEq/L = 3.1 mg/dL.) The serum concentrations of calcium and phosphate are reciprocally related under normal circumstances, and the calcium × phosphate product approximates 30. Intracellularly, cytosolic-free phosphate concentrations approximate those in serum—3 to 6 mg/dL.

The serum phosphate concentration is regulated by oral intake, intestinal absorption and excretion, renal tubular reabsorption and excretion of filtered phosphate, and release from bone by dissolution of hydroxyapatite by PTH and calcitriol, and it fluctuates with age, gender, growth rate, diet, and serum calcium levels (see Figure 8-2).[1,48,49] Inasmuch as phosphate is found in all cells and foods, dietary deficiency is unusual. Dietary phosphate is absorbed across the intestinal brush border as HPO_4^{2-} in direct proportion to its intake, principally in the duodenum and jejunum. Intestinal phosphate is absorbed by both passive paracellular diffusion related to the luminal concentration of this anion and by an active transcellular mechanism stimulated by calcitriol. The latter is an energy-requiring transport process with sodium through the type IIb Na^+-HPO_4^{2-} cotransporter protein (NPT2b; encoded by *SLC34A2*) with low affinity for phosphate whose activities are maintained by calcitriol-dependent Na^+,K^+-ATPase. Decreased intestinal absorption of phosphate is compensated by increased renal tubular reabsorption of this anion.[47] Phosphate is also secreted into the intestinal tract. When dietary phosphate intake falls below 310 mg/day in the adult, net phosphate absorption is negative. At low phosphate intakes, absorption is active in the duodenum, jejunum, and distal ileum, whereas at high intakes 60% to 80% of ingested phosphate is absorbed primarily by diffusion. Phosphate absorption can be impaired by its intraluminal precipitation as an aluminum or calcium salt and by intestinal malabsorption disorders.

The kidney regulates moment-to-moment phosphate homeostasis. Phosphate is filtered in the renal glomerulus and reabsorbed primarily in the proximal renal tubules. It is actively transported across the luminal membrane against an electrochemical gradient through renal specific type II Na^+-HPO_4^{2-} cotransporter proteins expressed on the apical (tubular) surface of cells in the proximal renal tubule—NPT2a encoded by *SLC34A1* and NPT2c encoded by *SLC34A3*—with the aid of a Na^+,K^+-ATPase pump.[1,47,50] Expression of *SLC34A1* is regulated by serum phosphate levels (hypophosphatemia increases expression), PTH, PTHrP, and fibroblast growth factor (FGF)-23 (discussed later). NPT2a actively transports approximately 70% and NPT2c 30% of proximal renal tubular reabsorbed phosphate in the mouse; in the human, NPT2c may be the major renal tubular transporter of phosphate. The Na^+-H^+ exchanger regulatory factor 1 (NHERF1; encoded by *SLC9A3R1*) is a scaffolding protein that is essential for trafficking of NPT2a and NPT2c to the luminal membrane of cells in the proximal renal tubule; NHERF1 also interacts with PTH1R.[50] Another family of phosphate transporters (PiT2, encoded by *SLC20A2*, OMIM ID: 158378) has also been identified in the renal proximal tubule. Phosphate exits the proximal

renal tubular cell with Na^+ by cation exchange for K^+ through channels located on the cell's basolateral membrane. The maximal tubular reabsorption of phosphate approximates the filtered load. Tubular phosphate reabsorption is increased by low phosphate intake and hypophosphatemia (due to decrease in filtered load), hypercalcemia (by decrease in the glomerular filtration rate), depletion of extracellular fluid volume, and metabolic alkalosis. Renal tubular reabsorption of phosphate is depressed by high phosphate intake, PTH (and PTHrP), and phosphatonins (discussed later) and is due to rapid internalization and subsequent destruction of NPT2a and NPT2c proteins.[47] The PTH-dependent degradative processes are mediated through the type 1 PTH/PTHrP receptor and transduced by PKA, PKC, and MAPK. Fibroblast growth factor-23 (FGF23) (a phosphatonin), calcitriol, glucocorticoids, and thiazide diuretics also decrease renal tubular reabsorption of phosphate. Phosphate is deposited in bone as hydroxyapatite dependent on local levels of calcium, phosphate, and alkaline phosphatase activity and reabsorbed by osteoclasts whose activity is stimulated by PTH, calcitriol, and other osteoclast activating factors (discussed later). Serum concentrations of phosphate are highest in infancy and early childhood (4 to 7 mg/dL) and then decline during mid-childhood and adolescence to adult values (2.5 to 4.5 mg/dL).

Phosphatonins

Phosphatonins are substances that regulate serum concentrations of phosphate by controlling its rate of renal tubular reabsorption and of calcitriol by regulating its rate of synthesis (see Figures 8-1 and 8-2). In addition to FGF23, other phosphatonins are matrix extracellular phosphoglycoprotein (encoded by *MEPE*), Frizzled related protein-4 (encoded by *FRP4*), and FGF7, all of which were first identified by analysis of tumors associated with hypophosphatemic osteomalacia.[49,51] In addition to many patients with tumor-induced osteomalacia, increased serum concentrations of FGF23 are found in patients with X-linked hypophosphatemic rickets (XHR) due to loss-of-function mutations in the membrane-bound, 749-amino-acid endopeptidase encoded by *PHEX* (phosphate-regulating gene with homologies to endopeptidases located on the X chromosome, OMIM ID: 307800), autosomal dominant hypophosphatemic rickets (ADHR—OMIM ID: 193100) due to activating mutations in *FGF23*, and autosomal recessive hypophosphatemic rickets (ARHR—OMIM ID: 241520/613312) due to loss of function mutations in *DMP1* (encoding dextrin matrix acidic phosphoprotein 1) or *ENPP1*(encoding endonucleotide pyrophosphatase/phosphodiesterase 1).[48,49] By inhibiting renal phosphate transport, phosphatonins lead to hyperphosphaturia and hypophosphatemia. They also decrease transcription of *CYP27B1*, thereby depressing activity of renal tubular 25-hydroxyvitamin D-1α-hydroxylase resulting in decreased synthesis of calcitriol and thus inappropriately normal or low serum concentrations of calcitriol and in impaired intestinal absorption of phosphate and calcium.[47,52-54] FGF23 is generated as a 251-amino-acid protein with a 24-amino-acid signal sequence; for optimal biologic function, FGF23 must be posttranslationally glycosylated by N-acetylgalactosaminyl transferase 3 encoded by *GALNT3*. FGF23 is proteolytically degraded by intracellular subtilisin-like proprotein convertase acting between amino acids 179 and 180 (. . . Arg_{176}-His_{177}-Thr_{178}-Arg_{179}-Ser_{180} . . .) that separates the amino terminal FGF-like domain from the carboxyl terminal tail. Spontaneously occurring (gain-of-function) mutations within this sequence of *FGF23* slow the inactivation of FGF23, thus prolonging its biologic life resulting in ADHR. *FGF23* is expressed primarily by osteoprogenitor cells, osteoblasts, and osteocytes and to a lesser extent by the thymus, brain, thyroid, parathyroid glands, cardiac muscle, liver, and small intestines. In osteoblasts and osteocytes, expression of *FGF23* is stimulated by high serum or dietary phosphate, PTH, calcitriol acting through the VDR, and in some patients parenteral infusion of iron.[47,55,56]

FGF23 induces renal phosphate excretion by down-regulating expression of *SLC34A1* (encoding NPT2a) and *SLC34A3* (encoding NPT2c), the Na^+-HPO_4^{2-} cotransporters that are expressed in the apical (luminal) membrane of cells of the proximal renal tubule (see Figure 8-2). FGF23 down-regulates expression of proximal renal tubular 25-hydroxyvitamin D-1α-hydroxylase encoded by *CYP27B1* and thus limits synthesis of calcitriol; FGF23 also increases the expression of 25-hydroxyvitamin D-24-hydroxylase (encoded by *CYP24A1*), thereby enhancing the catabolism of calcitriol—the two processes summating to lower calcitriol synthesis.[57] Inasmuch as calcitriol promotes synthesis of FGF23, there is a reciprocal feedback relationship between calcitriol and FGF23. FGF23 acts through binding to the c isoform of tyrosine kinase FGF receptors (FGFR) 1, 2, and 3. The genes encoding the FGFRs consist of 19 exons that may be alternatively spliced to include or to exclude either exon 8 or 9 (encoding the third extracellular immunoglobulin-like domain of the FGF receptor, which partially specifies the ligand bound by the receptor); when exon 8 is included in the mRNA transcript, the b isoform of the FGFR is formed; when exon 9 is included in the transcript, the c isoform is produced. Although it is likely that FGF23 binds to multiple FGFR c isoforms, it is the multifunctional protein α-klotho that serves as a coreceptor thereby converting FGFR into specific FGF23 receptors in the parathyroid glands and kidney.[49,50,58] FGFR1 mediates the effects of FGF23 on renal phosphate transport, whereas FGFR3 and FGFR4 translate the effects of FGF23 on renal calcitriol synthesis. There are several isoforms of α-klotho encoded by *KL*. As a coreceptor for FGF23, α-klotho is a 130-kDa transmembrane protein with a very short (10 amino acids) intracellular domain; without the transmembrane domain (encoded by exon 3), it is an 80 kDa secreted protein whose levels are measurable in serum; removal of the sequence of amino acids that tethers the extracellular domain to the cell membrane results in another form of circulating α-klotho.[47] Interestingly, *KL* is expressed in the renal distal convoluted tubule, not the proximal tubule, implying that it is circulating α-klotho that serves as the coreceptor for FGF23/FGFR1(IIIc) in the proximal renal tubule where phosphate is reabsorbed.[47]

KL is also expressed in the parathyroid gland; there, FGF23 interacting with α-klotho and FGFR1(IIIc) decreases transcription of *PTH* and thereby reduces PTH synthesis and secretion. Reciprocally, PTH increases synthesis of FGF23 by bone cells, establishing yet another feedback loop regulating FGF23 synthesis and secretion.[54] After binding of the FGF ligand to the FGFR, the receptors dimerize, autophosphorylate, and then phosphorylate intracellular proteins involved in signal transduction such as phospholipase-Cγ (PLCγ), growth factor receptor bound (GRB)-14, son of sevenless (SOS), and FGF receptor substrate (FRS)-2, a docking protein that links the activated FGFR to the phosphoinositide-3-kinase (PI3K), MAPK, and ERK1/2 signaling pathways.[59] Immunoreactive species of FGF23 are measurable in normal adult sera, but values vary depending on the epitopes recognized by the antibodies employed (i.e., whether they are specific for the full-length FGF23 protein or only for its carboxyl terminal fragment) and on the assay methodology (e.g., enzyme-linked immunosorbent assay or chemiluminescent immunoassay).[60-62] In assays that recognize full-length FGF23 and carboxyl terminal fragments of FGF23, intact FGF23 values varied diurnally and there was marked interindividual variability in FGF23 levels.[61] The serum FGF23 concentration rises when dietary phosphate increases (or during infusion of phosphate) and declines with phosphate restriction.[49]

Serum values of FGF23 are increased in patients with XHR, ADHR, ARHR, tumor-induced osteomalacia, and fibrous dysplasia associated with hypophosphatemia. In patients with ADHR, gain-of-function mutations (e.g., Arg179Trp) in *FGF23* result in resistance to degradation of FGF23, a protein that is normally cleaved between Arg179 and Ser180; in subjects with tumor-induced osteomalacia, the production of FGF23 is greatly increased. In familial tumoral calcinosis (OMIM ID: 211900), loss-of-function mutations in *FGF23* lead to accelerated intracellular degradation of FGF23 that prevents secretion of intact protein resulting in decreased renal excretion of phosphate, hyperphosphatemia, relatively increased calcitriol levels, and diffuse ectopic calcification.[49,63]

Tumors associated with hypophosphatemic osteomalacia also express the phosphatonins encoded by *FRP4* (Frizzled related protein-4), *MEPE* (matrix extracellular phosphoglycoprotein), and *FGF7*.[49,51] FRP4 is a secreted 346-amino-acid glycosylated protein that shares the structure of the extracellular domain of transmembrane Frizzled receptors. The natural ligands of Frizzled receptors are WNT proteins, and their coreceptors are cell surface low-density lipoprotein receptor-related protein (LRP) 5/6; these heterotrimeric complexes stabilize intracellular β-catenin and the attendant signal transduction systems that are essential for bone formation (discussed later). Secreted FRP4 serves as a decoy receptor competing with the Frizzled receptor for binding to WNT, thus inhibiting the function of the Frizzled receptor. *FRP4* is expressed in bone cells and in large amounts by tumors with associated osteomalacia. FRP4 inhibits sodium-dependent renal tubular phosphate reabsorption by inhibition of WNT signaling leading to hypophosphatemia; it also reduces expression of *CYP27B1*, the gene encoding 25-hydroxyvitamin D_3-1α-hydroxylase.[52] *MEPE* is primarily expressed by osteoblasts, osteocytes, and odontoblasts as well as by tumors associated with hypophosphatemic osteomalacia. It encodes a 525-amino-acid, 58-kDa protein, a matrix extracellular phosphoglycoprotein that is a member of the short-integrin-binding, ligand-interacting glycoprotein (SIBLING) family (discussed later). Expression of *MEPE* is inhibited by calcitriol. MEPE modulates osteoblast and osteoclast function and may both inhibit and support bone mineralization.[49] Although MEPE is able to inhibit sodium-dependent renal tubular phosphate reabsorption, its role in phosphate metabolism may be more complex inasmuch as knockout of *Mepe* in mice results in increased bone mass without altering serum phosphate or calcitriol values.[64] MEPE contains an acidic serine aspartate-rich MEPE-associated motif (ASARM) that binds to hydroxyapatite where it inhibits mineralization; ASARM is the physiologic substrate for PHEX, which degrades it, enabling further hydroxyapatite formation; loss-of-function mutations in *PHEX* lead to X-linked hypophosphatemic rickets (XHR).[54,65]

The short integrin-binding ligand-interacting glycoproteins (SIBLINGs) are related noncollagenous matrix proteins: MEPE, dentin-matrix acidic phosphoprotein 1 (DMP1), dentin sialo-phosphoprotein (DSPP), osteopontin (also termed secreted phosphoprotein 1 [SPP1]), and bone sialoprotein (BSP), whose encoding genes are clustered on chromosome 4q.[54] These proteins share several common structures including an acidic serine aspartate-rich MEPE associated motif (ASARM) near their carboxyl terminals and an Arg-Gly-ASP (RGD) tripeptide motif that interacts with and binds to integrins on the surface of interacting cells. The basic ASARM motif consists of 23 amino acids; several of the serine residues are specifically phosphorylated by casein serine kinase. ASARM is released from MEPE by cathepsins B and K; phosphorylated ASARM from MEPE binds avidly to hydroxyapatite and prevents further deposition of calcium and phosphate; it also inhibits phosphate uptake by both the intestinal tract and the renal tubule. (MEPE also is involved in the osteocytic response to a mechanical load [discussed later].) Phosphorylated and nonphosphorylated ASARMs released by MEPE, DMP1, DSPP, and SPP1 are resistant to proteolysis except by the zinc metalloendopeptidase PHEX (phosphate regulating gene with homologies to endopeptidases on the X chromosome). Thus, inactivating mutations of *PHEX* result in X-linked hypophosphatemic rickets, in part, by inability to degrade the ASARM peptide coating of hydroxyapatite.[54] PHEX may also be involved in the expression of *FGF23* and the stability of FGF23.[54] Carboxyl to the ASARM motif of DMP1 is an additional sequence of 35 amino acids that is termed the minfostin motif; a mutation in this region of *DMP1* results in one form of ARHR. It has been suggested that in the normal osteocyte, the ASARM region of DMP1 binds to PHEX expressed on its plasma membrane, thereby suppressing expression of *PHEX* and that displacement of binding by excessive free ASARM peptides (as in patients with XHR, ARHR) increases *FGF23* expression.[54] PHEX, DMP1, and FGF23 may also be involved in the regulation of energy metabolism (discussed later).

MAGNESIUM

Magnesium (Mg^{2+}) is the fourth most abundant of the body cations. Approximately 1% of body Mg^{2+} is present in the ECF compartment, ~14% in muscle and soft tissue, and ~85% in bone where it is found on the surface of the hydroxyapatite crystal and of which ~50% is freely exchangeable.[1,3,66] In blood, magnesium (1.7 to 2.4 mg/dL = 0.7 to 1 mmol/L) is partially bound to proteins (~30%), complexed to phosphate and other anions (~15%), and free Mg^{2+}_e (~55%). As with Ca^{2+}_e, Mg^{2+}_e levels rise as pH falls (increased acidity). Intracellularly, magnesium (0.5 mmol/L) is bound to ATP and other molecules; ~10% is in the ionic form and ~60% is within mitochondria. Mg^{2+}_e is a cofactor in many enzymatic reactions, including those that consume or produce ATP. Mg^{2+}_e alters free radicals and influences nitric oxide synthase, cyclic guanosine monophosphate generation, endothelin production, and immune function. Mg^{2+}_e decreases membrane excitability in nerve and muscle cells and blocks the excitatory N-methyl D-aspartate receptor. It is a necessary cofactor for the regulation of neuromuscular excitability, nerve conduction, oxidative metabolism by mitochondria, glycolysis, phosphorylation, and transcription and translation of DNA. It is essential for the secretion (but not the synthesis) of PTH by the parathyroid chief cell. Net intestinal magnesium absorption is directly related to dietary intake and independent of calcitriol.[1] Magnesium is passively absorbed by diffusion through voltage-dependent paracellular channels in the small intestine and colon in proportion to the intestinal luminal concentration of this cation. In addition, a small component of active, transcellular magnesium absorption takes place in the colon through transient receptor potential melastatin (TRPM)-6 cation transport channels complexed with TRPM7 channels.[22,67] Loss-of- function mutations in *TRPM6* result in autosomal recessive hypomagnesemia with secondary hypocalcemia due to impairment of intestinal (and renal tubular) absorption of this cation. The intestinal absorption of magnesium may be impaired by high phosphate intake, intestinal dysfunction, or chronic laxative abuse. Magnesium is also excreted into the intestinal tract.

Seventy percent of serum Mg^{2+}_e is ultrafiltrable and passes through the renal glomerular membrane; ~95% of filtered Mg^{2+} is reabsorbed—~15% in the proximal tubule and ~70% in the cortical TALH by passive paracellular means and ~10% in the distal convoluted tubule—in the latter site by an active transcellular reabsorption process.[1,66] Approximately 3% to 5% of filtered Mg^{2+} is excreted in the urine. In the proximal renal tubule, the reabsorption of water results in an increased intratubular Mg^{2+} concentration that facilitates its paracellular reabsorption.[66] In the TALH, Mg^{2+} reabsorption occurs through a paracellular pathway that is impermeable to water and is propelled by a transepithelial voltage gradient that is lumen positive and generated by a Na^+-K^+-ATPase.[66] The voltage gradient requires the integrated action of a Na^+-K^+-Cl^--coupled cotransporter (NKCC2 encoded by *SLC12A1*), a Cl^- channel (CLC-Kb encoded by *CLCNKB*), and a K^+ channel (ROMK encoded by *KCNJ1*). Barttin encoded by *BSND* is an essential beta subunit of the ROMK Cl^- channel.

Mg^{2+} is conducted from the lumen of the TALH to the interstitial space and into the vasculature by tight junction proteins encoded by the claudin gene family, specifically claudins 16 and 19. Tight junction proteins bridge intercellular gaps and regulate the movement of ions across an epithelial sheet. Paracellin-1 (encoded by *CLDN16*) is a 305-amino-acid, tight junction claudin protein whose gene is expressed primarily in the renal cortical TALH and distal convoluted tubule; paracellin-1 has four transmembrane domains with intracellular carboxyl and amino terminals.[16] Paracellin-1 is also utilized for paracellular reabsorption of Ca^{2+} in the TALH. Homozygous or compound heterozygous loss-of-function mutations of *CLDN16* lead to familial, autosomal recessive renal hypomagnesemia (type 3) characterized by renal wastage of Mg^{2+} in association with hypercalciuria and renal calcification (OMIM ID: 248250).[68] Claudin 19 is a 224-amino-acid tight junction protein. Biallelic inactivating mutations in *CLDN19* result in renal wasting of Mg^{2+}, hypomagnesemia, hypercalciuria, and nephrocalcinosis leading to renal failure; additionally, because *CLDN19* is expressed in the eye, affected subjects have decreased vision due to retinal malformation (hypomagnesemia type 5, OMIM ID: 248190).

It is primarily in the distal convoluted tubule—the last segment of the nephron in which Mg^{2+} is reabsorbed—that Mg^{2+} is actively transported through apical (luminal) membrane TRPM6 channels.[22,66,69] The renal tubular expression and intracellular movement of TRPM6 is regulated by dietary magnesium, estrogens, acid-base balance, and pro-epidermal growth factor (pro-EGF). TRPM7 (OMIM ID: 605692) is also expressed on the luminal surface of renal cells in the distal convoluted tubule where it too facilitates reabsorption of Mg^{2+}.[69] Other magnesiotropic proteins expressed in the distal convoluted tubule are the thiazide sensitive Na^+:Cl^- cotransporter, potassium channels Kv1.1 and Kir4.1, and hepatocyte nuclear factor 1B (HNF1B). Biallelic inactivating mutations in *TRPM6*—encoding an ion channel positioned on the luminal membranes of colonic cells and cells in the renal distal convoluted tubule—result in hypomagnesemia type 1 with secondary hypocalcemia (OMIM ID: 602014); this disorder presents in early infancy with tetany and seizures and is due to both impaired absorption of intestinal Mg^{2+} and reabsorption of renal tubular Mg^{2+}. Pro-EGF regulates the availability and activity of TRPM6 by enabling its movement from intracellular sites of storage to the luminal plasma membrane of renal distal convoluted tubular cells and colonic epithelial cells; biallelic loss of *EGF* leads to hypomagnesemia type 4 (OMIM ID: 611718). Loss of *SLC12A3* and its encoded product—the thiazide sensitive Na^+:Cl^- cotransporter—leads to reduced TRPM6 activity or abundance and the Gitelman syndrome (OMIM ID: 263800) of hypomagnesemia, hypocalciuria, and hypokalemic metabolic alkalosis. Loss of the voltage-gated K^+ channel Kv1.1 (encoded by *KCNA1*) that colocalizes with TRPM6 alters the polarization of the renal epithelial cell of the distal convoluted tubule, a functional abnormality that impairs activity of TRPM6 and hence of Mg^{2+} transport resulting in autosomal

dominant hypomagnesemia with episodic ataxia, tetany, muscular cramping, and myokymia (involuntary, localized quivering of a few muscle fibers within a muscle) (OMIM ID: 160120). The pathogenesis of hypomagnesemia (OMIM ID: 612780) that results from the loss-of-function mutations in *KCNJ10* encoding the inwardly rectifying K^+ channel, Kir4.1, is similar to that described for loss of *KCNA1*. Inactivating mutations of *KCNJ10* not only lead to hypomagnesemia but are also associated with the SESAME syndrome (seizures, sensory neural deafness, ataxia, mental retardation, electrolyte imbalance, OMIM ID: 612780). (In addition to the described Mg^{2+} transport proteins, there are several other well-characterized Mg^{2+} membrane transporters and solute carriers.[69])

PTH increases Mg^{2+} reabsorption in the renal TALH and distal convoluted tubule; hypermagnesemia and hypercalcemia (acting through the CaSR) decrease renal tubular magnesium reabsorption, as do expansion of extracellular fluid volume, metabolic alkalosis, phosphate depletion, loop diuretics, aminoglycoside antibiotics, and impaired renal function.[1]

ALKALINE PHOSPHATASE

The gene (*ALPL*) encoding tissue-nonspecific alkaline phosphatase (TNSALP) is expressed in bone (synthesized by the osteoblast), liver, kidney, and skin fibroblasts. Bone ALPL is a 507-amino-acid protein that is generated by alternative processing of *ALPL* utilizing one of two leading exons during transcription and translation, permitting the osteoblast to synthesize and release a specific bone isoform. Although TNSALP circulates as a homodimer, in tissue it is a homotetrameric ectoenzyme (ectophosphatase) located on the osteoblast's cell surface anchored through its carboxyl terminal to cell membrane phosphatidylinositol-glycan. In bone, alkaline phosphatase (1) binds to collagen type I and prepares skeletal matrix for mineralization; (2) hydrolyzes organic phosphates, principally pyrophosphate and other polyphosphates (discussed later) as well as phosphoethanolamine and pyridoxyl-5'-phosphate, the major circulating form of vitamin B_6, thereby increasing the local concentration of phosphate to values that exceed the calcium × phosphate product encouraging deposition of calcium phosphate as hydroxyapatite on collagen type I fibers; (3) transports inorganic phosphate and Ca^{2+} into the cell; and (4) inactivates pyrophosphate and other inhibitors of mineralization (e.g., phosphorylated ASARM derived from MEPE) by removing their phosphate moieties. The hepatic form of TNSALP is formed by alternative splicing of dual exons 1 of *ALPL*. Hepatic alkaline phosphatase converts pyridoxal-5′-phosphate to pyridoxal, a compound essential for normal synthesis of neural γ-aminobutyric acid (GABA), an inhibitory neurotransmitter; without pyridoxal-5′ phosphate central nervous system levels of GABA are low and seizures may occur. Loss-of-function mutations in *ALPL* lead to infantile, childhood, and adult forms of hypophosphatasia. Three genes encoding tissue-specific intestinal, placental, and germ cell alkaline phosphatase isoenzymes are clustered at chromosome 2q34-q37.

PARATHYROID HORMONE; PARATHYROID HORMONE–RELATED PROTEIN; PTH/PTHRP RECEPTORS

Parathyroid Hormone

Parathyroid hormone increases concentrations of plasma Ca^{2+}_e, lowers serum phosphate values, stimulates synthesis of calcitriol, and exerts both anabolic and catabolic effects on bone. PTH is synthesized and secreted by the chief cells of four paired parathyroid glands that are derived from the endoderm of the dorsal segments of the third (paired inferior glands) and fourth (paired superior glands) pharyngeal pouches; occasionally, there may be a fifth parathyroid gland embedded within the substance of the thyroid gland or in the mediastinum.[70] The thymus is formed by the endoderm of the third pharyngeal pouch and calcitonin-synthesizing parafollicular (C) cells of the thyroid by that of the fourth pharyngeal pouch. A critical transcription factor for the development of the parathyroid glands is the nuclear DNA binding transcription factor encoded by *GCM2* (OMIM ID: 603716). Although essential for neural glial cell development in insects, *Gcm* is not expressed in mammalian brain but is expressed primarily in the rodent placenta and thymus (*Gcm1*) and in the parathyroid gland (*Gcm2*); the pharyngeal pouch expression of *Gcm2* is maintained by a number of homeobox and transcription factors including those encoded by *Hoxa3*, *Pax1*, *Pax9*, and *Eya1*. The human homologue of *Gcm2* (*GCM2* or *GCMB*) is expressed in the parathyroid glands and in intrathymic PTH-secreting adenomas but not by normal human thymus.[71] Intragenic deletion or missense mutations of *GCM2* have been identified in subjects with familial, autosomal recessive hypoparathyroidism.

PTH is synthesized as a 115-amino-acid preprohormone that is translated into a full-length peptide of 84 amino acids ($PTH^{1\text{-}84}$). Its synthesis and secretion by chief cells of the parathyroid glands is regulated by Ca^{2+}_e concentrations acting through the CaSR (discussed earlier) that either enhance (when Ca^{2+}_e concentrations are low) or repress (when they are high) transcription of *PTH* and secretion of PTH; Ca^{2+}_e values also modulate the rate of chief cell proliferation, a response mediated by the CaSR. Calcimimetic drugs have inhibitory effects on PTH secretion, a property that has made these agents useful in the management of patients with excess secretion of PTH such as mild hyperparathyroidism and autosomal dominant familial hypocalciuric hypercalcemia. Higher serum phosphate values enhance production of PTH not only indirectly by lowering of Ca^{2+}_e values but also by stabilizing PTH mRNA permitting greater translation of PTH and by stimulation of chief cell replication. 1α,25-Dihydroxyvitamin D_3 (calcitriol) and its synthetic analog inhibit transcription of *PTH*, secretion of PTH, and proliferation of chief cells; FGF23 also decreases PTH release.[70] In response to acute hypocalcemia, PTH stored in secretory vesicles is rapidly released; when hypocalcemia is prolonged, the secretion of $PTH^{1\text{-}84}$ is augmented by a decrease in its rate of intracellular degradation and an increase in the rate of transcription of *PTH*; when hypocalcemia is extended, PTH synthesis and

secretion are amplified by increase in chief cell number. When Ca^{2+}_e values rise, the secretion of PTH is decreased acutely by degradation of stored $PTH^{1\text{-}84}$ by calcium-responsive proteases within the parathyroid glands; if prolonged, hypercalcemia leads to a decrease in the expression of *PTH*, possibly by destabilization of PTH mRNA, and to a decline in the number of chief cells.[70] *PTH* is composed of three exons; exon 1 is transcribed but not translated; the second and third exons of *PTH* encode the prepro-PTH sequence of 115 amino acids.

The stability, translation, and intracellular translocation of PTH mRNA are regulated by binding of cytosolic proteins to the 3′-untranslated region of PTH mRNA; these proteins include a member of the dynein complex that also binds to microtubules within the parathyroid chief cell and heterogeneous nuclear ribonucleoprotein D (HNRPD or AUF1) that directs mRNA into the proteasomal pathway of degradation.[72] The amino terminal 25-amino-acid signal sequence (encoded by exon 2) of 115-amino-acid prepro-PTH is removed by furin, a prohormone convertase, as the polypeptide leaves the endoplasmic reticulum forming the 90-amino-acid pro-PTH (encoded by exon 3) that is then further processed in the Golgi apparatus by furin to mature human 84-amino-acid PTH. $PTH^{1\text{-}84}$ is stored in secretory vesicles and granules from which it is either secreted by an exocytic mechanism or degraded by calcium-sensitive proteases (cathepsins B and H co-localized within the vesicles) to smaller amino and carboxyl terminal fragments of PTH. PTH degradation represents an important mechanism that regulates the release of intact bioactive $PTH^{1\text{-}84}$ and is accelerated when the plasma Ca^{2+}_e concentration is elevated. The full biologic activity of $PTH^{1\text{-}84}$ is found in its first 34-amino-acid sequence and all of its skeletal and renal affects (discussed later) are localized within the segment $PTH^{1\text{-}31}$. Amino acids numbers 1 and 2 (ser-val) comprise an activation sequence that is essential for bioactivity of the amino terminal portion of $PTH^{1\text{-}84}$. Amino acids 20 through 26 constitute a core sequence necessary for binding to the amino-terminal ectodomain of the PTH receptor (discussed later); within this 7-amino-acid sequence, Arg^{20} and Trp^{23} are critically important for binding of the PTH ligand to its receptor.[73] In addition to intact $PTH^{1\text{-}84}$, the parathyroid glands secrete a phosphorylated form of $PTH^{1\text{-}84}$ and carboxyl terminal PTH fragments of varying length, but they do not secrete amino terminal fragments of $PTH^{1\text{-}84}$.[74,75] The half-life of circulating $PTH^{1\text{-}84}$ is approximately 2 minutes; it is rapidly metabolized by the liver and excreted by the kidney. In the hepatic Kupffer cells, $PTH^{1\text{-}84}$ is cleaved usually either between amino acids 33 and 34 or between amino acids 36 and 37 to carboxyl terminal PTH peptides with half-lives of approximately 15 to 20 minutes. In the kidney, intact and carboxyl terminal fragments of PTH are filtered by the glomerulus, reabsorbed across the apical membrane of proximal renal tubular cells, and then degraded to smaller fragments. Megalin, a multifunctional low-density lipoprotein-related receptor (encoded by *LRP2*) expressed in coated pits on the luminal/apical surface (as well as endocytic vacuoles and lysosomes) of proximal renal tubular cells specifically recognizes intact $PTH^{1\text{-}84}$ and amino terminal fragments of PTH and mediates the renal tubular endocytosis of intact $PTH^{1\text{-}84}$ that has been filtered through the glomerulus.

The classical functions of $PTH^{1\text{-}84}$ as well as its shorter amino peptide derivatives $PTH^{1\text{-}34}$ and $PTH^{1\text{-}31}$ upon regulation of calcium and phosphate homeostasis are carried out through the seven-transmembrane, G-protein coupled type 1 PTH/PTHrP receptor (discussed later) expressed in the renal tubule and osteoblast. Thus, with equal potency $PTH^{1\text{-}84}$, $PTH^{1\text{-}34}$, and $PTH^{1\text{-}31}$ (1) inhibit renal tubular reabsorption of phosphate thereby increasing its urinary excretion; (2) increase renal tubular reabsorption of Ca^{2+}; (3) augment renal synthesis of calcitriol by enhancing expression of *CYP27B1* (encoding 25-hydroxyvitamin D_3-1α-hydroxylase) thereby amplifying intestinal absorption of calcium; (4) stimulate osteoblast production of receptor activator of nuclear factor κB-ligand (RANKL), thus enhancing osteoclastogenesis and osteoclastic reabsorption of calcium; and (5) mediate the anabolic effects of PTH on the volume and microarchitecture of bone.[76] $PTH^{1\text{-}84}$, $PTH^{1\text{-}34}$, and $PTH^{1\text{-}31}$ stimulate intracellular signal transduction through several G protein–coupled pathways including the $G_s\alpha$ subunit-adenylyl cyclase-cyclic adenosine monophosphate (AMP)-PKA and the G_{q11}-phospholipase C (PLC)-IP_3/diacylglycerol (DAG)-Ca^{2+}-PKC-MAPK signal transduction pathways. Furthermore, $PTH^{1\text{-}84}$ and $PTH^{1\text{-}34}$ can induce cell function through β-arrestin that does not involve a G protein (discussed later) that in turn activates intracellular signaling pathways mediated by MAPK, protein kinase B (PKB), and PI3K.[75,77]

As noted, multiple carboxyl-terminal peptides derived from $PTH^{1\text{-}84}$ circulate; they are either secreted directly by the parathyroid chief cells or return to the circulation after metabolism of intact $PTH^{1\text{-}84}$ by hepatic Kupffer cells.[75] Indeed, carboxyl terminal fragments of $PTH^{1\text{-}84}$ are secreted in greater abundance from the parathyroid glands than is intact $PTH^{1\text{-}84}$, and the proportion of carboxyl terminal fragments secreted increases as the ambient Ca^{2+} concentration rises. Amino terminal fragments generated by hepatic degradation of $PTH^{1\text{-}84}$ are degraded further within the liver and do not recirculate. $PTH^{1\text{-}84}$ and the carboxyl terminal fragments of PTH are filtered by the kidneys; the carboxyl terminal fragments are reabsorbed by the renal tubules and further degraded intracellularly. The kidneys are not a major source of circulating carboxyl terminal fragments of PTH. Among the carboxyl terminal fragments of PTH found in circulation are $PTH^{7\text{-}84}$,$PTH^{24\text{-}84}$,$PTH^{34\text{-}84}$,$PTH^{37\text{-}84}$,$PTH^{41\text{-}84}$,$PTH^{43\text{-}84}$. They are also extracted from serum by the kidneys, muscle, and bone.

In addition to their classical effects on mineral homeostasis, several nonclassical actions of $PTH^{1\text{-}84}$ have been identified, including (1) rapid and direct stimulation of intestinal calcium absorption independent of its effects on vitamin D metabolism, (2) stimulation of hepatic gluconeogenesis, (3) acute natriuresis and calciuresis, and (4) enhancement of neutrophil movement in vitro.[75] Inasmuch as many of these nonclassical biologic effects of intact PTH are not replicated by the amino terminal fragment $PTH^{1\text{-}34}$, it has been suggested that they may be related to the carboxyl terminal portion of the protein. Indeed, the carboxyl terminal fragments of PTH may

have biologic functions that oppose those of the amino terminal portion of PTH—namely, hypocalcemic properties that are perhaps mediated by pro-apoptotic effects on both osteoclasts and osteocytes.[78] Thus, $PTH^{7\text{-}84}$ lowers serum calcium levels in parathyroidectomized rats maintained eucalcemic by diet and antagonizes $PTH^{1\text{-}84}$-stimulated increase in calcium concentrations, urinary phosphate excretion, and bone turnover.[55] $PTH^{7\text{-}84}$ directly lowers the rate of bone resorption and suppresses the bone resorbing effects of $PTH^{1\text{-}34}$, calcitriol, prostaglandin E_2, and interleukin (IL)-11. However, $PTH^{39\text{-}84}$ and $PTH^{53\text{-}84}$ augment the biologic effects of $PTH^{1\text{-}34}$. $PTH^{7\text{-}84}$ does not bind to the PTH/PTHrP receptor nor does it inhibit $PTH^{1\text{-}84}$–mediated increase in cyclic AMP generation implying that $PTH^{7\text{-}84}$ likely acts through a receptor other than PTH1R (discussed later).

Because there are multiple circulating forms of PTH, its immunologic measurement is dependent on the specificity of the antibody(ies) employed in the assay. When a first-generation polyclonal PTH radioimmunoassay is utilized, both intact and carboxyl terminal fragments of PTH are usually measured. Use of second-generation immunoradiometric and immunochemiluminometric assays employing dual monoclonal antibodies, the first directed toward an epitope within the amino terminal of PTH (amino acids 20 to 25) and the second specific for an epitope in the carboxyl terminal of PTH (e.g., amino acids 59 to 78), improves the specificity of immunologic assays for intact $PTH^{1\text{-}84}$. Nevertheless, most of these assays detect both intact and selected carboxyl-terminal fragments of PTH and consequently measured PTH values may be inappropriately high, particularly in patients with chronic renal insufficiency in whom circulating levels of carboxyl terminal fragments of PTH are high.[79,80,81] Furthermore, the comparability of PTH assays from different commercial sources is often limited.[82,83] Employing a two site immunochemiluminescent assay, serum PTH concentrations average approximately 11 to 13 pg/mL and range between 2.3 and 24.5 pg/mL in children and adolescents 2 to 16 years of age; values do not vary with age but are a bit higher in girls than boys.[84] Development of "third-generation" of PTH assays that employ antibodies that are directed to an epitope of the first four amino acids of PTH has improved the specificity and stability of assays for $PTH^{1\text{-}84}$, but their clinical advantage is uncertain.[85,86] A "fourth generation" of PTH assays is under development that employs immunoaffinity, in situ digestion, liquid chromatography, and tandem mass spectrometry to separate $PTH^{1\text{-}84}$ from smaller PTH fragments.[81]

The synthesis and secretion of $PTH^{1\text{-}84}$ and its various fragments are modulated for the most part by the serum $Ca^{2+}{}_e$ concentration acting through the CaSR expressed on the plasma membrane of the parathyroid chief cell. Because a change in serum $Ca^{2+}{}_e$ concentration sensed by the parathyroid chief cell CaSR is quickly reflected in changes in cytosolic $Ca^{2+}{}_i$ levels, the release of PTH can be regulated on a minute-to-minute basis. Rapidly declining as well as steady-state low serum concentrations of $Ca^{2+}{}_e$ increase PTH secretion by accelerating its release from storage sites in secretory vesicles; hypocalcemia also raises intracellular levels of PTH mRNA by increasing the transcription rate of *PTH* and enhancing the stability of PTH mRNA by its posttranscriptional binding to cytosolic proteins.[3,70] Hypercalcemia decreases *PTH* transcription and destabilizes and thence lowers cellular levels of PTH mRNA. The serum $Ca^{2+}{}_e$ concentration also determines the form of PTH released by the parathyroid gland; during hypocalcemia, $PTH^{1\text{-}84}$ is the predominant form secreted; in hypercalcemic states, carboxyl terminal fragments of PTH are released in abundance.

Low serum phosphate concentrations exert an independent and direct inhibitory effect on the transcription of *PTH*, posttranscriptional PTH mRNA stability, PTH secretion, and proliferation of parathyroid chief cells; hyperphosphatemia increases PTH secretion by lowering plasma concentrations of Ca^{2+} and by enhancing the stability of PTH mRNA.[70] Prolonged hyperphosphatemia contributes to hyperplasia of the parathyroid glands frequently encountered in patients with chronic renal disease. Calcitriol directly inhibits *PTH* transcription acting through the VDR and the vitamin D response element (VDRE) in the 5′-untranslated region of *PTH* that associates with a transcriptional repressor factor (discussed later). Calcitriol also controls expression of *CASR* and of *VDR* and decreases proliferation of parathyroid cells. However, chronic hypocalcemia overcomes the suppressive effects of calcitriol on *PTH* transcription by decreasing the VDR number in the parathyroid chief cell. FGF23 released by osteocytes and osteoblasts directly impedes synthesis and secretion of PTH. Both hypomagnesemia and hypermagnesemia inhibit release but not synthesis of PTH. Other agents that increase PTH release include β-adrenergic agonists, dopamine, prostaglandin E, potassium (by decreasing cytosolic $Ca^{2+}{}_i$ levels within the parathyroid chief cell), prolactin, lithium (by resetting the set-point for PTH release), glucocorticoids, estrogens, and progestins. Prostaglandin $F_{2\alpha}$, α-adrenergic agonists, and fluoride suppress PTH release by increasing $Ca^{2+}{}_i$ values.

PTH regulates the serum concentration of $Ca^{2+}{}_e$ directly by stimulating its reabsorption in the distal renal tubule and from the skeleton and indirectly by augmenting the intestinal absorption of calcium by increasing the synthesis of calcitriol. In bone, PTH enhances osteoclast differentiation and maturation indirectly by acting on and through stromal cells and osteoblasts to increase synthesis of RANKL, an activator of osteoclastogenesis, and to suppress that of osteoprotegerin, a decoy receptor for RANKL (discussed later). When administered intermittently, $PTH^{1\text{-}84}$ and $PTH^{1\text{-}34}$ exert anabolic effects on skeletal mass by increasing osteoblast number by accelerating their differentiation from progenitor cells and from inactive bone-lining cells. They do so in part by decreasing the stimulatory effects of the peroxisome proliferator activator receptor gamma (PPARγ) on mesenchymal stem cell differentiation into adipocytes permitting their differentiation into osteoblasts, by augmenting the rate of osteoblast proliferation, and by reducing the rate of apoptosis of osteoblasts.[76,87] The anabolic effects of PTH on bone formation are further mediated by enhancing synthesis and release of matrix-embedded growth factors such as locally generated insulin-like growth factor-I

(IGF-I), which has positive effects on differentiation and survival of osteoblasts, by stimulating the WNT-Frizzled-β-catenin pathway of osteoblastogenesis, and by inhibiting antagonists to bone formation such as sclerostin. Additionally, through promotion of β-arrestin–mediated intracellular signal transduction, PTH increases trabecular number and volume.[76,77] Thus, PTH stimulates anabolism of bone mineralization, whereas bisphosphonates exert an antiresorptive effect.[88] PTH stimulates calcitriol synthesis by increasing renal tubular expression of *CYP27B1*, the gene encoding 25-hydroxyvitamin D_3-1α-hydroxylase, the enzyme that catalyzes the synthesis of calcitriol from calcidiol (discussed later). PTH depresses the proximal and distal renal tubular reabsorption of phosphate by decreasing the expression of *SLC34A1*, encoding a Na^+-HPO_4 cotransporter, thus increasing the urinary excretion of this anion (discussed previously). In addition, PTH directly stimulates secretion of bone-derived FGF23, thereby further increasing urinary phosphate excretion; PTH also indirectly stimulates FGF23 release by lowering serum phosphate concentrations and increasing those of calcitriol.[89]

Parathyroid Hormone–Related Protein

Parathyroid hormone–related protein (PTHrP) was initially identified as a major cause of humoral hypercalcemia of malignancy. PTH and PTHrP have evolved from a common ancestral gene through gene duplication. They share 8 of their first 13 amino acids (the site of the activating domain for the common PTH/PTHrP receptor); although their amino acid sequences diverge thereafter, the predicted three-dimensional structures of the next 21 amino acids are similar.[90,91] Both peptides bind to the PTH/PTHrP receptor (PTH1R), although their receptor binding domains are distinct; PTH binds more avidly to PTH1R and elicits a greater hypercalcemic response than does PTHrP.[91] PTHrP is synthesized by many fetal and adult tissues (cartilage, bone, smooth, cardiac and skeletal muscle, skin, breast, intestines, parathyroid glands, pancreatic islets, pituitary, placenta, and central nervous system) and plays a crucial role in chondrocyte differentiation and maturation, formation of the mammary gland and eruption of teeth, epidermal and hair follicle growth, and other developmental events.[90,91] Whereas PTH acts as an endocrine hormone on tissues distant from its source—the parathyroid glands, except in patients with humoral hypercalcemia of malignancy when PTHrP or segments thereof are secreted into the circulation and during lactation—PTHrP is synthesized locally and acts primarily as a paracrine, autocrine, or intracrine messenger. Although the secretion of PTH is regulated by Ca^{2+}_e levels and fluctuates rapidly, the production of PTHrP is constitutive and is controlled at the point of expression of its encoding gene (*PTHLH*). *PTHLH* is transcribed and translated into 139-, 141-, and 173-amino-acid isoforms.[3,87] In addition, amino ($PTHrP^{1\text{-}36}$) and midregion and carboxyl terminal ($PTHrP^{38\text{-}94}$,$^{38\text{-}95}$,$^{38\text{-}101}$,$^{107\text{-}138}$,$^{109\text{-}138}$) products of *PTHLH* are formed by the actions of prohormone convertases during posttranslational processing in specific tissues.[90,91] The smaller peptides ($PTHrP^{1\text{-}36}$,$^{38\text{-}95}$,$^{38\text{-}101}$,$^{108\text{-}138}$,$^{109\text{-}138}$) may act as paracrine factors or be secreted into the circulation. PTHrP secreted by fetal parathyroid glands and the $PTHrP^{37\text{-}94}$ midregion fragment synthesized by the placenta increase placental calcium transport. Intact PTHrP binds only to PTH1R, through which receptor it exerts its biologic effects on mineral homeostasis. Carboxyl terminal fragments of PTHrP may act through as yet unidentified receptors. Because transcription of *PTHLH* can begin in an exon downstream of the exon that encodes the signal peptide of this protein, after synthesis PTHrP can remain in the cytoplasm and be directly imported into the nucleus.[91] (A nuclear localization sequence is present between amino acids 84 and 93 in the PTHrP molecule.) PTHrP is shuttled into the nucleus bound to importin β1 where it binds to and perhaps regulates RNA trafficking, protein translation, and ribosomal dynamics, thereby influencing cell proliferation and survival.[90,91] Acting through the vitamin D nuclear receptor, calcitriol impairs the synthesis of PTHrP by down-regulating transcription of *PTHLH* and decreasing stability of PTHrP mRNA, thereby increasing the rate of intracellular degradation of the PTHrP protein.

Although the effects of $PTHrP^{1\text{-}36}$,$^{1\text{-}141}$ on calcium, phosphate, and vitamin D metabolism are similar to those of PTH, the major roles of this and other segments of PTHrP in many developmental processes, including those of breast, teeth, cartilage, and endochondral bone, distinguish PTHrP from PTH (Table 8-4). In the fetus, periarticular chondrocytes located at the ends of long bones synthesize PTHrP in response to Indian hedgehog (IHH), a protein secreted by late proliferative–early prehypertrophic chondrocytes that functions through its receptor, Patched 1 (please refer to Figure 8-9, presented later in the chapter). PTHrP enters into and diffuses through the growth plate and signals prehypertrophic chondrocytes through PTH1R to slow their rate of differentiation to hypertrophic chondrocytes—thus prolonging the stage of proliferation and delaying ossification, thereby increasing the length of the cartilaginous growth plate and the long bone.[90,91] In addition to stimulating the secretion of PTHrP, IHH increases the rate of growth plate chondrocyte proliferation, which also directs differentiation of perichondrial cells to osteoblasts. In the postnatal mouse, PTHrP is produced by the proximal resting/undifferentiated and early proliferating chondrocytes in response to IHH.[92] Biallelic loss of PTHrP in knockout mice is lethal due to bony malformations. In $Pthlh^{-/-}$ mice, there is decrease in the number of resting and proliferating chondrocytes; the columnar organization of the growth plate is disrupted, there is premature acceleration of chondrocyte maturation and apoptosis, and inappropriate ossification resulting in a dwarfing phenotype (a domed and foreshortened cranium, short limbs, small thorax) that is similar to that of Blomstrand chondrodysplasia (OMIM ID: 215045), a disorder associated with loss-of-function mutations of *PTH1R*.[93] Mice in which expression of *Pthlh* has been maintained only in chondrocytes survive but display small stature, cranial chondrodystrophy, and failure of tooth eruption. In the heterozygous state ($Pthlh^{+/-}$), mouse fetal development is normal but by 3 months of

TABLE 8-4 **Parathyroid Hormone–Related Protein: Sites of Expression and Action**

Site	Action
Mesenchyma	
Subarticular cells/ prechondrocytes	PTHrP depresses the rate of differentiation of late proliferating chondrocytes to hypertrophic chondrocytes and delays ossification, thus permitting increased longitudinal growth of the cartilaginous growth plate
Bone	Enhances or depresses bone resorption
Smooth muscle	Relaxation
Vascular system	
Myometrium	
Urinary bladder	
Cardiac muscle	Positive chronotropic and inotropic effects
Skeletal muscle	
Epithelia	
Skin	Regulates proliferation of keratinocytes
Breast	Induces ductal branching and formation of breast epithelium, secreted into milk, drives mobilization of calcium from maternal bone for transfer to nursing infant
Teeth	Stimulates resorption of overlying bone enabling eruption
Endocrine system	
Parathyroid glands	Stimulates transport of calcium
Pancreatic islets	Stored and cosecreted with insulin
Placenta	Enhances calcium transport from mother to fetus
Central nervous system	Neuroprotective by antagonizing excessive calcium-related excitotoxicity

Compiled and adapted from Wysolmerski, J. J. (2008). Parathyroid hormone-related protein. In C. J. Rosen (Ed.), Primer on the metabolic bone diseases and disorders of mineral metabolism *(pp. 127–133) (7th ed.). Washington DC: The American Society of Bone and Mineral Metabolism.; Wysolmerski, J. J. (2012). Parathyroid hormone-related protein: an update.* J Clin Endocrinol Metab, 97, *2947–2956.*

age the trabeculae of the long bones are osteopenic; a similar bone phenotype is noted when loss of *Pthlh* expression is confined to osteoblasts.[94] For comparison, in mice in which *Pth* has been knocked out, there is decreased mineralization of cartilage matrix, expression of vascular endothelial growth factor (VEGF) and neovascularization, osteoblast number, and trabecular bone volume.[95] Thus, both PTH and PTHrP are necessary for normal fetal endochondral bone development. Cortical thickness of long bones is increased in both *Pth*- and *Pthlh*-null mouse models, indicating that the regulation of endochondral and periosteal osteoblast function differs. The production of PTHrP by mice osteoblasts in vitro can be increased by mechanical deformation, suggesting that PTHrP may play a role in the response to skeletal loading.[90]

The placental transport of calcium is dependent on PTHrP produced by the placenta itself, as the maternal-fetal calcium gradient is lost in its absence and may be restored by administration of a midmolecular fragment.[90,91,94] Both PTH and PTHrP are required for normal mineral homeostasis in utero. In mice that lack parathyroid glands, fetal serum calcium and magnesium concentrations are low and phosphate levels are elevated; to a lesser extent similar changes occur with loss of expression of *Pthlh*.[96] PTH does not regulate placental calcium transfer, whereas PTHrP is critically important for this process.[91] PTHrP is essential for normal nipple and mammary gland development in utero; this protein promotes differentiation of mesenchymal tissue surrounding the budding epithelial mammary ducts; PTHrP is also important for terminal differentiation of the mammary system during puberty.[90,91] During lactation, mammary expression of *PTHLH* and secretion of PTHrP increases whereas production of estrogens declines, permitting unopposed PTHrP-induced mobilization of maternal skeletal calcium necessary for the breastfed infant but substantially decreasing maternal bone mineral content, a process that is reversed when lactation ceases.[97] PTHrP also is expressed and presumably functional in smooth muscle (stomach, urinary bladder, vasculature), cardiac muscle, pancreatic islet cells, and central and peripheral nervous systems; it is essential for the eruption of developing teeth.[90] Serum concentrations of PTHrP are low except when it is secreted by tumors leading to humoral hypercalcemia of cancer. There are high levels of PTHrP in breast milk and low concentrations of PTHrP may be measurable in the serum of lactating women.

Parathyroid Hormone and Parathyroid Hormone–Related Protein Receptors

PTH and PTHrP bind with equal affinity to a common receptor (PTH1R) through which most of the classical mineral homeostatic physiologic functions of these peptides are exerted. PTH1R is a 585-amino-acid protein that is a member of the class B family of GPCRs (whose ligands are calcitonin, growth hormone releasing hormone, secretin, glucagon, glucagon-like peptide 1, gastric inhibiting polypeptide, vasoactive intestinal polypeptide, corticotropin-releasing hormone, and pituitary

adenylate cyclase activating protein 1) that are characterized by long extracellular amino terminal domains (~160-amino-acid) residues) with multiple cysteine residues forming disulfide bridges. PTH1R has an extracellular domain, 7 transmembrane spanning segments connected by three extracellular and three intracellular loops, and an intracellular domain with which the signaling G-protein interacts. *PTH1R* is composed of 15 exons; there are three promoters that regulate expression of *PTH1R*. As a result of alternative mRNA splicing, there are several isoforms of the PTH/PTHrP receptor. The amino terminal sequences of PTH and PTHrP are essential for activation of PTH1R and for binding to the extracellular domain of PTH1R.[3,75,98] The amino terminal extracellular domain of PTH1R contains six conserved cysteine residues that form three disulfide bonds; clustered near the first transmembrane domain are four glycosylated asparagine residues. Prior to association with its ligands, PTH1R is present as a homodimer—the carboxyl portion of the amino terminal extracellular domain (amino acids 29-187, the core domain linking the extracellular domain to the first transmembrane helix) of one PTH1R molecule forms an α helix that mimics the receptor binding structure(s) involving amino acids 20-26 of PTH/PTHrP that is recognized by and binds to the ligand binding groove of the extracellular domain of its companion PTH1R, thus forming a homodimer of two PTH1Rs.[98] Of critical importance to PTH1R dimerization are amino acid residues Arg179 and Val183, as their experimental loss renders PTH1R incapable of dimerization but still able to bind PTH and signal through $G_{s\alpha}$. Interaction with ligand disrupts the homodimeric structure of PTH1R that then acts as a monomer to activate intracellular signal transduction.

The most important core region for binding of PTH to the ectodomain of PTH1R lies between PTH:Arg20-Lys26 and requires both Arg20 and Trp23.[73] Trp23 interacts with Ile38, Leu41, and Ile135 within the extracellular domain of PTH1R. The first 10 amino acids of PTH within which is the PTH1R activating region do not interact with the ectodomain of PTH1R but rather with its extracellular loops (also termed the "J" or juxtamembrane domains). Interaction of PTH with PTH1R activates both G_{as} and G_{aq11} proteins and their respective signal transduction pathways.[3] After inducing intracellular signaling, PTH1R is phosphorylated by GPCR kinases (GRKs), thereby facilitating binding of β-arrestin (encoded by *ARBB2*, OMIM ID: 197841) leading to inactivation of PTH1R, its internalization, and subsequent destruction or reutilization.[77] The latter processes require ubiquitination of PTH1R and β-arrestin and proteasomal processing. By an alternate pathway, carboxyl terminal PTH peptides may also promote endocytosis of PTH1R.[75] Once internalized, PTH1R may be degraded, recycled to the cell membrane, or directed to the nucleus by importins-α_1 and -β where it is found in the nucleoplasm.[99] The role of nuclear PTH1R in relaying the biologic effects of PTH and PTHrP is unknown.

Interaction of PTH with PTH1R activates $G_{s\alpha}$, resulting in adenylate cyclase–mediated cyclic AMP production leading to intracellular signal transduction through PKA. Binding of PTH to PTH1R also activates $G_{q/11}$ signal transduction through PLC, leading to the formation of IP_3 and DAG from cell membrane-bound phosphoinositide, mobilization of Ca^{2+} from the endoplasmic reticulum, and activation of PKC.[77] (In the kidney, PTH1R is expressed on the apical and basolateral surfaces of cell in the proximal renal tubule; binding of PTH to apical PTH1R preferentially signals through cyclic AMP-PKA, whereas interaction of PTH with basolateral PTH1R activates the PLC-PKC signal transduction pathway.) Through both PKA and PKC, PTH/PTH1R activates the MAPK signal transduction system of extracellular signal-regulated kinases (ERK)-1,2 allowing regulation of cell differentiation, division, growth, and apoptosis. In addition to participating in the internalization of the PTH-PTH1R complex, β-arrestin enables it to stimulate intracellular signaling in a manner that is *independent* of G-proteins; the GRK-mediated patterns of proline phosphorylation of PTH1R and β-arrestin likely determine their conformations and functional activities.[100] Thus, interaction of PTH-PTH1R with β-arrestin may also activate the MAPK signaling system and facilitate PI3K and PKB (AKT) intracellular signal transduction pathways.[77] PTH-PTH1R activation of the MAPK pathway through the G-protein pathway is rapid and brief, lasting only a few minutes, whereas activation through the β-arrestin pathway is slow and sustained over hours. Another mechanism through which PTH/PTH1R interaction affects cellular function is by the binding of PTH1R to the Na^+-H^+ exchanger regulatory factor 2 (NHERF2, encoded by *SLC9A3R2*) with consequent stimulation of PLC activity and inhibition of adenylyl cyclase activity.[101] In the kidney, PTH1R binds to NHERF1 (encoded by *SLC9A3R1*), an essential factor for trafficking of the phosphate channel NPT2a to the luminal membrane of cells in the proximal renal tubule.[50] In the proximal renal tubule, linking of PTH to PTH1R increases cyclic AMP production leading to a decrease in the internalization and expression of the sodium-phosphate cotransporters and an increase in urinary phosphate excretion; association of PTH1R with NHERF1 blunts the phosphaturic effect of PTH.[50] Therefore, disruption of PTH1R-NHERF1 interaction leads to renal wasting of phosphate. Agonists of PTH have been synthesized that can bias the selection of the signal transduction pathway utilized; a biased agonist might stabilize a receptor conformation that stimulates a specific signal transduction sequence or activate one pathway while simultaneously inhibiting another.[77,100] Thus, $PTH^{1\text{-}34}$ stimulates $G_{s\alpha}$, G_{aq11}, and β-arrestin intracellular signaling systems, whereas $PTH^{1\text{-}31}$ preferentially enhances signaling through $G_{s\alpha}$ and $PTH^{3\text{-}34}$ through $G_{q/11}$. D-Trp^{12}, Tyr^{34} -$PTH^{7\text{-}34}$ is a PTH1R agonist that acts through the β-arrestin pathway.

PTH1R is expressed in renal tubular cells and osteoblasts, skin, breast, heart, and pancreas, among other tissues—the latter sites reflecting the paracrine targets of PTHrP. PTH, PTHrP, and calcitriol decrease expression of *PTH1R*. Targeted loss of *PTH1R* is accompanied by impaired proliferation of chondrocytes and acceleration of chondrocyte maturation and calcification, an outcome mimicked by targeted loss of $G_{s\alpha}$ in chondrocytes.[75,94]

Clinically, inactivating mutations in *PTH1R* result in hypocalcemia and Blomstrand chondrodysplasia (OMIM ID: 215045), whereas constitutively activating mutations of *PTH1R* lead to hypercalcemia and Jansen metaphyseal chondrodysplasia (OMIM ID: 156400). The abnormalities of chondrocyte maturation seen experimentally with inactivating mutations of *Pth1r* are mimicked to an extent by loss-of-function mutations of *Pthlh* as well. The loss of PTH results in an aberrant formation of primary spongiosa of long bone and defective mineralization.[95]

A second PTH receptor (PTHR2) is selectively activated by PTH but does not recognize PTHrP; PTH specificity is determined by Ile[5] and Trp[23] in native PTH, sites that affect activation and binding, respectively.[75] *PTHR2* encodes a 539-amino-acid GPCR with 50% homology to PTH1R that activates adenylyl cyclase; it is expressed predominantly in brain, testis, placenta, and pancreas, but not in bone or kidney; its physiologic role is uncertain. In response to $PTH^{1\text{-}84}$, PTHR2 enhances both cyclic AMP generation and Ca^{2+} mobilization. However, the naturally occurring endogenous ligand for PTHR2 is not PTH but the 39-amino-acid PTH-related hypothalamic tuberoinfundibular peptide (TIP39); this protein is also expressed in the testis and various central nervous system regions.[91] A third PTH receptor that recognizes amino terminal sequences of PTH has been identified in zebra fish and termed type-3 PTH receptor (zPTHR3); rat PTH binds to this receptor and activates adenylyl cyclase[75]. Specific receptors recognizing the amino terminal sequences of PTHrP have been found in brain and skin; PTHrP stimulates the release of arginine vasopressin from the supraoptic nucleus in vitro.[75]

Although as yet not molecularly characterized, receptors for carboxyl terminal fragments of $PTH^{1\text{-}84}$ have been identified as renal and bone cell binding sites for $PTH^{1\text{-}84}$ from which intact hormone can be only partially displaced by $PTH^{1\text{-}34}$ but can be further displaced by $PTH^{53\text{-}84}$ and $PTH^{69\text{-}84}$.[75] In addition, in osteocytes, osteoblasts, and chondrocytes from which the PTH/PTHrP receptor has been "knocked out" and to which intact $PTH^{1\text{-}84}$ but not $PTH^{1\text{-}34}$ binds, labeled $PTH^{1\text{-}84}$ can be displaced by carboxyl terminal $PTH^{19\text{-}84}$,$^{28\text{-}84}$,$^{39\text{-}84}$ fragments. Important determinants for binding of PTH to the carboxyl terminal selective receptor(s) appear to be amino acids 24-27 (Leu-Arg-Lys-Lys) and 53-54 (Lys-Lys). Furthermore, carboxyl terminal fragments of PTH exert biologic effects in intact and *PTH1R*-null cells such as regulation of alkaline phosphatase activity in osteosarcoma cells and osteoblasts (but not generation of collagen type I), stimulation of Ca^{2+} uptake by osteosarcoma cells and chondrocytes, and cell survival in vitro. Thus, the physiologic actions of PTH likely reflect the integrated sum of the individual functions of the intact hormone and its carboxyl terminal fragments. The membrane sites that bind carboxyl terminal fragments of PTH do not recognize carboxyl terminal fragments of PTHrP.

CALCITONIN

Calcitonin is a 32-amino-acid peptide that in mammals is secreted by the neural crest-derived parafollicular (C) cells of the thyroid gland in response to rising concentrations of $Ca^{2+}{}_e$.[3] Calcitonin lowers concentrations of $Ca^{2+}{}_e$ by inhibiting osteoclastic bone resorption. After binding of calcitonin to its GPCR expressed on the osteoclast cell membrane, the morphology of the ruffled membrane of the functioning osteoclast flattens causing it to withdraw from the bone reabsorption site, thereby lowering $Ca^{2+}{}_e$ concentrations.[102] Calcitonin also decreases renal tubular reabsorption of filtered calcium. Calcitonin is encoded by a six-exon gene (*CALCA*) that by alternative transcription and translation forms a 141-amino-acid protein from which calcitonin (exon 4) and katacalcin, a 21-amino-acid hypocalcemic peptide adjacent to the carboxyl terminus of calcitonin, are derived, and a 128-amino-acid protein from which is gleaned the 37-amino-acid calcitonin gene–related peptide-α (exon 5), a vasodilator and neurotransmitter that also interacts with the calcitonin receptor. Calcitonin is also expressed by cells in the adenohypophysis and brain and by neuroendocrine cells in the lung and elsewhere. Calcitonin is produced in excess by medullary carcinoma of the thyroid and, at times, by other neuroendocrine tumors. The calcitonins of multiple species share similar structures including five of the first seven amino terminal amino acids, a disulfide bridge between amino acids 1 and 7, glycine at amino acid residue 28, and a proline amide residue at carboxyl terminal amino acid 32. In the interior of the peptide, species other than human have several basic amino acids that make them more stable and easily recognized by the human calcitonin receptor and thus more biologically potent (e.g., therapeutically useful salmon calcitonin).

Calcitonin secretion is stimulated primarily by increasing concentrations of $Ca^{2+}{}_e$ transduced by the CaSR expressed on the plasma membrane of the C cell.[3] Members of the gastrin-cholecystokinin intestinal peptide hormone family (gastrin, glucagon, pancreozymin), glucocorticoids, and β-adrenergic agonists are also potent calcitonin secretagogues[3,102]. Calcium, pentagastrin, and glucagon are effective stimuli employed to assess calcitonin secretion clinically. Somatostatin, calcitriol, and chromogranin $A^{1\text{-}40}$ inhibit and chromogranin $A^{403\text{-}428}$ stimulates calcitonin secretion. Calcitonin secretion falls as the $Ca^{2+}{}_e$ concentration declines. The half-life of calcitonin is brief; it is metabolized primarily by the kidney and also by liver, bone, and thyroid gland. Serum levels of calcitonin are high in the fetus (when serum total calcium concentrations are normally 12 to 13 mg/dL) and newborn, fall rapidly after birth as $Ca^{2+}{}_e$ values decline and then fall more slowly until 3 years of age, remaining relatively constant thereafter (< 12 pg/mL). After 10 years of age, serum concentrations of calcitonin are higher in males than in females. The physiologic role of calcitonin is unclear as serum calcium concentrations are normal in patients with both decreased (primary congenital or acquired hypothyroidism) and increased (medullary carcinoma of the thyroid) secretion of this peptide. However, disposal of a calcium load is slower in the calcitonin-deficient subject. Immunoassayable concentrations of calcitonin are increased in patients with medullary carcinoma of the thyroid, chronic renal insufficiency, and pycnodysostosis.[102] Individual commercial immunoassays for

calcitonin may detect differing epitopes, have altered intra-assay dynamics, and provide inconsistent measurements.[103]

The biologic effects of calcitonin are mediated through its 490-amino-acid class B GPCR encoded by *CALCR*. Intracellular signaling of the CALCR is transduced through the $G_{s\alpha}$ adenylyl cyclase-cyclic AMP-PKA and $G_{q\alpha}$-PLC-IP_3 signal transduction pathways. Alternative splicing of *CALCR* results in two isoforms of the calcitonin receptor, one of which has an additional 16 amino acids inserted into its first intracellular loop between transdomains I and II. Accessory proteins that modulate function of the calcitonin receptor have also been described. The calcitonin receptor is expressed in osteoclasts; when exposed to calcitonin the osteoclast shrinks and bone-resorbing activity declines quickly. Thus, calcitonin lowers serum calcium and phosphate levels particularly in patients with hypercalcemia. In lactating women, serum levels of calcitonin rise and calcitonin is excreted in breast milk. Women who are breastfeeding their infants lose 5% to 10% of their trabecular bone mineral during 6 months of lactation; it is recouped rapidly when lactation ceases, much more quickly than when bone mass is lost because of other problems (e.g., glucocorticoid excess, bed rest). Experimentally, in the female mouse in which the gene encoding calcitonin has been eliminated, loss of calcitonin has no effect on maternal bone mineralization during pregnancy or on the rate of skeletal remineralization after weaning of the pups. However, 21 days of lactation are associated with much more marked demineralization of the spine of the nursing mother without calcitonin, a response that is reversible by the administration of exogenous calcitonin during the interval of lactation.[104] Thus, in mammals calcitonin may be essential for protection of maternal skeletal mass during lactation

VITAMIN D

Synthesis and Biologic Activity of Vitamin D

Cholecalciferol (vitamin D_3) is synthesized in skin from cholesterol: 7-dehydrocholesterol is converted to previtamin D_3 by ultraviolet B radiation at 290 to 315 nm and heat (37° C) from sunlight and thence to cholecalciferol (Figure 8-3).[105,106] Previtamin D_3 is also metabolized into products such as lumisterol$_3$ and tachysterol$_3$ and to inactive photoproducts when exposure to sunlight is excessive, thereby preventing vitamin D intoxication by exposure to sunlight alone. Cutaneously synthesized vitamin D_3 enters the circulation directly. Vitamin D_3 is present in oily fish such as wild salmon, herring, cod, and mackerel and in shiitake mushrooms. Ergocalciferol (vitamin D_2) is derived from the plant and yeast sterol—ergosterol. Vitamin D_2 differs structurally from vitamin D_3 by the presence of a double bond between carbons 22 and 23 and a methyl group on carbon 24. Vitamins D_3 and D_2 exert similar biologic effects when ingested in equivalent physiologic doses; however, the increase in the serum concentration of 25-hydroxyvitamin D (calcidiol) is less after ingestion of vitamin D_2 than vitamin D_3 when quantitatively similar amounts of both vitamins are consumed.[105,107] The latitude, season of the year, and time of day influence the rate of cutaneous synthesis of vitamin D_3 in response to exposure to sunlight; in higher latitudes the path through which ultraviolet B radiation from the sun travels is longer and more of the ultraviolet radiation is absorbed by the ozone layer; thus, less reaches the surface of the earth. Exposure of the back of a white adult to intense summer sun (mid-July) for 10 to 12 minutes in the northeastern United States generates ~10,000 to 20,000 IU of vitamin D_3 over the next 24-hour interval.[108] (For persons of color, 30 to 120 minutes of exposure to sunlight may be required for comparable effects.) Sun blocking topical creams and aging also decrease the cutaneous formation of cholecalciferol in response to sunlight. Both forms of vitamin D undergo similar chemical modifications to bioactive metabolites. Orally ingested vitamin D is packaged into chylomicrons and absorbed into the intestinal lymphatic system and then the circulation. Both vitamins D_3 and D_2 are transported to the liver linked to vitamin D–binding protein (DBP), a polymorphic variant of the serum α2-globulin synthesized by the liver termed Gc (group-specific component) encoded by *GC* (see Table 8-3). DBP binds vitamin D and its hydroxylated metabolites with high affinity and capacity.[109] In the liver, vitamin D is hydroxylated to 25-hydroxyvitamin D (25OHD; calcidiol is 25OHD_3) primarily by the vitamin D-25-hydroxylase encoded by *CYP2R1*, a 501-amino-acid microsomal cytochrome P450 enzyme.[106] A homozygous missense mutation in *CYP2R1* has been identified in a patient with vitamin D–dependent rickets due to a defect in vitamin D-25 hydroxylation.[110] Additionally, there are three other widely expressed vitamin D 25-hydroxylases encoded by *CYP2J2*, *CYP3A4*, and *CYP27A1* whose activities affect bile acid and drug metabolism.[106,111] Calcidiol exerts only a minimal inhibitory effect on its production; thus, serum concentrations of 25OHD reflect available body stores of vitamin D.

Because of decreased exposure to sunlight and marginal dietary intake of vitamin D, body stores of this vitamin have been considered to be deficient or insufficient in many North American subjects.[108] Thus, in populations living in sun-rich environments, the lower normal concentration of serum 25OHD has been reported to be 32 ng/mL with an upper limit of 100 ng/mL. However, there is substantial controversy concerning this stated normal range of 25OHD values (discussed later). Physiologic data pertaining to calcium homeostasis and skeletal health such as the relationship between serum concentrations of 25OHD and a plateau value for PTH values, intestinal absorption of calcium, and optimal bone mineralization have been utilized to advocate the position that a serum 25OHD level of approximately 30 ng/mL is the minimum normal value in adult men and that 25OHD concentrations between 10 and 30 are insufficient.[112,113] If a serum 25OHD value of 30 ng/mL were the actual lower normal limit, then approximately 50% of white, Hispanic, and African-American youth and 31% of white, 63% of Hispanic, and 82% of African-American adults would be vitamin D deficient.[113,114]

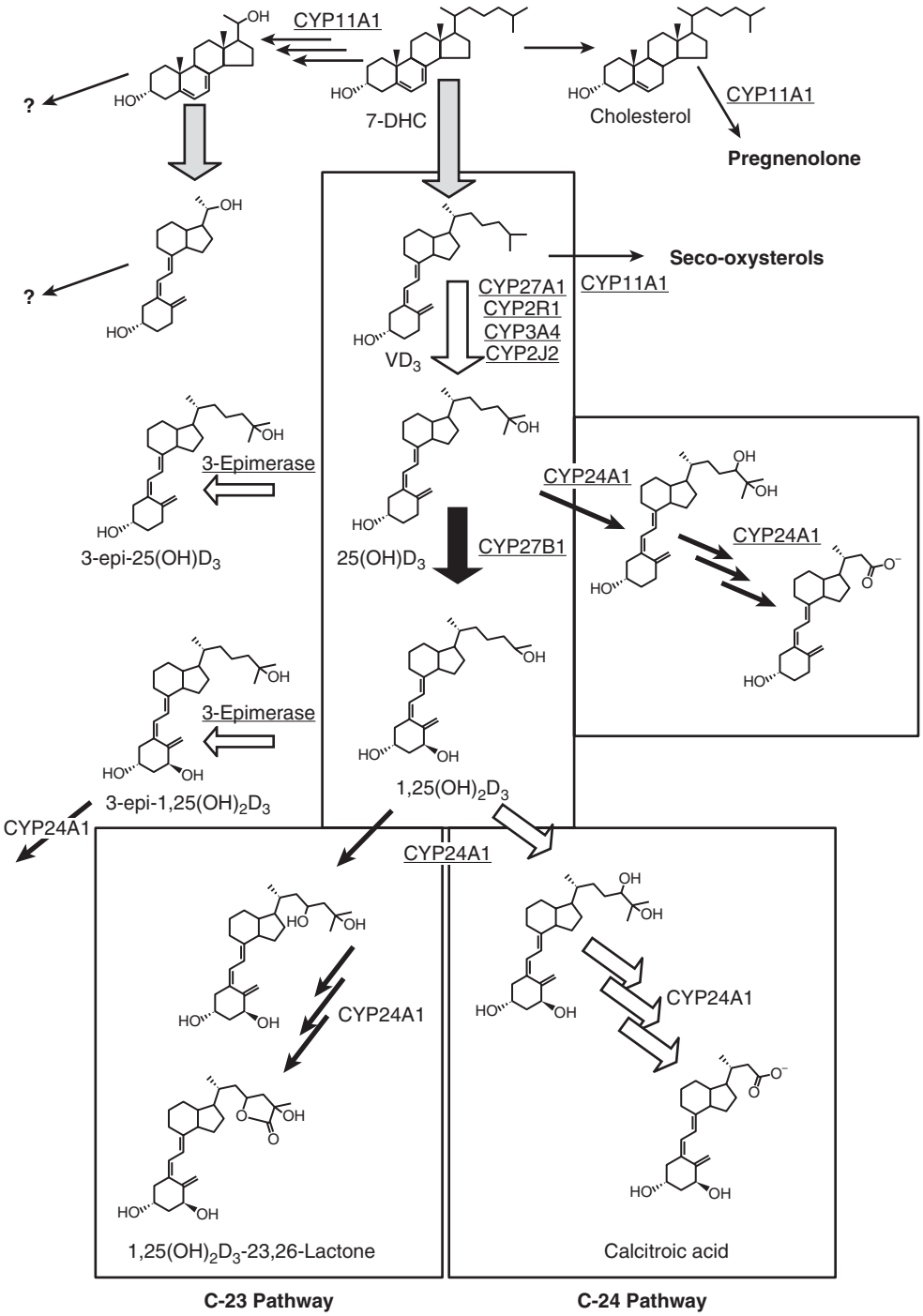


FIGURE 8-3 ■ Genetic regulation of the synthesis and metabolism of vitamin D. In skin, 7-dehydrocholesterol (7-DHC) is metabolized to cholecalciferol (vitamin D_3) that is then hydroxylated at carbon 25 in the liver to form calcidiol ($25OHD_3$) and at carbon 1 in the kidneys to form calcitriol [$1,25(OH)_2D_3$]. Calcitriol is inactivated by hydroxylation at carbon 24 in the kidney (see text). (Reproduced from Schuster, I. (2011). Cytochromes P450 are essential players in the vitamin D signaling system. *Biochim Biophys Acta, 1814,* 186–199, with permission.)

However, further data in adults, children, and adolescents indicate that the relationship between serum concentrations of 25OHD and PTH is linear and that there is no one clearly identifiable inflection point for suppression of PTH values relative to levels of 25OHD.[115,116] Additionally, there are substantial differences (often > 5 ng/mL) in 25OHD values measured by different commercially available calcidiol assays.[117] Critical analysis of these data has led the American Academy of Pediatrics and the Institute of Medicine to define the lower limit of normal of the serum 25OHD concentration as 20 ng/mL (50 mmol/L).[30,118] Utilizing the 25OHD level of 20 ng/mL as the lower limit of normal, investigators have concluded that the reported prevalence of vitamin D deficiency has been vastly overestimated. Some authors have concluded that vitamin D deficiency should be defined as a 25OHD

concentration below 12 ng/mL (< 30 mmol/L) and that 25OHD values above 16 ng/mL (40 mmol/L) are adequate to maintain calcium homeostasis and bone health.[119] Guidelines for calcium and vitamin D intakes in children and adolescents as recommended by the Institute of Medicine and the Endocrine Society are presented in Table 8-3. Clinically, oral intake of 400 U/day of vitamin D per day in healthy infants is sufficient to prevent vitamin D deficiency if administered conscientiously.[120] The recommended upper limit of vitamin D intake is 4000 IU/day for all age groups above 8 years. Serum values of 25OHD above 50 ng/mL (125 mmol/L) are presently considered excessive.[119]

Bound to DBP, 25OHD is filtered through the glomerular membrane and reabsorbed by cells in the proximal renal tubule by the multifunctional receptor, megalin (LRP2), expressed in coated pits on the luminal/apical surface, endocytic vacuoles, and lysosomes of proximal tubular cells.[121] After uptake by the lysosomes, the megalin-calcidiol complex is disrupted and calcidiol is released into the cytosol. It then enters mitochondria where it is further hydroxylated to the biologically active metabolite 1,25-dihydroxyvitamin D_3—$1,25(OH)_2D_3$ is calcitriol—by the cytochrome P450 mono-oxygenase 25-hydroxyvitamin D-1α-hydroxylase encoded by *CYP27B1*.[106] *CYP27B1* has nine exons that encode a 507-amino-acid protein with a mitochondrial signal sequence at its amino terminal and ferredoxin and heme-binding sites within its structure. As a class I mitochondrial cytochrome P450 enzyme, 25OHD-1α-hydroxylase requires for its catalytic activity electrons from reduced nicotinamide adenine dinucleotide phosphate (NADPH) that are ferried to the enzyme protein by the electron transport proteins—NADPH-ferredoxin and ferredoxin reductase.[111] Although *CYP27B1* is expressed primarily in renal proximal convoluted and straight tubular cells, it is also expressed by keratinocytes and hair follicles, osteoblasts, placental decidual and trophoblastic cells, the gastrointestinal and central nervous systems, lung, testes, breast, and pancreatic islets as well as monocytes and macrophages. In proximal convoluted renal tubular cells, expression of *CYP27B1* is stimulated by PTH and PTHrP acting through PTH1R-adenylyl cyclase-cyclic AMP-PKC. Phosphorylation of methyl-CpG-binding domain protein 4 (MBD4, OMIM ID: 603574) by PKC enables this demethylase to remove transcription-repressing methyl groups from the CpG island in the 5′ promoter region of *CYP27B1*, thus enabling gene expression.[122] The 1α-hydroxyl group of calcitriol is essential for its binding to the vitamin D receptor (VDR).[123]

In the straight renal tubules, *CYP27B1* expression is increased by calcitonin by a pathway independent of cyclic AMP—one that involves the C/EBPβ transcription factor and the SWI/SWF helicase chromatin remodeling complex.[111] Expression of *CYP27BI* and hence activity of 25OHD-1α-hydroxylase are also increased by hypocalcemia and hypophosphatemia, 24R,25-dihydroxyvitamin D, growth hormone, IGF-I, and prolactin. In monocytes and macrophages, expression of *CYP27B1* may be induced by inflammatory cytokines such as interferon-γ but is not regulated by concentrations of $Ca^{2+}{}_e$ or phosphate or by PTH or calcitriol.[106,124] Increased serum and tissue levels of Ca^{2+} and phosphate directly suppress expression of *CYP27B1* and thus depress 25OHD-1α-hydroxylase activity. Calcitriol exerts a direct autoinhibitory effect on renal *CYP27B1* expression acting through the VDR and thus on its own synthesis (discussed later); it also exerts an indirect inhibitory effect on *CYP27B1* expression by impairing PTH synthesis in the parathyroid gland.[105,125] (In activated monocytes, calcitriol actually enhances transcription of *CYP27B1*.[124]) The osteocyte product FGF23 depresses 25OHD-1α-hydroxylase activity, whereas calcitriol up-regulates *FGF23* expression, effectively establishing a reciprocal control system for these compounds.[54,55,126] Biallelic inactivating mutations of *CYP27B1* result in vitamin D–dependent rickets type I (OMIM ID: 264700), a disorder associated with hypocalcemia, hypophosphatemia, secondary hyperparathyroidism, severe rickets, and often also alopecia.[127]

Calcitriol is inactivated in bone, intestine, liver, and kidney by glucuronidation, sulfation, multisite (carbons-23, -24, -26) hydroxylation, and lactone formation to water-soluble compounds (such as calcitroic acid) that are excreted in urine and bile.[105,106,125] In the kidney, 25OHD and $1,25(OH)_2D$ are converted to $24R,25(OH)_2D$ and $1,24R,25(OH)_3D$, respectively, by mitochondrial 25OHD-24-hydroxylase encoded by *CYP24A1*, the first in a series of hydroxylations that increase the water solubility of the products enabling their biliary and renal excretion (see Figure 8-3). The expression of *CYP24A1* is increased by hypercalcemia, hyperphosphatemia, FGF23, and calcitriol as well as retinoic and lithocholic acids, rifampicin, carbamazepine, and phenobarbital and suppressed by hypocalcemia and PTH.[111] *CYP24A1* is also expressed by fibroblasts, lung, intestine, oocytes, brain, thyroid, and other tissues.[106] Cleavage of the side chain between carbons 23 and 24 that have been hydroxylated by hepatic cytochrome P450-3A (*CYP3A4*)–dependent enzymes generates water-soluble calcitroic acid that is excreted in bile.[105,106] Phenobarbital, phenytoin, carbamazepine, and rifampicin impair bone mineralization by inactivating calcitriol and do so by increasing its state of hydroxylation by binding to and activating the nuclear pregnane X receptor (*NR112*) that in turn increases expression of *CYP3A4*.[128] Calcitriol may also induce expression of hydroxylases that utilize cytochrome P450-3A, thereby enhancing its own hydroxylation and degradation. Inactivating mutations of *CYP24A1* result in some forms of idiopathic hypercalcemia of infancy by reducing the rate of degradation and thereby prolonging the biologic life of calcitriol.[129]

Although emphasis has been placed on the vitamin D endocrine system in the regulation of mineral homeostasis and bone health, its role as a paracrine factor may also be considered. For example, epidermal keratinocytes within the basal layer of skin express all of the enzymes necessary for genesis of calcitriol (discussed earlier), and this locally synthesized compound is critical for regulation of the proliferation and terminal differentiation of keratinocytes and hair follicles.[106] Adipocytes not only store excessive vitamin D, but these cells also express vitamin D-25-hydroxylase and 1α-hydroxylase activities, suggesting that fat may be a site of calcidiol and calcitriol synthesis.[106] The bulk of circulating calcitriol is bound to

DBP, but it is its free fraction that is biologically active. Indeed, the serum level of DBP does not affect the calcitriol pool that enters the cell and regulates gene transcription.[111] Approximately 0.04% of calcidiol and 0.4% of calcitriol are present in free form in serum. Normal ranges of calcitriol concentrations are neonates, 8 to 72 pg/mL; infants and children, 15 to 90 pg/mL; and adults, 21 to 65 pg/mL.

Calcitriol exerts its biologic effects by binding to the VDR, a nuclear transcription factor, after which it regulates gene expression (discussed later). Through binding to the nuclear VDR, calcitriol regulates the expression of more than 900 target genes involved in mineral and bone metabolism and processes that likely influence cell growth, musculoskeletal, cardiovascular, immune, skin, and pancreatic islet cell functions and energy metabolism.[113,123,125,130] The nuclear effects of calcitriol take place over hours to days as the processes of transcription, translation, posttranslational modifications to the encoded protein(s), storage, and secretion occur in multiple cytosolic compartments (discussed later). The VDR also associates with caveolae of the cell plasma membrane, flask-shaped invaginations of the membrane composed of sphingolipids and cholesterol where, after binding to ligand, the VDR is able to interact with intracellular signal transduction systems to exert rapid functional cell responses.[131,132] The rapid cell responses to calcitriol generated by association with the caveolar VDR are evident within seconds to minutes after contact and include immediate intestinal absorption of calcium (transcaltachia), opening of voltage-gated calcium and chloride channels in osteoblasts, endothelial cell migration, and pancreatic β cell secretion of insulin. It is the flexible three-dimensional configuration of calcitriol that enables this ligand to exert both genomic and nongenomic (rapid response) actions.[123,131] The protean three-dimensional structure of calcitriol is the result of (1) rotation of side chain carbon pairs 17-20, 20-22, 22-23, 23-24, and 24-25; (2) rotation of the carbon 6-7 bond around the B ring; or (3) chair-chair interconversion with formation of either an α- or a β- configuration of the cyclohexane-like A ring (Figure 8-4). Rotation about the carbon 6-7 bond of the B ring allows calcitriol to assume either an extended 6-s-*trans* configuration or a 6-s-*cis* conformation. It is the *trans* form of calcitriol with the β-chair configuration of the A ring that is recognized and bound by the hydrophobic pocket of the nuclear VDR. Ligand-bound VDR then interacts with the retinoid X receptor (RXR), forming a heterodimer that is recognized by specific hexameric sequences of DNA bases (the vitamin D response element [VDRE]) in the regulatory 5′ region of target genes (discussed later).[106] The calcitriol/VDR-RXR heterodimer recruits transcription-activating or transcription-repressing coregulatory proteins enabling its genomic transcriptional effects. The *cis* form of calcitriol permits its rapid cell membrane–related actions. For binding to DBP, calcitriol assumes yet another shape.[131]

Calcitriol primarily controls intestinal, skeletal, and renal function by regulating expression of genes encoding calcium transporters (calbindin-D_{28K}, calbindin-D_{9K}), calcium channels (TRPV5, expressed primarily in the kidney; TRPV6, expressed predominantly in the intestinal tract), bone matrix proteins (osteocalcin, osteopontin, type I collagen, alkaline phosphatase), and osteoclast activators (RANKL). Calcitriol promotes endochondral bone formation; it increases length of the long bones by amplifying volume, proliferation, and differentiation of epiphyseal chondrocytes and promotes mineralization of cartilage matrix.[133] Calcitriol increases trabecular and cortical bone formation by augmenting osteoblast number and function; it increases alkaline phosphatase activity, osteocalcin synthesis, and type I collagen formation and represses bone resorption by osteoclasts. It does so by a direct cellular effect independent of endogenous PTH as it is active in the mouse in which both *Cyp27b1* and *Pth* have been "knocked out."[133] Calcitriol directly suppresses transcription of *PTH* in the parathyroid gland

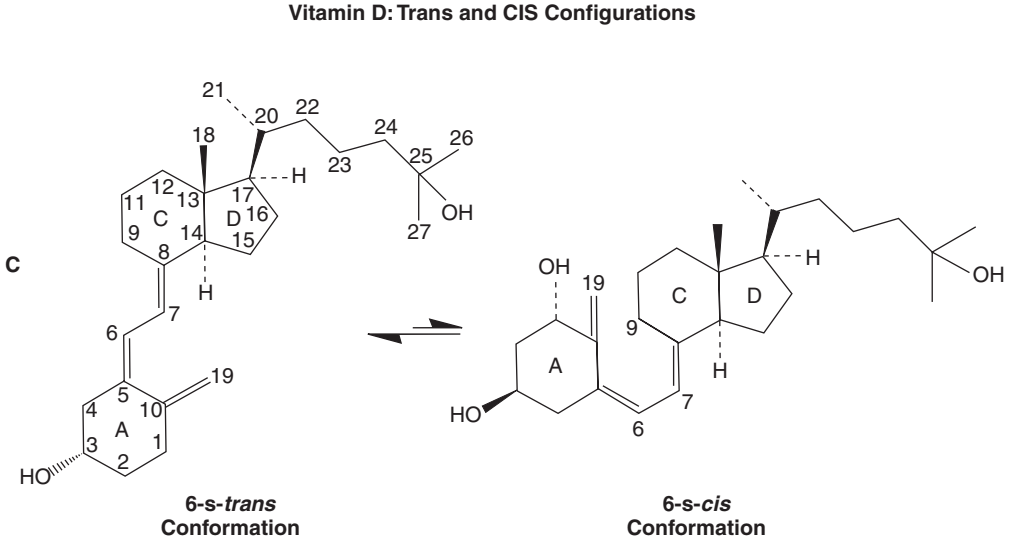


FIGURE 8-4 ■ **Cis and trans structures of calcitriol.** (Reproduced from Norman, A. W. (2006). Minireview: vitamin D receptor: new assignments for an already busy receptor. *Endocrinology*, *147*, 5542–5548, with permission.)

acting through the VDR. Vitamin D is also important for normal muscle development and strength.

Mediated by the nuclear VDR, calcitriol stimulates the absorption or reabsorption of calcium in the intestines, bone, and kidney. In the duodenum and proximal small intestine, calcitriol increases the efficiency of calcium uptake from the intestinal lumen by increasing the number of epithelial calcium transport channels (TRPV6) in enterocytes, its movement through the cytoplasm, and its transfer across the basal lateral membrane into the circulation—in part by the induction of calbindin$_{9k}$ (a calcium binding protein), alkaline phosphatase, Ca-ATPase, calmodulin, and other proteins.[134] Calcitriol also increases jejunal and ileal absorption of phosphate through a transcellular mechanism utilizing the type II Na^+-HPO_4^{2-} cotransporter protein (NPT2) (encoded by *SLC34A1*) expressed on the luminal surface of the enterocyte. When vitamin D stores are replete, 40% of dietary calcium and 80% of dietary phosphate may be absorbed. Even greater efficiency of mineral absorption is realized during periods of rapid growth, pregnancy, and lactation. A major task of calcitriol is to maintain calcium and phosphate concentrations in blood at levels sufficient to sustain mineralization of osteoid (collagen) containing bone matrix synthesized by osteoblasts. Paradoxically, in states of calcium deficiency, calcitriol acts indirectly within bone to induce monocytic stem cell differentiation into osteoclasts by stimulating osteoblast/stromal cell synthesis of osteoclast activating factors such as RANKL (discussed later). During periods of calcium deficiency, calcitriol is able to promote bone resorption through increased osteoblast production of osteopontin, a bone matrix noncollagenous protein to which osteoclast cell surface integrin receptors bind that leads to bone destruction. Calcitriol also stimulates osteoblasts to produce osteocalcin, bone specific alkaline phosphatase, osteoprotegerin, and various cytokines.

A number of synthetic vitamin D_3 analogs have been synthesized, including alfacalcidol [1α-(OH)D_3], calcipotriol [1α,25-(OH)$_2$-24-cyclopropyl-D_3], maxacalcitol [1α,25-(OH)$_2$-22-oxa-D_3], and tacalcitol [1α,24R-(OH)$_2D_3$]. These vitamin D analogs have been engineered to retain the noncalcemic actions of the parent compound while reducing their calcemic properties. Calcitriol and vitamin D_3 analogs exert many immunomodulatory effects.[135] In animal models of autoimmune diseases such as experimental autoimmune encephalitis, systemic lupus erythematosus, autoimmune thyroiditis, autoimmune diabetes mellitus, and inflammatory bowel disease, calcitriol exerts a protective effect. Calcitriol and its analogs inhibit T-lymphocyte differentiation into Th1 cells that secrete IL-2, tumor necrosis factor (TNF)-α, and interferon-γ, thereby experimentally modifying disease induction, course, and severity. Calcitriol and analogs also exert differentiating and antiproliferative effects on a variety of cells; thus, calcitriol induces differentiation of promyelocytes into monocytes and macrophages. These agents enhance differentiation of keratinocytes and when administered orally or topically to patients with psoriasis vulgaris effectively ameliorate the disease; they are particularly effective when coadministered with a topical glucocorticoid. In vitro, calcitriol and its analogs inhibit growth of prostate, breast, and colon cancer cell lines; in vivo, experimentally calcitriol and its analogs prevent or reduce mammary tumor formation, whereas in the absence of the VDR mammary tumor growth is enhanced.[135] Calcitriol prevents hypertension and cardiac hypertrophy in mice in whom *VDR* or *CYP27B1* have been knocked out. In humans, inferential but not clinically proven data suggest that vitamin D plays a role in the pathophysiology of several nonskeletal disorders including hypertension and cardiovascular disease, autoimmune disorders, multiple sclerosis, and infectious illnesses such as tuberculosis, leprosy, and influenza.[113,130,136]

Vitamin D Receptor

The vitamin D receptor is a 427-amino-acid nuclear transcription factor that is encoded by the 11 exon gene, *VDR*. Because there are two potential start site codons for transcription of *VDR* in exon 2, there is a second isoform of VDR with 423 amino acids. At its 5′ terminus, *VDR* has three noncoding exons (1A, 1B, 1C) followed by exons 2 through 9 that encode the active protein enabling transcription of three unique mRNA isoforms depending on the splicing pattern of exons 1B and 1C. As a member of the steroid-thyroid–vitamin D receptor gene superfamily of nuclear transcription activating factors, the VDR has multiple domains: a short amino-terminal segment of 24 amino acids (domains A and B) that houses a ligand-independent transcription activating function termed activation function-1 (AF-1) that interacts with the general transcription factor IIB, a DNA binding domain (C) with two zinc fingers (exons 2, 3), a "hinge" region (D), and a long carboxyl terminal domain (E) with ligand and retinoid X receptor α (RXRα) binding sites and a second transcription activating sequence (AF-2) (exons 7, 8, 9). Two nuclear localization signals are found within and immediately distal to the zinc finger DNA binding domain, the first of which specifically recognizes the vitamin D response element (VDRE), whereas the second zinc finger permits heterodimerization with RXRα (discussed later).[130] Structurally, the E domain is composed of 12 α-helices (H1-H12) and has two ligand-dependent transactivating regions—E1 between amino acids 232-272 and AF2 between amino acids 416-424—that recruit transcriptional coactivating factors when the VDR is activated by binding to calcitriol; it also contains sequences that enable heterodimerization with RXRα.[130]

Among the factors that enhance transcription of *VDR* are calcitonin, retinoic acid, estrogen, and transcription factor SP1. β-catenin potentiates the transcriptional activity of VDR by binding to its carboxyl terminal AF2 segment. In part, estrogens increase expression of *VDR* through binding to estrogen receptors present in the caveolae of the cell membrane that then activate the MAPK signal transduction pathway.[137] PTH downregulates expression of *VDR*. The VDR is widely expressed in the intestinal tract, distal renal tubule, osteoblast, keratinocyte, hair follicle, fibroblast, smooth and cardiac muscle, lung, bladder, thyroid, parathyroid, pancreas, adrenal cortex and medulla, pituitary, placenta, uterus,

ovary, testis, prostate, activated T and B lymphocytes, macrophages, monocytes, spleen, thymus and tonsil, brain, spinal cord, and sensory ganglia. Inactivating mutations of *VDR* result in vitamin D–resistant rickets (vitamin D–dependent rickets, type 2A, OMIM ID: 277440).

Whereas most members of the superfamily of nuclear receptor transactivating factors pair as homodimers to bind to their specific hormone response elements in the 5′-untranslated region of the target gene, the calcitriol-VDR complex teams through its E (ligand binding) domain with its obligate partner, unliganded RXRα (encoded by *RXRA*), to form a heterodimer that then binds to a VDRE. The endogenous ligand for RXRα is 9-cis-retinoic acid. RXRβ and RXRγ also bind to the VDR. When unliganded, the bulk of the VDR is cytoplasmic; binding of calcitriol to the VDR leads to heterodimerization with RXRα and translocation of the tripartite complex to the nucleus. Unliganded VDR can also be guided to the VDRE in the 5′ region of its target gene by interaction with the Williams syndrome transcription factor (WSTF, encoded by *BAZ1B*), a component of a multiprotein, chromatin-remodeling complex termed WINAC (WSTF including nucleosome assembly complex); there the VDR remains inactive until bound to calcitriol (discussed later).[138,139] There are three classes of VDREs located in the 5′ upstream promoter region of target genes: designated generic positive, conventional negative, and E-box–like negative.[122] The generic positive or transcription activating VDRE is composed of two repeated hexanucleotide sequences linked by three nonspecific nucleotide bases: 5′ . . . (A/G)GGTCA-nnn-AGGACA . . . 3′.[109,122] RXRα binds to the 5′ half of the VDRE and VDR to its 3′ segment. The conventional negative or transcription repressing VDRE is composed of a single copy of the generic positive VDRE with the nucleotide sequence, 5′ . . . (A/G) G(G/T)TCA . . . 3′; it is through the conventional negative VDRE that calcitriol inhibits expression of *PTH* and *PTHLH*. The nucleotide sequence of the E-box–like negative VDRE is 5′ . . . CANNTG . . . 3′. After binding to the designated VDRE, the calcitriol-VDR-RXRα-WINAC complex recruits coactivating or corepressing modulating proteins (e.g., steroid receptor coactivators [SRC]-1, -2, -3/p160 family with histone acetyl transferase activity, the chromatin remodeling complex—SWI/SNF, and other comodulators of transcription such as C/EBPβ, TRIP/SUG1, CPB/p300, TIF1) into the promoter site as well as the general transcription activating apparatus of the target gene (TFIIA, -B, TAF family).[109,122,140] Histones are lysine-containing protein components of chromatin, a complex of protein and DNA of which a chromosome is composed; 147 base pairs of DNA wrap around a histone octamer comprised of two copies each of histones H2A, H2B, H3, and H4 forming a nucleosome, a structure that regulates replication, repair, and expression of genes.[139] Epigenetic regulation of gene expression is achieved, in part, by histone modifying enzyme complexes that remodel chromatin by altering the state of histone methylation, acetylation, phosphorylation, ubiquitination, sumoylation, and proline isomerization.[141] WSTF is a component of three ATP-dependent chromatin remodeling complexes that alter nucleosome position and thus affect gene exposure to the primary transcription apparatus and accordingly gene expression or silencing; they are directed to sites of action by specific histone modifications.[139] Within the WSTF protein are a chromatin-binding domain (PHD), a tyrosine kinase domain that enables it to phosphorylate histone tyrosine residues, and a binding site for the VDR. In addition to WSTF, the WINAC ATP-dependent chromatin remodeling complex is composed of 12 other subunits; it plays a role in chromatin assembly and cell cycle progression and facilitates activation of *CYP24A1* encoding 25-hydroxyvitamin D-24-hydroxylase while repressing *CYP27B1* encoding 25-hydroxyvitamin D-1α-hydroxylase (Figure 8-5).

Gene activation by the calcitriol-VDR-RXR-WINAC complex is initiated by recruitment of a histone acetyl transferase–helicase complex (e.g., SRC/SWI/SNF) to the promoter segment of the target gene; acetylation of a lysine residue within a histone protein destabilizes the region enabling DNA to unwind, thereby granting basal transcription factors and RNA polymerase II access to the transcription start site, thus bringing about RNA modeling of the target gene. Another VDR coactivator is the vitamin D interacting protein (DRIP, encoded by *MED4*) that links the VDRE and the initiation start site of the target gene to RNA polymerase II and its cofactors.[130] Corepressors cause chromatin to compact by removal of acetyl or addition of methyl groups to lysine residues within a histone protein, thereby silencing gene expression. Inasmuch as the E-box–negative VDRE lacks the common hexameric VDRE, this VDRE binds to and gene transcription is inhibited (e.g., *CYP24A1* encoding vitamin D–24-hydroxylase) by the interaction of unliganded VDR with the VDR-interacting repressor (VDIR encoded by *TCF3*), a process that involves recruitment of a histone deacetylase. Calcitriol inhibits activation of *CYP27B1* encoding 25-hydroxyvitamin D-1α-hydroxylase by binding to VDR that links to the VDIR-WINAC complex and replacing histone acetyltransferase with a histone deacetylase, thus halting transcription activation (see Figure 8-5).[122,139] In addition to regulatory effects on gene expression by alterations of histone lysine residues, calcitriol-VDR-RXR–induced methylation of DNA cytosine residues at CpG sites in the promoter region 5′ to *CYP27B1* (another epigenetic modification) also represses expression of this gene.[122] Positive and negative regulation of target gene expression by the VDR may also be achieved by interaction of the VDR complex with other transcription factors.[140] As illustrated in Figure 8-5, WINAC is involved in both calcitriol-VDR-VDIR mediated gene transcription and repression.[122] Deletion or inactivating mutations of *BAXBI* (encoding WSTF) impair the linkage between calcitriol, VDR, and WINAC and thus impede the inhibitory effects of calcitriol on transcription of *CYP27B1*, likely resulting in increased synthesis of 25-hydroxyvitamin D-1α-hydroxylase and excessive synthesis of calcitriol, perhaps the pathophysiologic process that leads to hypercalcemia in patients with Williams syndrome.

Some of the more than 900 genes whose expression is regulated by the VDR are listed in Figure 8-6.[106,140] That there may be approximately 2000 VDREs (vitamin D response elements) within the human genome indicates the potentially widespread effects exerted by the vitamin D system.[142] Calcitriol acting through the VDR stimulates transcription of genes encoding calcium transport proteins (TRPV5/6), bone matrix proteins (osteopontin, osteocalcin), bone resorption factors (RANKL), and 25OHD-24-hydroxylase and represses those that encode PTH, PTHrP, and 25-hydroxyvitamin

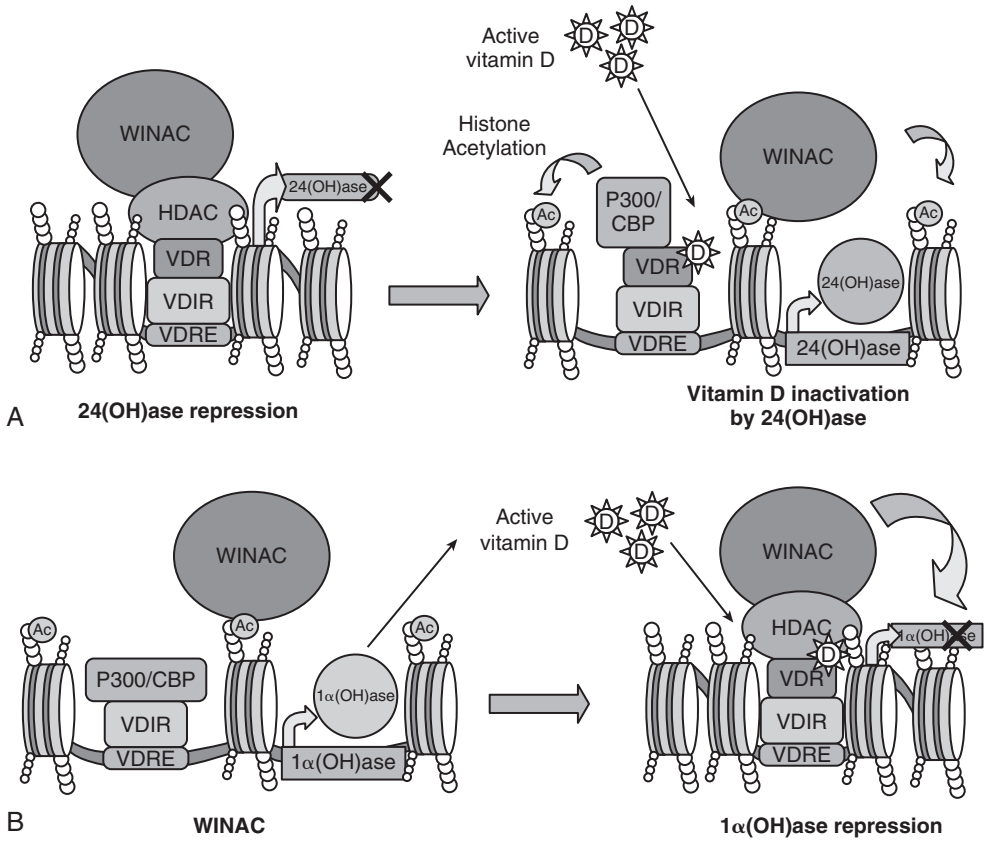


FIGURE 8-5 ■ Regulation of vitamin D receptor (VDR) action by interaction with WINAC (WSTF including nucleosome assembly complex) and VDIR (VDR-interacting repressor). **A,** Under basal conditions, histone deacetylase (HDAC), unliganded VDR-RXR, and VDIR bind to the vitamin D response element (VDRE) of *CYP24A1* encoding 25-hydroxyvitamin D-24-hydroxylase and suppress gene expression; after calcitriol binds to VDR-RXR, HDAC is replaced by a histone acetyltransferase activation complex (p300/CBP) leading to active transcription of *CYP24A1*. **B,** The autoregulatory effect of calcitriol on its own synthesis is mediated by its interaction with the VDR-RXR-VDIR-WINAC complex and replacement of histone acetyltransferase by histone deacetylase. (Reproduced from Barnett, C., & Krebs, J. E. (2011). WSTF does it all: a multifunctional protein in transcription, repair and replication. *Biochem Cell Biol*, *89*, 12–23, with permission.)

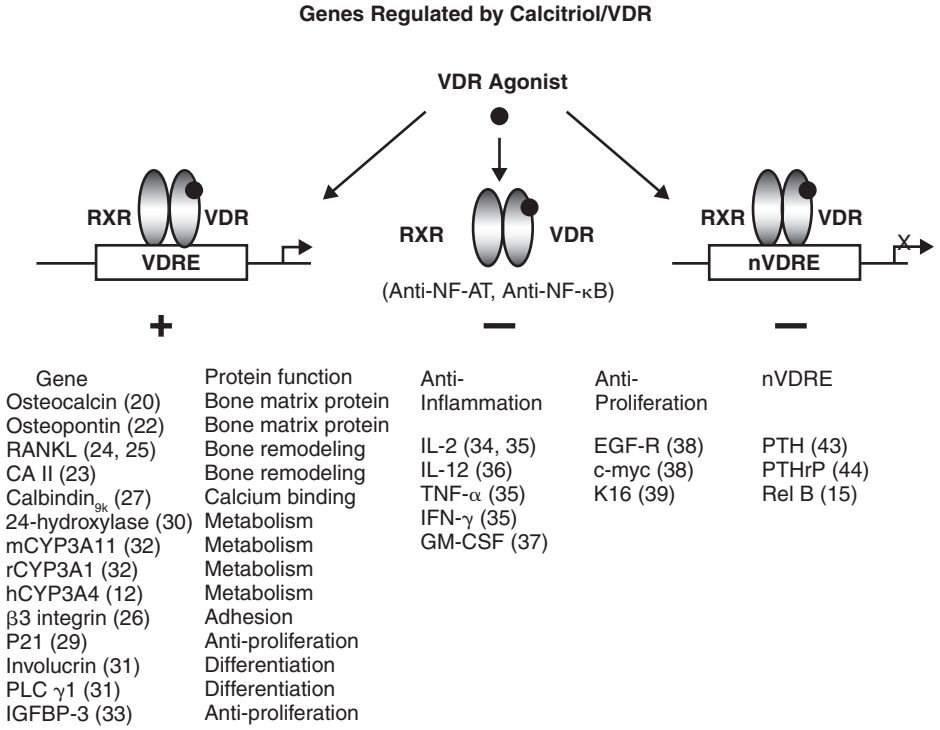


FIGURE 8-6 ■ **Genes regulated by calcitriol and the vitamin D receptor.** (Reproduced from Nagpal, S., Na, S., & Rathnachalam, R. (2005). Noncalcemic actions of vitamin D receptor ligands. *Endocr Rev*, *26*, 662–687, with permission.)

D-1α-hydroxylase. The calcitriol-VDR complex also suppresses expression of multiple cytokines (IL-2, interferon-γ, granulocyte macrophage-colony stimulating factor) by negatively interacting with transcription factors that enhance their transcription (e.g., NF-κB).

Although the VDR is necessary for the actions of calcitriol in the intestines, kidney, bone, skin, and elsewhere, its loss does not interfere with embryogenesis and fetal development in humans with loss-of function mutations in *VDR* and vitamin D resistance or in mice in which *Vdr* has been knocked out. Newborn mice homozygous for targeted deletions that result in truncation of the VDR survive fetal life, appear normal at birth, and grow well for the first 24 days of life.[143] However, after weaning their rates of weight gain and linear growth and serum $Ca^{2+}{}_e$ and phosphate levels decline and serum PTH concentrations and parathyroid gland weights increase. In $Vdr^{-/-}$ mice as young as 15 days of age osteoid surface is increased, bone mineralization decreased, and epiphyseal plate cartilage formation disorganized with irregular columns of chondrocytes, increased matrix, and excessive vascularity relative to wild-type or heterozygous ($Vdr^{+/-}$) mice.[143] In addition, hair loss begins at 4 weeks of age and is complete by 4 months of age in homozygous $Vdr^{-/-}$ animals and is due to a defect in the growth cycle of hair follicles, a phenocopy of the generalized alopecia associated with loss of hairless (*HR*), a transcriptional corepressor that interacts with the VDR.[140] Maintenance of normal serum concentrations of calcium and phosphate by the feeding of high calcium-phosphate diets to $Vdr^{-/-}$ pups beginning at 16 days of age prevents the development of all of the skeletal abnormalities, indicating that the main physiologic effects of calcitriol and the VDR are on the intestinal absorption of calcium and phosphate and the maintenance of normal serum concentrations of these ions.[143]

As discussed, calcitriol also has rapid, nongenomic effects mediated through plasma membrane caveolae-associated VDRs (Figure 8-7).[132] A membrane protein that binds calcitriol—1,25-dihydroxyvitamin D membrane associated rapid response steroid-binding protein (1,25-dihydroxyvitamin D-MARRSBP encoded by *TXNIP*)—has been identified that may interact with the classic VDR to bring about the rapid responses to calcitriol.[130] After binding of calcitriol to the plasma membrane-associated VDR, its signal is transmitted by several classical intracellular signal transduction systems including (1) adenylyl cyclase induction of cyclic AMP and PKA; (2) PLC- and PLD-mediated increase in phosphoinositide turnover, resulting in generation of DAG and IP_3 that increases the permeability of Ca^{2+} channels and releases Ca^{2+} from storage sites in the endoplasmic reticulum; (3) G_q-protein through PLC-β1 activation and intracellular redistribution of PKC isoforms (α, β, δ); and (4) Jun-activated kinase and the MAPK pathway.[144] Within minutes after exposure of a vitamin D responsive tissue (e.g., intestine, chondrocyte, osteoblast) to calcitriol, there is increase in the intracellular concentration of $Ca^{2+}{}_i$ (transcaltachia) and activation of PLC, PKC, and MAPK.[131] In osteoblasts from $VDR^{-/-}$ mice and in fibroblasts from patients with inactivating mutations of *VDR*, the rapid actions of calcitriol are lost as is the association of the VDR with caveolae, evidence of the importance of the VDR for membrane-initiated responses to calcitriol. In chondrocytes, there is MARRS-dependent enhancement of calcium flux, PKC activity, and matrix vesicle

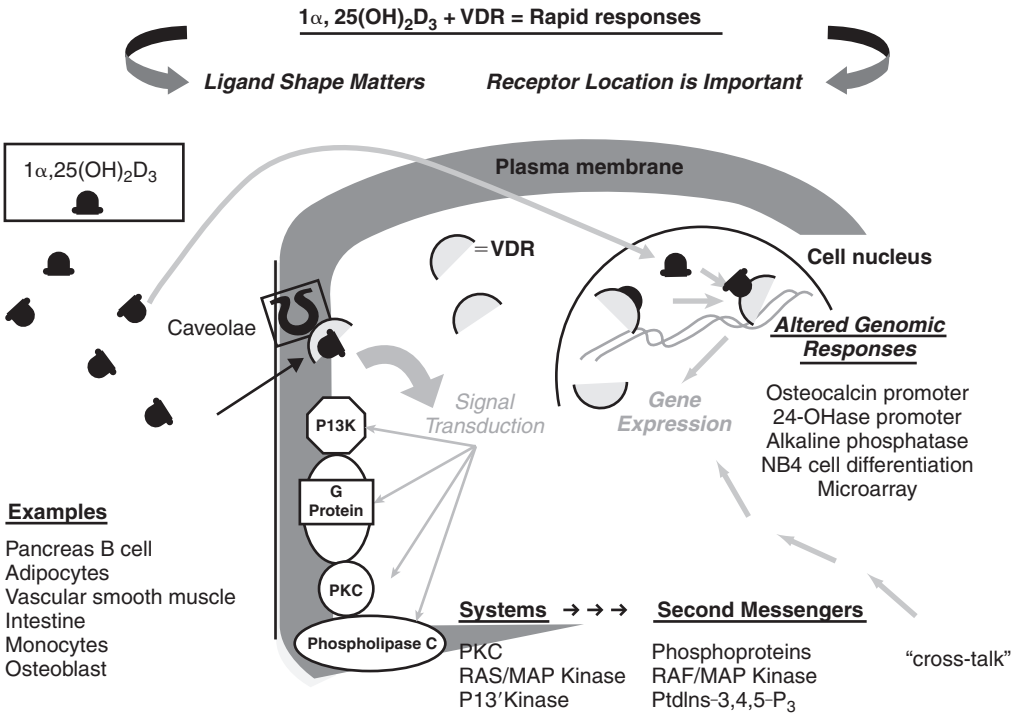


FIGURE 8-7 ■ Genomic and nongenomic (rapid) responses to calcitriol. (Reproduced from Norman, A. W., & Bouillon, R. [2010]. Vitamin D nutritional policy needs a vision for the future. *Exp Biol Med, 235*, 1034–1045, with permission.)

mineralization.[145] The plasma membrane caveolae-associated VDR may link to $G_{s\alpha}$ and thence to a calcium channel or adenylyl cyclase or PLC or with caveolin, a protein that interacts with the nonreceptor tyrosine kinase (Src) and in turn with PLC or the kinase h-Ras.[144,146] The rapid effects of vitamin D may optimize its genomic effects by phosphorylation of proteins required by the VDR transcriptional complex.

SKELETON: CARTILAGE AND BONE

Cartilage and Bone Differentiation and Formation

The skeleton is the framework of the body. It is composed of cartilage and bone, specialized cells and forms of connective tissue that provide (1) mechanical support for muscle/tendon insertion that enables movement; (2) protective shield for soft tissue organs; (3) repository for bone marrow; (4) reserve source of calcium, phosphate, magnesium, and other metabolically important ions; and it serves as (5) an endocrine organ as the secreted products of bone cells regulate the function of distant organs—for example, the osteocyte secretes sclerostin, an inhibitor of bone formation, and FGF23, a factor that depresses renal tubular reabsorption of phosphate and the synthesis of calcitriol.[147,148] Bone differentiation begins in utero when transformation of mesenchymal cells into chondrocytes that secrete collagen type II form the skeletal anlagen; the shape of the skeleton is complete by the ninth week of gestation following which there are many fold increases in skeletal dimensions, volume, and mass.[148-151] In the normal full-term neonate, skeletal weight approximates 200 to 300 g, 30 g of which is calcium. There are two primary bone shapes: flat bones (e.g., cranium, scapula, pelvis) and long bones (e.g., humerus, radius, ulna, metacarpal, femur, tibia, fibula, metatarsal).[152] Flat bones develop by membranous bone formation in which embryonic mesenchymal cells differentiate directly into osteoblasts, which then form bone; long bones are derived by both endochondral and membranous bone formation. Endochondral bone formation is the process by which the cartilaginous pattern of a bone is replaced by bone tissue.[149] The external surface of bone is enveloped by periosteum (containing blood vessels, nerve terminals, osteoblasts, and osteoclasts), whereas the interior of bone next to marrow is lined by endosteum. Long bones consist of a central hollow shaft (diaphysis) distal to which are the metaphyses, cartilaginous growth plates, and epiphyses. The diaphysis is composed of cortical bone, and the metaphysis/epiphysis consists of trabecular bone enveloped by cortical bone. Eighty percent of the adult skeleton is dense cortical bone; 20% is cancellous and composed of a network of trabeculae.

In addition to its cellular constituents (osteoblasts, osteocytes, osteoclasts, stromal and hematopoietic cells), bone is composed of solid mineral—hydroxyapatite = $Ca_{10}(PO_4)_6(0H)_2$—that has been deposited on collagen fibrils within extracellular matrix.[153] Bone extracellular matrix is comprised primarily of the secreted products of the osteoblasts and is composed of collagenous fibrils (collagen types I, III, V) on which the mineral phase of bone is deposited. Also within the extracellular matrix are noncollagenous, multifunctional proteins that organize, regulate, and coordinate mineralization, serum proteins (e.g., albumin, α2HS-glycoprotein, growth factors; e.g., IGF-I), proteoglycans (proteins with acidic polysaccharide side chains; e.g., chondroitin sulfates such as aggrecan, perlecan, glypican), glycosylated proteins with cell attachments (e.g., alkaline phosphatase, osteonectin), SIBLING proteins (e.g., osteopontin, BSP, DMP1, MEPE) (discussed earlier), fibronectin, fibrillins 1 and 2, and γ-carboxylated (gla) proteins (e.g., osteocalcin, matrix gla protein, protein S). Type I collagen and alkaline phosphatase promote bone mineralization, a process modulated by osteocalcin and matrix gla protein.[153]

Modeling of bone is the process that takes place during growth in which the shape and size of a bone are determined. *Remodeling* of bone is a continual process in which portions of formed bone are periodically reabsorbed and replaced by new bone; remodeling occurs in both the growing child and the adult. During early embryogenesis, bone is formed by condensation of mesenchymal cells in genetically determined patterns of position, arrangement, size, and shape.[154,155] Individual mesenchymal cells then differentiate either into chondrocytes that secrete a matrix of collagen type II in the anlagen of endochondral bones or directly into osteoblasts in precursor regions of intramembranous bone where they secrete a matrix rich in collagen type I. Osteoprogenitor pluripotent stromal mesenchymal stem cells provide a continuous supply of bone-forming osteoblasts, the network of osteocytes embedded throughout bone that monitors bone integrity and strength, and bone surface lining cells. Osteoclasts derived from hematopoietic precursor cells mediate bone resorption. Chondroblasts, osteoblasts, adipocytes, myoblasts, and fibroblasts are derived from a common mesenchymal cell (Figure 8-8).[156] Bone morphogenetic proteins (BMP) are members of the transforming growth factor-β (TGFβ) superfamily that direct the transformation of a pluripotent mesenchymal cell into the pathway leading to formation of chondrocytes and osteoblasts. BMP-2, -4, and -7 are important factors that direct the process of osteoblast differentiation, although there is substantial redundancy in the system and other BMPs also participate. The BMPs act through designated cell membrane threonine/serine kinase receptors (e.g., BMPRIA; BMPR2); depending on the interaction of the BMP receptors involved, intracellular signaling is transduced by the SMAD (mothers against decapentaplegic homolog) or MAPK pathways and induces synthesis of specific transcription factors that further the differentiation process.[157]

Chondrogenesis

In cartilaginous bones, longitudinal growth occurs by differentiation, proliferation, and hypertrophy of chondrocytes and by formation of extracellular matrix. Chondrocytes secrete an extracellular matrix that in

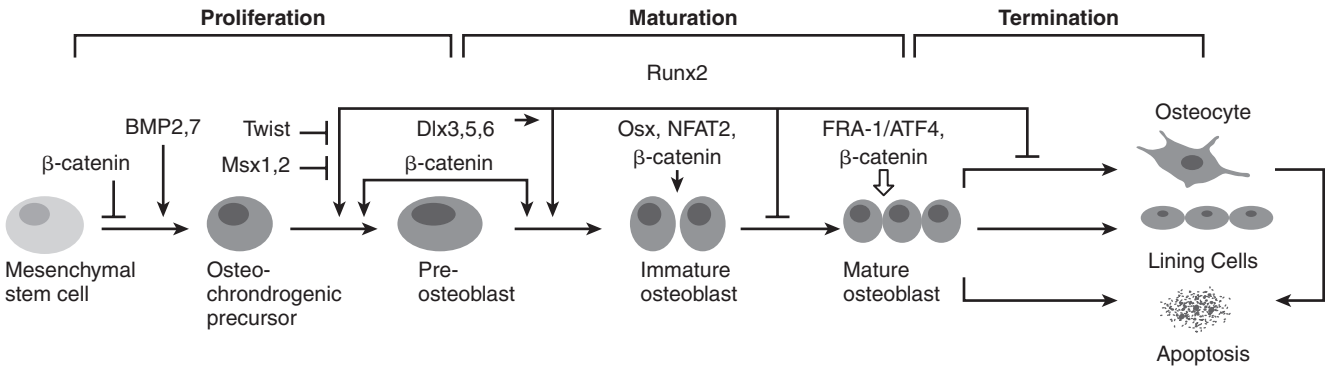


FIGURE 8-8 ■ Osteoblastogenesis. A common mesenchymal stem cells gives rise to chondroblasts, osteoblasts, myoblasts, fibroblasts, and adipocytes in response to specific differentiating factors. Bone morphogenetic proteins (BMP) are involved in the earliest steps leading to the differentiation of the common mesenchymal precursor cell into the osteoblast-chondrocyte lineage. RUNX2 and β-catenin are necessary for osteoblastic differentiation of the common progenitor cell of chondrocytes and osteoblasts and for further maturation of osteoblasts. (SOX9 is critically important for the differentiation and function of chondroblasts. PPARγ2 [peroxisome proliferator-activated receptor γ2] stimulates the differentiation of adipocytes. Osteoblasts and adipocytes may be interconverted depending on whether RUNX2 or PPARγ2 is the activated transcription factor. MyoD is a muscle-specific transcription factor necessary for development of myoblasts.) (Reproduced from Krause, C., de Gorter, D. J. J., Karperien, M., & ten Dijke, P. (2008). Signal transduction cascades controlling osteoblast differentiation. In C. J. Rosen (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (pp. 10–16) (7th ed.). Washington, DC: American Society for Bone and Mineral Research, with permission.)

addition to collagen type II is composed of large aggregates of aggrecan and the glycosaminoglycan hyaluronan, the latter two products enabling cartilage to compress and expand.[149] Other components of the extracellular matrix of cartilage include collagens types VI, IX, X, XI, and XIV; the large proteoglycan perlecan; cartilage oligomeric protein (COMP; also termed thrombospondin-5); and matrilins-1 and -3. COMP, matrilin-3, and collagen type IX are essential for normal growth plate structure as mutations in the genes encoding each of these proteins lead to pseudohypochondroplasia (*COMP*) or forms of multiple epiphyseal dysplasia. Expression of many of the genes encoding components of cartilage extracellular matrix (including those encoding collage type II and aggrecan) is dependent on the transcription factor SOX9.

The expression of *SOX9* is stimulated by FGF signaling through the MAPK pathway. SOX9 is a 509-amino-acid protein with an SRY homology domain that is also expressed in the testis where it is responsible for differentiation of Sertoli cells. Inactivating mutations of *SOX9* lead to camptomelic dysplasia and sex reversal in males (OMIM ID: 114290). Target genes of SOX9 include those that encode collagen type II(α1) and aggrecan, a chondroitin sulfate proteoglycan core protein; a mutation in the gene-encoding aggrecan leads to an autosomal dominant form of spondyloepiphyseal dysplasia associated with premature degenerative arthropathy (OMIM ID: 608361). *SOX9* is expressed not only in chondrocytes in the resting phase but also those in the proliferative phase but not in chondrocytes in the hypertrophic phase of maturation.[155]

The *pattern* of endochondral bone development is directed by factors that are independent of bone formation (e.g., in transgenic mice in which *Runx2* is inactivated, the cartilage skeleton forms normally but is not ossified).[158] (In humans, heterozygous inactivating mutations in *RUNX2* result in cleidocranial dysplasia [OMIM ID: 119600], manifested by growth retardation, hypoplasia of the clavicle and pelvis, delayed closure of cranial sutures, and defective tooth eruption.) Vertebrae evolve from the condensation and segmentation of paraxial mesoderm into somites under the direction and control of multiple genes including *NOTCH1*, *SHH*, and *PAX1* and Notch ligands encoded by *DLL3* and *JAG1*.[158] The appearance of limb buds, proliferating mesenchymal cells that grow out from the lateral body wall and are capped by an apical ectodermal ridge, heralds development of the cartilage anlagen of the long bones, a process of segmentation directed experimentally by homeobox genes (*Hoxa13*, *H0xd13*), *Sbh*, *Wnt7a*, *Gli3*, *Tgf*β, *Fgf4*, *Fgfr1*, *Fgfr2*, *Bmp2*, *Bmp4*, *Bmp6*, *Bmp*7, *Lmx1b*, *Pitx1*, *Tbx4*, *Tbx5*, *Twist*, and other signaling, receptor, and transcription regulating factors involved in differentiation, paracellular communication, and cell-to-cell interaction.[158,159] Mitochondrial RNA-processing endoribonuclease encoded by *RMRP* is a ribonucleoprotein that is essential for assembly of ribosomes and cyclin-dependent cell cycle activity as well as chondrocyte proliferation and differentiation. Inactivating mutations of *RMRP* result in anauxetic dysplasia (OMIM ID: 607095), a spondylometaepiphyseal dysplasia characterized by intrauterine and postnatal growth retardation with adult stature < 85 cm.[160] Histologically, the growth plates of these patients are depleted of chondrocytes. Different mutations of *RMRP* result in varying clinical manifestations (e.g., cartilage hair hypoplasia [OMIM ID: 250250] and metaphyseal dysplasia without hypotrichosis [OMIM ID: 250460]).

Chondrocyte differentiation within the resting zone of the cartilaginous growth plate is stimulated by growth hormone that also prompts local synthesis of IGF-I that then mediates chondrocyte proliferation and hypertrophy acting through the IGF type 1 receptor (encoded by *IGF1R*). Chondrocyte differentiation and proliferation is also enhanced by IHH, a 336-amino-acid product of prehypertrophic and early hypertrophic chondrocytes that

acts through its cell membrane receptor Patched 1 (encoded by *PTCH1*) and coreceptor Smoothened (*SMOH*) to stimulate the synthesis of PTHrP. Differentiation of proliferating chondrocytes into prehypertrophic chondrocytes and of the latter into hypertrophic chondrocytes is inhibited in paracrine fashion by PTHrP functioning through PTH1R; PTHrP is secreted in utero by cells in the proximal periarticular perichondrium and postnatally by chondrocytes in the resting zone of the cartilaginous growth plate and diffuses into the zone of proliferating chondrocytes where it delays further differentiation, thus maintaining their replicative capacity (Figure 8-9).[92,161,162] Ultimately, the more distal proliferating chondrocytes escape from the inhibitory effects of PTHrP, undergo hypertrophy, and then die. In humans, inactivating mutations of *PTH1R* result in rapid chondrocyte maturation and Blomstrand chondrodystrophy (OMIM ID: 215045), whereas activating mutations in *PTH1R* lead to impaired chondrocyte maturation and Jansen metaphyseal chondrodysplasia (OMIM ID: 156400). Thus, the rate of prehypertrophic and terminal hypertrophic differentiation of chondrocytes is regulated locally by both PTHrP and IHH, the latter by encouraging chondrocyte hypertrophy.[161] IHH is also able to stimulate the conversion of perichondrial cells into osteoblasts.[162] The synthesis of IHH is increased by several BMPs. During the process of chondrocyte hypertrophy, individual cell length increases 6- to 10-fold; as the chondrocyte dies collagenous fibers are enzymatically digested, the extracellular matrix calcifies, and vascular endothelial growth factor (VEGF) is produced.[163] Surrounding the cartilage anlagen is a periosteal collar of bone deposited by recently differentiated osteoblasts; in response to VEGF blood vessels, chondroclasts, osteoclasts, osteoblasts, stromal, marrow, and other cells then invade the underlying cartilage and establish a primary center of ossification. A secondary ossification center forms at the distal ends of long bones (defining the epiphyses) with intervening areas of cartilaginous growth plates that permit bone lengthening by continued proliferation and hypertrophy of chondrocytes and formation of extracellular matrix.

As the chondrocyte progresses through its maturational stages of differentiation, proliferation, hypertrophy, and death, the patterns of expression of hundreds of genes change as do the components of secreted extracellular matrix.[149,164,165] For example, experimentally the mesenchymal stem cell expresses, among many other genes,

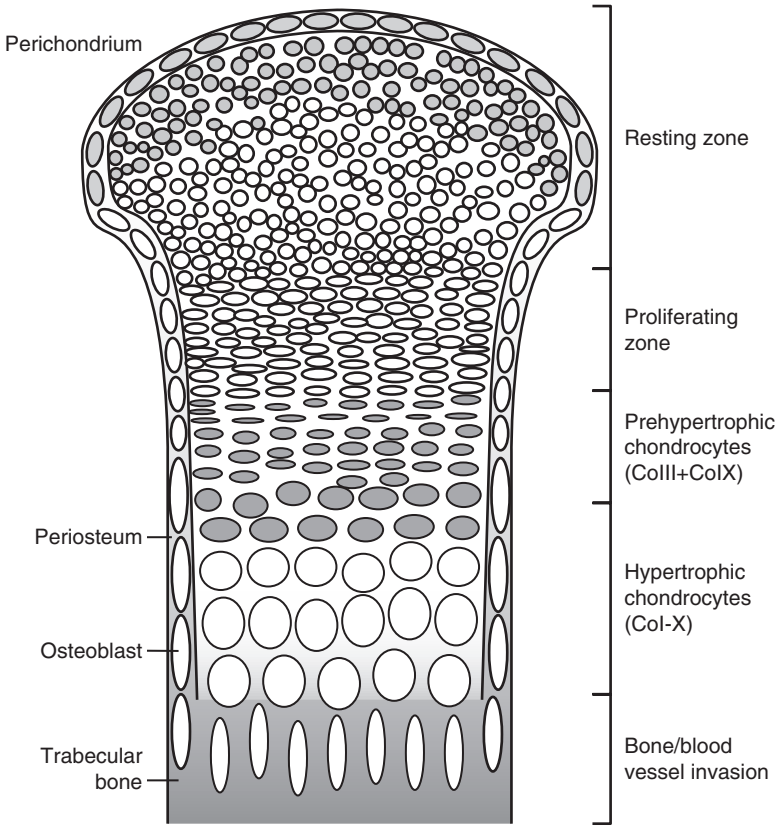


FIGURE 8-9 ■ The epiphyseal cartilage growth plate is composed of zones of resting, proliferating, prehypertrophic, and hypertrophic chondrocytes that develop longitudinally and a transitional zone in which apoptotic hypertrophic chondrocytes and surrounding matrix are replaced by bone. Indian hedgehog (IHH) is synthesized by prehypertrophic chondrocytes. Receptors for parathyroid hormone–related protein are expressed by proliferating and transitional chondrocytes. IHH stimulates secretion of parathyroid hormone–related protein (PTHrP) by periarticular cells and this in turn blocks further differentiation and maturation of late proliferating chondrocytes to hypertrophic chondrocytes, thus prolonging the period of cartilage growth. IHH also acts through bone morphogenetic proteins (BMP) and the WNT-β-catenin pathway to enhance chondrocyte hypertrophy. Fibroblast growth factors (FGF) also influence chondrocyte proliferation and maturation. (Reproduced from Yang Y: Skeletal morphogenesis and embryonic development. IN Rosen CJ: Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism (8th ed.). New York: John Wiley & Sons, 2013. Fig 1-4. © 2013 American Society for Bone and Mineral Research.)

Sox9 and *Col2a1*; the resting chondrocyte expresses *Srfp5*, *Sox9*, *Col2a1*, *Agc1*, and *Pthlh*; the proliferating chondrocyte expresses *Sox9*, *Col2a1*, *Agc1*, *Fgfr3*, and *Runx2*; the prehypertrophic chondrocyte expresses *Bmp*, *Col2a1*, *Col10a1*, *Agc1*, *Pth1r*, *Runx2*, and *Vegf*; the hypertrophic chondrocyte expresses *Col10a1*, *Alpl*, *Ihh*, *Runx2*, and *Vegf*; and the terminal hypertrophic chondrocyte expresses *Col10a1*, *Vegf*, and *Mmp13*. During the transition from the resting to the proliferating chondrocyte, functional gene pathways that are involved include the VDR/RXR and BMP signaling systems: in the transition from the proliferative to the hypertrophic chondrocyte prominent functional gene systems include BMP signaling and components of cellular growth and the cell cycle such as p53; during the senescent phase of chondrocyte maturation the most prominently expressed signaling pathways are those involving VDR/RXR, MAPK, and WNT/β-catenin.[164] When the postproliferative chondrocyte begins to hypertrophy, the production of collagen type II declines and that of collagen type IX increases, whereas late hypertrophic and dying chondrocytes synthesize the collagenase, matrix metalloproteinase 13. In addition to IHH, transition from a proliferative to a hypertrophic chondrocyte is stimulated by triiodothyronine working in concert with IGF-I and FGFR3. Triiodothyronine acts through nuclear thyroid hormone receptor α to increase intracellular signaling through the WNT4/β-catenin pathway. Triiodothyronine also inhibits expression of *Pthlh*, thus shortening the proliferative phase of chondrogenesis. Increase in activity of FGFR3 accelerates the hypertrophic process. As discussed, IHH inhibits chondrocyte hypertrophy acting through PTHrP that in turn down-regulates expression of *RUNX2* and impedes its regulation of hypertrophy associated genes.[149]

FGF-1, -2, -6, -7, -9, and -18 acting through one of four FGF receptors are also important for normal chondrocyte differentiation and development.[163] Normal chondrogenesis is dependent on the balance between the positive regulation of chondrogenesis exerted through FGFR2 and FGFR4 and the negative regulation transmitted through FGFR1 and FGFR3. Mutations in *FGFR1* and *FGFR2* have been associated with syndromes of premature craniosynostosis (Jackson-Weiss, Pfeiffer, Crouzon, and Apert syndromes), whereas alterations in *FGFR1* have also been identified in patients with hypogonadotropic hypogonadism. Gain-of-function mutations in *FGFR3*, expressed in chondrocytes in utero and postnatally, are associated with achondroplasia, hypochondroplasia, and related chondrodystrophies. Interestingly, mutations in *FGFR4* have not been identified in patients with osteochondrodystrophies to date. BMPs affect chondrogenesis by increasing production of IHH, thus increasing chondrocyte proliferation, but also advance chondrocyte maturation.[162]

Chondrocytes within the late hypertrophic zone of the cartilage growth plate disintegrate and discharge their contents as they die. Blood vessels with their cellular cargo invade the extracellular matrix; chondroclasts and osteoclasts digest the extracellular matrix that has been degraded by proteolysis by matrix metalloproteinases, and crystals of calcium phosphate are deposited in the debris. These are later reabsorbed by osteoclasts after which osteoblasts secrete a matrix of collagen type I into which hydroxyapatite crystals are deposited to form bone. Ultimately, growth plate cartilage disappears as primary and secondary centers of ossification meet and fuse, a process regulated by estrogen, which appears to accelerate the senescent process in late hypertrophic chondrocytes.[149]

Although Runt-related transcription factor 2 (encoded by *RUNX2*) inhibits the initial differentiation of the multipotential mesenchymal stem cell into the chondrocyte pathway, it is essential for progression of the chondrocyte through its later stages of differentiation as well as for the early stages of osteoblastogenesis.[166] RUNX2 is the alpha subunit of a transcription factor complex that binds to the promoter region of target genes such as *COLA1* and *MMP13*.

Osteoblastogenesis

During osteoblastogenesis, RUNX2 guides pluripotent mesenchymal stem cells into the osteoblast lineage and up-regulates the expression of genes encoding collagen 1A1, osteocalcin, and several other osteoblast products (see Figure 8-8). After initial osteoblast differentiation, the expression of *RUNX2* declines as the mature osteoblast evolves. Stromal mesenchymal stem cells differentiate not only into chondroblasts (under the direction of SOX9) and osteoblasts (through WNT-induced signaling of RUNX2, DLX5, and SP7 [osterix]), but also into adipocytes (through PPARγ2), myoblasts, and fibroblasts. Committed osteoblasts and adipocytes are capable of redifferentiating into the other cell type depending on whether the expression of *RUNX2* or *PPARG* is paramount; this process is directed by WNT10b, which enhances expression of *RUNX2*, *DLX5*, and *SP7* while suppressing that of *PPARG*.[156,167] The nuclear NAD-dependent protein deacetylase encoded by *SIRT1* also inhibits the adipocyte differentiating effects of PPARγ2 by docking corepressors to this transcription factor, thereby diverting mesenchymal stem cells into the osteoblastogenic pathway.[168] BMP -2, -4, and -7 induce osteoblastogenesis acting through their heterodimeric cell surface receptors and transduce their intracellular signals through receptor-regulated Smads 1, 5, and 8 that heterodimerize with DNA-binding SMAD 4 to induce expression of *RUNX2*, *DLX5*, and SP7; TGFβ signals through Smads 2 and 3 to promote *RUNX2* expression; platelet-derived growth factor, FGFs, and IGF-I also enhance the proliferation and further differentiation of committed osteoblast precursors.[156]

Differentiation of the pluripotent mesenchymal stem cell into the osteoblast lineage is also under the guidance of WNT-stimulated intracellular signaling transduction pathways (Figure 8-10).[158,169,170] (WNT is a term derived by combining the names of the *Drosophila* gene *Wingless* with the corresponding mouse gene *Int*.) Secreted WNT cytokines, a 19-member family of lipid-modified signaling glycoproteins with 350 to 400 amino acids and a conserved sequence of 22 cysteine residues (e.g., WNT1, WNT3a, WNT5a, WNT7b, WNT9a, WNT10b), bind to the GPCR Frizzled receptors (encoded by *FZD1*) and to low-density lipoprotein receptor-related proteins (LRP) 5/6 coreceptors (encoded by *LRP5*, *LRP6*), both of which are long chain proteins with a single transmembrane domain situated on the mesenchymal stem cell's

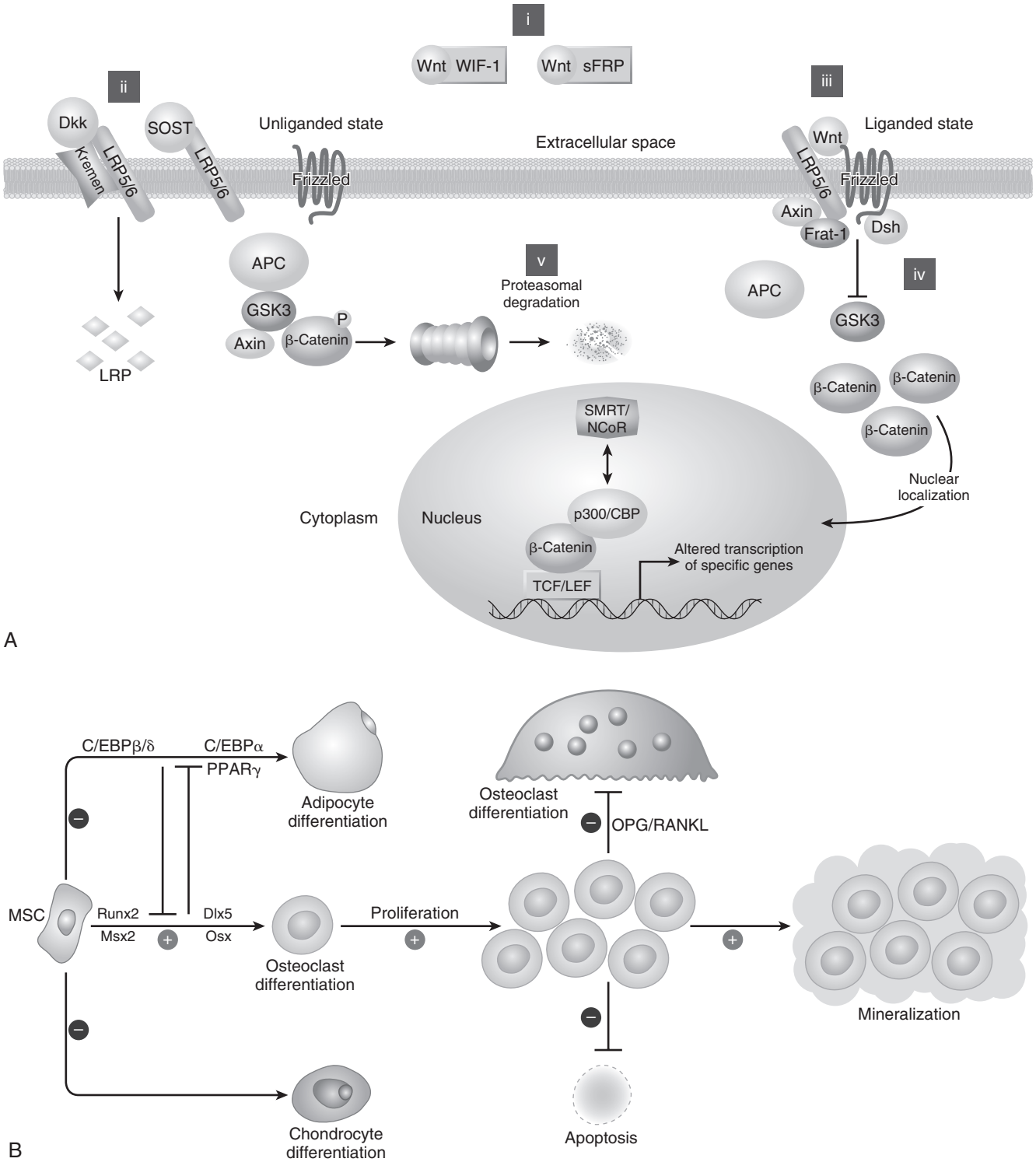


FIGURE 8-10 ■ Influence of WNT-β-catenin signal transduction pathway on osteoblastogenesis. When a WNT ligand binds to its Frizzled receptor and its coreceptors lipoprotein receptor-related proteins (LRP) 5/6, phosphorylation, and degradation of β-catenin cease, enabling its transport into the nucleus where it serves as a cofactor for the TCF/LEF transcription factor complex and enhances osteoblast differentiation. The WNT signaling system can be inhibited by binding of sclerostin (SOST) and Dikkopf (DKK) to the coreceptors and of WNT inhibitory factor (WIF1) and secreted frizzled-related protein (sFRP) to the WNT ligand. (See text for details.) (Reproduced from Krishnan, V., Bryant, H. U., & MacDougald, O. A. (2006). Regulation of bone mass by Wnt signaling. *J Clin Invest*, *116*, 1202–1209, with permission.)

plasma membrane. WNT binding to Frizzled and LRP 5/6 leads to intracellular signaling through canonical (β-catenin) and noncanonical (the $G\alpha_q$-protein and PLC) signal transduction pathways. The canonical signal transduction pathway is mediated by increasing cytosolic concentrations of β-catenin (encoded by *CTNNB1*) (see Figure 8-10).[149,169,171] β-Catenin is a transcription enhancing factor that in its free state enters the nucleus and associates with the T cell factor/lymphoid enhancer binding factor (TCF/LEF) transcription activating complex. In the inactive state in cytosol, β-catenin is bound to a multiprotein complex of adenomatous polyposis coli (APC), axin, and glycogen synthase kinase 3 (GSK3). Phosphorylation of β-catenin by GSK3 directs it into the degradative ubiquitin/proteasomal pathway. Binding of a WNT ligand to its Frizzled receptor and to its LRP 5/6 coreceptors leads to linking of the intracellular protein Disheveled-1 (encoded by *DVL1*) to the Frizzled receptor and to inhibition of GSK3-mediated phosphorylation of β-catenin, which slows its rate of degradation and releases it from binding to axin (encoded by *AXIN1*) increasing cytoplasmic levels of β-catenin and accelerating its transport into the nucleus.[169,172] In the nuclei of mesenchymal stem cells, β-catenin serves as a nuclear transcriptional cofactor by binding to TCF/LEF (thus displacing transcriptional corepressors and recruiting coactivating factors) and thereby stimulating expression of *RUNX2* (also termed core-binding factor alpha subunit 1 or CBFA1) and other target genes. RUNX2 activity may also be enhanced by WNT acting through a signaling pathway that is independent of β-catenin—that is, prostaglandin E_2 (PGE_2) is also able to activate *RUNX2* transcription.[170] In turn, RUNX2 serves as a transcription factor that stimulates the expression of *SP7* (encoding osterix). RUNX2 and SP7/osterix, a 431-amino-acid nuclear transcription factor, are essential for further differentiation of mesenchymal stem cells into osteoblasts and for their synthesis of osteocalcin (*BGLAP*) and collagen type I(α1) (*COL1A1*).[158,166] Osteocalcin plays an important role in normal mineralization of bone matrix. Other gene targets of RUNX2 include those encoding BMP4, FGFR1, Dickkopf, WNT10a, WNT10b, FGF18, and TGFβR1.[173] RUNX2 is not only central to initiation of osteoblastogenesis but also to its maintenance. WNT signaling increases the production of osteoprotegerin (OPG) by the stromal cell/osteoblast, a protein that inhibits osteoclastogenesis, thereby further increasing bone mass (discussed later).

Also essential for osteoblast differentiation and proliferation and, hence, normal bone development is FGF18. Expression of *FGF18* is directly stimulated by the WNT-Frizzled-LRP 5/6-β-catenin pathway through TCF/LEF.[174] Interestingly, the 5′ promoter site for TCF/LEF of *FGF18* also harbors a RUNX2 binding site. Thus, the WNT-Frizzled-LRP 5/6-β-catenin signal transduction system and RUNX2 coordinately enhance *FGF18* expression. FGF18 affects chondrogenesis, osteogenesis, and osteoclastogenesis. FGF18 inhibits chondrocyte proliferation and antagonizes the effects of BMPs acting through its cell surface tyrosine kinase receptor (FGFR3).[155,158] RUNX2 increases expression of the gene encoding TGFβ type 1 receptor through which TGFβ enhances the transcriptional activity of TCF/LEF.[170]

The WNT signaling pathway is opposed by several factors: secreted Frizzled-related proteins and WNT inhibitory factor 1 (encoded by *WIF1*) bind to WNT, thus interfering with ligand binding to its Frizzled receptor and to LRP 5/6; also, the complex of Dickkopf (encoded by *DKK*)/Kremen and sclerostin (encoded by *SOST*) binds to LRP 5/6, increasing their internalization and degradation and thus decreasing the number of LRP 5/6 coreceptors available for linkage to WNT and to its Frizzled receptor. Additionally, the axin-binding function of LRP5 is inhibited by binding of its extracellular domain to Dickkopf. WNT signaling results in an increase in the rates of osteoblast differentiation and proliferation, a decrease in the rate of osteoblast apoptosis, repression of mesenchymal stem cell differentiation into chondrocytes or adipocytes, and a decrease in osteoclastogenesis—the various processes summing to increase osteoblast number and hence the tempo of bone formation and remodeling.

Activating mutations of *LRP5* are associated with an autosomal dominant form of relatively benign high bone mass (OMIM ID: 601884) or, in some patients, autosomal dominant osteopetrosis type 1 (OMIM ID: 607634), whereas inactivating mutations of *LRP5* result in the autosomal recessive osteoporosis-pseudoglioma syndrome (OMIM ID: 259770) in which, in addition to decreased bone formation due to subnormal rate of osteoblast proliferation, the hyaloid vessels of the embryonic eye fail to regress.[175] (Some experimental data have suggested that it is expression of *LRP5* in the enterochromaffin cells of the intestinal tract [rather than in bone itself] that modulates bone mass through regulation of serotonin production by these cells.[176,177] In this proposed pathway, intestinal LRP5 negatively controls the production of tryptophan hydroxylase and consequently the synthesis of serotonin; thus, a loss of LRP5 results in increased serotonin synthesis and secretion by the enterochromaffin cells that is transported through the circulation to the bone osteoblasts where it inhibits osteoblast proliferation. However, these observations have not been supported by the studies of other investigators; currently, it has been concluded that LRP5 primarily acts locally in bone to regulate bone differentiation and mineralization.[178]) Experimentally, a loss of *Lrp6* also results in low bone mass; however, it but does not do so by impairing osteoblast function but rather by increasing osteoclastogenesis. LRP4, a third member of the LRP family, exercises an *inhibitory* influence on skeletal mineralization by enabling Dickkopf and sclerostin to interact with LRP 5/6.

Osteoblasts have a life span of 3 months. They are heterogeneous and express diverse genes that may be dependent or independent of the stage of the cell cycle and extent of differentiation.[167] The heterogeneity of osteoblasts may relate to the many different bone architectures and microenvironments in which they reside. Actively bone-forming osteoblasts have a large nucleus, plentiful Golgi apparatus, and abundant endoplasmic reticulum. When the rate of bone formation is low, osteoblasts are small and quiescent and incorporated into the endosteum separating bone mineral from marrow or into the undersurface of the periosteum. Differentiated, mature osteoblasts secrete collagenous and noncollagenous proteins including

collagen type I, bone-specific alkaline phosphatase, and the calcium and phosphate binding proteins—osteocalcin, osteopontin, and osteonectin—thus making bone matrix competent for mineralization.[179] Osteoblasts control mineralization of matrix by regulating local concentrations of phosphate through synthesis of cell membrane-bound alkaline phosphatase, which frees organically bound phosphate, and by reducing levels of inhibitors of bone formation such as pyrophosphates and the acidic serine aspartate-rich MEPE-associated motif (ASARM) peptide (discussed later). Calcium and phosphate can then precipitate in bone matrix as hydroxyapatite crystals.

When an osteoblast is surrounded by bone matrix, it is transformed into an osteocyte embedded in a bony lacuna, a process influenced by matrix metalloproteinases, dentin-matrix protein 1 (DMP-1), and other factors.[180] Within mature bone, osteocytes form a network of interconnected long cytoplasmic processes that lie within canaliculi that link with one another across gap junctions and connect deep osteocytes with newly formed osteocytes and with surface lining cells.[172] Osteocytes do not divide but have a half-life of approximately 25 years. They are capable of synthesizing a number of proteins including TGFβ, PHEX, DMP-1, matrix extracellular phosphoglycoprotein (MEPE), FGF23, and sclerostin. Although possibly capable of transiently destroying bone locally by osteocytic osteolysis, the primary roles of the osteocyte is to sense the mechanical load placed on the skeleton and the resultant deformation and strain (discussed later).[181] The osteocyte does so by monitoring movement of fluid and pressure within canaliculi within a basic multicellular unit (BMU) of osteoblasts and osteoclasts. In response to a strain, osteocytes synthesize a number of substances, some of which bring about the movement of osteoclasts and osteoblasts to the site of bone stress (e.g., microfracture) allowing the removal of injured bone and its replacement by new bone (bone remodeling), whereas others play an anabolic or catabolic role in the bone's response to mechanical loading and stress.[175]

Sclerostin (encoded by *SOST*) is a 213-amino-acid protein that binds LRP 5/6 and BMPs, thereby inhibiting WNT-LRP5/6-β-catenin–mediated osteoblast differentiation (see Figure 8-10) and suppressing bone formation; secondarily, sclerostin increases expression *RANKL*, thereby promoting osteoclastogenesis.[182] Sclerostin is primarily expressed and secreted by cortical and trabecular osteocytes in response to a decrease in mechanical forces ("unloading") exerted on the skeleton; as a consequence, the rate of bone formation decreases and that of bone resorption increases.[183] In response to mechanical loading, osteocyte expression of *SOST* decreases and the rate of bone formation increases. The inhibitory effect of mechanical loading on osteocyte production of sclerostin may be mediated by paracrine factors such as prostaglandins, nitric oxide, or oncostatin M (OMIM ID: 165095). In addition to mechanical forces, the expression of *SOST* or synthesis of sclerostin is suppressed by PTH functioning through PTH1R. (There is a high density of binding sites for carboxyl fragments of PTH on osteocyte membranes suggesting that these sequences may play a role in the mechanosensory activity of the osteocyte network.[75]) Estrogens, cytokines produced by osteoblasts and osteoclasts (oncostatin M, leukemia inhibitory factor, cardiotrophin-1, IL-33), prostaglandin E2, and hypoxia also suppress expression of *SOST*. Calcitonin, osterix, and TNFα exert a direct stimulatory effect on osteocyte expression of *SOST* and synthesis of sclerostin.

The "mechanostat" function of the osteocyte is indispensable for maintenance of optimal bone strength, mass, size, and shape. The stimulus for the functional adaptation of bone is mechanical strain ("the deformation of bone tissues that occurs with loading").[184,185] The magnitude of a strain is determined by its force, frequency, and distribution and is also dependent on the site on which the strain is exerted and by genetic characteristics of the individual on whom the mechanical load is placed. In response to mechanical deformation, fluid shifts in the lacunae and canaliculi provoke the osteocyte to release nitric acid and PGE_2 that recruit osteoblasts to the strain site where new bone is formed beneath the periosteum of long bones and on existing trabeculae (*formation modeling*). When mechanical force on bone is reduced (e.g., immobilization, bed rest, space flight), the rate of osteocyte apoptosis increases by uncertain mechanisms. The dying osteocyte releases both macrophage colony-stimulating factor (M-CSF) and soluble receptor activator of nuclear factor κB-ligand (sRANKL) leading to an increase in osteoclastogenesis and bone resorption (discussed later) primarily on the endosteal surface of long bones without a comparable increase in osteoblast-directed bone formation resulting in diminished cortical thickness and bone strength (*resorption modeling*). In sites of microfractures, signals (M-CSF, sRANKL) from dying osteocytes recruit osteoclasts that, in turn, attract osteoblasts for removal and reformation of bone, respectively (*targeted remodeling*).[185] Interestingly, bisphosphonates, sex steroids, and PTH prevent osteocytic apoptosis, a property that partially underlies the positive physiologic effects of these compounds on bone mass. The strain placed on long bones by muscular exertion and other forces (e.g., jumping) exerts a positive anabolic effect through stimulation of osteocyte function. The functional muscle-bone unit confers the ability of bone to modify its strength, mass, and shape in response to muscular force.[186]

$PTH^{1\text{-}84}$ functioning through PTH1R stimulates growth of osteoblast progenitor cells and inhibits apoptosis of osteoblasts and osteocytes, thereby enhancing bone formation and accounting for its therapeutic usefulness in treatment of osteopenic states; however, $PTH^{1\text{-}84}$ also promotes bone resorption by stimulating osteoblast production of RANKL, an osteoclast activating factor (discussed later). In osteoblasts, carboxyl terminal fragments of PTH affect the generation of alkaline phosphatase, procollagen I, and apoptosis, some of which effects may be opposite to those of $PTH^{1\text{-}84}$. Calcitriol, functioning through the nuclear VDR, increases osteoblast synthesis of several noncollagenous matrix proteins including osteocalcin. Glucocorticoids decrease bone mineralization by depressing osteoblast differentiation, function, and life span and by promoting osteocyte apoptosis. Glucocorticoids also enhance osteoclastogenesis and prolong their bone resorbing activity.[187] Estrogens stimulate osteoblast proliferation and synthesis of collagen type I and inhibit apoptosis of osteoblasts and osteocytes. Estrogens enhance trabecular and endosteal bone growth and exert a biphasic effect on

periosteal bone growth: in prepubertal children, small amounts of estrogens promote periosteal bone growth; in pubertal and adult subjects, estrogens oppose this process; estrogens also accelerate growth plate fusion and inhibit bone resorption—the latter by enhancing osteoclast apoptosis. Androgens primarily promote mineralization by conversion to estrogens, as evidenced by the osteopenia noted in adult males with aromatase deficiency or loss-of-function mutations in estrogen receptor-α (ERα).[188] Androgens also have a direct effect on bone mineralization; acting through the androgen receptor they increase periosteal bone growth during puberty in both males and females; however, the stronger bones of men compared to women reflect not increased volumetric bone mineral density but rather increased bone size due to greatly expanded periosteal bone width.[189] Increased periosteal bone width in males is due in part to the androgen-induced effect of increased muscle mass, strain, and mechanical loading on bones.[190] Nevertheless, estrogens too are necessary for normal periosteal bone growth as despite normal testosterone secretion the aromatase-deficient male has decreased periosteal bone width, a situation that can be reversed by administration of estrogen acting through ERα. A portion of the anabolic effects of estrogens and PTH on bone mineralization may be mediated through their stimulation of the GH/IGF-I axis. GH enhances the proliferation and differentiation of osteoblast precursors, whereas IGF-I increases osteoblast function and trabecular and cortical bone volume and decreases osteoblast and osteocyte apoptosis.[109,167] The endogenous GH secretagogue ghrelin promotes proliferation and differentiation of osteoblasts and bone mineralization in vivo.[191] Leptin secreted by adipocytes or osteoblasts acting locally in bone exerts anabolic effects and promotes bone formation. However, centrally active (hypothalamic ventromedial nucleus) leptin impairs bone formation (discussed later).[147]

Cortical or compact bone is present in the cranium, scapula, mandible, ilium, and shafts of the long bones; both its periosteal and endosteal surfaces are lined with layers of osteogenic cells. Cancellous (trabecular or spongy) bone is located in the vertebrae, basal skull, pelvis, and ends of the long bones. Because only 15% to 25% of trabecular bone volume is calcified (compared to 80% to 90% of cortical bone volume) and thus has a far greater surface area, trabecular bone is metabolically quite active; it has a high turnover rate, making it more vulnerable to disorders that adversely affect bone mineralization. In flat bones (skull, ilium, mandible), intramembranous ossification begins with the local condensation of mesenchymal cells that differentiate directly into preosteoblasts and osteoblasts and initiate the formation of irregularly calcified (woven) bone that is then replaced by mature lamellar bone. Membranous bones grow by apposition, a process supported by the development of new blood vessels induced by VEGF, a protein that also enhances bone formation.[192] The periosteum is a fibrous network in which osteoblasts synthesize peripheral compact bone; cortical bone reinforces bone strength and complements and extends that provided by trabecular and endosteal bone. Tendons and ligaments insert and are fixed into cortical bone.

Osteoclastogenesis

In addition to their pivotal role in bone formation, osteoblasts and bone marrow stromal cells regulate bone resorption by controlling the differentiation, maturation, and function of osteoclasts (Figures 8-11A and 8-11B). Osteoclasts are multinucleated giant cells that adhere to

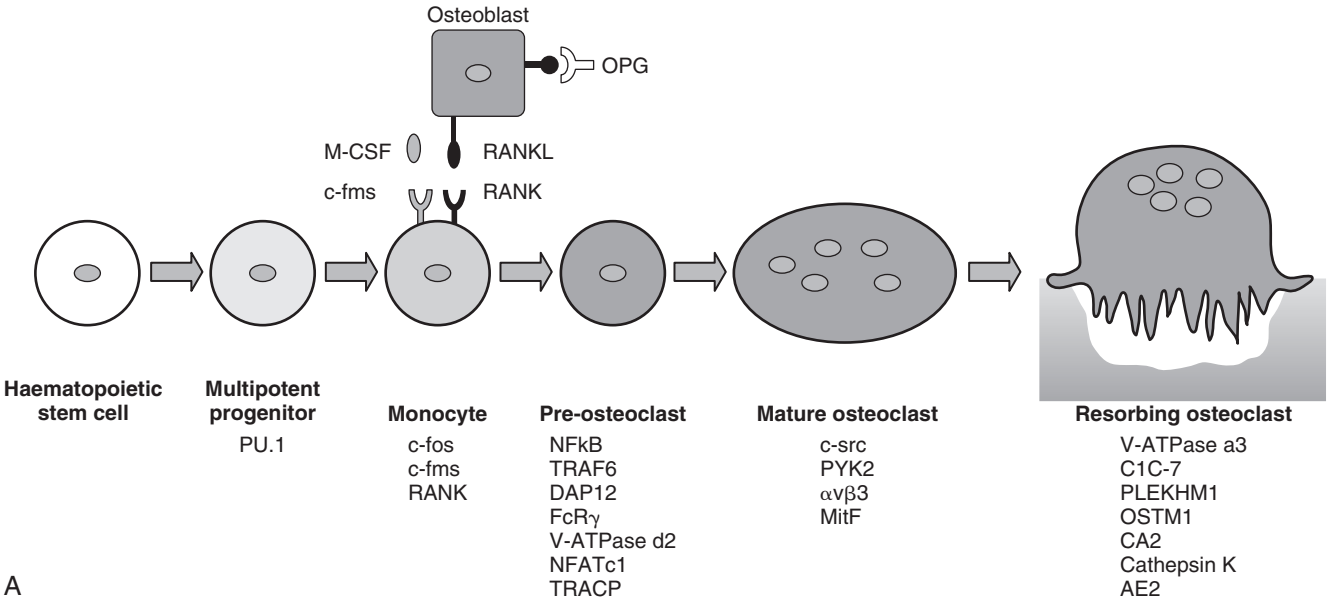
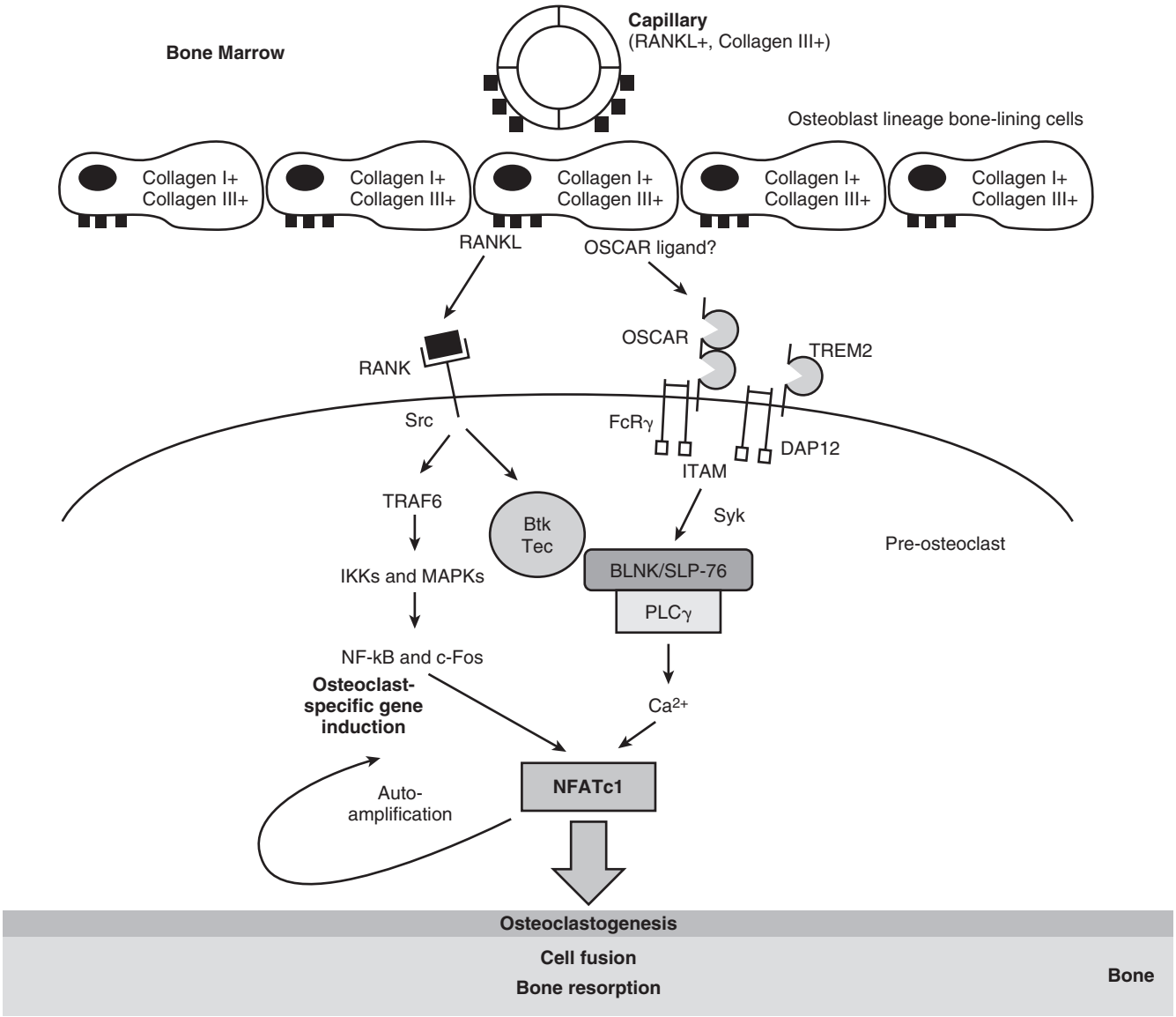


FIGURE 8-11 ■ **A,** Genetic regulation of osteoclastogenesis. After initial differentiation following exposure of a mesenchymal stem cell to M-CSF, RANKL synthesized by stromal cells and osteoblasts binds to RANK expressed on the plasma membrane of preosteoclasts which signals through SRC, TRAF6, and MAPK to enable NFκB and c-Fos to translocate NFATC1 to the nucleus where it assists in the maturation of the preosteoclast into a mature osteoclast and serves as a transcription factor for osteoclast-specific proteins (tartrate-resistant acid phosphatase, cathepsin K, β_3-integrin, calcitonin receptor).



B

FIGURE 8-11, cont'd ■ B, RANKL produced by osteoblasts and stromal cells binds to RANK expressed on the surface of the osteoclast precursor cell; signal transduction trough TRAF6 and NFκB enables the translocation of NFATC1 into the nucleus and further maturation of the osteoclast. The costimulatory stimulus for osteoclastogenesis proceeds through OSCAR and TREM2, membrane proteins that recognize as ligands amino acid sequences within the structures of collagen types I and III. These receptors then signal through ITAM adaptors to stimulate PLCγ that converts membrane bound phosphatidylinositol to inositol triphosphate (and diacylglycerol) resulting in mobilization of Ca^{2+} from storage sites in the endoplasmic reticulum and increase in cytosolic Ca^{2+} concentrations that also activate NFATC1 and stimulate osteoclastogenesis. (See text for details.) BLNK, B-cell linker protein; ITAM, immunoreceptor tyrosine-based activation motif; NFATC1, nuclear factor of activated T cells, cytoplasmic, calcineurin-dependent 1; OSCAR, osteoclast-associated receptor; PLCγ, phospholipase Cγ; RANK, receptor activator of nuclear factor κB; RANKL, RANK ligand; LP-76, SH2 domain-containing leukocyte protein, 76 kD;TREM2, triggering receptor expressed on myeloid cells-2. (**A,** Reproduced from Henricksen R, Bollerslev J, Everts V, Karsdal MA [2001]. Osteoclast activity and subtypes as a function of physiology and pathology: implications for future treatments of osteoporosis. Endocr Rev 32:31-63, with permission. **B,** Reproduced from Barrow, A. D., Raynal, N., Andersen, A. L., et al. (2011). OSCAR is a collagen receptor that costimulates osteoclastogenesis in DAP12- deficient humans and mice. *J Clin Invest, 121*, 3505–3516, with permission.)

the surface of bone and form a subosteoclastic lacuna into which the osteoclast secretes hydrochloric acid to dissolve the mineral phase of bone (hydroxyapatite) and cathepsin K and matrix metalloproteinases to digest organic matrix.[193] Osteoclasts develop from hematopoietic stems cells of monocyte-macrophage lineage. In response to PTH (or PTHrP), calcitriol, IL-6 and -11, and several other cytokines (e.g., TNFα), and prostaglandins (discussed later), osteoblasts and bone marrow stromal cell synthesize both M-CSF and RANKL, essential stimuli for osteoclast differentiation.[187] Osteoblasts and stromal cells also synthesize a decoy acceptor protein for RANKL that inhibits osteoclastogenesis, osteoprotegerin (OPG). M-CSF, a cytokine that functions through its receptor, CSF1R/c-Fms, whose expression is induced by the transcription factor PU.1 (OMIM ID: 165170),

enables hematopoietic stem cells to differentiate into macrophage colony-forming units from which are derived macrophages and osteoclasts. M-CSF/CSF1R, signaling through the MAPK and PI3K3 transduction pathways, facilitates microphthalmia-associated transcription factor (MITF)-stimulated expression of the gene encoding B-cell leukemia/lymphoma 2 (Bcl-2), an anti-apoptotic factor that permits survival of osteoclast precursors. Subsequently, RANKL, a member of the TNF ligand superfamily encoded by *TNFSF11* and expressed on the surface of bone marrow stromal cells and osteoblasts, binds to M-CSF induced RANK expressed on the surface of osteoclast progenitor cells where it induces their further differentiation and activation.[187] RANKL is a 317-amino-acid protein composed of cytoplasmic (48 amino acids), transmembrane (21 amino acids), and extracellular (248 amino acids) domains with the binding site for RANK extending between amino acids 137 and 158. In the promoter region of *TNFSF11* is a response element for RUNX2, the osteoblast differentiating transcription factor. *RANKL* is also expressed in lymphoid tissue (where it is critical for development of the immune system), striated and cardiac muscle, lung, intestines, placenta, thyroid, prechondroblast mesenchymal cells, and hypertrophic chondrocytes. In addition to furthering osteoclast differentiation, RANKL enhances function of the mature osteoclast and inhibits its apoptosis. RANKL stimulates transcription of osteoclast-specific proteins such as tartrate-resistant acid phosphatase (TRAP), matrix metalloproteinase 9, carbonic anhydrase II, cathepsin K, the a3 subunit of vacuolar $[H^+]$-ATPase (encoded by *TCIRG1*), chloride channel 7 (encoded by *CLCN7*), osteopetrosis-associated transmembrane protein 1 (encoded by *OSTM1*), $\alpha_v\beta_3$-integrin, and the calcitonin receptor. RANKL also enhances the development of calcium resorption lacunae and pits (discussed later).[187] In RANKL knockout mice, loss of osteoclasts leads to osteopetrosis; because RANKL also affects differentiation and function of the immune system, these animals have thymic hypoplasia and lymph node agenesis. In humans, inactivating mutations of *TNFSF11* (encoding RANKL) and of *TNFRSF11A* (encoding RANK) result in osteopetrosis due to decreased osteoclast formation (OMIM ID: 259710 and 612310, respectively).[187] BMPs too stimulate osteoclast formation and function.[194]

By cell-to-cell interaction, RANKL expressed on the cell membrane of the stromal cell/osteoblast binds to RANK expressed on the cell membrane of osteoclast precursor cells. RANK, a member of the TNF receptor superfamily, is a 616-amino-acid protein with a signal peptide (28 amino acids), cytoplasmic domain (383 amino acids), transmembrane domain (21 amino acids), and extracellular domain (184 amino acids) that is expressed in osteoclasts, fibroblasts, and B and T lymphocytes. Binding of RANKL to RANK leads to transmission of an intracellular signal through SRC (OMIM ID: 190090), adaptor proteins that associate with RANK including TNF receptor-associated factor (TRAF) 6 (OMIM ID: 602642) and GRB2-associated binding protein (GAB) 2 (OMIM ID: 606203), and subsequently through c-Jun N-terminal kinases (JNK; members of the MAPK family) to activate NFκB and c-Fos (encoded by *FOS*), the latter a component of the activator protein-1 (AP-1) transcription factor complex (see Figure 8-11B).[195] The expression of c-Fos is regulated by Ca^{2+}/calmodulin-dependent protein kinases (CaMKs, OMIM ID: 604998) and cyclic AMP-responsive element binding protein (CREB, OMIM ID: 123810). NFκB, a family of five dimeric transcription factors of which NFκB1 and NFκB2 are crucial for osteoclastogenesis, is activated by degradation of the inhibitor of κB (IκB, OMIM ID: 164008), a protein that traps NFκB in the cytoplasm and is itself destroyed through the ubiquitination/proteasomal pathway after phosphorylation of its serine residues. Free NFκB then acts via c-Fos and AP-1 to stimulate osteoclastogenesis in concert with the 827-amino-acid master osteoclastogenic transcription factor–nuclear factor of activated T cells, cytoplasmic, calcineurin-dependent 1 (NFATC1, OMIM ID: 600489).[193,195]

Movement of NFATC1 from the cytoplasm into the nucleus is facilitated by calcineurin, a serine/threonine phosphatase activated by cytosolic Ca^{2+}; calcineurin is composed of a Ca^{2+}-binding protein and a calmodulin-binding catalytic subunit (encoded by *PPP3CA*, OMIM ID: 114105). Exposure of the nuclear localization signal of NFATC1 by dephosphorylation of its serine residues enables its translocation into the nucleus. Nuclear NFATC1 then interacts with AP.1, PU.1, MITF, and CREB in a transcriptional complex that stimulates expression of genes encoding osteoclast-specific proteins (e.g., TRAP, cathepsin K, β3 integrin, calcitonin receptor, OSCAR) and leads to terminal differentiation of osteoclasts.[195] RANKL/RANK signaling also stimulates tyrosine kinases (BTK [OMIM ID: 300300], TEC [OMIM ID: 600583]) that form an osteoclastogenic signaling complex with the adaptor protein BLNK (OMIM ID: 604515) that activates PLCγ leading to cleavage of membrane phosphatidylinositol-4,5- bisphosphate into DAG and IP_3, the latter mobilizing Ca^{2+} from intracellular storage sites within the endoplasmic reticulum resulting in increased cytosolic Ca^{2+} levels and activation of calcineurin and NFATC1.[196] In the preosteoclast, there is an essential costimulatory signaling system of immunoglobulin-like receptors through which NFATC1 is also activated. The osteoclast-associated receptor (OSCAR) and the triggering receptor expressed on myeloid cells (TREM)-2 are transmembrane costimulatory receptors expressed on the plasma membrane of preosteoclasts whose ligands are sequences of amino acids (. . . GPOGPX(GFX(. . .) derived from collagens types I, II, and III of extracellular matrix to which preosteoclasts are exposed at sites of bone formation (see Figure 8-11B).[195] The costimulatory receptors associate with intracellular adapter proteins that contain an immunoreceptor tyrosine-based motif (ITAM) such as the Fc receptor common γ subunit (FcRγ, OMIM ID: 147139) and DNAX-activating protein of 12 kDa (DAP 12, OMIM ID: 604142) that are phosphorylated by RANKL/RANK signaling. The complex of costimulatory receptors and ITAM-containing intracellular factors contribute to activation of NFATC1 by enhancing PLCγ activity working through spleen tyrosine kinase (SYK, OMIM ID: 600085) and BLNK, thus integrating the functions of the RANKL/RANK and OSCAR/ITAM signal transduction

systems.[193,195,196] Interestingly, NFATC1 autoamplifies its own expression by linking to an NFAT-binding site within the promoter region of *NFATC1*, a site that is activated by epigenetic mechanisms that regulate histone acetylation and methylation.[193]

During osteoclast differentiation, preosteoclast cell membranes fuse, forming the mature multinucleated giant cell osteoclast. Maturing osteoclasts develop circumferential podosomes (adhesion structures with abundant actin that can degrade matrix) that are critical for cell-cell fusion, a process requiring tyrosine phosphorylation of the adaptor proteinTks5 by PI3K and Src.[197,198] Syncytin-1 (OMIM ID: 604659), a plasma membrane protein, also plays a role in osteoclast fusion, as do f-actin and adhesion factors such as E-cadherin and integrins.[199,200] In addition to the contribution of the endoplasmic stores of Ca^{2+} to cytosolic Ca^{2+} concentrations, extracellular Ca^{2+} enters the differentiating and mature osteoclast through transient receptor potential (TRP) cation channels 4 and 5.[193] TRPV4 plays a role in osteoclast differentiation, whereasTRPV5 is important for the functional activity of the mature osteoclast. In the subosteoclast resorption lacuna, Ca^{2+} levels exceed 40 mM (serum Ca^{2+} values range between 1.1 and 1.3 mM); consequently, as intraosteoclast Ca^{2+} concentrations rise, bone resorbing activity is inhibited, and the osteoclast ages and dies. Concurrently, the high Ca^{2+} environment around the resorption lacuna attracts osteoblasts that produce the bone needed to fill the lacuna. Thus, the processes of bone resorption and formation are likely coupled by lacunar levels of Ca^{2+}.

OPG (OMIM ID: 602643) is a member of the TNF receptor superfamily and is synthesized and secreted by the stromal cell/osteoblast; it acts as a "decoy" receptor by binding to RANKL, thus inhibiting the interaction of RANKL and RANK and thereby osteoclastogenesis.[201] The 5 exon gene (*TNFRSF11B*) encoding human OPG is expressed also in the lung, liver, heart, kidney, intestinal cells, brain, thyroid, lymphocytes, and monocytes. Human OPG is synthesized as a 401-amino-acid propeptide; after cleavage of the 21-amino-acid signal peptide the mature protein of 380 amino acids contains 4 cysteine-rich amino terminal domains and two carboxyl terminal "death" domains; it is glycosylated and released into the paracellular space as a disulfide-linked homodimer. The synthesis of OPG is enhanced by IL-1α and -1β, TNFα, TNFβ, BMP-2, TGFβ, and estrogen, and it is antagonized by calcitriol, glucocorticoids, and PGE_2. Through binding to RANKL on the surface of the stromal cell/osteoblast or to its circulating secreted form, OPG inhibits the osteoclast activating and bone reabsorbing effects of calcitriol, PTH, and the interleukins. Overexpression of OPG in transgenic mice leads to osteopetrosis, whereas its knockout is associated with loss of cortical and trabecular bone and osteoporosis, multiple fractures, and hypercalcemia; the latter model is the experimental counterpart of juvenile Paget disease (OMIM ID: 239000).[202] OPG also binds to and neutralizes the TNF-related apoptosis-inducing ligand (TRAIL), a product of T lymphocytes that transduces apoptotic signals, and thus OPG is also likely to be a cell survival factor.[201]

When mature osteoclasts attach to bone, the inferior surface of the osteoclast develops a ring structure composed of β-actin filaments and $\alpha_v\beta_3$ integrins that bind to matrix-embedded osteopontin and other components that contain the amino acid sequence—Arg-Gly-Asp (RGD)—thereby forming a sealed zone and creating an isolated microenvironment between the osteoclast's inferior cell membrane and the outer bone surface, the resorption lacuna (Figure 8-12).[187] The inferior or apical surface of the osteoclast within the sealed zone develops an irregular ruffled border through which are secreted products of the

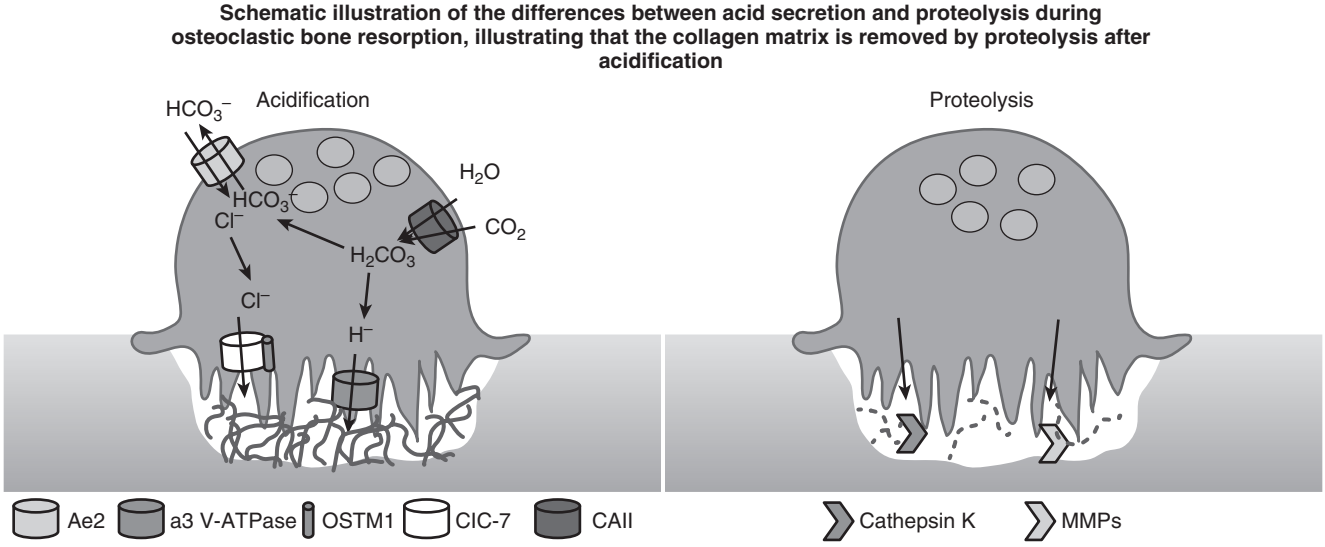


FIGURE 8-12 ■ Differentiated osteoclasts form a ruffled border by adhering to bone surface through αvβ3 integrin receptors. A subosteoclast lacuna is formed by the dissolution of bone mineral by osteoclast secreted hydrochloric acid (left panel) and the resorption of organic bone matrix by cathepsin K and matrix metalloproteins (right panel). Subsequently, osteoblasts are attracted to this pit (by the high local Ca^{2+} concentrations) as new bone is formed in the continuing process of bone remodeling. (Reproduced from Henricksen, R., Bollerslev, J., Everts, V., & Karsdal, M. A. (2011). Osteoclast activity and subtypes as a function of physiology and pathology: implications for future treatments of osteoporosis. *Endocr Rev, 32*, 31–63, with permission.)

osteoclast. Into the isolated sealed resorption lacuna the osteoclast pumps (1) acid (H^+ or protons) generated from carbon dioxide by carbonic anhydrase II (encoded by *CA2*) and actively transported via a vacuolar type ATPase proton pump (encoded by *TCIRG1*) and chloride ions passively transmitted through a chloride channel (encoded by *CLCN7*) that functions as a proton-chloride antiporter to form a highly acidic (pH 4.5) milieu that dissolves hydroxyapatite, the mineral phase of bone, and (2) lysosomal proteolytic enzymes (such as the cysteine proteases—cathepsins K, B, and L—and collagenases such as matrix metalloproteinase [MMP] −9 [OMIM ID: 120361]) that digest osteoid, the protein matrix of bone.[187,203] Osteopetrosis-associated transmembrane protein 1 (encoded by *OSTM1*) is a 334-amino-acid protein that is essential for normal processing of CLCN7 and hence for normal acidification of the subosteocytic resorption lacuna and consequent dissolution of hydroxyapatite. Osteopetrosis associated with large numbers of osteoclasts develops in patients with loss-of-function mutations in the genes encoding carbonic anhydrase II (OMIM ID: 259730), the vacuolar ATPase proton pump (OMIM ID: 259700), the chloride channel (OMIM ID: 166600, 611490), or its associated protein, OSTM1 (OMIM ID: 259720), whereas inactivating mutations in the gene encoding cathepsin K lead to pycnodysostosis (OMIM ID: 265800).[187] After the osteoclast contacts bone, it functionally polarizes into two realms: the inferior portion of the osteoclast above the ruffled membrane transports protons, chloride ions, and enzymes from the interior of the cell into the subcellular space and reabsorbs the products degraded by these agents; the superior portion of the osteoclast processes and excretes the reabsorbed materials. TRPV5, the calcium channel required for intestinal and renal tubular absorption/reabsorption of calcium, is also found on the ruffled membrane of the osteoclast.[22] Experimental disruption of *Trpv5* leads to osteoclast dysfunction and impaired ability to reabsorb calcium from bone. Paradoxically, however, *Trpv5*$^{-/-}$ mice are not osteopetrotic; rather, they are osteopenic due to excessive renal loss of calcium. *Trpv4* is also expressed in osteoclasts, and *Trpv4*$^{-/-}$ mice are osteopetrotic as osteoclast maturation is impeded. Multiple osteoclast subtypes have been described whose individual function depends on the genes the osteoclast expresses, the anatomic location of the osteoclast, and the functional response of the osteoclast to exogenous agents.[187] Thus, there are subtle distinctions between endochondral and membranous bone osteoclasts, trabecular and cortical osteoclasts, and osteoclasts involved in targeted and stochastic bone remodeling. (Targeted bone remodeling occurs locally and involves replacement of damaged bone by new bone in order to maintain bone strength; stochastic bone remodeling occurs systemically in response to PTH released as the Ca^{2+}_e concentration falls; it maintains and perhaps increases bone integrity.)

$PTH^{1\text{-}84}$, PTHrP, calcitriol, thyroid hormone, IL-1β, -3,-6, and -11, TNFα, PGE_2, and glucocorticoids stimulate expression of RANKL and M-CSF and depress that of OPG and hence favor osteoclast development and bone resorption. Calcitonin, estrogen, interferon-γ, IL-4, -10, and -18, TGFβ, and bisphosphonates antagonize these processes.[204] Estrogens, acting through nuclear ERα, maintain and augment bone mass by inhibiting its dissolution by suppressing T-cell production of osteoclast activating cytokines such as IL-1, -6, and TNFα and by suppressing expression of RANKL and increasing that of TGFβ. Additionally, estrogens interfere with RANKL/RANK–stimulated intracellular signal transduction through MAPK and also down-regulate expression of *ITGB3* (encoding $\alpha_v\beta_3$ integrin).[187] Calcitonin acting through its GPCR dissociates the processes of bone resorption and bone formation by transiently depressing the bone-resorbing activity of osteoclasts; it does so by impeding formation of the ruffled membrane. Although osteoclasts do not express *PTH1R*, $PTH^{1\text{-}84}$ enhances osteoclastogenesis by stimulating osteoblast and stromal cell production of RANKL. However, when $PTH^{1\text{-}34}$ is administered intermittently, it increases osteoblast activity and the rate of bone formation and slows the rate of osteoblast apoptosis, functioning in part through IGF-I. Interestingly, carboxyl terminal fragments of $PTH^{1\text{-}84}$ can inhibit osteoclast formation and function and antagonize the osteoclast stimulating effects of $PTH^{1\text{-}84}$, calcitriol, prostaglandins, and interleukins.[75] GH acting through IGF-I increases bone mass both by increasing bone size as well as the size and strength of muscles; it also stimulates intestinal calcium absorption (through activation of 25-hydroxyvitamin D-1α-hydroxylase and synthesis of calcitriol) and renal phosphate retention.

Bone modeling is accomplished by the independent action of osteoblasts and osteoclasts and is not dependent on prior bone resorption.[152] Bone remodeling is the process during which the strength, structure, and function of bone are renewed; bone resorption and deposition are sequentially linked. Bone remodeling is accomplished within the BRU of designated osteoclasts and osteoblasts; it is a continuous process in which old cancellous and cortical bone is reabsorbed and replaced by new bone and takes place in the growing as well as the mature skeleton. The BRU is 1 to 2 mm in length, 0.2 to 0.4 mm in width, led by osteoclasts, and trailed by osteoblasts; in the adult skeleton the life span of the BRU is 6 to 9 months and 10% of the skeleton is turned over each year. The site selected for remodeling is targeted by osteocytes sensing a mechanical or stress defect. Bone remodeling occurs in four stages: (1) *activation*, in which osteoclast precursor cells target a resting bone surface, evolve into osteoclasts, convert the area into a BRU composed of a subosteocytic bone resorption lacuna, and initiate (2) bone *resorption*, during which the mineral phase of bone is solubilized by acid and the protein component degraded by proteases; when complete the osteoclast dies; this phase is followed by (3) *reversal*, in which monocytes, osteocytes, and osteoblasts attracted to the BRU through their detection of the high Ca^{2+} concentrations in the resorption lacunae and by growth factors (IGF-I and -II, TGFβ, BMP) released from the matrix or secreted by osteoclasts enter the area of reabsorbed bone and initiate (4) renewed bone *formation*, by secreting osteoid, increasing local concentrations of calcium and phosphate to levels that exceed their solubility and degrading ASARM peptides, pyrophosphates, and proteoglycans that inhibit mineralization. The majority of osteoblasts and osteoclasts in the BRU are eliminated by programmed cell death, apoptosis, whereas some osteoblasts develop into osteocytes.

Bone Extracellular Matrix and Mineralization

Organic matrix proteins comprise 35% of bone, and type I collagen makes up 90% of these proteins.[179] Type I collagen is composed of a coiled triple helix of two polypeptide chains of collagen type I(α1) and one of type II(α2) that are cross-linked intra-molecularly by disulfide bonds and intermolecularly at the amino (N) and carboxyl (C) telopeptides by pyridinium compounds that permit bundling of collagen molecules into fibrils and fibers (Figures 8-13 and 8-14).[205,206] Every third amino acid in the collagen α peptides is glycine, which permits the chains to coil; proline, hydroxyproline and hydroxylysine are also incorporated in large quantities. Proline is hydroxylated to 4-hydroxyproline and 3-hydroxyproline by prolyl 4-hydroxylase and prolyl 3-hydroxylase-1 (P3H1), respectively; lysine is hydroxylated by lysyl hydroxylase and some hydroxylysine residues are glycosylated. P3H1 (also termed leprecan, encoded by *LEPRE1*) is an enzyme

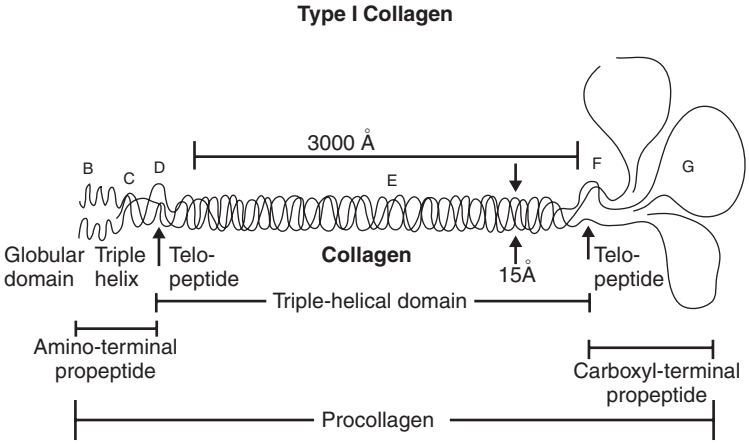


FIGURE 8-13 ■ Type I collagen is synthesized in the endoplasmic reticulum of osteoblasts as a larger procollagen molecule with amino (N) and carboxyl (C) terminal extensions that are cleaved into carboxyl (PICP) and amino (PINP) terminal propeptides after secretion of procollagen type I into extracellular matrix. Mature type I collagen is a coiled triple helix of two polypeptide chains of collagen α1(I) and one of α2(I) that are cross-linked intramolecularly by disulfide bonds and intermolecularly at the N and C terminals by nonhelical pyridinium telopeptides. (Reproduced from Byers, P. H. (1995). Disorders of collagen biosynthesis and structure. In C. R. Scriver, A. L. Beaudet, W. S. Sly, D. Vale (Eds.), *The metabolic and molecular bases of inherited disease* (pp. 4029–4077) (7th ed.). New York: McGraw-Hill, with permission.)

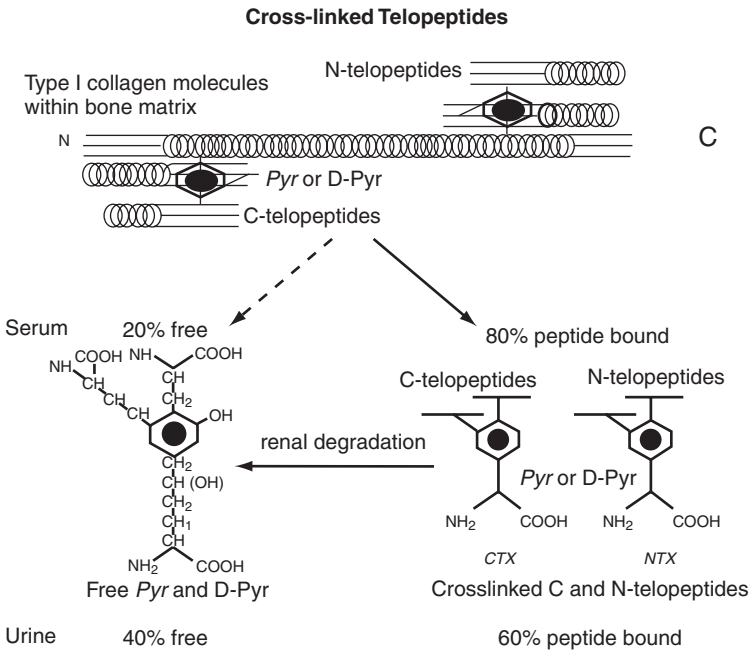


FIGURE 8-14 ■ Pyridinium and telopeptides of collagen. Pyridinoline (Pyr, hydroxylysyl-pyridinoline) and deoxypyridinoline (D-Pyr or Dpd, lysyl-pyridinoline) form nonreducible pyridinium cross-links between mature collagen fibers rendering them insoluble. Amino (N-) and carboxyl (C-) terminal regions (NTX, CTX) of collagen are proteolytically removed during degradation of mature collagen and secreted into extracellular space and serum. The carboxyl terminal telopeptide of type I collagen (ICTP) is a marker of collagen type I degradation. (Reproduced from Garnero, P., & Delmas, P. D. (1998). Biochemical markers of bone turnover: applications for osteoporosis. *Endocrinol Metab Clin NA, 27*, 303–323, with permission.)

that specifically hydroxylates *only* the proline residue at codon 986 in bone collagen type I(α1), a reaction that requires interaction of P3H1 with cartilage-associated protein (encoded by *CRTAP*) and cyclophilin B (encoded by *PIPB*). *CRTAP* is expressed in the proliferative zone of developing cartilage and at the chondro-osseous junction; cyclophilin B is a peptidyl-prolyl cis-trans isomerase. That posttranslational modifications of collagen type I(α1) are essential for normal bone formation is evidenced by the association of inactivating mutations of *CRTAP* with osteogenesis imperfecta types IIB (OMIM ID: 610854) and VII (OMIM ID: 610682) and of *LEPRE1* with osteogenesis imperfecta type VII.[207,208,209] Amino and carboxyl terminal extensions of the propeptides of collagen are removed by proteolysis during formation of the mature collagen molecule and are partially secreted into extracellular space and serum. Pyridinoline (PYR; hydroxylysyl-pyridinoline) and deoxypyridinoline (DPD; lysyl-pyridinoline) form nonreducible pyridinium crosslinks between mature collagen fibers thus making them insoluble (see Figure 8-14). Type I collagen predominates in bone but is also present in ligaments, tendons, fascia, and skin. Type II cartilage is composed of three procollagen type II(α1) chains and is primarily deposited in cartilage. Type III collagen (three procollagen type III[α1] chains) is present in bone, tendons, arteries, and intestine, and type IV (three procollagen type IV[α1]) cartilage is a component of cell basement membranes.

Measurement of serum (and urine) concentrations of markers of bone formation and bone resorption provide information about bone turnover in adults and children (Figure 8-15).[210] Assessment of serum levels of soluble (s) RANKL and OPG reflect osteoblast function as related to osteoclastogenesis.[211] Serum levels of soluble RANKL are higher in male children and adolescents and increase with age in both genders; the median concentration in males is 0.27 pmol/L (range nondetectable to 0.94) and in females it is 0.08 pmol/L (range nondetectable to 1.42). Serum concentrations of OPG do not vary with age or gender in children and adolescents; the median concentration of OPG is 3.7 pmol/L (range 1.02 to 6.63). Determination of the procollagen I extension peptides

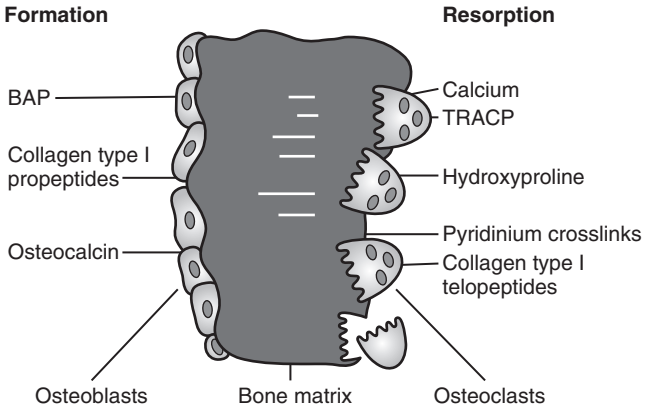


FIGURE 8-15 ■ Biochemical markers of bone formation and resorption. (Reproduced from Jurimae, J. (2010). Interpretation and application of bone turnover markers in children and adolescents. *Curr Opin Pediatr, 22,* 494–500, with permission.)

procollagen type I carboxyl terminal propeptide (PICP) and procollagen type I amino terminal propeptide (PINP) reflect collagen synthesis and thus osteoblast function, as does determination of the osteoblast products: osteocalcin and bone-specific alkaline phosphatase. Degradation of mature bone matrix collagen type I by osteoclast-secreted cathepsin K and matrix metalloproteinases releases pyridinoline (PYR), deoxypyridinoline (DPD), and the amino terminal (NTX) and carboxyl terminal (ICTP) telopeptides of type I collagen. The urinary excretion of hydroxyproline and hydroxylysine and measurements of PYR, DPD, NTX, CTX, and ICTP in urine or serum reflect catabolism of collagen type I and thus bone resorption. Osteoclast activity is also reflected by measuring serum concentrations of tartrate-resistant acid phosphatase type 5b isoform (TRAP5b).[210] In the normal pregnant woman, serum concentrations of sRANKL, OPG, and ICTP are highest in the second trimester, whereas osteocalcin values are maximal in the first trimester, suggesting that early in normal pregnancy the rate of bone formation is increased, whereas in the second trimester, the rate of bone resorption is amplified.[212] Serum levels of markers of both bone formation and resorption are higher in the fetus than mother (Table 8-5).[212-214] Fetal umbilical cord plasma PICP concentrations are highest in midgestation and decline in the last trimester to term values; after birth PICP values fall in preterm neonates during the first 3 days of life and then increase steadily to peak values at 36 weeks postconceptual age; PICP levels in cord plasma are higher in males than females and correlate with gestational age and birth weight.[214] In general, values of bone formation and resorption markers are highest in infants, fall during childhood, increase slightly during adolescence, and then decline to adult levels (Tables 8-6A and 8-6B).[210,215-220] In children and adolescents, serum concentrations of markers of bone turnover are unrelated to the body mass index (BMI); however, age and gender significantly influence values of serum bone markers with higher levels in younger subjects and earlier decline in values in females than in males.[218]

Serum concentrations of sclerostin reflect the function of osteocytes but vary with the assay employed.[221] Values of serum sclerostin are generally higher in healthy adult males than females (50 versus 37 pmol/L) and rise two to fourfold with aging; depending on age and gender, serum concentrations of sclerostin may correlate positively with bone mineral content and density (BMC, BMD) and negatively with serum levels of calcium, bone alkaline phosphatase, PINP, osteocalcin, and ICTP. Sclerostin values are decreased by physical activity and increased by immobilization. They are elevated in patients with hypoparathyroidism and decline after intermittent administration of PTH[1-34].[222-226] Serum sclerostin concentrations are higher in boys (median ~23 pmol/L) than girls (median ~19 pmol/L), peak at 10 years of age in girls and 14 years in boys, and fall during puberty in both sexes.[219]

The adult skeleton is composed of mineral (50% to 70%), organic matrix (20% to 40%), water (5% to 10%), and lipids (< 3%). Ten to 15 percent of bone matrix is composed of noncollagenous peptides secreted by the osteoblast including proteoglycans (chondroitin sulfate,

TABLE 8-5 **Markers of Bone Formation and Resorption in Pregnant Women and Neonates**

Bone Formation					
	Trimester			**Full-Term Cord**	
	1	2	3		
Osteocalcin	4.47	2.02	1.56	3.3	
ng/mL ± SD	2.75			0.3	
Bone alkaline	273	69			
Phosphatase IU/L	11	12			
				M	F
PICP				822	824
ng/mL (range)				475-1420	246-1450
Bone Resorption					
sRANKL	0.59	1.17	0.76		
pmol/L (< SD)	0.38	0.54	0.41		
OPG	3.50	6.64	3.52		
pmol/L (< SD)	1.51	0.12	0.36		
ICTP	1.23	1.71	0.89	2.7	4.6
ng/mL (< SD)	0.41	0.56	0.29	0.2	0.1

Compiled and adapted from Jurimae, J. (2010). Interpretation and application of bone turnover markers in children and adolescents. Curr Opin Pediatr, 22, *494–500; Wasilewska, A., Rybi-Szuminska, A. A., & Zoch-Zwierz, W. (2009). Serum osteoprotegerin (OPG) and receptor activator of nuclear factor κB (RANKL) in healthy children and adolescents.* J Pediatr Endocrinol Metab, 22, *1099–1104; Dorota, D., Bogdan, K. G., Mieczyslaw, G., et al. (2012). The concentrations of markers of bone turnover in normal pregnancy and preeclampsia.* Hypertens Pregnancy, 31, *166–176; Yamaga, A., Taga, M., Hashimoto, S., & Ota, C. (1999). Comparison of bone metabolic markers between maternal and cord blood.* Horm Res, 51, *277–279; Seibold-Weiger, K., Wollmann, H. A., Ranke, M. B., & Speer, C. P. (2000). Plasma concentrations of the carboxyterminal propeptide of type I procollagen (PICP) in preterm neonates from birth to term.* Pediatr Res, 48, *104–108.*

heparan sulfate), glycoproteins, growth stimulating proteins (BMP, TGFβ, IGF-I), cell attachment peptides (integrin ligands: osteopontin, osteonectin, fibronectin), and γ-carboxylated (Gla) proteins or proteins derived from serum (e.g., albumin).[179] Macromolecular proteoglycans are composed of glycosaminoglycans (acidic polysaccharide side chain) linked to a core protein and are important for normal synthesis of collagen and bone development. Osteonectin (encoded by *SPARC*) is a phosphorylated, 32-kDa glycoprotein that binds to Ca^{2+} in hydroxyapatite and to collagen fibrils enabling calcification of bone matrix. Bone alkaline phosphatase is an isoform of tissue nonspecific alkaline phosphatase (encoded by *ALPL*), an 80-kDa glycoprotein essential for bone mineralization (discussed earlier). Osteopontin (also termed bone sialoprotein or secreted phosphoprotein 1 and encoded by *SPP1*) is a 75-kDa sulfated and phosphorylated glycoprotein that contains the amino acid sequence—Arg-Gly-Asp (RGD)—necessary for linkage to integrins and hence for attachment of osteoclasts to bone; it also binds Ca^{2+} and hydroxyapatite and may play a role in the initiation of bone matrix mineralization. Osteopontin is secreted by osteoblasts in response to calcitriol. Osteocalcin (encoded by *BGLAP*) is a 49-amino-acid, γ-carboxylic acid–containing 6-kDa peptide that plays an essential role in bone mineralization; it is produced only by osteoblasts in response to BMP-7 and calcitriol acting through RUNX2, whereas its posttranslational synthesis is dependent on vitamin K. Interestingly, osteocalcin also regulates synthesis of testosterone by the testicular Leydig cell and stimulates release of insulin by pancreatic beta cells and adiponectin by white adipose tissue.[177,227,228]

Bone Mineralization

Although calcium and phosphate are costored in extracellular and intracellular fluid compartments, they are prevented from precipitating by inhibitors of precipitation such as polyphosphates (polymeric groups of phosphate anions linked together by oxygen bridges in linear chain or cyclic ring structures), pyrophosphate (the first member of a series of polyphosphates that is composed of two phosphate groups linked by an oxygen bridge), organic ion chelators (oxalate, citrate), and proteins (e.g., osteocalcin, osteopontin, SIBLING proteins).[6] (Pyrophosphate is generated by ectonucleotide pyrophosphatase/phosphodiesterase 1 [encoded by *ENPP1*] mediated cleavage of nucleotides.) Controlled formation and deposition of hydroxyapatite into the extracellular matrix of cartilage (collagen types II and X) and bone (collagen type I) occurs when the inhibitors of biomineralization are degraded locally. In the first phase of bone formation, hypertrophic chondrocytes and osteoblasts initiate bone crystal formation by generating 100-nm subsurface matrix vesicles containing calcium, phosphate, TNSALP, calbindin-D_{9K}, carbonic anhydrase, pyrophosphatases, osteocalcin, and osteopontin. TNSALP, synthesized by chondrocytes and osteoblasts and packaged within vesicles, is tethered

TABLE 8-6A Markers of Bone Formation and Resorption in Male Children and Adolescents (2.5 to 97.5 percentiles)

Bone Formation					
Age (years)	6-15	> 15			
Alk Ptase (U/L)	136-414	50-251			
Age (years)	6-9	> 9-11	> 11-15	> 15	
BAP (U/L)	51-164.3	65.6-138.2	45.5-208.4	13.1-80	
Age (years)	6-9	> 9-15	> 15		
OC (ng/mL)	56.5-152.1	48.2	226.4		
Age (years)	6-11	> 11-14	> 14-15	> 15	
TPINP (ng/mL)	407-1079	339-1399	> 1217	61-718	
Bone Resorption					
Age (years)	6-9	> 9-15	> 15		
β-CTX (ng/mL)	2.05-2.38	1-2.9	0.50-2.43		
Bone age (years)	6-8	9-11	12-14	15-17	18-21
PINP (ng/mL)	295	311	319	251	124
(∀ = SE)	13	10	11	14	10
β-CTX (ng/mL)	1.9	1.8	2.4	1.8	1.2
(∀ = SE)	0.2	0.2	0.2	0.2	0.1
PTH (pg/mL)	37.2	26.7	36.8	36.4	24.7
(∀ = SE)	2.9	3.3	4.3	6.2	3.5
25OHD	31.2	30.8	26.8	27.9	29.2
(∀ = SE)	4	1.3	1.2	2	2.8

Alk Ptase, alkaline phosphatase; BAP, bone alkaline phosphatase; OC, osteocalcin; TPINP, total procollagen N-terminal peptide; ICTP (β-CTX); β-Crosslaps, carboxyl terminal cross-link telopeptide of type I collagen.

Compiled and adapted from Huang, Y., Eapen, E., Steele, S., & Grey, V. (2011). Establishment of reference intervals for bone markers in children and adolescents. Clin Biochem, 44, *771–778; Kirmani, S., Christen, D., van Lenthe, G. H., et al. (2009). Bone structure at the distal radius during adolescent growth.* J Bone Miner Res, 24, *1033–1042.*

TABLE 8-6B Markers of Bone Formation and Resorption in Female Children and Adolescents (2.5 to 97.5 percentiles)

Bone Formation					
Age (years)	6-11	> 11-14	> 14-15	> 15	
Alk Ptase (U/L)	157-359	60-401	50-232	26-110	
Age (y)	6-11	> 11-14	> 14-15	> 15	
BAP (U/L)	23.5-151.1	20.8-172.3	12.6-105.8	8.1-43.9	
Age (years)	6-10	> 10-14	> 14-15	> 15	
OC (ng/mL)	61.4-136.2	24.1-232.1	17.8-119.6	21.1-76.7	
Age (years)	6-10	> 10-11	> 11-14	> 14-15	> 15
TPINP (ng/mL)	411-1022	— 1451	109-1346	38-510	49-277
Bone Resorption					
Age (years)	6-10	> 10-14	> 14-15	> 15	
ICTP (ng/mL)	0.82-2.06	0.49-2.76	0.12-1.73	> 1.59	
Bone age (years)	6-8	9-11	12-14	15-17	18-21
PINP (ng/mL)	273	275	263	143	89.1
(∀ = SE)	5	9	8	15	8
β-CTX (ng/mL)	2.3	2,2	2.3	1.1	0.8
(∀ = SE)	0.1	0.1	0.2	0.1	0.1
PTH (pg/mL)	30.8	24.6	38.8	29.4	28.4
(∀ = SE)	3.5	2	4.2	4.6	3.5
25OHD	25.5	23.9	19.9	22.4	22.1
(∀ = SE)	1.8	1.4	0.9	1.6	1.9

Alk Ptase, alkaline phosphatase; BAP, bone alkaline phosphatase; OC, osteocalcin; TPINP, total procollagen N-terminal peptide; ICTP (β-CTX); β-crosslaps, carboxyl terminal cross-link telopeptide of type I collagen.

Compiled and adapted from Huang, Y., Eapen, E., Steele, S., & Grey, V. (2011). Establishment of reference intervals for bone markers in children and adolescents. Clin Biochem, 44, *771–778; Kirmani, S., Christen, D., van Lenthe, G. H., et al. (2009). Bone structure at the distal radius during adolescent growth.* J Bone Miner Res, 24, *1033–1042.*

to the vesicular wall through the complex glycolipid, glycosylphosphatidylinositol. After the multipass transmembrane protein ANK (OMIM ID: 605145) transports pyrophosphate into the extracellular matrix, TNSALP cleaves inorganic phosphate from pyrophosphate and other polyphosphates, thereby increasing local phosphate concentrations. Phospho 1 (encoded by *PPP1R1B*) is a phosphatase present within matrix vesicles that when extruded from the matrix vesicle raises local levels of phosphate by releasing it from phosphoethanolamine and phosphocholine. After initial precipitation of calcium and phosphate as amorphous calcium phosphate within the matrix vesicles themselves, its extrusion into the extracellular matrix, and further increases in local concentrations of phosphate and Ca^{2+}; the structural properties of collagen fibrils direct the formation of the hydroxyapatite crystal.[6]

Mineralization of bone is partially dependent on the extent of phosphorylation of osteopontin; when 40% of the phosphorylation sites of osteopontin are phosphorylated bone mineralization is inhibited; when 95% of its sites are phosphorylated hydroxyapatite formation is promoted.[179,229] Matrix GLA protein, an 84-amino-acid vitamin K–dependent peptide containing γ-carboxylated glutamate that is related to but distinct from osteocalcin and encoded by *MGP*, has great affinity for Ca^{2+} and inhibits precipitation of calcium and phosphate; *MGP* is expressed in arteries and chondrocytes but not in osteoblasts; in patients with biallelic loss-of-function nonsense mutations in *MGP*, there is extensive calcification of cartilage (Keutel syndrome, OMIM ID: 245150).

The strength of the bone is determined by its size (height, width, depth), mineral mass, macro- and microarchitecture, and material properties (e.g., elasticity) of collagen that in turn are regulated not only by endocrine hormones and paracrine growth factors but also by mechanical forces exerted on the skeleton by the environment (gravity) and by the muscular system.[186,230,231] Bone mass and strength are determined in part by the loads placed on bone by biomechanical forces exerted by muscles—the mechanostat model. In this model, osteocytes monitor the stresses and strains (deformations) that are the result of mechanical forces placed on it. In response to mechanical force, (1) osteocytes synthesize less sclerostin and their rate of apoptosis declines and the rate of WNT-stimulated bone formation increases, and (2) stromal cells decrease their expression of RANKL and thus the process of osteoclastogenesis slows. Additionally, in response to mechanical stress multiple growth factors generated by the osteoblast (FGFs, IGF-I, TGFβ) act in an autocrine/paracrine manner on their respective tyrosine kinase receptors expressed in the cell membrane of the osteoblast to activate the PI3K, PKB, and MAPK signal transduction systems; prostaglandins activate GPCRs, adenylyl cyclase and PKA and the cyclic AMP responsive transcription factors; PLC generation leads to increase in cytosolic $Ca^{2+}{}_i$ and osteoblast function as does influx of Ca^{2+} through L-type calcium channels in the cell membrane.[232] One of the target genes affected by mechanical stimulation is *RUNX2* (discussed earlier) whose product is essential for osteoblast differentiation and expression and synthesis of osteoblast-specific proteins (discussed earlier). Repetitive bone strain (an applied deforming force that might be compressive, lengthening, or angulating) leads to enhanced quantity and quality of bone (bone strength). It is this property that enables various exercises to increase bone mineralization at all ages and states of mobility. The mechanisms that lead to increased bone mass are inactivated by decreased weight bearing such as immobilization or decreased gravity (e.g., space flight) and lead to bone loss (disuse osteoporosis). Although bone strength is in part dependent on bone mineralization, it is the size and integrity of bone that primarily determine its strength.[233] Clinically, this paradox is illustrated by the increased rate of fractures in children with osteopetrosis or marble bone disease despite extremely dense bones with thick cortices and trabeculae.

Skeletal accretion of calcium begins early in fetal life and progresses through childhood and puberty. The skeleton accumulates 25 to 30 g of calcium in utero and accrues 1300 g by adulthood. The average total body bone mineral content (BMC) of the adult male approximates 2800 g and of the adult female 2200 g with greater values in black than white individuals. Approximately 60% of total adult bone calcium is acquired during adolescence, and 25% is acquired in the 2 years prior to and after the peak velocity of BMC accrual; 30% of the mean BMC of the adult female lumbar spine (60 g) is deposited during this interval.[234] In both males and females, peak bone mass achieved in young adulthood is inversely related to the age of pubertal onset.[235]

As evidenced by the close concordance of bone mineral status between mothers and daughters, identical and fraternal twins and siblings, 60% to 80% of the peak or maximum adult bone mass is determined by genetic factors.[236] Candidate genes through which this parental relationship might be exercised include those encoding the vitamin D, calcium-sensing, estrogen, low-density lipoprotein-related, leptin and β-adrenergic receptors, cytokines (e.g., IL-6, TGFβ) and growth factors (BMPs, IGF-I), and bone matrix proteins such as type I collagen and osteocalcin emphasizing the fact that the regulation of bone mineralization is genetically complex and heterogeneous.[237] Genome-wide association studies have identified numerous genes that likely influence bone mineralization including those encoding corticotropin-releasing hormone receptor, β-catenin, RANK, and sclerostin.[238] In addition to intrafamilial factors, race, sex, body size, and composition are important determinants of bone calcium content. In black male and female youths, there is higher whole body BMC and BMD and lumbar spine, one-third radius, total hip, and femoral neck BMD than in white, Asian, or Hispanic youth.[239] Asian female children and adolescents have lower whole body and femoral neck BMD than do white and Hispanic subjects. Hispanic males have lower lumbar spine BMD than do white and Asian youth. Radial and femoral neck (peripheral) and vertebral (axial) BMDs correlate with sex, age, height, weight, body mass index, pubertal and postpubertal hormonal status, calcium intake, and exercise in children, adolescents, and adults.[240] In young women only 16% to 21% of peak vertebral and femoral bone mass can be accounted for by weight, height, physical activity as an adolescent, and the VDR genotype, emphasizing once more the essential role of multiple factors in this process. Because adult peak bone mass is inversely related to the risk

of osteopenia and osteoporosis in later adulthood (in adults, a 10% increase in BMD reduces the risk of femoral neck fracture by 50%), it is essential that bone mass be maximized during childhood and adolescence.[241]

The rate of accumulation of bone mass increases during puberty, and peak bone mass is achieved early in the third decade of life; calcium intake accounts for perhaps 5% of accrued peak bone mass, whereas exercise may contribute 10% to 22% of peak bone mass and is, therefore, one the factors that can be modified in the pursuit of the goals of attaining and maintaining maximum peak bone mass.[242] Thirty minutes of programmed weight bearing exercise thrice weekly increases BMC of the femoral neck and lumbar spine in prepubertal boys and girls as may resistance training of moderate intensity performed 20 to 40 minutes once or twice per day, 5 days per week; high-impact, weight-bearing exercises (ballet, tennis, volleyball, gymnastics, jumping, running, soccer, rugby, ice hockey) increase mass of weight-bearing bones, particularly in children and adolescents, an effect that may long outlast the period of exercise itself.[242,243] Weight-bearing/high-impact exercises do so in part by augmenting periosteal bone acquisition and increasing the cortical thickness of long bones particularly of the legs at their most distal extremes closest to the ground where weight bearing is maximal. At peak BMC velocity, the annual gain and total accumulation (over 2 years) of total body bone mineral in active boys and girls are 80 g/year and 120 g/year, respectively, greater than in inactive adolescents. One year after peak BMC velocity, the total body, femoral neck, and lumbar spine BMCs are 9% to 17% greater in active than in relatively inactive subjects. Even greater normal activities (such as walking longer distances to school) convey long-lasting salutary effects on bone mineralization and later fracture risks.[244] The effects of diminished physical activity on bone growth and strength are clearly illustrated by children with neurologic insults that prevent normal motion (Erb paresis, hemiplegia, spinal cord insults) and thus restrain limb growth. An abrupt decrease in the mechanical load on bone (e.g., bed rest, space flight) evokes rapid loss of bone mineralization as the rate of bone formation declines, whereas that of bone resorption is maintained. In patients with limited physical activity due to neurologic or muscular insults, bone density may be increased by high-frequency, low-amplitude mechanical stimulation.[242]

Systemic hormones and growth factors (GH, IGF-I, PTH, leptin, thyroid and sex hormones, glucocorticoids) have substantial effects on chondrocyte proliferation, maturation, and function.[245] GH acting through the GH receptor increases the synthesis of BMPs, directly enhances differentiation of prechondrocytes within the resting zone of the cartilaginous growth plate, supports proliferation of chondrocytes in the reserve or resting zone, and increases local expression of *IGF1*. GH receptors are expressed in chondrocytes in the reserve, proliferative, and hypertrophic zones and mediate chondrocyte proliferation and maturation and IGF-I synthesis through the Janus kinase 2-signal transduction and transcription 5b (JAK2-STAT5b) signaling system.[246] IGF-I, expressed predominantly in proliferating chondrocytes, functioning through the IGF type 1 receptor expressed in chondrocytes in the reserve, proliferative, and hypertrophic zones stimulates the clonal expansion of committed chondrocytes.[247] IGF-I coordinates chondrocyte proliferation and inhibits their apoptosis; it modulates their differentiation and maturation and their synthesis of matrix heparan sulfate proteoglycan, a matrix component that is necessary for efficient signaling of FGFs and their receptors.[248] IGF-I also influences the interaction of Ihh and PTHrP. IGF-binding proteins (IGFBP)-1 through -6 are synthesized by growth plate chondrocytes where they regulate levels of bioactive IGF-I as well as exert direct stimulatory/inhibitory effects on chondrocyte proliferation depending on the stage of chondrocyte differentiation.[249] In utero, both IGF-I and IGF-II are essential for normal fetal growth as denoted by the in utero growth retardation experienced by the fetus with a loss-of-function mutation in *IGF1*, *IGF2*, or *IGF1R*.[245,248] Neither GH nor IGF-I is necessary for patterning of the skeleton, however. Systemic loss of GH secretion or IGF-I production or inactivation of the IGF-I receptor greatly impairs linear growth of long bones postnatally. Selective loss of hepatic IGF-I production lowers total circulating levels of IGF-I to 25% of normal but does not adversely affect growth in transgenic mice, indicating that it is IGF-I synthesized by the cartilage growth plate that affects chondrocyte division by a paracrine mechanism.[250] In patients with inactivating mutations of the GH receptor or deletion of the gene encoding IGF-I, administration of IGF-I enhances linear growth indicating that this growth factor is able to stimulate cartilage proliferation without the initial differentiating effect of GH but does so to a lesser extent than does GH in the GH deficient subject; thus, sufficient numbers of differentiated prechondrocytes are necessary for optimal IGF-I effect.[251] The GH secretagogue, ghrelin, is also synthesized and secreted by chondrocytes and affects their intracellular metabolism.[252]

Through expression of the GH receptor by osteoblasts, GH stimulates their differentiation, proliferation and function—enhancing synthesis and secretion of osteocalcin, bone-specific alkaline phosphatase, and type I collagen. GH also increases expression of IGF-I by the osteoblast as do estrogen, PTH, cortisol, and calcitriol; IGF-I is essential for GH-induced osteoblast proliferation in vitro; it also decreases expression of the GH receptor, whereas estrogen stimulates its expression.[253] In response to GH, IGFBP-3 and IGFBP-5 are synthesized by (rat) osteoblasts; IGFBP-4 expression is decreased by GH in rat and human osteoblasts. The IGFBPs may augment or restrict activity of IGF-I and IGF-II; thus, IGFBP-5 binds to bone cells, matrix, and hydroxyapatite and enhances the actions of IGF-I on bone. Expression of the GH receptor by the osteoblast is up-regulated by IGFBP inhibition of IGF-I activity. Acting through the osteoblast, GH increases osteoclast proliferation and activity; human osteoclasts express receptors for IGF-I, which also enhances osteoclast formation and activation.

In children with GH deficiency and adults with childhood-onset or adult-onset GH deficiency, BMC and areal and volumetric BMDs are decreased and increase when GH is administered.[253] Administration of

GH to the GH deficient subject is followed by increase in serum and urine levels of markers of bone formation and resorption (osteocalcin, bone-specific alkaline phosphatase, PICP, ICTP, PYR, DPD, and NTX) with maximal values achieved 3 to 6 months after beginning treatment. There is a biphasic response of bone mass during GH treatment; for approximately the first 6 months of GH administration BMD declines as bone resorption exceeds formation; BMD increases steadily to positive values over the next 6 to 12 months. In subjects with GH receptor deficiency or IGF-I gene deletion, BMC and BMD are decreased relative to control subjects but volumetric BMD is not, suggesting that bone size but not bone mineral acquisition is impaired by isolated IGF-I deficiency. Nevertheless, administration of IGF-I to subjects with deletion of *IGF1* enhances osteoblast function as documented by increased serum concentrations of osteocalcin and bone specific alkaline phosphatase, BMC, and areal and volumetric BMDs. In acromegalic patients there is increased bone turnover, and variably increased lumbar spine and femoral neck BMD and iliac crest cortical and trabecular bone mass.

Thyroid hormone receptors (TRα, TRβ) are expressed in the reserve and proliferative zones of the growth cartilage. Triiodothyronine, acting primarily through TRα, enables the differentiation of resting chondrocytes and their entrance into the proliferative phase; but there, thyroid hormone inhibits further chondrocyte proliferation and promotes differentiation to terminal hypertrophic chondrocytes and secretion of collagen type X. They do so in part by disrupting the reciprocal interaction of Ihh and PTHrP thereby accelerating chondrocyte maturation, effects mediated by FGFR3 and the STAT signaling pathway and by down-regulation of IGF-I expression in chondrocytes.[254] Thyroid hormone is essential also for vascular invasion of the growth plate's hypertrophic zone and induction of metaphyseal trabecular bone formation. Thyroid hormones are necessary for fusion of the epiphyseal cartilage plate, although fusion may occur in the absence of thyroid hormone through estrogenic action. Through osteoblast-expressed receptors for thyroid hormone, triiodothyronine increases osteoblast production of osteocalcin, bone-specific alkaline phosphatase, and IGF-I. Thyroid hormones increase the rate of bone remodeling by expanding the number of osteoclasts and sites of bone resorption and the amount of bone resorptive surface; urinary calcium excretion is increased by thyroid hormones; in excess, thyroid hormones lead to net bone loss.

Estrogen and androgens promote chondrocyte maturation.[245] Although many of the effects of androgens are mediated by their conversion to estrogens (discussed later), nuclear androgen receptors are expressed by chondrocytes, and nonaromatizable androgens stimulate chondrocyte proliferation and long bone growth. Estrogens acting through ERα and ERβ expressed in chondrocytes exert a biphasic effect on chondrocyte proliferation, increasing its rate at low doses and reducing it at higher doses; by decreasing chondrocyte proliferation and accelerating the rates of chondrocyte maturation, senescence, and apoptosis, estrogens lead to epiphyseal fusion.[255] Complete maturation and fusion of the growth plate are mediated primarily by estrogens as evidenced by the failure of growth plate fusion in young adult males with inactivating mutations of the genes encoding aromatase (the enzyme that converts androgens to estrogens) or ERα, despite adult levels of testosterone. Chondrocytes may also be capable of synthesizing estrogens from androgens as aromatase activity has been found in growth plate chondrocytes.[245] This observation suggests that locally produced as well as systemic estrogens may contribute to chondrocyte maturation and growth plate fusion. Sex hormones play major roles in the accretion of bone mineral in both females and males as the bulk of adult bone calcium stores are deposited during puberty when the peak rate of accrual of total body BMC occurs in boys and girls 0.7 years after attainment of peak height velocity (PHV) and 0.4 to 0.5 years after peak accrual of lean body mass (a surrogate measurement of muscle mass).[234,256] After controlling for size, total body and femoral neck peak BMC velocity and BMC accrual over 2 years around the PHV are greater in males than females; there is no gender effect on accrual of BMC of the lumbar spine, however. Both androgens (in part by conversion to estrogen) and estrogens markedly influence rates of bone formation and resorption, although it is the effect of estrogen that predominates as evidenced by (1) the marked osteopenia of adult males with androgen sufficiency but estrogen deficiency related to loss-of-function mutations in the genes encoding aromatase and ERα, (2) the beneficial effects of estrogen but not of testosterone on BMD in males with aromatase deficiency, (3) the very close association of BMD and serum levels of bioavailable estrogen in elderly men, and (4) the significant correlation in adult men treated with testosterone between changes in BMD and increases in serum concentrations of estradiol but not of testosterone.[255] Nevertheless, the osteopenia of adult (46XY) females with complete androgen insensitivity due to loss-of-function mutations of the X-linked androgen receptor despite elevated serum testosterone and (endogenous or exogenous) estradiol concentrations and the fragile bone structure of the (*Tfm*) mouse counterpart of this disorder indicates that androgens, too, increase bone mineralization. Furthermore, nonaromatizable dihydrotestosterone has a direct anabolic effect on bone, as it stimulates the proliferation and maturation of osteoblasts, increases the production of procollagen I(α1), and prevents bone loss in orchidectomized rats.

Estrogens increase bone mass primarily by suppressing bone resorption; they do so through inhibition of osteoclastogenesis and down-regulation of osteoblast production of osteoclast activating factors such as IL-6 (and its receptor), TNFα, and M-CSF, increasing production of osteoprotegerin, and accelerating apoptosis of mature osteoclasts.[257] Estrogens also prolong the life span of osteoblasts and osteocytes. During adolescence in the female not only does the rate of bone deposition increase, but that of bone resorption declines. There is maturation-related increase in BMC and areal and volumetric BMD and in metacarpal length, width, and cortical thickness due, in part, to decline in width of the marrow cavity. Data suggest that the pubertal increase in production of GH and IGF-I mediates, in part, longitudinal and periosteal skeletal growth and mineral acquisition during puberty. Estrogen may decrease endosteal bone resorption through inhibition of IL-6 generation, thereby

contributing to increase in cortical bone mass. Estrogens likely account for part of the pubertal growth spurt of girls and boys acting both indirectly by increasing the secretion of GH and the systemic and local production of IGF-I and directly on the chondrocyte.

The nuclear glucocorticoid receptor is expressed in chondrocytes in the proliferative and hypertrophic zones; cortisol exerts an inhibitory effect on chondrocyte proliferation, hypertrophy, synthesis of cartilage matrix, and maturation, but also delays senescence of the growth plate thereby permitting catch-up growth when excess glucocorticoid exposure is temporary.[258,259] Glucocorticoids act in part by depressing expression of the genes encoding the GH receptor, IGF-I, and IGF1R in growth plate chondrocytes and by regulating synthesis of IGFBPs and thereby indirectly function of IGF-I. Paradoxically, glucocorticoids also increase expression of *SOX9* and the earliest phase of chondrocyte differentiation. Glucocorticoids suppress osteoblastogenesis and accelerate the rate of apoptosis of osteoblasts and osteocytes, in part by suppressing expression of *BMP2* and *RUNX2*, thus depressing the rate of bone formation.[257] Glucocorticoids transiently accelerate osteoclastogenesis by promoting osteoblast synthesis of RANKL and depressing expression of *TNFRSF11B* (encoding OPG).[187] In excess, glucocorticoids decrease trabecular bone and osteoid volumes contributing to bone weakness and collapse.

Transcripts of C-type natriuretic peptide (CNP, encoded by *NPPC*) and its receptor (encoded by *NPR2*) are expressed by chondrocytes; these peptides stimulate the growth of proliferative and hypertrophic chondrocytes, enhance osteoblast function, and induce endochondral ossification.[260] CNP increase the thickness of the growth plate by enlarging chondrocyte size, signaling through the MAPK pathway; it does not affect differentiation of chondrocytes.[261] Plasma concentrations of the amino-terminal pro-C-type natriuretic peptide are positively related to growth velocity in normal children and adolescents.[262] Biallelic loss-of-function mutations in *NPR2* have been identified in patients with acromesomelic dysplasia–Maroteaux type (OMIM ID: 602875).[263] In this disorder, there is shortening and deformation of the forearms, forelegs, and vertebrae resulting in severely compromised adult stature. Increased expression of *NPPC* has been associated with an overgrowth phenotype.[261] In an experimental mouse model of achondroplasia due to a gain-of-function mutation in *Fgfr3*, increase in serum levels of CNP can promote substantial growth.[261]

After genetic influences, the factor to which bone mass is most closely related is weight. Obese children, adolescents, and adults have higher BMC and BMD values than do slimmer subjects—levels that are directly related to both lean body mass (i.e., muscle) and to fat mass.[264,265] Chondroblasts, osteoblasts, and adipocytes arise from a common mesenchymal stem cell and can be interconverted depending on which transcription factor is expressed in the stem cell (e.g., PPARγ promotes adipogenesis); consequently, the fat cell synthesizes and secretes a number of adipokines that affect bone development. Among these products is leptin (encoded by *LEP*), a 16-kDa protein with anorexigenic properties that acts within the ventromedial hypothalamic nucleus to depress appetite, increase the rate of energy utilization, enable fertility by enhancing gonadotropin secretion, and regulate thyrotropin secretion; leptin also decreases bone mineralization.[177,228] Within the central nervous system, leptin is linked to osteoblasts through the sympathetic nervous system; mediated by the β_2-adrenergic receptor expressed on the osteoblast cell membrane, leptin-stimulated increase in sympathetic input both inhibits osteoblast proliferation, hence decreasing bone formation, and increases osteoblast expression of *RANKL*, thereby favoring osteoclastogenesis. The neurotransmitter serotonin—synthesized by neurons in the nuclei of the dorsal and ventral raphe of the brainstem and transported by their axons to the ventromedial hypothalamic nucleus—there stimulates the local expression of genes whose products increase appetite such as proopiomelanocortin and the melanocortin-4-receptor that oppose the anorexigenic effects of leptin; serotonin also decreases sympathetic tone, thus antagonizing the skeletal effects of leptin and increasing bone formation in the periphery. It has been suggested that acting on the serotonin synthesizing neurons of the dorsal and ventral raphe, leptin inhibits serotonin production (or release) and thereby depresses both the accrual of bone mass and appetite.[177,228] The dual central actions of leptin on appetite and bone accrual may thus link the processes of energy metabolism and bone accretion. Another association between bone and energy metabolism is the relationship between osteocalcin and insulin; undercarboxylated osteocalcin stimulates insulin release from the pancreatic β-cell and facilitates insulin sensitivity in muscle, fat, and liver; reciprocally, insulin increases osteoblast synthesis of undercarboxylated osteocalcin.[228] Osteocalcin also stimulates Leydig cell synthesis of testosterone, acting through its GPCR and the cyclic AMP response element binding protein to increase steroidogenesis, a complementary physiologic effect to that of luteinizing hormone.[227] Adiponectin (OMIM ID: 605441), a 28-kDa product of the white adipocyte whose plasma values are inversely related to visceral fat mass, modulates osteoclastogenesis by inducing osteoblast expression of RANKL and inhibiting that of its antagonist, osteoprotegerin.[266] Experimentally, loss-of-function mutations in the gene encoding adiponectin are associated with increase in bone mass.[265] Resistin (OMIM ID: 605565) stimulates both osteoblastogenesis and osteoclastogenesis.[265] MEPE and ASARM peptides influence energy metabolism and fat mass.[54] *Phex* deficient HYP mice are hyperglycemic and hypoinsulinemic, and the rate of gluconeogenesis is increased in HYP osteoblasts. Further, acidic ASARM peptides enhance decarboxylation of osteocalcin.[54]

Assessment of Bone Mass and Strength

Bone stress is determined by the three-dimensional size (volume) of the bone, its mineral content, and its material properties such as elasticity. Bone mineralization may be directly assessed by bone biopsy and histomorphometric analysis of bone formation and resorption.[267,268] Undecalcified transiliac biopsies permit limited assessment of bone modeling (changes in bone size, shape, mass) but detailed analysis of remodeling (bone renewal) as the iliac

crest biopsy is primarily composed of trabecular bone with a circumscribed amount of cortical bone. During bone modeling, osteoblasts and osteoclasts are active on opposite bone surfaces across from one another. Thus, the bone surface may change position, size, or mass during the modeling process. Usually modeling is associated with gain in bone mass as the rate of osteoblastic deposition of bone is more rapid than is the rate of osteoclastic resorption. During bone remodeling, osteoclastic resorption of bone is followed by osteoblastic replacement of the reabsorbed bone at the same surface with a net change of zero in bone mineral at the remodeling site under normal circumstances. Histomorphometry enables quantitation of structural parameters of bone size and amount (cortical width, trabecular number, and thickness), static bone formation (thickness and surface of osteoid or unmineralized bone matrix, osteoblast surface), dynamic bone formation after labeling with a fluorochrome such as tetracycline (mineral apposition and bone formation rates), and static resorption (osteoclast number and appearance and extent of eroded surfaces). Iliac trabecular thickness but not trabecular number increases substantially between 2 and 20 years of age, whereas remodeling activity peaks in young children, declines, and then increases again during puberty.[267] Noninvasive bone biopsy is now achievable with the use of high-resolution peripheral quantitative computed tomography (HRpQCT) employing a voxyl size of 82 μm that permits construction of microfinite element (μFE) models of bone strength and enabling quantitation of both cortical and trabecular bone dimensions (discussed later).[269,270] Micro-magnetic resonance imaging (μMRI) is another noninvasive technique that allows "virtual bone biopsy."[271]

Noninvasive methods of assessment of skeletal mineralization include bone x-rays (of limited value), radiographic absorptiometry or photodensitometry, single photon or single x-ray absorptiometry, dual photon or dual energy x-ray absorptiometry (DEXA), spinal and peripheral quantitative computed tomography (pQCT), quantitative ultrasonography (QUS), high-resolution quantitative MRI, quantitative μMRI, and magnetic resonance microscopy.[233] DEXA is the most frequently employed method for quantifying axial (skull, spine) and peripheral (appendicular) bone mass and bone area as well as body composition because of its relatively low radiation dosage (5 μSv), ease of use and applicability for infants, rapidity, accuracy, precision, and reproducibility under controlled circumstances.[233,272] The ratios of attenuation of x-rays of two energies (70 kV, 140 kV) traversing the same pathway through the patient reflect the density and mass of the tissue through which the x-rays have crossed; computer analysis of these captured energies then reconstructs the boundaries, density, and mass of the tissue. Because of the variability between instruments and analytic software programs (infant, pediatric, adult) employed for DEXA, the report of the DEXA scan should include not only the recorded data but also the type of DEXA instrument and the software version employed for analysis. Because relatively low BMDs in children make it more difficult to distinguish clearly bone edges, specific infant and pediatric software must be used to address this problem.

DEXA does not measure true BMD, the mass within a volume of uniform composition that is expressed as grams of hydroxyapatite/cm^3 (g/cm^3); rather DEXA measures the two-dimensional areal or surface mass of mineral within a region of bone of nonuniform composition (cortex, trabeculae, osteoid, marrow); it is expressed as grams of hydroxyapatite/cm^2 (g/cm^2). Because DEXA does not take into account the depth of a bone, it underestimates BMD in small children and overestimates it in large subjects.[273] In children and adolescents, bone size (volume) increases with growth and maturation; the larger the three-dimensional structure of bone, the greater is the recorded areal BMD, even though the actual or volumetric (v) BMD may not change substantially. Therefore, calculated or apparent (v) BMDs (BMAD) (g/cm^3) data have been generated in an attempt to correct this problem. Volumetric BMD increases as the cortex thickens, the number and width of trabeculae per unit volume rises, and the amount of hydroxyapatite per unit of trabecular volume accrues. Although during childhood and adolescence areal BMD of the femoral shaft increases with age, its vBMD remains relatively constant. On the other hand, vBMD of the lumbar spine increases in late puberty and early adulthood because of increasing thickness of trabeculae that is not gender specific but is greater in blacks than in whites after puberty.[274] Cross-sectional areas of cortical and trabecular bone increase with age with males achieving greater increase in periosteal apposition than do females during puberty. Cortical cross-sectional area is similar in white and black subjects.[275] Included in measurement of whole body areal BMD is the skull; this structure characteristically has twice the areal BMD of that of the rest of the skeleton and may comprise as much as 20% to 50% of the total BMD in young children; whole body areal BMD correlates better with height when the skull is excluded.[276] Consequently, there are reference data for DEXA whole body BMC and BMD with inclusion or exclusion of the skull.[239,277] The influence of the skull on BMC is illustrated by the observations that in non-black 10-year-old boys, the 50th percentile for whole body BMC including the skull is 1109 g; excluding the skull the BMC of this cohort is 804 g; in non-black males at age 17 years the 50th percentile for whole body BMC including the skull is 2532 g; without the skull the 50th percentile BMC is 2055 g. Inasmuch as DEXA BMC and BMD values increase with height in both boys and girls, DEXA-derived bone mass and density data should be adjusted for height (height age) when interpreting study results in children and adolescents (and very short and very tall adults as well).[278,279]

Whole or total body BMC/BMD, lumbar spine BMC/BMD, and femoral BMD are the most commonly measured indices of bone mass in childhood by DEXA and recorded as g/cm^2. Data are often reported as a Z score, the number of standard deviations about the mean of peers matched for chronologic age, gender, and stage of pubertal development. (In adults, the DEXA T score is commonly reported; the T score is the number of standard deviations about the mean of

maximal or peak bone mass recorded in healthy young adults aged 20 to 29 years. The T score should *not* be utilized in children and adolescents.) In neonates and infants, it is recommended that whole body BMC be the primary measurement when assessing bone mineralization.[280] In neonates with appropriate weight for gestational age (AGA), whole body BMC by DEXA doubles between 32 and 40 weeks and increases 3.5-fold between birth weights of 1000 and 4000 g; small for gestational age (SGA) neonates have lower whole body BMC than do AGA neonates of comparable gestational age but they are similar to those of AGA infants with the same birth weights (Table 8-7).[281,282] In young adults (16 to 19 years) born prematurely with birth weights < 1.5 kg, total body BMC is less than that of subjects born at term with normal birth weights but appropriate for their smaller stature.[283] Areal BMD of the lumbar spine (L2 to L4) is low in prematurely born infants but "catches up" to that of full-term infants by 2 years of age.[284]

Whole body BMC by DEXA increases approximately threefold between 7 and 17 years of age and is greater in males than females and in black relative to non-black youth (Tables 8-8A and 8-8B).[239,277,285] Whole body BMC is greater in adult males than females because of the larger size of male bones. Lumbar vertebral areal BMD doubles between 5 and 17 years of age in both boys and girls (Tables 8-9A and 8-9B).[239,277] The BMD of the femoral neck increases 1.5-fold between 7 and 17 years of age in both genders.[239] The BMC of the distal one-third radius approximately doubles and its BMC increases 1.5-fold between 7 and 17 years.[239,270] At all skeletal sites, BMC and BMD assessed by DEXA are higher in males than females and in black relative to non-black children and adolescents.[239] In females, the rate of maximal increase in whole body BMC occurs in the year of menarche and follows the year of peak height velocity (Figure 8-16).[234,286] In girls with menarche before 12 years, there is greater peak BMD (at age 18 to 19 years) than in females with menarche after 14 years. BMD is greater in children with premature adrenarche than in preadrenarchal peers, but appropriate for the tall stature of the adrenarchal subjects.[278] In adult males with a history of delayed puberty, there is lower lumbar spine BMD than in those with pubertal onset at 11 to 12 years. DEXA measurements of bone mineralization are affected by the composition of soft tissues that surround the axial skeleton; variations in fat about the bone may significantly influence the recorded DEXA measurements, thus limiting the use of DEXA

TABLE 8-7 **Whole Body Bone Mineral Content (BMC, g) and Density (g/cm²) by Pencil Beam Dual Energy X-Ray Absorptiometry in Prematurely Born and Full-Term Appropriate or Small for Gestational Age Infants (AGA, SGA)**

GA	BMC				BMD	
	AGA	SD	SGA	SD	AGA	
32-33	21.9	(9.7)				
34-35	32	(11.3)				
36-37	39.4	(15.9)	24.9	(7.4)		
38-39	45.5	(18.4)	27	(11.1)		
Birth Weight		**Range**				**Range**
1001-1500	22.9	(21.7-24.1)			0.146	(0.141-0.150)
1501-2000	31.8	(30.9-32.8)			0.162	(0.158-0.165)
2001-2500	42.2	(41.7-43.2)			0.178	(0.175-0.181)
2501-3000	54.6	(53.9-55.3)			0.199	(0.196-0.202)
3001-3500	66.9	(66-67.9)			0.220	(0.217-0.224)
3501-4000	77.6	(76.3-78.8)			0.234	(0.229-0.238)
Postnatal age		**SD**				**SD**
9-90	103	(10)			0.238	(0.022)
91-150	137	(20)			0.259	(0.024)
150-270	196	(27)			0.302	(0.018)
271-390	253	(41)			0.335	(0.029)

GA, gestational age (weeks), birth weight (g), postnatal age (days) (95% range).

Compiled and adapted from Lapillone, A., Braillon, P., Claris, O., et al. (1997). Body composition in appropriate and in small for gestational age infants. Acta Paediatr, 86, *196–200; Koo, W. W. K., Walters, J., Bush, A. J., et al. (1996). Dual-energy x-ray absorptiometry studies of bone mineral status in newborn infants.* J Bone Miner Res, 11, *1997-1002; Koo, W. W. K., Bush, A. J., Walters, J., & Carlson, S. E. (1998). Postnatal development of bone mineral status during infancy.* J Am Coll Nutr, 17, *65–70.*

TABLE 8-8A Whole Body DEXA Bone Mineral Content (BMC, g) and Bone Mineral Density (BMD, g/cm^2) in Black and Non-Black Male Children and Adolescents

	Non-Black		Black	
Whole Body with Skull				
	BMC	BMD	BMC	BMD
7	834 (688-1036)	0.723 (0.636-0.828)	911 (732-1054)	0.778 (0.664-0.859)
9	1021 (838-1265)	0.797 (0.706-0.906)	1119 (874-1331)	0.857 (0.736-0.954)
11	1207 (986-1498)	0.855 (0.761-0.968)	1363 (1034-1689)	0.918 (0.794-1.027)
13	1534 (1214-1960)	0.926 (0.822-1.053)	1741 (1283-2308)	0.987 (0.846-1.126)
15	2086 (1592-2754)	1.038 (0.907-1.205)	2228 (1649-3053)	1.107 (0.921-1.314)
17	2532 (1965-3322)	1.151 (0.996-1.358)	2648 (2046-3454)	1.201 (1.027-1.400)
Whole Body Less Skull				
5	362 (268-490)		443 (309-569)	
7	563 (418-763)		637 (458-824)	
9	728 (540-986)		829 (605-1085)	
11	895 (664-1213)		1045 (757-1409)	
13	1211 (898-1642)		1411 (996-2022)	
15	1720 (1276-2331)		1918 (1381-2744)	
17	2055 (1524-2785)		2264 (1717-3023)	
20	2189 (1624-2967)		2400 (1866-3115)	

Years of age; 50th percentile (3rd to 97th percentiles).

Compiled and adapted from Kalkwarf, H. J., Zemel, B. S., Gilsanz, V., et al. (2007). The bone mineral density in childhood study: bone mineral content and density according to age, sex, and race. J Clin Endocrinol Metab, 92, *2087–2099; Zemel, B. S., Kalkwarf, H. J., Gilsanz, V., et al. (2011). Revised reference curves for bone mineral content and areal bone mineral density according to age and sex for black and non-black children: results of the Bone Mineral Density in Childhood Study.* J Clin Endocrinol Metab, 96, *3160–3169.*

TABLE 8-8B Whole Body (DEXA) Bone Mineral Content (BMC, g) and Bone Mineral Density (BMD, g/cm^2) in Black and Non-Black Female Children and Adolescents

	Non-Black		Black	
Whole Body with Skull				
CA	***BMC***	***BMD***	***BMC***	***BMD***
7	800 (659-1006)	0.695 (0.612-0.805)	884 (743-1083)	0.753 (0.657-0.834)
9	969 (798-1220)	0.767 (0.674-0.884)	1042 (856-1306)	0.817 (0.713-0.914)
11	1210 (954-1602)	0.847 (0.740-0.977)	1380 (1076-1729)	0.912 (0.796-1.036)
13	1641 (1192-2159)	0.963 (0.830-1.042)	1819 (1412-2282)	1.025 (0.895-1.192)
15	1953 (1483-2418)	1.059 (0.919-1.173)	2070 (1622-2665)	1.098 (0.960-1.304)
16	2025 (1570-2466)	1.079 (0.939-1.224)	2137 (1673-2774)	1.117 (0.977-1.336)
Whole Body Less Skull				
5	362 (268-490)		443 (309-569)	
7	563 (418-763)		637 (458-824)	
9	728 (540-986)		829 (605-1085)	
11	895 (664-1213)		1045 (757-1409)	
13	1211 (898-1642)		1411 (996-2022)	
15	1720 (1276-2331)		1918 (1381-2744)	
17	2055 (1524-2785)		2264 (1717-3023)	
20	2189 (1624-2967)		2400 (1866-3115)	

Years of age; 50th percentile (3rd to 97th percentiles).

Compiled and adapted from Kalkwarf, H. J., Zemel, B. S., Gilsanz, V., et al. (2007). The bone mineral density in childhood study: bone mineral content and density according to age, sex, and race. J Clin Endocrinol Metab, 92, *2087–2099; Zemel, B. S., Kalkwarf, H. J., Gilsanz, V., et al. (2011). Revised reference curves for bone mineral content and areal bone mineral density according to age and sex for black and non-black children: results of the Bone Mineral Density in Childhood Study.* J Clin Endocrinol Metab, 96, *3160–3169.*

TABLE 8-9A **Lumbar Spine DEXA Bone Mineral Content (g) and Lumbar Spine, Total Hip, and One-Third Radius Bone Mineral Density (BMD, g/cm²) in Black and Non-Black Male Children and Adolescents**

	Non-Black		Black	
Lumbar Spine				
CA	BMC	BMD	BMC	BMD
7	18.7 (14.1-24.3)	0.527 (0.423-0.643)	19.3 (14.1-24.5)	0.549 (0.415-0.642)
9	22,7 (17.1-29.7)	0.574 (0.459-0.703)	23.8 (17.3-30.9)	0.608 (0.458-0.727)
11	26.7 (19.9-35)	0.618 (0.489-0.758)	28.2 (20.6-38.1)	0.651 (0.492-0.794)
13	34.9 (24.6-48)	0.707 (0.540-0.865)	37.7 (27.1-55.9)	0.758 (0.580-0.961)
15	52.1 (35.2-73.2)	0.873 (0.674-1.087)	54.3 (36.7-79.6)	0.924 (0.722-1.185)
17	66.1 (46.6-89.8)	1.003 (0.800-1.273)	63.8 (44.5-90.7)	1.012 (0.866-1.287)
Total Hip				
7		0.651 (0.516-0.769)		0.720 (0.566-0.830)
9		0.709 (0.572-0.847)		0.772 (0.601-0.903)
11		0.761 (0.623-0.913)		0.829 (0.641-0.988)
13		0.838 (0.680-1.024)		0.933 (0.713-1.154)
15		0.982 (0.766-1.219)		1.053 (0.822-1.349)
17		1.078 (0.832-1.336)		1.132 (0.909-1.477)
One-Third Radius				
7		0.452 (0.393-0.515)		0.481 (0.408-0.555)
9		0.491 (0.428-0.559)		0.535 (0.455-0.622)
11		0.525 (0.457-0.600)		0.573 (0.485-0.669)
13		0.577 (0.495-0.671)		0.619 (0.517-0.721)
15		0.658 (0.558-0.774)		0.693 (0.572-0.792)
17		0.735 (0.637-0.845)		0.753 (0.629-0.841)

CA = Years of age; 50th percentile (3rd to 97th percentiles).
Compiled and adapted from Kalkwarf, H. J., Zemel, B. S., Gilsanz, V., et al. (2007). The bone mineral density in childhood study: bone mineral content and density according to age, sex, and race. J Clin Endocrinol Metab, 92, *2087–2099.*

in extremely thin or obese children.[287] Generally, BMC, areal BMD, or BMAD below −2 SDs for age and gender are considered abnormally low. However, DEXA measurements must always be interpreted in relationship to the patient's clinical findings.[273]

QCT measures volumetric BMD of both trabecular and cortical bone at any site but has been commonly applied to the lumbar spine.[274] However, the radiation dose delivered to the spine by this method is high (~30 μSv). During childhood, lumbar spine vBMD measured by QCT is similar in black and white youth; during puberty black males and females gain twice the vBMD recorded in whites with no gender difference. Although BMD values of the lumbar spine acquired by DEXA and QCT are reasonably well related, osteopenia is reported in children far more often by DEXA than by QCT, unless DEXA measurements take into account body height and bone size.[287] Peripheral (p) QCT enables quantitation of bone mineralization in distal or proximal radial, femoral, or tibial sites (regions of both cortical and trabecular bone); the delivered radiation dose (10 μSv) is low. Peripheral QCT may be determined in the non-dominant radius two thirds of the distance between its proximal and distal ends and (ultra) distally at a point that is 4% of forearm length with a reference line being drawn through the most distal aspect of the cartilage growth plate when it is open or through the middle of the ulnar border of the articular cartilage when the growth plate is closed.[288] pQCT permits measurement of total and cortical bone area, cortical thickness, BMC (mg/mm), cortical and trabecular vBMD, periosteal and endosteal circumferences, and marrow area (Table 8-10).

High-resolution pQCT (HRpQCT) measurements are obtained at the ultradistal radius in the interval between 1 and 10 mm above the proximal limit of the epiphyseal growth plate of the nondominant wrist (the site in which the majority of adolescent fractures occur) and deliver a local absorbed radiation dose of 0.065 cGy and a total radiation exposure of < 0.01 mSv.[270] HRpQCT enables quantitative measurement of cortical and trabecular bone including cortical bone vBMD, cortical bone area, cortical bone thickness (Ct.Th [μm]), periosteal and endosteal (endocortical) circumferences (μm), cortical pore volume (cortical areas of inverse density) and the "cortical porosity index" (cortical pore volume/cortical bone volume ratio), trabecular bone vBMD, trabecular

TABLE 8-9B **Lumbar Spine DEXA Bone Mineral Content (g) and Lumbar Spine, Total Hip, and One-Third Radius Bone Mineral Density (BMD, g/cm²) in Black and Non-Black Female Children and Adolescents**

	Non-Black		Black	
CA	BMC	BMD	BMC	BMD
Lumbar Spine				
7	17.6 (13.7-23.8)	0.528 (0.431-0.668)	18.5 (14.6-25.2)	0.570 (0.446-0.698)
9	21.4 (16.2-29.6)	0.578 (0.467-0.730)	22 (16.7-30.5)	0.618 (0.486-0.760)
11	27.7 (20.0-39.7)	0.660 (0.508-0.861)	31.7 (22.2-45)	0.737 (0.584-0.912)
13	41.8 (28.2-59.2)	0.833 (0.622-1.051)	45.9 (32.4-63.6)	0.911 (0.727-1.135)
15	52 (36.5-70.1)	0.958 (0.769-1.164)	54 (39.2-74.3)	1.011 (0.811-1.268)
16	54.1 (38.8-71.9)	0.982 (0.799-1.185)	56.2 (41.4-77)	1.040 (0.836-1.307)
Total Hip				
7		0.603 (0.504-0.716)		0.670 (0.570-0.804)
9		0.651 (0.543-0.785)		0.712 (0.599-0.863)
11		0.727 (0.598-0.919)		0.807 (0.667-1.004)
13		0.868 (0.675-1.085)		0.936 (0.766-1.178)
15		0.948 (0.744-1.165)		0.995 (0.800-1.283)
16		0.962 (0.758-1.178)		1.004 (0.803-1.302)
One-Third Radius				
7		0.466 (0.386-0.508)		0.477 (0.414-0.537)
9		0.485 (0.418-0.558)		0.511 (0.439-0.583)
11		0.532 (0.449-0.611)		0.570 (0.492-0.653)
13		0.610 (0.512-0.696)		0.643 (0.556-0.733)
15		0.655 (0.569-0.740)		0.682 (0.594-0.763)
16		0.668 (0.585-0.751)		0.694 (0.606-0.770)

CA = Years of age; 50th percentile (3rd to 97th percentiles).
Compiled and adapted from Kalkwarf, H. J., Zemel, B. S., Gilsanz, V., et al. (2007). The bone mineral density in childhood study: bone mineral content and density according to age, sex, and race. J Clin Endocrinol Metab, 92, *2087–2099.*

bone vBMD/total bone volume ratio (BV/TV [%]), trabecular number (Tb.N [mm^{-1}]), trabecular thickness (Tb.Th [μm]), and trabecular spacing (Tb.SP [μm]) (Table 8-11). Determination of the "cortical porosity index" affords an estimate of strength of the distal wrist. HRpQCT also permits construction of microfinite element (μFE) models of bone strength enabling calculation of strain energies and loads supported by cortical and trabecular bone and "failure loads."[270,289] Utilizing μFE models, graphic representations of the architecture of the cortex and trabeculae can be constructed. Figure 8-17 (see the website for the full color image) depicts visually three-dimensional reconstructions of cortical and trabecular bone as derived by HRpQCT examinations.[270] In boys, cortical bone vBMD, Ct.Th, BV/TV, and Tb.Th increase in late puberty. In girls, cortical bone vBMD and Ct.Th decline in midpuberty and then increase. Total bone strength increases in males and female through puberty. The percentage of load borne by cortical bone decreases and the cortical bone porosity index increases in midpuberty, coincident with the point of peak incidence of adolescent radial fractures.[270]

Quantitative ultrasonography (QUS) measures the speed of a longitudinal sound (SOS) wave as it is propagated along a bone.[233] The rate of movement of sound through bone is dependent on its microstructural and macrostructural characteristics, mineral density, and elasticity and is thought to be a measure of bone strength. It is an attractive method for assessment of bone because it does not utilize radiation, is low in cost, and the measuring equipment is portable. Transmitter and receiver ultrasound transducers placed on either side of the examination site (os calcis, patella, tibia, radius, phalanges) quantitate transmission velocity or signal attenuation and convert these observations to SOS. In a study of 1085 children and adolescents, SOS increased steeply at the tibia and radius during the first 5 years of life, more slowly between 6 and 11 years of age, and then again more rapidly during pubertal development.[290] In 3044 healthy subjects ages 2 to 21 years, phalangeal QUS SOS and bone transmission time (BTT) increased over time and with advancing adolescent development and were related to gender, age, height, and weight.[291] QUS SOS is higher in postmenarchal than in premenarchal girls; adiposity (BMI) and serum leptin levels are inversely related to SOS in pubertal girls.[292] The overlap of QUS data between various ages makes interpretation of a single SOS measurement problematic;

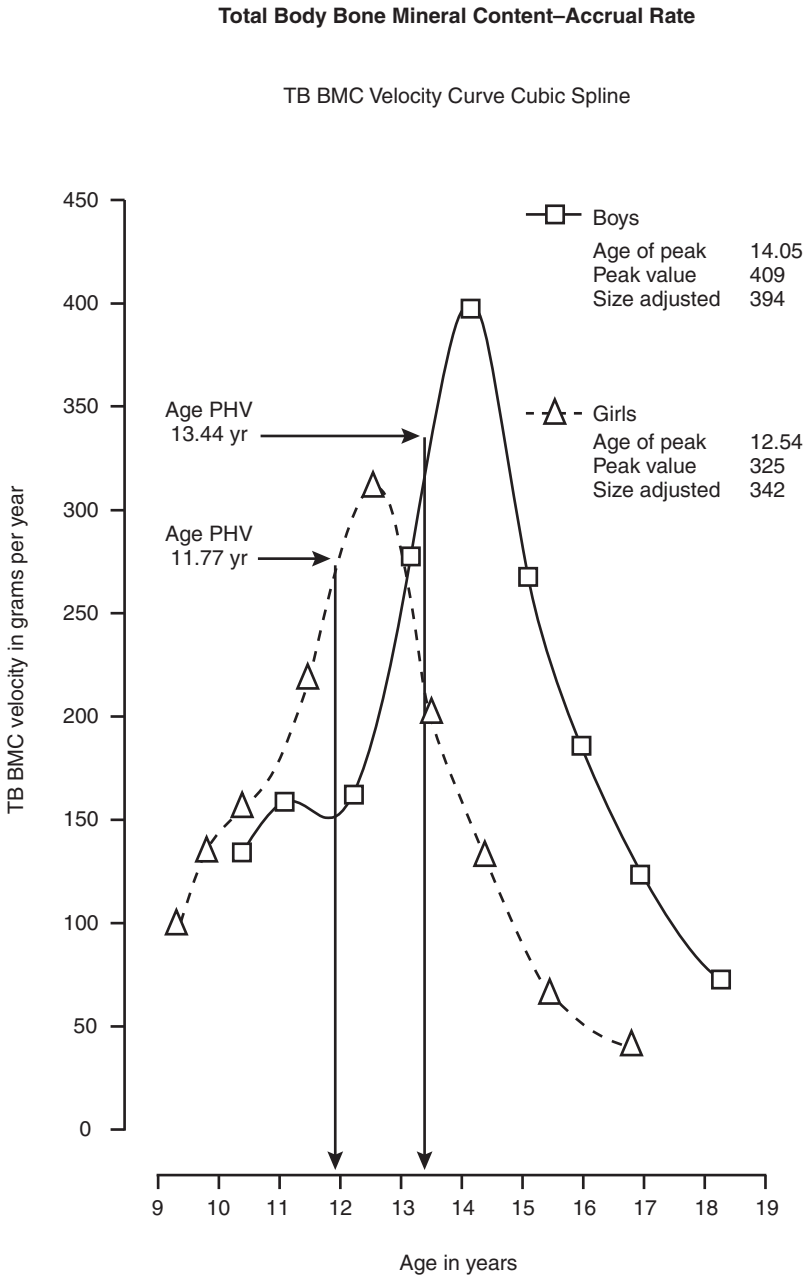


FIGURE 8-16 ■ During adolescence the peak rate of accrual of total body bone mineral content (BMC) occurs in boys and girls 0.7 years after attainment of peak height velocity (PHV). (Reproduced from Bailey, D. A., McKay, H. A., Mirwald, R. L., et al. (1999). A six-year longitudinal study of the relationship of physical activity to bone mineral accrual in growing children: the University of Saskatchewan bone mineral accrual study. *J Bone Miner Res, 14,* 1672-1679, with permission.)

serial assessment may be useful. Thus, in a cohort of 29 preterm infants, tibial SOS values declined over time in neonates whose gestational ages were less than 29 weeks, suggesting progressive loss of bone strength in this population and consistent with the development of osteopenia of prematurity.[293] Although there is marginal correlation between vBMD determined by pQCT and SOS measurements in children and adolescents, QUS may complement but is unlikely to replace assessment of mineralization by radiographic methods at this time.

Magnetic resonance imaging of the skeleton may also be utilized to assess bone geometry and estimate bone strength.[294] Bone high-resolution magnetic resonance imaging (hrMRI) of the wrist and tibia measures cortical and trabecular bone structure including trabecular number (mm^{-1}), thickness (mm), bone fraction (%), and separation (mm).[295] Further, micro MRI can be employed to measure whole bone and trabecular bone stiffness (an indicator of whole bone mechanical competence, quality, and strength) and to obtain virtual bone biopsies.[271,296]

TABLE 8-10 **Peripheral Quantitative Computed Tomography—Non-Dominant Distal Radius (mean < SD)**

Age	vBMD-tot[1]	vBMD-trab[1]	vBMD-cort[1]	CSA[2]
Males				
6-7	306 (34)	206 (32)	388 (42)	174 (31)
8-9	294 (34)	189 (34)	380 (41)	211 (31)
10-11	290 (33)	194 (32)	368 (41)	245 (37)
12-13	292 (38)	201 (36)	366 (47)	289 (47)
14-15	293 (35)	201 (33)	369 (47)	351 (70)
16-17	349 (56)	217 (30)	458 (86)	358 (49)
18-23	401 (60)	220 (42)	549 (83)	377 (64)
Adults	438 (56)	224 (46)	594 (81)	374 (45)
Pubertal Stage				
I	299 (32)	198 (31)	381 (41)	212 (47)
II	288 (40)	186 (31)	372 (52)	269 (43)
III	286 (33)	197 (36)	359 (37)	293 (44)
IV	296 (42)	210 (35)	367 (50)	334 (65)
V	361 (72)	215 (40)	481 (109)	377 (59)
Females				
6-7	290 (36)	191 (31)	370 (45)	164 (30)
8-9	283 (22)	186 (23)	362 (32)	185 (25)
10-11	281 (36)	191 (36)	355 (44)	237 (39)
12-13	295 (39)	197 (32)	376 (54)	260 (55)
14-15	303 (37)	179 (25)	407 (53)	297 (32)
16-17	350 (57)	186 (26)	483 (95)	300 (45)
18-23	371 (50)	195 (35)	516 (74)	295 (42)
Adults	395 (46)	182 (34)	569 (69)	281 (37)
Pubertal Stage				
I	284 (30)	187 (29)	363 (39)	188 (38)
II	277 (34)	190 (34)	348 (37)	239 (57)
III	288 (44)	204 (44)	356 (50)	250 (47)
IV	291 (43)	197 (32)	375 (56)	282 (30)
V	347 (54)	190 (28)	476 (87)	295 (43)

[1]mg/cm^3 [2]mm^2 CSA, Cross-sectional area.

Compiled and adapted from Neu, C. M., Manz, F., Rauch, .F, et al. (2001). Bone densities and bone size at the distal radius in healthy children and adolescents: a study using peripheral quantitative computed tomography. Bone, 28, *227–232; Rauch, F., & Schonau, E. (2005). Peripheral quantitative computed tomography of the distal radius in young subjects: new reference data and interpretation of results.* J Musculoskelet Neuronal Interact, 5, *119–126.*

MINERAL HOMEOSTASIS DURING THE LIFE CYCLE

During the first trimester of pregnancy, maternal serum concentrations of total calcium decline and remain low through gestation, whereas serum concentrations of Ca^{2+}_e remain relatively constant.[297] PTH values decline to 10% to 30% of the nonpregnant range in the first trimester of pregnancy and then rise to mid nonpregnant levels in the latter half of gestation. The secretion of PTHrP by the placenta, amnion, decidua, umbilical cord, breast, and fetal parathyroid glands increases several-fold beginning early in the first trimester, and maternal levels rise throughout gestation. Maternal calcitonin values also increase during gestation. Serum concentrations of calcidiol do not change, but maternal levels of calcitriol, synthesized primarily by the maternal kidney under the influence of PTHrP but also in part by the placenta, decidua, and fetal kidneys, increase more than twofold, enabling increased calcium absorption by the maternal small intestine. The rate of maternal bone resorption increases through gestation, whereas the rate of bone formation declines during the first trimester and then increases in the third trimester (see Table 8-5).[212,298] During a normal 40-week gestation, maternal whole body BMD does not change; cortical BMD increases (arms +2.8%, legs +1.8%), whereas trabecular BMD declines (vertebrae −4.5%, pelvis −3.2%); these affects are reversible after completion of pregnancy.[297]

TABLE 8-11 **High-Resolution Peripheral Quantitative Computed Tomography (HRpQCT) in Midadolescent Males and Young Adult Females**

Males (N = 89)		**Females (N = 15)**	
Age (in years)	15.2 ∀ = 0.5	Age (in years)	19.4 ∀ = 1.2
DEXA		DEXA	
Radial metaphysis BMD (mg/cm^2)	383 ∀ = 54	Femoral neck BMD (mg/cm^2)	830 ∀ = 110
Radial diaphysis BMD (mg/cm^2)	660 ∀ = 67	Total hip BMD (mg/cm^2)	940 ∀ = 70
Femoral neck BMD (mg/cm^2)	901 ∀ = 133	L2-L4 BMD (mg/cm^2)	1010 ∀ = 130
Total hip BMD (mg/cm^2)	992 ∀ = 139	HRpQCT	
Femoral diaphysis BMD (mg/cm^2)	1682 ∀ = 171	Ultradistal radius	
L2-L4 BMD (mg/cm^2)	918 ∀ = 135	Total area (mm^2)	265.4 ∀ = 54.1
HRpQCT		Total vBMD (mm^3)	298.2 ∀ = 52.6
Ultradistal radius		Cortical vBMD (mm^3)	825.8 ∀ = 64.6
Total vBMD (mg/cm^3)	257 ∀ = 38	Trabecular vBMD (mm^3)	158.1 ∀ = 26.6
Cortical vBMD (mg/cm^3)	637 ∀ = 73	Trabecular area (mm^2)	212.3 ∀ = 53.3
Trabecular vBMD (mg/cm^3)	195 ∀ = 27	Trabecular area (%)	79.4 ∀ = 5
BV/TV (%)	16.2 ∀ = 2.3	Tb.N (mm^{-1})	1.96 ∀ = 0.26
Tb.N (mm^{-})	2.23 ∀ = 0.20	Tb.Th (μm)	70.0 ∀ = 10
Tb.Th (μm)	72.6 ∀ = 8.4	Tb.Sp (μm)	450.0 ∀ = 70
Tb.Sp (μm)	379 ∀ = 41	Cortical area (mm^2)	47.9 ∀ = 13.2
Ct.Th (μm)	388 ∀ = 219	Cortical area (%)	18.7 ∀ = 5.8
CSA (mm^2)	333 ∀ = 61	Ct.Th (μm)	710.0 ∀ = 20
Stiffness (kN/mm)	87.2 ∀ = 21.6	Cortical perimeter (mm)	67.6 ∀ = 6.6
Failure load (N)	4239 ∀ = 996	Distal tibia	
Distal tibia		Total area (mm^2)	700.6 ∀ = 104.6
Total vBMD (mg/cm^3)	272 ∀ = 45	Total vBMD (mm^3)	308.1 ∀ = 39.6
Cortical vBMD (mg/cm^3)	730 ∀ = 56	Cortical vBMD (mm^3)	870.7 ∀ = 31.3
Trabecular vBMD (mg/cm^3)	205 ∀ = 27	Trabecular vBMD (mm^3)	192.3 ∀ = 24.7
BV/TV (%)	17.1 ∀ = 2.3	Trabecular area (mm^2)	583.0 ∀ = 106
Tb.N (mm^{-})	2.13 ∀ = 0.31	Trabecular area (%)	83.0 ∀ = 3.7
Tb.Th (μm)	81.1 ∀ = 10.6	Tb.N (mm^{-1})	1.77 ∀ = 0.26
Tb.Sp (μm)	398 ∀ = 62	Tb.Th (μm)	90.0 ∀ = 20
Ct.Th (μm)	851 ∀ = 336	Tb.Sp (μm)	480.0 ∀ = 70
CSA (mm^2)	888 ∀ = 151	Cortical area (mm^2)	116.1 ∀ = 20.5
Stiffness (kN/mm)	259.6 ∀ = 54.7	Cortical area (%)	16.9 ∀ = 4
Failure load (N)	12430 ∀ = 2559	Ct.Th (mm)	1.14 ∀ = 0.22
		Cortical perimeter (mm)	102.8 ∀ = 7.6
		(Mean < SD)	

Compiled and adapted from Ackerman, K. E., Nazem, T., Chapko, D., et al. (2011). Bone microarchitecture is impaired in adolescent amenorrheic athletes compared with eumenorrheic athletes and nonathletic controls. J Clin Endocrinol Metab, 96, *3123–3133; Chevalley, T., Bonjour, J. P., van Rietbergen, B., et al. (2011). Fractures during childhood and adolescence in healthy boys: relation with bone mass, microstructure, and strength.* J Clin Endocrinol Metab, 96, *3134–3142.*

Thus, early in gestation the pregnant woman meets fetal demand for calcium by increasing the rate of resorption of stored bone calcium, whereas the calcium requirement of the more mature fetus is met by a substantially increased rate of maternal intestinal calcium absorption.

During lactation, the nursing mother daily transfers to her suckling infant 280 to 400 mg of calcium mobilized from her skeleton in response to PTHrP secreted primarily by the breast.[297] Calcium concentrations are low in colostrum, approximately 25 mg/dL in breast milk during the first 6 months of lactation and 21 mg/dL during months 6 to 12 of nursing.[299] Maternal total calcium, calcitriol, and calcitonin values are normal, whereas $Ca^{2+}{}_e$, phosphate, and PTHrP levels increase during lactation. Urinary levels of markers of bone resorption and serum values of markers of bone formation are both elevated during lactation, implying rapid turnover of maternal bone mineral during nursing. Maternal bone mineralization declines 3% to 10% during lactation only to reaccrue rapidly after weaning.[300] It has been recommended that pregnant and lactating women receive 600 IU of vitamin D and 1300 mg of calcium daily and that their neonates and infants receive 400 IU of vitamin D daily whether breast or formula fed (see Table 8-3).

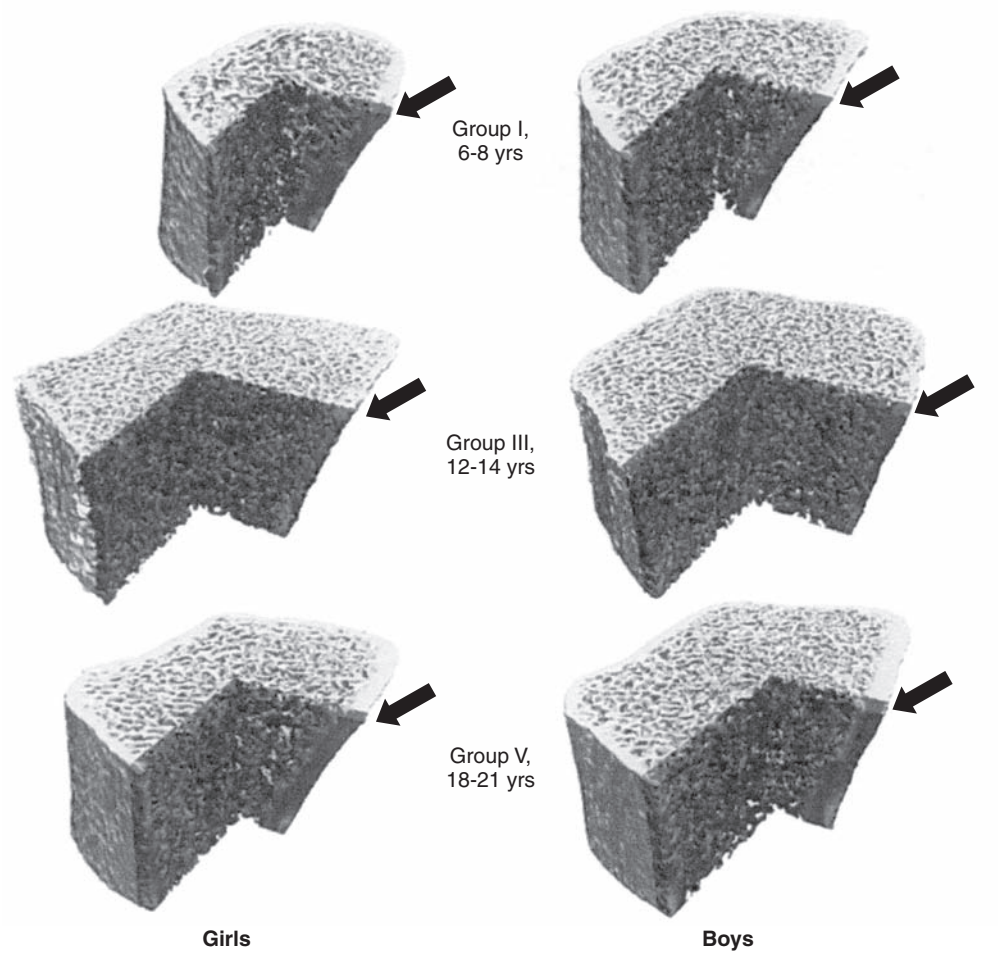


FIGURE 8-17 ■ Three-dimensional (3D) reconstructions of cortical and trabecular bone of ultradistal radius. Arrows point to cortical bone. (Reproduced from Kirmani, S., Christen, D., van Lenthe, G. H., et al. (2009). Bone structure at the distal radius during adolescent growth. *J Bone Miner Res, 24*, 1033–1042, with permission.)

During gestation, the fetus accrues 30 to 35 g of calcium beginning late in the first trimester with development of primary ossification centers in the long bones and vertebrae between the eighth and twelfth weeks of pregnancy; approximately 80% of calcium accretion occurs in the third trimester.[150,301] At 28 weeks' gestation calcium is deposited into the fetal skeleton at the rate of 100 mg/day, whereas at 35 weeks calcium is deposited at the rate of 250 mg/day.[298,302] At birth, whole body bone mineral content (BMC) is positively related to gestational age, body length, and most closely to body weight, as are lumbar spine (L1-L4) BMC and BMD.[302,303] The fetal skeleton serves two roles: (1) it is a metabolically important source of calcium mobilized by fetal PTH and PTHrP acting through PTH1R when the supply of calcium from the mother is limited; (2) it provides a rigid structural and protective framework for fetal soft tissues. From at least 15 weeks' gestation, serum concentrations of total calcium and particularly Ca^{2+}_e are substantially higher in the human fetus than in the mother (1.4:1), the physiologic significance of which is unknown. Fetal serum calcium levels are established independently of and are not directly related to maternal calcium concentrations. Additionally, fetal serum concentrations of magnesium and phosphate are greater than maternal values. The parathyroid glands are essential for maintenance of normal fetal calcium concentrations. By the tenth week of gestation, they secrete PTH and possibly PTHrP as well, and both peptides function additively to maintain fetal serum calcium levels. PTH does not stimulate placental calcium transport, but it is secreted by the fetal parathyroid gland in response to hypocalcemia, and fetal mice in which the expression of PTH has been ablated (e.g., *Hoxa3* null mice) are hypocalcemic and skeleton mineralization is impaired.[96,301] Fetal PTH increases fetal renal tubular and bone mineral reabsorption. Maternal hypercalcemia suppresses and maternal hypocalcemia stimulates secretion of fetal PTH. Both amino and mid-molecule fragments of PTHrP (e.g., $PTHrP^{1\text{-}86}$, $PTHrP^{67\text{-}86}$) produced by the fetal parathyroid gland, placenta, amnion, chorion, and umbilical cord maintain high fetal serum calcium concentrations

by stimulating active maternal-to-fetal transport of calcium across the placental syncytiotrophoblast against a concentration gradient. The CaSR is expressed in the human placenta in the first trimester and is involved in placental calcium transport.[304] Calcium selective ion channels (TPRV5, TPRV6) located at the apical surface of synctiotrophoblast cells facilitate maternal-fetal transplacental transfer of calcium. Ca^{2+} enters the syncytiotrophoblast through a calcium channel and exits via a Ca^{2+}-ATPase channel (encoded by *ATP2B1* or related gene whose expression is up-regulated by calcitriol). The effect of PTHrP on placental calcium transport is mediated in part by receptors that recognize mid-molecule or carboxyl terminal fragments of PTHrP, as in fetal mice in which *Pth1r* has been ablated placental calcium transport remains active while fetal serum calcium levels are low. In fetal mice in which the expression of *Pthlh* has been lost, serum calcium levels are lower than control values and maintained by fetal PTH at values comparable to those of the mother; in Pth1r null fetal mice placental calcium transfer is decreased, and chondrocyte maturation and bone development are abnormal.[301] Serum concentrations of PTHrP are high in the human fetus (term cord blood 2 to 5 pmol/L) and increase still further when fetal plasma calcium values decline; PTH levels are less than those in maternal serum. In utero, calcitonin concentrations are elevated in response to the increased serum calcium levels of the fetus, but this peptide does not have a major impact on fetal calcium homeostasis.[301]

Fetal serum calcitriol concentrations are a bit lower than those of the mother. Experimentally, fetal serum calcium values and mineralization of the fetal skeleton are normal in the presence of maternal vitamin D deficiency or an inactive VDR and in the VDR-null fetal mouse, if the mother ingests a diet enriched with calcium and phosphate, indicating that the fetus does not have an absolute requirement for calcitriol or the VDR for normal mineral metabolism.[301,305] In humans, the fetal cartilaginous skeleton is present by the eighth week of gestation; primary ossification centers appear in the long bones and vertebrae by the twelfth week and secondary centers at the femoral ends are noted by the thirty-fourth week.[150] Fetal PTHrP secreted by juxta-articular cells of the long bone regulates the orderly rate of chondrocyte maturation, whereas fetal PTH maintains serum calcium and phosphate values appropriate for bone mineralization. Magnesium is actively transported across the placenta. In utero fetal magnesium concentrations exceed maternal values and are inversely related to gestational age, reflecting the third trimester decline in maternal magnesium concentrations.

Calcium levels in cord blood correlate with gestational age and exceed maternal values by 1 to 2 mg/dL as a result of the active placental calcium pump. When the neonate is abruptly removed from the transplacental infusion of maternal calcium, total calcium and Ca^{2+}_e concentrations decline rapidly in the first 6 to 12 hours after delivery to nadir values (from 12 to 9 mg/dL and 1.45 to l.20 mmol/L, respectively) by 24 to 72 hours of age.[301,306] Calcium levels are a bit lower and PTH values higher in neonates delivered by cesarean section than in those delivered vaginally. After birth, PTHrP values decline rapidly; thus, in order to maintain mineral homeostasis, the neonate becomes dependent on endogenous PTH, exogenous vitamin D, ingested and absorbed calcium, renal tubular resorption of calcium, and bone calcium stores for its calcium needs. In response to the fall in Ca^{2+}_e values, serum levels of PTH begin to increase on the first day of life followed by rise in calcitriol concentrations and slow decline (after an initial postnatal rise) in calcitonin values.[307] For the first 2 to 4 weeks after birth, there is increased efficiency of intestinal calcium absorption by passive means that are independent of vitamin D, perhaps due to the lactose content of milk which affects paracellular transport of Ca^{2+}.[301] Later in the neonatal period, vitamin D–dependent intestinal calcium absorption increases. Renal tubular handling of calcium and the response to PTH matures during the first several weeks of life. Bone calcium accretion continues at the rate of 150 mg/kg/day for several months after birth, a vitamin D–dependent process. Due to both decreased glomerular filtration and increased tubular reabsorption, serum concentrations of phosphate are maximum in the neonate. Serum phosphate concentrations increase after delivery from cord values of 3.8 to 8.1 mg/dL to levels that range between 4.5 and 9 mg/dL during the first week of life and then stabilize at values between 4.5 and 6.7 mg/dL through the first year of life. In preterm or acutely ill neonates, the fall in calcium values is often exaggerated and more prolonged, and bone mineralization is frequently impaired in very preterm newborns and infants. Maternal hypercalcemia suppresses and maternal hypocalcemia stimulates secretion of fetal PTH, effects that may carry over to the neonate for several days. Serum magnesium concentrations are quite stable in infants and children ranging between 1.5 to 2.2 mg/dL through four months of age and between 1.7 to 2.3 mg/dL through 5 years of age. Human breast milk contains on average: calcium 28 mg/dL, phosphate 13 mg/dL, and vitamin D15-50 IU/L.[299] Cow milk formulas contain approximately 40 to 60 mg/dL calcium, 30 to 40 mg/dL phosphate, and 30 to 40 IU/dL vitamin D. The bioavailability of calcium in prepared formulas depends on its source and the formula's content and source of protein, fat, and carbohydrates. Infants fed formulas that contain palm olein absorb less calcium than those receiving a formula fortified with another form of fat or human breast milk, and these infants have lower total body bone mineral content.[308] Phytate (inositol hexaphosphate) present in soy formulas and infant cereals chelates calcium, and oxalate, a constituent of spinach, precipitates calcium thereby reducing intestinal absorption of this mineral, a difficulty overcome by increasing dietary calcium content.

During childhood and adolescence serum concentrations of total calcium (8.8 to 10.8 mg/dL depending on the analytic laboratory) and magnesium (1.7 to

2.2 mg/dL) remain relatively constant.[307] Serum phosphate levels are higher in children (4.5 to 6.2 mg/dL) than in adults (2.5 to 4.5 mg/dL) and attain maximum values several months before the peak height velocity of adolescence is achieved; the rise has been attributed to augmentation of the renal tubular reabsorption of phosphate due to the combined effects of the increased secretion of growth hormone, IGF-I, and sex hormones, factors that contribute to the pubertal growth spurt. Total serum alkaline phosphatase activity is also higher in children than in adults and increases transiently during the pubertal growth spurt. Levels of bone-specific alkaline phosphatase increase between 12 and 9 months before peak height velocity, reach maximum levels at Tanner male genital stage 3, and are directly related to the secretion of testosterone in boys. In females, serum bone-specific alkaline phosphatase activity peaks at Tanner stage 3 breast development and correlates with serum osteocalcin concentrations. Serum concentrations of PTH fluctuate very little during adolescence, whereas levels of calcitriol rise transiently.

REFERENCES

1. Favus, M. J., & Goltzman, D. (2008). Regulation of calcium and magnesium. In C. J. Rosen, (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (pp. 104–108) (7th ed.). Washington, DC: The American Society of Bone and Mineral Metabolism.
2. Heaney, R. P. (2011). The nutrient problem, as seen through the lens of calcium. *J Clin Endocrinol Metab*, *96*, 2035–2037.
3. Bringhurst, F. R., Demay, M. B., & Kronenberg, H. M. (2010). Hormones and disorders of mineral metabolism. In H. M. Kronenberg, S. Melmed, K. S. Polonsky, P. R. Larsen, (Eds.), *Williams Textbook of Endocrinology* (pp. 1203–1268) (11th ed.). Philadelphia: Saunders/Elsevier.
4. Cahalan, M. D. (2010). How to STIMulate calcium channels. *Science*, *330*, 43–44.
5. Dewitte, K., Stockl, D., & Thienpont, L. M. (1999). pH dependency of serum ionized calcium. *Lancet*, *354*, 1793–1794.
6. Kornak, U. (2011). Animal models with pathologic mineralization phenotypes. *Joint Bone Spine*, *78*, 561–567.
7. Gill, D. L., Spassova, M. A., & Soboloff, J. (2006). Calcium entry signals: trickles and torrents. *Science*, *313*, 183–184.
8. Halling, D. B., Aracena-Parks, P., & Hamilton, S. L. (2005). Regulation of voltage-gated Ca^{2+} channels by calmodulin. *Sci STKE*, re15.
9. Abernethy, D. R., & Schwartz, J. B. (1999). Calcium-antagonist drugs. *N Engl J Med*, *341*, 1447–1457.
10. Vig, M., Peinelt, C., Beck, A., et al. (2006). CRACM1 is a plasma membrane protein essential for store-operated Ca^{2+} entry. *Science*, *312*, 1220–1223.
11. Park, C. Y., Shcheglovitov, A., & Dolmetsch, R. (2010). The CRAC channel activator STIM1 binds and inhibits L-type voltage-gated calcium channels. *Science*, *330*, 101–105.
12. Wang, Y., Deng, X., Mancarella, S., et al. (2010). The calcium store sensor, STIM1, reciprocally controls Orai and CaV1.2 channels. *Science*, *330*, 105–109.
13. Feske, S., Gwack, Y., Prakriya, M., et al. (2006). A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. *Nature*, *441*, 179–185.
14. Byun, M., Abhyankar, A., Lelarge, V., et al. (2010). Whole-exome sequencing-based discovery of STIM1 deficiency in a child with fatal classic Kaposi sarcoma. *Exper Med*, *207*, 2307–2312.
15. Dellis, O., Dedos, S. G., Tovey, S. C., et al. (2006). Ca^{2+} entry through plasma membrane IP_3 receptors. *Science*, *313*, 229–233.
16. Wong, V., & Goodenough, D. A. (1999). Paracellular channels! *Science*, *285*, 62.
17. Abramson, J., Paz, A., & Philipson, K. D. (2012). It's all in the symmetry. *Science*, *335*, 669–670.
18. Liao, J., Li, H., Zeng, W., et al. (2012). Structural insight into he ion-exchange mechanism of the sodium/calcium exchanger. *Science*, *335*, 686–688.
19. McMurray, J. J. V., & Smith, G. L. (2011). Calcium handling in the failing heart and SUMO: weighing the evidence. *N Engl J Med*, *365*, 1738–1739.
20. Stigler, J., Ziegler, F., Gieske, A., et al. (2012). The complex folding network of single calmodulin molecules . Science, *334*, 512–516.
21. Bronner, F. (2008). Recent developments in intestinal calcium absorption. *Nutrition Rev*, *67*, 109–113.
22. Dimke, H., Hoenderop, J. G. J., & Bindels, R. J. M. (2011). Molecular basis of epithelial Ca^{2+} and Mg^{2+} transport: insights from the TRP channel family. *J Physiol*, *589*. 7, 1535–1542.
23. Tiosano, D., Hadad, S., Chen, Z., et al. (2011). Calcium absorption, kinetics, bone density, and bone structure in patients with hereditary vitamin D-resistant rickets. *J Clin Endocrinol Metab*, *96*, 3701–3709.
24. Ajibade, D. V., Dhawan, P., Fechner, A. J., et al. (2010). Evidence for a role of prolactin in calcium homeostasis: regulation of intestinal transient receptor potential vanilloid type 6, intestinal calcium absorption, and the 25-hydroxyvitamin D_3 gene by prolactin. *Endocrinology*, *151*, 2974–2984.
25. Akhter, S., Kutuzova, G. D., Christakos, S., & DeLuca, H. F. (2007). Calbindin D_{9k} is not required for 1,25-dihydroxyvitamin D_3-mediated Ca^{2+} absorption in small intestine. *Arch Biochem Biophys*, *460*, 227–232.
26. Kutuzova, G. D., Sundersingh, F., Vaughan, J., et al. (2008). TRPV6 is not required for 1α,25-dihydroxyvitamin D_3-induced intestinal calcium absorption in vivo. *PNAS*, *105*, 19655–19659.
27. Fujita, H., Sugimoto, K., Inatomi, S., et al. (2008). Tight junction proteins claudin-2 and -12 are critical for vitamin D-dependent Ca^{2+} absorption between enterocytes. *Mol Biol Cell*, *19*, 1912–1919.
28. Lanou, A. J., Berkow, S. E., & Barnard, N. D. (2005). Calcium, dairy products, and bone health in children and young adults: a reevaluation of the evidence. Pediatrics, 115, 736–743.
29. Greer, F. R., & Krebs, N. F. (2006). Optimizing bone health and calcium intakes of infants, children, and adolescents. *Pediatrics*, *117*, 578–585.
30. Ross, A. C., Manson, J. E., Abrams, S. A., et al. (2011). The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. *J Clin Endocrinol Metab*, *96*, 53–58.
31. Hill, K. M., Braun, M. M., Egan, K. A., et al. (2011). Obesity augments calcium-induced increases in skeletal calcium retention in adolescents. *J Clin Endocrinol Metab*, *96*, 2171–2177.
32. Greer, F. R. (2005). Bone health: it's more than calcium intake. *Pediatrics*, *115*, 792–794.
33. Alexander, R. T., Woudenberg-Vrenken, T. E., Buurman, J., et al. (2009). Klotho prevents renal calcium loss. *J Am Soc Nephrol*, *20*, 2371–2379.
34. Chang, Q., Hoefs, S., van der Kempt, A. W., et al. (2005). The beta glucuronidase klotho hydrolyzes and activates the TRPV5 channel. *Science*, *310*, 490–493.
35. De Groot, T., Kovalevskaya, N. V., Verkaart, S., et al. (2011). Molecular mechanisms of calmodulin action on TRPV5 and modulation by parathyroid hormone. *Molec Cell Biol*, *31*, 2845–2852.
36. Brown, E. M. (2008). Ca^{2+}-sensing receptor. In C. J. Rosen (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (pp. 134–141) (7th ed.). Washington, DC: The American Society of Bone and Mineral Metabolism.
37. Magno, A. L., Ward, B. K., & Ratajczak, T. (2011). The calcium-sensing receptor: a molecular perspective. *Endocr Rev*, *32*, 3–30.
38. Pidasheva, S., Grant, M., Canaff, L., et al. (2006). Calcium-sensing receptor dimerizes in the endoplasmic reticulum: biochemical

and biophysical characterization of the CASR mutants retained intracellularly. *Hum Molec Genet*, *15*, 2200–2209.
39. Yamaguchi, T., Chattopadhay, N., & Brown, E. M. (2000). G protein-coupled extracellular Ca^{2+} (Ca^{2+}_o)-sensing receptor (CaR): roles in cell signaling and control of diverse cellular functions. In B. W. O'Malley (Ed.), *Hormones and signaling* (pp. 209–253) New York: Academic Press.
40. Mizobuchi, M., Ritter, C. S., Krits, I., et al. (2009). Calcium-sensing receptor expression is regulated by glial cells missing-2 in human parathyroid cells. *J Bone Miner Res*, *24*, 1173–1179.
41. Scillitani, A., Guarnieri, V., de Geronimo, S., et al. (2004). Blood ionized calcium is associated with clustered polymorphisms in the carboxyl-terminal tail of the calcium-sensing receptor. *J Clin Endocrinol Metab*, *89*, 5634–5638.
42. Renkema, K. Y., Bindels, R. J. M., & Hoenderop, J. G. J. (2011). Role of the calcium-sensing receptor in reducing the risk for calcium stones. *Clin J Am Soc Nephrol*, *6*, 2076–2082.
43. Chang, W., Tu, C., Chen T.-H., et al. (1999). Expression and signal transduction of calcium-sensing receptors in cartilage and bone. *Endocrinology*, *140*, 5883–5893.
44. Zaidi, M., & Adebanjo, O. A. (2000). Calcium handling by the osteoclast. *Endocrinologist*, *10*, 155–163.
45. Steddon, S. J., & Cunningham, J. (2005). Calcimimetics and calcilytics: fooling the calcium receptor. *Lancet*, *365*, 2237–2239.
46. Dong, B. J. (2005). Cinacalcet: an oral calcimimetic agent for the management of hyperparathyroidism. *Clin Therapeutics*, *27*, 1725–1751.
47. Farrow, E. G., & White, K. E. (2010). Recent advances in renal phosphate handling. *Nature Rev Nephrol*, *6*, 207–215.
48. Bergwitz, C., Collins, M. T., Kamath, R. S., & Rosenberg, A. E. (2011). Case 33–2011: a 56 year-old man with hypophosphatemia. *N Engl J Med*, *365*, 1625–1635.
49. White, K. E., Larsson, T. E., & Econs, M. J. (2006). The roles of specific genes implicated as circulating factors in normal and disordered phosphate homeostasis: Frizzled related protein-4, matric extracellular phosphoglycoprotein, and fibroblast growth factor 23. *Endocr Rev*, *27*, 221–241.
50. Prie, D., & Friedlander, G. (2010). Genetic disorders of renal phosphate transport. *N Engl J Med*, *362*, 2399–2409.
51. Carpenter, T. O., Ellis, T. K., Insogna, R. L., et al. (2004). Fibroblast growth factor 7: an inhibitor of phosphate transport derived from oncogenic osteomalacia-causing tumors. *J Clin Endocrinol Metab*, *90*, 1012–1020.
52. Jan de Beur, S. M. (2006). Tumor-induced osteomalacia. In M. J. Favus (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (pp. 345–351) (6th ed.). Washington, DC: American Society for Bone and Mineral Research.
53. Quarles, L. D. (2012). Skeletal secretion of FGF-23 regulates phosphate and vitamin D metabolism. *Nat Rev Endocrinol*, *8*, 276–286.
54. Rowe, P. S. (2012). Regulation of bone-renal mineral and energy metabolism: the PHEX, FGF23, DMP1, MEPE ASARM pathway. *Crit Rev Eukaryotic Gene Express*, *22*, 61–86.
55. Kolek, O. I., Hines, E. R., Jones, M. D., et al. (2005). 1α, 25-Dihydroxyvitamin D_3 upregulates FGF23 gene expression in bone: the final link in a renal-gastrointestinal-skeletal axis that controls phosphate transport. *Am J Physiol Gastrointest Liver Physiol*, *289*, G1036–G1042.
56. Masuyama, R., Stockmans, I., Torrekens, S., et al. (2006). Vitamin D receptor in chondrocytes promotes osteoclastogenesis and regulates FGF23 production in osteoblasts. *J Clin Invest*, *116*, 3150–3159.
57. Hori, M., Shimizu, Y., & Fukumoto, S. (2011). Minireview: fibroblast growth factor 23 in phosphate homeostasis and bone metabolism. *Endocrinology*, *152*, 4–10.
58. Yu, X., Ibrahimi, O. A., Goetz, R., et al. (2005). Analysis of the biochemical mechanisms for the endocrine actions of fibroblast growth factor-23. *Endocrinology*, *146*, 4647–4656,.
59. Hebert, J. M. (2011). FGFs: neurodevelopment's Jack-of-all-trades: how do they do it? *Front Neurosci*, *5*, 133.
60. Shimizu, Y., Fukumoto, S., & Fujita, T. (2012). Evaluation of a new automated chemoluminescence immunoassay for FGF23. *J Bone Miner Met*, *30*, 217–221.
61. Smith, E. R., Cai, M. M., McMahon, L. P., & Holt, S. G. (2012). Biological variability of plasma intact and C-terminal FGF23 measurements. J Clin Endocrinol Metab, *97*, 3357–3365.
62. White, K. E., & Econs, M. J. (2008). Fibroblast growth factor-23. In C. J. Rosen (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (pp. 112–117) (7th ed.). Washington, DC: The American Society of Bone and Mineral Metabolism.
63. Benet-Pages, A., Orlik, P., Strom, T. M., & Lorenz-Depiereux, B. (2005). An FGF23 missense mutation causes familial tumor calcinosis with hyperphosphatemia. *Hum Molec Genet*, *14*, 385–390.
64. Gowen, L. C., Petersen, D. N., Mansolf, A. L., et al. (2003). Targeted disruption of the osteoblast/osteocyte factor 45 gene (OF45) results in increased bone formation and bone mass. *J Biol Chem*, *278*, 1998–2007.
65. Addison, W. N., Nakano, Y., Loisel, T., et al. (2008). MEPE-ASARM peptides control extracellular matrix mineralization by binding to hydroxyapatite: an inhibition regulated by PHEX cleavage of ASARM. *J Bone Miner Res*, *23*, 1638–1649.
66. San-Cristobal, P., Dimke, H., Hoenderop J. G. J., & Bindels, R. J. M. (2010). Novel molecular pathways in renal Mg^{2+} transport: a guided tour. *Curr Opin Nephrol Hypertens*, *19*, 456–462.
67. Chubanov, V., Waldegger, S., Maderos y Schnitzler, M., et al. (2004). Disruption of TRPM6/TRPM7 complex formation by a mutation in the TRPM6 gene causes hypomagnesemia with secondary hypocalcemia. *Proc Nat Acad Sci USA*, *101*, 2894–2899.
68. Kausalya, R. J., Amasheh, S., Gunzel, D., et al. (2006). Disease associated mutations affect intracellular traffic and paracellular Mg^{2+} transport function of Claudin16. *J Clin Invest*, *116*, 878–891.
69. Ferre, S., Hoenderop J. G. J., & Bindels, R. J. M. (2011). Insight into renal Mg^{2+} transporters. *Curr Opin Nephrol Hypertension*, *20*, 169–176.
70. Nissenson, R. A., & Juppner, H. (2008). Parathyroid hormone. In C. J. Rosen (Ed.). *Primer on the metabolic bone diseases and disorders of mineral metabolism* (pp. 123–127) (7th ed.). Washington, DC: The American Society of Bone and Mineral Metabolism.
71. Maret, A., Bordeaux, I., Ding, C., et al. (2004). Expression of GCM by intra thymic parathyroid hormone-secreting adenomas indicates their parathyroid cell origin. *J Clin Endocrinol Metab*, *89*, 8–12.
72. Epstein, E., Sela-Brown, A., Ringel, I., et al. (2000). Dynein light chain binding to a 3'-untranslated sequence mediates parathyroid hormone mRNA association with microtubules . *J Clin Invest*, *105*, 505–512.
73. Drechsler, N., Frobel, J., Jahreis, G., et al. (2011). Binding specificity of the parathyroid hormone receptor. *Biophys Chem*, *154*, 66–72.
74. D'Amour, P., Brossard, J., Rousseau, L., et al. (2005). Structure of non-(1–84) PTH fragments secreted by parathyroid glands in primary and secondary hyperparathyroidism. *Kidney Int*, *68*, 998–1007.
75. Murray, T. M., Rao, L. G., Divieti, P., & Bringhurst, P. R. (2005). Parathyroid hormone secretion and action: evidence for discrete receptors for the carboxyl-terminal region and related biological actions of carboxyl-terminal ligands. *Endocr Rev*, *26*, 78–113.
76. Lombardi, G., Di Somma, C., Rubino, M., et al. (2011). The roles of parathyroid hormone in bone remodeling: prospects for novel therapeutics. *J Endocrinol Invest*, *34*(Suppl. 7), 18–22.
77. Bohine, B. N., & Gesty-Palmer, D. (2011). β-arrestin-biased agonism at the parathyroid hormone receptor uncouples bone formation from bone resorption. *Endocrine Metab Immune Dis Drug Targets*, *11*, 112–119.
78. Scillitani, A., Guarnieri, V., Battista, C., et al. (2011). Carboxyl-terminal parathyroid hormone fragments; biological effects. *J Endocrinol Invest*, *34*(Suppl. 7), 23–26.
79. Salusky, I. B., J& uppner, H. (2004). New PTH assays and renal osteodystrophy. *Pediatr Nephrol*, *19*, 709–713.
80. Silverberg, S. J., & Bilezikian, J. P. (2008). Primary hyperparathyroidism. In C. J. Rosen (eEd.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (pp. 302–306) (7th ed.). Washington, DC: The American Society of Bone and Mineral Metabolism.

81. Vieira, J. G. H. (2012). PTH assays: understanding what we have and forecasting what we will have. *J Osteopor*, *2012*, 523246.
82. Cantor, T., Yang, Z., Caraiani, N., & Ilamathi, E. (2006). Lack of comparability of intact parathyroid hormone measurements among commercial assays for end-stage renal disease patients: implication for treatment decisions. *Clin Chem*, *52*, 1771–1776.
83. Zimmerman, M. K. (2012). Discrepant intact parathyroid hormone result by immunoassay. *Clin Chim Acta*, *413*, 344–345.
84. Vietri, M. T., Sessa, M., Pilla, P., et al. (2006). Serum osteocalcin and parathyroid hormone in healthy children assessed with two new automated assays. *J Pediatr Endocrinol Metab*, *19*, 1413–1419.
85. Cavalier, E., Cartisi, A., Bekaert, A. C., et al. (2012). New insights on the stability of parathyroid hormone as assayed by an automated 3rd generation PTH assay. *Clin Chim Acta*, *413*, 253–254.
86. Terry, A. H., Orrock, J., & Meikle A. W. (2003). Comparison of two third-generation parathyroid hormone assays. *Clin Chem*, *49*, 336–337.
87. Brown, E. M., & Juppner, H. (2006). Parathyroid hormone: synthesis, secretion, and action. In M. J. Favus (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (pp. 90–99) (6th ed.). Washington, DC: American Society for Bone and Mineral Research.
88. Dempster, D. W., Zhou, H., Recker, R. R., et al. (2012). Skeletal histomorphometry in subjects on teriparatide or zolendronic acid therapy (SHOTZ) study: a randomized controlled trial. *J Clin Endocrinol Metab*, *97*, 2799–2808.
89. Lopez, I., Rodriguez-Ortiz, M. E., Almaden, Y., et al. (2011). Direct and indirect effects of parathyroid hormone on circulating levels of fibroblast growth factor 23 in vivo. Kidney *Intern*, *80*, 475–482.
90. Wysolmerski, J. J. (2008). Parathyroid hormone-related protein. In C. J. Rosen (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (pp. 127–133) (7th ed.). Washington, DC: The American Society of Bone and Mineral Metabolism.
91. Wysolmerski, J. J. (2012). Parathyroid hormone-related protein: an update. *J Clin Endocrinol Metab*, *97*, 2947–2956.
92. Chau, M., Forcinito, P., Andrade, A. C., et al. (2011). Organization of the Indian hedgehog: parathyroid hormone-related protein system in the postnatal growth plate. *J Molec Endocrinol*, *47*, 99–107.
93. Nissenson, R. A. (1998). Editorial: parathyroid hormone (PTH)/PTHrP receptor mutations in human chondrodysplasia. *Endocrinology*, *139*, 4753–4755.
94. Broadus, A. E., & Nissenson, R. A. (2006). Parathyroid hormone-related protein. In M. J. Favus (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (pp. 99–106) (6th ed.). Washington, DC: American Society for Bone and Mineral Research.
95. Miao, D., He, B., Karaplis, A. C., & Goltzman, D. (2002). Parathyroid hormone is essential for normal fetal bone formation. *J Clin Invest*, *109*, 1173–1182.
96. Kovacs, C. S., Chafe, L. L., Fudge, N. J., et al. (2001). PTH regulates fetal blood calcium and skeletal mineralization independently of PTHrP. *Endocrinology*, *142*, 4983–4993.
97. Buhimschi, C. S. (2004). Endocrinology of lactation. *Obstet Gynecol Clin North Am*, *31*, 963–979.
98. Pioszak, A. A., Harikimar, K. G., Parker, N. R., et al. (2010). Dimeric arrangement of the parathyroid hormone receptor and a structural mechanism for ligand-induced dissociation. *J Biol Chem*, *285*, 12435–12444.
99. Pickard, B. W., Hodsman, A. B., Fraher, L. J., & Watson, P. H. (2006). Type 1 parathyroid hormone receptor (PTH1R) nuclear trafficking: association of PTH1R with importin α_1 and β. *Endocrinology*, *147*, 3326–3332.
100. Gough, N. R. (2011). Focus issue: cracking the G protein-coupled receptor code. *Sci Signal*, *4*, eg7.
101. Mahon, M. J., Donowitz, M., Yun, C. C., & Segre, G. V. (2002). Na^+/H^+ exchanger regulatory factor 2 directs parathyroid hormone 1 receptor signaling. *Nature*, *417*, 858–861.
102. Adam, S. (2008). Calcitonin. In C. J. Rosen (Ed). *Primer on the metabolic bone diseases and disorders of mineral metabolism* (pp. 250–251) (7th ed.). Washington, DC: The American Society of Bone and Mineral Metabolism.
103. Leboeuf, R., Langlois, F., Martin, M., et al. (2006). "Hook effect" in calcitonin immunoradiometric assay in patients with metastatic medullary thyroid carcinoma: case report and review of the literature. *J Clin Endocrinol Metab*, *91*, 361–364.
104. Woodrow, J. P., Sharpe, C. J., Fudge, N. J., et al. (2006). Calcitonin plays a critical role in regulating skeletal mineral metabolism during lactation. *Endocrinology*, *147*, 4010–4021.
105. Holick, M. F. (2011). Vitamin D: evolutionary, physiological and health perspectives. *Curr Drug Targets*, *12*, 4–18.
106. Schuster, I. (2011). Cytochromes P450 are essential players in the vitamin D signaling system. *Biochim Biophys Acta*, *1814*, 186–199.
107. Autier, P., Gandini, S., & Mullie, P. (2012). A systematic review: influence of vitamin D supplementation on serum 25-hydroxyvitamin D concentration. *J Clin Endocrinol Metab*, *97*, 2606–2613.
108. Hollis, B. W. (2005). Circulating 25-hydroxyvitamin D levels indicative of vitamin D sufficiency: implications for establishing a new effective dietary intake recommendation for vitamin D. *J Nutr*, *135*, 317–322.
109. Bickle, D., Adams J, & Christakos, S. (2008). Vitamin D: production, metabolism, mechanism of action, and clinical requirements. In C. J. Rosen (Ed). *Primer on the metabolic bone diseases and disorders of mineral* metabolism (pp. 141–149) (7th ed.). Washington, DC: The American Society of Bone and Mineral Metabolism.
110. Cheng, J. B., Levine, M. A., Bell, N. H., et al. (2004). Genetic evidence that the human CYP2R1 enzyme is a key vitamin D-25 hydroxylase. *Proc Nat Acad Sci USA*, *101*, 7711–7715.
111. Christakos, S., Ajibade, D. V., Dhawan, P., et al. (2010). Vitamin D: metabolism. *Endocrinol Metab Clin North Am*, *39*, 243–253.
112. Kuchuk, N. O., Pluijm, S. M., van Schoor, N. M., et al. (2009). Relationships of serum 25-hydroxyvitamin D to bone mineral density and serum parathyroid hormone and markers of bone turnover in older persons. *J Clin Endocrinol Metab*, *94*, 1244–1250.
113. Holick, M. F. (2011). The D-batable Institute of Medicine report: a D-lightful perspective. *Endocrine Pract*, *17*, 143–149.
114. Engelman, C. D. (2011). Vitamin D recommendations: the saga continues. *J Clin Endocrinol Metab*, *96*, 3065–3066.
115. Hill, K. M., McCabe, G. P., McCabe, L. D., et al. (2010). An inflection point of serum 25-hydroxyvitamin D for maximal suppression of parathyroid hormone is not evident from multi-site pooled data in children and adolescents. *J Nutr*, *140*, 1983–1988.
116. Rosen, C. J., Abrams, S. A., Aloia, J. F., et al. (2012). IOM committee members respond to Endocrine Society vitamin D guideline. *J Clin Endocrinol Metab*, *97*, 1146–1152.
117. Barake, M., Daher, R. T., Salti, I., et al. (2012). 25-Hydroxyvitamin D assay variations and impact on clinical decision making. *J Clin Endocrinol Metab*, *97*, 835–843.
118. Wagner, C. L., & Greer, F. R. (2008). Prevention of rickets and vitamin D deficiency in infants, children, and adolescents. *Pediatrics*, *112*, 1142–1152.
119. Aloia, J. F. (2011). The 2011 report on dietary reference intake for vitamin D: where do we go from here? *J Clin Endocrinol Metab*, *96*, 2987–2996.
120. Mutlu, G. Y., Kusdal, Y., Ozsu, E., et al. (2011). Prevention of vitamin D deficiency in infancy: daily 400 IU vitamin D is sufficient. Intern J Pediatr Endocrinol. Retrieved from www.ijpeonline.com/content/2011/1/4.
121. Friedman, P. A. (1999). Calcium transport in the kidney. *Curr Opin Nephrol Hypertension*, *8*, 589–595.
122. Kouzmenko, A., Ohtake, F., Fujiki, R., & Kato, S. (2010). Hormonal gene regulation through DNA methylation and demethylation. *Epigenomics*, *2*, 765–744.
123. Chiellini, G., & DeLuca, H. F. (2011). The importance of stereochemistry on the actions of vitamin D. *Curr Top Med Chem*, *11*, 840–859.
124. Overbaugh, L., Stoffels, K., Waer, M., et al. (2006). Immune regulation of 25-hydroxyvitamin D-1α-hydroxylase in human monocytic THP1 cells: mechanisms of interferon-γ-mediated induction. *J Clin Endocrinol Metab*, *91*, 3566–3574.
125. Holick, M. F. (2006). Resurrection of vitamin D deficiency and rickets. *J Clin Invest*, *116*, 2062–2072.

126. de Oliviera, R. B., & Moyses, R. M. A. (2010). FGF-23: state of the art. *J Bras Nefrol*, *32*, 316–323.
127. Wang, J. T., Lin C.-J., Burridge, S. M., et al. (1998). Genetics of vitamin D 1α-hydroxylase deficiency in 17 families. *Am J Hum Genet*, *63*, 1694–1702.
128. Zhou, C., Assem, M., Tay, J. C., et al. (2006). Steroid and xenobiotic receptor and vitamin D receptor crosstalk mediates CYP24 expression and drug-induced osteomalacia. *J Clin Invest*, *116*, 1703–1712.
129. Schlingmann, K. P., Kaufmann, M., Weber, S., et al. (2011). Mutations in CYP24A1 and idiopathic infantile hypercalcemia. *N Engl J Med*, *365*, 410–421.
130. Rosen, C. J., Adams, J. S., Bikle, D. D., et al. (2012). The nonskeletal effects of vitamin D: an Endocrine Society scientific statement. *Endocr Rev*, *33*, 456–492.
131. Norman, A. W. (2006). Minireview: vitamin D receptor: new assignments for an already busy receptor. *Endocrinology*, *147*, 5542–5548.
132. Norman, A. W., & Bouillon, R. (2010). Vitamin D nutritional policy needs a vision for the future. *Exp Biol Med*, *235*, 1034–1045.
133. Xue, Y., Karaplis, A. C., Hendry, G. N., et al. (2006). Exogenous 1,25-dihydroxyvitamin D_3 exerts a skeletal anabolic effect and improves mineral ion homeostasis in mice that are homozygous for both the 1α-hydroxylase and parathyroid hormone null alleles. *Endocrinology*, *147*, 4801–4810.
134. Holick, M. F., & Garabedian, M. (2006). Vitamin D photobiology, metabolism, mechanism of action, and clinical application. In M. J. Favus (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (pp. 106–114) (6th ed.). Washington, DC: American Society for Bone and Mineral Research.
135. Christakos, S., & DeLuca, H. F. (2011). Minireview: vitamin D: is there a role in extraskeletal health? *J Clin Endocrinol Metab*, *152*, 2930–2936.
136. DeLuca, H. (2008). Evolution of our understanding of vitamin D. *Nutrition Rev*, *66*(Suppl. 2), S73–S87.
137. Gilad, L. A., Bresler, T., Gnainsky, J., et al. (2005). Regulation of vitamin D receptor expression via estrogen-induced activation of the ERK 1/2 signaling pathway in colon and breast cancer cells. *J Endocrinol*, *185*, 577–592.
138. Kitagawa, H., Fujiki, R., Yoshimura, K., et al. (2003). The chromatin-remodel complex WINAC targets a nuclear receptor to promoters and is impaired in Williams syndrome. *Cell*, *113*, 905–917.
139. Barnett, C., & Krebs, J. E. (2011). WSTF does it all: a multifunctional protein in transcription, repair and replication. *Biochem Cell Biol*, *89*, 12–23.
140. Nagpal, S., Na, S., & Rathnachalam, R. (2005). Noncalcemic actions of vitamin D receptor ligands. *Endocr Rev*, *26*, 662–687.
141. Dawson, M. A., Kouzarides, T., & Huntly, B. J. P. (2012). Targeting epigenetic readers in cancer. *N Engl J Med*, *367*, 647–657.
142. Carlberg, C., Seuter, S., & Heikkinen, S. (2012). The first genome-wide view of vitamin D receptor locations and their mechanistic implications. *Anticancer Res*, *32*, 271–282.
143. Amling, M., Priemel, M., Holzmann, T., et al. (1999). Rescue of skeletal phenotype of vitamin D receptor-ablated mice in the setting of normal mineral ion homeostasis: formal histomorphometric and biomechanical analyses. *Endocrinology*, *140*, 4982–4987.
144. Fleet, J. C. (2004). Rapid, membrane-initiated actions of 1,25 dihydroxyvitamin D: what are they and what do they mean? *J Nutrition*, *134*, 3215–3218.
145. Boyan, B. D., & Schwartz, Z. (2004). Rapid vitamin D-dependent PKC signaling shares features with estrogen-dependent PKC signaling in cartilage and bone. *Steroids*, *69*, 591–597.
146. Huhtakangas, J. A., Olivera, C. J., Bishop, J. E., et al. (2004). The vitamin D receptor is present in caveolae-enriched plasma membranes and binds 1α,25$(OH)_2$-vitamin D_3 in vivo and in vitro. *Mol Endocrinol*, *18*, 2660–2671.
147. Cohen, M. M., Jr. (2006). The new bone biology: pathologic, molecular, and clinical studies. *Am J Med Genet*, *140A*, 2646–2706.
148. Lorenzo, J. A., Canalis, E., & Raisz, L. G. (2008). Metabolic bone disease. In H. M. Kronenberg, S. Melmed, K. S. Polonsky, P. R. Larsen (Eds.), *Williams textbook of endocrinology* (pp. 1269–1310) (11th ed.). Philadelphia: Saunders/Elsevier.
149. Mackie, E. J., Tatarczuch, L., & Mirams, M. (2011). The skeleton: a multi-functional complex organ. The growth plate chondrocyte and endochondral ossification. *J Endocrinol*, *211*, 109–121.
150. Rauch, F. (2008). Fetal and neonatal bone development. In C. J. Rosen (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (pp. 72–74) (7th ed.) Washington, DC: The American Society of Bone and Mineral Metabolism.
151. Yang, Y. (2008). Skeletal morphogenesis and embryonic development. In C. J. Rosen (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (pp. 2–10) (7th ed.). Washington, DC: The American Society of Bone and Mineral Metabolism.
152. Dempster, D. W. (2006). Anatomy and function of the adult skeleton. In M. J. Favus (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (pp. 7–11) (6th ed.). Washington, DC: American Society for Bone and Mineral Research.
153. Robey, P. G., & Boskey, A. L. (2008). The composition of bone. In C. J. Rosen (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (pp. 32–38) (7th ed.). Washington, DC: The American Society of Bone and Mineral Metabolism.
154. Karsenty, G. (2003). The complexities of skeletal biology. *Nature*, *423*, 316–318.
155. Kronenberg, H. M. (2003). Developmental regulation of the growth plate. *Nature*, *423*, 332–336.
156. Krause, C., de Gorter, D. J. J., Karperien, M., & ten Dijke, P. (2008). Signal transduction cascades controlling osteoblast differentiation. In C. J. Rosen (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (pp. 10–16) (7th ed.). Washington, DC: American Society for Bone and Mineral Research.
157. Kaplan, F. S., Fiori, J., Serrano de la Pena, L., et al. (2006). Dysregulation of the BMP-4 signaling pathway in fibrodysplasia ossificans progressiva. *Ann NY Acad Sci*, *1068*, 54–65.
158. Olsen, B. R. (2006). Bone embryology. In M. J. Favus (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (pp. 2–6) (6th ed.). Washington, DC: American Society for Bone and Mineral Research.
159. Marglies, E. H., & Innis, J. W. (2000). Building arms or legs with molecular models. *Pediatr Res*, *47*, 2–3.
160. Thiel, C. T., Horn, D., Zabel, B., et al. (2005). Severely incapacitating mutations in patients with extreme short stature identify RNA-processing endoribonuclease RMRP as an essential cell growth regulator. *Am J Hum Genet*, 77, 795–806.
161. Mak, K. K., Kronenberg, H. M., Chuang P.-T., et al. (2008). Indian hedgehog signals independently of PTHrP to promote chondrocyte hypertrophy. *Development*, *135*, 1947–1956.
162. Marino, R. (2011). Growth plate biology: new insights. *Curr Opin Endocrinol Diab Obes*, *18*, 9–13.
163. Spath S.-S., Andrade, A. C., Chau, M., & Nilsson, O. (2011). Local regulation of growth plate cartilage. In C. Camacho-Hubner, O. Nilsson, L. Savendahl (Eds.), *Cartilage and bone development and its disorders.* Endocr Dev Basel: Karger, 21, 12–22.
164. Lui, J. C. K., Anrade, A. A., Forcinito, P., et al. (2010). Spatial and temporal regulation of gene expression in the mammalian growth plate. *Bone*, *46*, 1380–1390.
165. Zuscik, M. J., Hilton, M. J., Zhang, X., et al. (2008). Regulation of chondrogenesis and chondrocyte differentiation by stress. *J Clin Invest*, *118*, 429–438.
166. Komori, T. (2010). Regulation of bone development and extracellular matrix protein genes by RUNX2. *Cell Tissue Res*, *339*, 189–195.
167. Aubin, J. E., et al. (2006). Bone formation: maturation and functional activities of osteoblast lineage cells. In M. J. Favus (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (pp. 20–29) (6th ed.). Washington, DC: American Society for Bone and Mineral Research.
168. Backesjo C.-M., Li, Y., Lindgren, U., & Haldosen L.-A. (2006). Activation of Sirt1 decreases adipocyte formation during

osteoblast differentiation of mesenchymal stem cells . *J Bone Miner Res*, *21*, 993–1002.
169. Krishnan, V., Bryant, H. U., & MacDougald, O. A. (2006). Regulation of bone mass by Wnt signaling. *J Clin Invest*, *116*, 1202–1209.
170. McCarthy, T. L., & Centrella, M. (2010). Novel links among Wnt and TGF-β signaling and Runx2. *Molec Endocrinol*, *24*, 587–597.
171. Nelson, W. J., & Nusse, R. (2004). Convergence of Wnt, β-catenin, and cadherin pathways. *Science*, *303*, 1483–1487.
172. Ott, S. M. (2005). Editorial: Sclerostin and Wnt signaling: the pathway to bone strength. J *Clin Endocrinol Metab*, *90*, 6741–6743.
173. James, M. J., Jarvinen, E., Wang, X.-P., & Thesleff, I. (2006). Different roles of Runx2 during early neural crest-derived bone and tooth development. *J Bone Miner Res*, *21*, 1034–1044.
174. Reinhold, M. I., & Naski, M. C. (2007). Direct interactions of Runx2 and canonical Wnt signaling induce FGF18. *J Biol Chem*, *282*, 3653–3663.
175. Johnson, M. L. (2012). LRP5 and bone mass regulations: where are we now? *Bonekey Rep 1*, Article number: 1; doi:10.1038/bonekey.
176. Yadav, V. K., & Ducy, P. (2010). Lrp5 and bone formation. *Ann NY Acad Sci*, *1192*, 103–109.
177. Karsenty, G., & Oury, F. (2010). The central regulation of bone mass. The first link between bone remodeling and energy metabolism. *J Clin Endocrinol Metab*, *95*, 4795–4801.
178. Cui, Y., Niziolek, P. J., MacDonald, B. T., et al. (2011). Lrp5 functions in bone to regulate bone mass. Nature Med, 17, 684–691.
179. Robey, P. G., & Boskey, A. L. (2006). Extracellular matrix and biomineralization of bone. In M. J. Favus (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (pp. 12–19) (6th ed.). Washington, DC: American Society for Bone and Mineral Research.
180. Noble, S. N. (2008). The osteocyte lineage. *Arch Biochem Biophys*, *473*, 106–111.
181. Tèti, A., & Zallone, A. (2009). Do osteocytes contribute to bone mineral homeostasis? Osteocytic osteolysis revisited. *Bone*, *44*, 11–16.
182. Rubin, J., & Rubin, C. (2010). Stand UP! *J Clin Endocrinol Metab*, *95*, 2050–2053.
183. Sims, N. A., & Chia, L. Y. (2012). Regulation of sclerostin expression by paracrine and endocrine factors. *Clinic Rev Bone Miner Metab*, *10*, 98–107.
184. Chen, J.-H., Liu, C., You, L., & Simmons, C. A. (2010). Boning up on Wolff's law: mechanical regulation of the cells that make and maintain bone. *J Biomech*, *43*, 108–118.
185. Hughes, J. M., & Petit, M. A. (2010). Biological underpinnings of Frost's mechanostat thresholds: the important role of osteocytes. *Musculoskelet Neuronal Interact*, *10*, 128–135.
186. Fricke, O., Beccard, R., Semler, O., & Schoenau, E. (2010). Analysis of muscular mass and function: the impact on bone mineral density and peak muscle mass. *Pediatr Nephrol*, *25*, 2393–2400.
187. Henriksen, R., Bollerslev, J., Everts, V., & Karsdal, M. A. (2011). Osteoclast activity and subtypes as a function of physiology and pathology: implications for future treatments of osteoporosis. *Endocr Rev*, *32*, 31–63.
188. Bilezikian, J. P. (2006). Editorial: What's good for the goose's skeleton is good for the gander's skeleton. *J Clin Endocrinol Metab*, *91*, 1223–1225.
189. Vanderschueren, D., Venken, K., Ophoff, J., et al. (2006). Clinical review: sex steroids and the periosteum: reconsidering the roles of androgens and estrogens in periosteal expansion. *J Clin Endocrinol Metab*, *91*, 378–382.
190. Ohlsson, C., Borjesson, A. E., & Vandenput, L. (2012). Sex steroids and bone health in men. *BoneKey Reports*, *1*, Article number: 2; doi: 10.1038/bonekey.2012. 3.
191. Fukushima, N., Hanada, R., Teranishi, H., et al. (2004). Ghrelin directly regulates bone formation. *J Bone Miner Res*, *20*, 790–798.
192. Zelzer, E., & Olsen, B. R. (2005). Multiple roles of vascular endothelial growth factor (VEGF) in skeletal development, growth, and repair. *Curr Topics Dev Biol*, *65*, 169–187.
193. Negishi-Koga, T., & Takayangi, H. (2009). Ca^{2+}-NFATc1 signaling is an essential axis of osteoclast differentiation. *Immunological Rev*, *231*, 241–256.
194. Okamoto, M., Murai, J., Yoshikawa, H., & Tsumaloi, N. (2006). Bone morphogenetic proteins in bone stimulate osteoclasts and osteoblasts during bone development. *J Bone Miner Res*, *21*, 1022–1033.
195. Barrow, A. D., Raynal, N., Andersen, A. L., et al. (2011). OSCAR is a collagen receptor that costimulates osteoclastogenesis in DAP12-deficient humans and mice. *J Clin Invest*, *121*, 3505–3516.
196. Shinora, M., Koga, T., Okamoto, K., et al. (2008). Tyrosine kinases Btk and Tec regulate osteoclast differentiation by linking RANK and ITAM signals. *Cell*, *132*, 794–806.
197. Oikawa, T., Itoh, T., & Takenawa, T. (2008). Sequential signals toward podosome in NIH-src cells. *J Cell Biol*, *182*, 157–169.
198. Oikawa, T., Oyama, M., Kozuka-Hata, H., et al. (2012). Tks5-dependent formation of circumferential podosomes/invadopodia mediates cell-cell fusion. *J Cell Biol*, *197*, 553–568.
199. Helming, L., & Gordon, S. (2009). Molecular mediators of macrophage fusion. *Trends Cell Biol*, *19*, 514–522.
200. Soe, K., Andersen, T. L., Hobolt-Pedersen, A. S., et al. (2011). Involvement of human endogenous retroviral syncytin-1 in human osteoclast fusion. *Bone*, *48*, 837–846.
201. Fili, S., Karalaki, M., & Schaller, B. (2009). Therapeutic implications of osteoprotegerin. *Cancer Cell Intern*, *9*, 26–33.
202. Whyte, M. P., Obrecht, S. E., Finnegan, P. M., et al. (2002). Osteoprotegerin deficiency and juvenile Paget's disease. *N Engl J Med*, *347*, 175–184.
203. Weinert, S., Jabs, S., Supanchart, C., et al. (2010). Lysosomal pathology and osteopetrosis upon loss of H^+-driven lysosomal Cl- accumulation. *Science*, *328*, 1401–1403.
204. Ross, F. P. (2006). Osteoclast biology and bone resorption. In M. J. Favus (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (pp. 30–35) (6th ed.). Washington, DC: American Society for Bone and Mineral Research.
205. Byers, P. H. (1995). Disorders of collagen biosynthesis and structure. In C. R. Scriver, A. L. Beaudet, W. S. Sly, D. Vale (Eds.), *The metabolic and molecular bases of inherited disease* (pp. 4029–4077) (7th ed.). New York: McGraw-Hill.
206. Garnero, P., & Delmas, P. D. (1998). Biochemical markers of bone turnover: applications for osteoporosis. *Endocrinol Metab Clin NA*, *27*, 303–323.
207. Barnes, A. M., Chang, W., Morello, R., et al. (2006). Deficiency of cartilage-associated protein in lethal osteogenesis imperfecta. *N Engl J Med*, *355*, 2757–2764.
208. Cabral, W. A., Chang, W., Barnes, A. M., et al. (2007). Prolyl 3-hydroxylase 1 deficiency causes a recessive metabolic bone disorder resembling lethal/severe osteogenesis imperfecta. *Nature Genet*, *39*, 359–365.
209. Morello, R., Bertin, T. K., Chen, Y., et al. (2006). CRTAP is required for prolyl 3-hydroxylation and mutations cause recessive osteogenesis imperfecta. *Cell*, *127*, 291–304.
210. Jurimae, J. (2010). Interpretation and application of bone turnover markers in children and adolescents. *Curr Opin Pediatr*, *22*, 494–500.
211. Wasilewska, A., Rybi-Szuminska, A. A., & Zoch-Zwierz, W. (2009). Serum osteoprotegerin (OPG) and receptor activator of nuclear factor κB (RANKL) in healthy children and adolescents. *J Pediatr Endocrinol Metab*, *22*, 1099–1104.
212. Dorota, D., Bogdan, K. G., Mieczyslaw, G., et al. (2012). The concentrations of markers of bone turnover in normal pregnancy and preeclampsia. *Hypertens Pregnancy*, *31*, 166–176.
213. Yamaga, A., Taga, M., Hashimoto, S., & Ota, C. (1999). Comparison of bone metabolic markers between maternal and cord blood. *Horm Res*, *51*, 277–279.
214. Seibold-Weiger, K., Wollmann, H. A., Ranke, M. B., & Speer, C. P. (2000). Plasma concentrations of the carboxyterminal propeptide of type I procollagen (PICP) in preterm neonates from birth to term. *Pediatr Res*, *48*, 104–108.
215. Crofton, P. M., Wade, J. C., Taylor, M. R. H., & Holland, C. V. (1997). Serum concentrations of carboxyl-terminal propeptide of type I procollagen, amino-terminal propeptide of type III procollagen, cross-linked carboxy-terminal telopeptide of type

I collagen, and their interrelationships in school children. *Clin Chem*, *43*, 1577–1581.
216. Mora, S., Pitukcheewanont, P., Kaufman, F. R., et al. (1999). Biochemical markers of bone turnover and the volume and the density of bone in children at different stages of sexual development. *J Bone Miner Res*, *14*, 1664–1671.
217. Gracia-Marco, L., Vicente-Rodriguez, G., Valtuena, J., et al. (2010). Bone mass and bone metabolism markers during adolescence. The HELENA study. *Horm Res Paediatr*, *74*, 339–350.
218. Huang, Y., Eapen, E., Steele, S., & Grey, V. (2011). Establishment of reference intervals for bone markers in children and adolescents. *Clin Biochem*, *44*, 771–778.
219. Kirmani, S., Amin, S., McCready, L. K., et al. (2012). Sclerostin levels during growth in children. *Osteoporosis Int*, *23*, 1123–1130.
220. Rauchenzauner, M., Schmid, A., Heinz-Erain, P., et al. (2007). Sex- and age-specific reference curves for serum markers of bone turnover in healthy children from 2 months to 18 years. *J Clin Endocrinol Metab*, *92*, 443–449.
221. McNulty, M., Singh, R. J., Li, X., et al. (2011). Determination of serum and plasma sclerostin concentrations by enzyme-linked immunoassays. *J Clin Endocrinol Metab*, *96*, E1159–E1162.
222. Amrein, K., Amrein, S., Drexler, C., et al. (2012). Sclerostin and its association with physical activity, age, gender, body composition, and bone mineral content in healthy adults. *J Clin Endocrinol Metab*, *97*, 148–154.
223. Costa, A. G., Cremers, S., Rubin, M. R., et al. (2011). Circulating sclerostin in disorders of parathyroid gland function. *J Clin Endocrinol Metab*, *96*, 3804–3810.
224. Drake, M. T., Srinivasan, B., Modder, U. I., et al. (2010). Effects of parathyroid hormone treatment on circulating sclerostin levels in postmenopausal women. *J Clin Endocrinol Metab*, *95*, 5056–5062.
225. Gaudio, A., Pennisi, P., Bratengeier, C., et al. (2010). Increased sclerostin serum levels associated with bone formation and resorption markers in patients with immobilization-induced bone loss. *J Clin Endocrinol Metab*, *95*, 2248–2253.
226. Modder, U. I., Hoey, K. A., Amin, S., et al. (2011). Relation of age, gender, and bone mass to circulating sclerostin levels in men and women. *J Bone Miner Res*, *26*, 373–379.
227. Karsenty, G. (2012). The mutual dependence between bone and gonads. *J Endocrinol*, *213*, 107–114.
228. Karsenty, G., & Oury, F. (2012). Biology without walls: the novel endocrinology of bone. *Annu Rev Physiol*, *74*, 87–105.
229. Gericke, A., Qin, C., Spevak, L., et al. (2005). Importance of phosphorylation for osteopontin regulation of biomineralization. *Calc Tiss Int*, *77*, 45–54.
230. Chavassieux, P., Seeman, E., & Delmas, P. D. (2007). Insights into material and structural basis of bone fragility from diseases associated with fractures. How determinants of the biomechanical properties of bone are compromised by disease. *Endocr Rev*, *28*, 151–164.
231. Rubin, C., & Rubin, J. (2006). Biomechanics and mechanobiology of bone. In M. J. Favus (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (pp. 36–42) (6th ed.). Washington, DC: American Society for Bone and Mineral Research.
232. Hughes-Fulford, M. (2004). Signal transduction and mechanical stress. *Sci STKE*, *2004*, re12.
233. Specker, L., & Schonau, E. (2005). Quantitative bone analysis in children: current methods and recommendations. *J Pediatr*, *146*, 726–731.
234. Bailey, D. A., McKay, H. A., Mirwald, R. L., et al. (1999). A six-year longitudinal study of the relationship of physical activity to bone mineral accrual in growing children: The University of Saskatchewan bone mineral accrual study. *J Bone Miner Res*, *14*, 1672–1679.
235. Gilsanz, V., Chalfant, J., Kalkwarf, H., et al. (2011). Age at onset of puberty predicts bone mass in adulthood. *J Pediatr*, *158*, 100–105.
236. Greenfield, E. M., & Goldberg, V. M. (1997). Genetic determination of bone density. *Lancet*, *350*, 1263–1254.
237. Center, J. R., & Eisman, J. A. (2008). Genetics of osteoporosis. In C. J. Rosen (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (pp. 213–219) (7th ed.). Washington, DC: The American Society of Bone and Mineral Metabolism.
238. Ralston, S. H., & Uitterlinden, A. G. (2010). Genetics of osteoporosis. *Endocr Rev*, *31*, 629–662.
239. Kalkwarf, H. J., Zemel, B. S., Gilsanz, V., et al. (2007). The bone mineral density in childhood study: bone mineral content and density according to age, sex, and race. *J Clin Endocrinol Metab*, *92*, 2087–2099.
240. Rubin, L. A., Hawker, G. A., Peltekova, V., et al. (1999). Determinants of peak bone mass: clinical and genetic analyses in a young female Canadian cohort. *J Bone Miner Res*, *14*, 633–643.
241. Weaver, C. M., Peacock, M., & Johnston, C. C., Jr. (1999). Adolescent nutrition in the prevention of postmenopausal osteoporosis. *J Clin Endocrinol Metab*, *84*, 1839–1843.
242. Pitukcheewanont, P., Punyasavatsut N, & Feuilee, M. (2010). Physical activity and bone health in children and adolescents. *Pediatr Endocrinol Rev*, 7, 275–282.
243. Morel, J., Combe, B., Francisco, J., & Bernard, J. (2001). Bone mineral density of 704 amateur sportsman involved in different physical activities. *Osteoporosis Int*, *12*, 152–157.
244. Rikkonen, T., Tuppurainen, M., Kroger, H., et al. (2006). Distance of walking in childhood and femoral bone density in perimenopausal women. *Eur J Appl Physiol*, *97*, 509–515.
245. Van der Eerden, B. C. J., Karperien, M., & Wit, J. (2003). Systemic and local regulation of the growth plate. *Endocr Rev*, *24*, 782–801.
246. Rosenfeld, R. (2006). Molecular mechanisms of IGF-I deficiency. *Horm Res*, *65*(Suppl. 1), 15–20.
247. Cruickshank, J., Grossman, D. I., Peng, R. K., et al. (2005). Spatial distribution of growth hormone receptor, insulin-like growth factor-1 receptor and apoptotic chondrocytes during growth plate development. *J Endocrinol*, *184*, 543–553.
248. Wang, Y., Nishida, S., Sakata, T., et al. (2006). Insulin-like growth factor-I is essential for embryonic bone development. *Endocrinology*, *147*, 4753–4761.
249. Cohen, P. (2006). Overview of the IGF-I system. *Horm Res*, *65*(Suppl. 1), 3–8.
250. Yakar, S., Liu, J.-L., Stannard, B., et al. (1999). Normal growth and development in the absence of hepatic insulin-like growth factor-I. *Proc Natl Acad Sci USA*, *96*, 7324–7329.
251. Savage, M. O., Attie, K. M., David, A., et al. (2006). Endocrine assessment, molecular characterization and treatment of growth hormone insensitivity. *Nat Clin Pract Endocrinol Metab*, *2*, 395–407.
252. Caminos, J. E., Gualillo, O., Lago, F., et al. (2005). The endogenous growth hormone secretagogue (ghrelin) is synthesized and secreted by chondrocytes. *Endocrinology*, *146*, 1285–1292.
253. Ohlsson, C., Bengtsson B-A, Isaksson O. G. P., et al. (1998). Growth hormone and bone. *Endocr Rev*, *19*, 55–79.
254. Barnard, J. C., Williams, A. J., Rabier, B., et al. (2005). Thyroid hormones regulate fibroblast growth factor receptor signaling during chondrogenesis. *Endocrinology*, *146*, 5568–5580.
255. Couse, J. F., & Korach, K. S. (1999). Estrogen receptor null mice: what have we learned and where will they lead us? *Endocr Rev*, *20*, 358–417.
256. Rauch, F., Bailey, D. A., Baxter-Jones, A., et al. (2004). The 'muscle-bone unit' during the pubertal growth spurt. *Bone*, *34*, 771–775.
257. Manolagas, S. C. (2000). Birth and death of bone cells: basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. *Endocr Rev*, *2*, 115–137.
258. Robson, H., Siebler, T., Shalet, S. M., & Williams, G. R. (2003). Interactions between GH, IGF-I, glucocorticoids, and thyroid hormones during skeletal growth. *Pediatr Res*, *52*, 137–147.
259. Lui, J. C., & Baron, J. (2011). Effects of glucocorticoids on the growth plate. *Endocr Dev*, *20*, 187–193.
260. Potter, L. R., Abbey-Hosch, A., & Dickey, D. M. (2006). Natriuretic peptides, their receptors, and cyclic guanosine monophosphate-dependent signaling functions. *Endocr Rev*, *27*, 47–72.

261. Lenz, A. (2011). Natriuretic peptides in children: physiology and clinical utility. *Curr Opin Pediatr*, *23*, 452–459.
262. Prickett, T. M.C. R., Lynne, A. M., Barrell, G. K., et al. (2005). Amino-terminal proCNP: a putative marker of cartilage activity in post natal growth. *Pediatr Res*, *58*, 334–340.
263. Bartels, C. F., Bukulmez, H., Padayatti, P., et al. (2004). Mutations in the transmembrane natriuretic peptide receptor NPR-B impair skeletal growth and cause acromesomelic dysplasia, type Maroteaux. *Am J Hum Genet*, *75*, 27–34.
264. Janicka, A., Wren, T. A. L., Sanchez, M. M., et al. (2007). Fat mass is not beneficial to bone in adolescents and young adults. *J Clin Endocrinol Metab*, *92*, 143–147.
265. Reid, I. R. (2010). Fat and bone. *Arch Biochem Biophys*, *503*, 20–27.
266. Luo, X.-H., Guo, L.-J,. Xie, H., et al. (2006). Adiponectin stimulates RANKL and inhibits OPG expression in human osteoblasts through the MAPK signaling pathway. *J Bone Miner Res*, *21*, 1648–1656.
267. Glorieux, F. H., Travers, R., Taylor, A., et al. (2000). Normative data for iliac bone histomorphometry in growing children. *Bone*, *26*, 103–109.
268. Rauch, F. (2006). Watching bones at work: what we can see from bone biopsies. *Pediatr Nephrol*, *21*, 457–462.
269. Khosla, S., Melton, L. J., Achenbach, S. J., et al. (2006). Hormonal and biochemical determinants of trabecular microstructure at the ultradistal radius in women and men. *J Clin Endocrinol Metab*, *91*, 885–891.
270. Kirmani, S., Christen, D., van Lenthe, G. H., et al. (2009). Bone structure at the distal radius during adolescent growth. *J Bone Miner Res*, *24*, 1033–1042.
271. Bhagat, Y. A., Rajapakse, C. S., Magland, J. F., et al. (2011). Performance of μMRI virtual bone biopsy for structural and mechanical analysis at the distal tibia at 7T field strength. *J Magn Reson Imaging*, *33*, 372–381.
272. Bachrach, L. K. (2005). Osteoporosis and measurement of bone mass in children and adolescents. *Endocrinol Metab Clin North Am*, *34*, 521–535.
273. Ward, K. A., Ashby, R. L., Roberts, S. A., et al. (2007). UK reference data for the Hologic QDR Discovery dual-energy x ray absorptiometry scanner in healthy children and young adults aged 6–17 years. *Arch Dis Child*, *92*, 53–59.
274. Gilsanz, V., Skaggs, D. L., Kovanlikaya, A., et al. (1998). Differential effects of race on the axial and appendicular skeletons of children. *J Clin Endocrinol Metab*, *83*, 1420–1427.
275. Miller, P. D., & Leonard, M. B. (2006). Clinical use of bone mass measurements in children and adults for the assessment and management of osteoporosis. In M. J. Favus (Eed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (pp. 150–161) (6th ed.). Washington, DC: American Society for Bone and Mineral Research.
276. Brismar, T. B., Lindgren A.-C., Ringertz, H., et al. (1998). Total bone mineral measurements in children with Prader-Willi syndrome: the influence of the skull's bone mineral content per area (BMA) and of height. *Pediatr Radiol*, *28*, 38–42.
277. Zemel, B. S., Kalkwarf, H. J., Gilsanz, V., et al. (2011). Revised reference curves for bone mineral content and areal bone mineral density according to age and sex for black and non-black children: results of the Bone Mineral Density in Childhood Study. *J Clin Endocrinol Metab*, *96*, 3160–3169.
278. Utriainen, P., Jaaskelainen, J., Saarinen, A., et al. (2009). Body composition and bone mineral density in children with premature adrenarche and the association of LRP5 gene polymorphisms with bone mineral density. *J Clin Endocrinol Metab*, *94*, 4144–4151.
279. Zemel, B. S., Leonard, M. B., Kelly, A., et al. (2010). Height adjustment in assessing dual energy x-ray absorptiometry measurements of bone mass and density in children. *J Clin Endocrinol Metab*, *95*, 1265–1273.
280. Koo, W. W. K. (2000). Body composition measurements during infancy. *NY Acad Sci*, *904*, 383–392.
281. Koo, W. W. K., Walters, J., Bush, A. J., et al. (1996). Dual-energy x-ray absorptiometry studies of bone mineral status in newborn infants. *J Bone Miner Res*, *11*, 1997–1002.
282. Lapillone, A., Braillon, P., Claris, O., et al. (1997). Body composition in appropriate and in small for gestational age infants. *Acta Paediatr*, *86*, 196–200.
283. Weiler, H. A., Yuen, C. K., & Seshia, M. M. (2002). Growth and bone mineralization of young adults weighing less than 1500 grams at birth. *Early Hum Devel*, *67*, 101–1012.
284. Yeste, D., Aslmar, J., Clemente, M., et al. (2004). Areal bone mineral density of the lumbar spine in 80 premature newborns: a prospective and longitudinal study. *J Pediatr Endocrinol Metab*, *17*, 959–966.
285. Zemel, B. S., Leonard, M. B., Kalkwarf, H. J., et al. (2004). Reference data for whole body, lumbar spine and proximal femur for American children relative to age, gender and body size. *J Bone Miner Res*, *19*(Suppl. 1), S231.
286. McKay, H. A., Bailey, D. A., Mirwald, R. L., et al. (1998). Peak bone mineral accrual and age at menarche in adolescent girls: a 6-year longitudinal study. *J Pediatr*, *133*, 682–687.
287. Wren, T. A. L. H., Liu, X., Pitukcheewanont, P., & Gilsanz, V. (2005). Bone densitometry in pediatric patients: discrepancies in the diagnosis of osteoporosis by DXA and CT. *J Pediatr*, *146*, 776–779.
288. Rauch, F., & Schonau E (2005). Peripheral quantitative computed tomography of the distal radius in young subjects: new reference data and interpretation of results. *J Musculoskelet Neuronal Interact*, *5*, 119–126.
289. Chevalley, T., Bonjour, J. P., van Rietbergen, B., et al. (2011). Fractures during childhood and adolescence in healthy boys: relation with bone mass, microstructure, and strength . J Clin Endocrinol Metab, *96*, 3134–3142.
290. Zadik, Z., Price, D., & Diamond, G. (2003). Pediatric reference curves for multi-site quantitative ultrasound and its modulators. *Osteoporos Int*, *14*, 857–862.
291. Baroncelli, G. I., Bertelloni, S., Ceccarelli, C., & Saggese G (1998). Measurement of volumetric bone mineral density accurately determines degree of lumbar undermineralization in children with growth hormone deficiency. *J Clin Endocrinol Metab*, *83*, 3150–3154.
292. Klentrou, P., Ludwa, I. A., & Falk, B. (2011). Factors associated with bone turnover and speed of sound in early and late-pubertal females. *Appl Physiol Nutr Metab*, *36*, 707–714.
293. Ashmeade, T., Pereda, L., Chen, M., & Carver, J. D. (2007). Longitudinal measurements of bone status in preterm infants. *J Pediatr Endocrinol Metab*, *20*, 415–424.
294. Majumdar, S. (2008). Magnetic resonance imaging of bone. In C. J. Rosen (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (pp. 163–166) (7th ed.). Washington, DC: The American Society of Bone and Mineral Metabolism.
295. Wehrli, F. W. (2007). Structural and functional assessment of trabecular and cortical bone by micro magnetic resonance imaging. *J Magn Reson Imaging*, *25*, 390–409.
296. Liu, X. S., Zhang, X. H., Rajapakse, C. S., et al. (2010). Accuracy of high-resolution in vivo micro magnetic resonance imaging for measurements of microstructural and mechanical properties of human distal tibial bone. *J Bone Miner Res*, *25*, 2039–2050.
297. Kovacs, C. S., & Kronenberg, H. M. (2008). Pregnancy and lactation. In C. J. Rosen (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (pp. 90–95) (7th ed.). Washington, DC: American Society for Bone and Mineral Research.
298. Black, A. J., Topping, J., Durham, B., et al. (2000). A detailed assessment of alterations in bone turnover, calcium homeostasis, and bone density in normal pregnancy. *J Bone Miner Res*, *15*, 557–563.
299. Koo, W. W. K., & Warren, L. (2003). Calcium and bone health in infants. Neonatal Netw, *22*, 23–37.
300. Weisman, Y. (2003). Maternal, fetal, and neonatal vitamin D and calcium metabolism during pregnancy and lactation. In Z. Hochberg (Ed.), *Vitamin D and rickets. Endocr Dev*, *6*, 34–49.
301. Kovacs, C. S. (2008). Fetal calcium metabolism. In C. J. Rosen (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (pp. 108–112) (7th ed.). Washington, DC: American Society for Bone and Mineral Research.
302. Rigo, J., De Curtis, M., Pieltain, C., et al. (2000). Bone mineral metabolism in the micropremie. *Clin Perinatol*, *27*, 147–170.

303. Koo, W. W. K., & Hockman, E. M. (2000). Physiologic predictors of lumbar spine bone mass in neonates. *Pediatr Res*, *48*, 485–489.
304. Bradbury, R. A., Cropley, J., Kifor, O., et al. (2002). Localization of the extracellular Ca^{2+}-sensing receptors in the human placenta. *Placenta*, *23*, 192–200.
305. Rummens, K., van Cromphaut, S. J., Carmeliet, G., et al. (2003). Pregnancy in mice lacking the vitamin D receptor: normal maternal skeletal response, but fetal hypomineralization rescued by maternal calcium supplementation. *Pediatr Res*, *54*, 466–473.
306. Carpenter, T. O. (2006). Neonatal hypocalcemia. In M. J. Favus (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (pp. 224-227) (6th ed.). Washington, DC: American Society for Bone and Mineral Research.
307. Hsu, S. C., & Levine, M. A. (2004). Perinatal calcium metabolism: physiology and pathophysiology. *Semin Neonatol*, *9*, 23–36.
308. Koo, W. W., Hockman, E. M., & Dow, M. (2006). Palm olein in the fat blend of infant formulas: effect on the intestinal absorption of calcium and fat, and bone mineralization. *J Am Coll Nutr*, *25*, 117–122.

QUESTIONS

1. In response to changes in the extracellular concentration of ionized calcium, what does the calcium sensing receptor do?
   a. Stimulates synthesis of parathyroid hormone
   b. Transduces its intracellular signal through activation of tyrosine kinase
   c. Impairs production of calcitriol (1,25-dihydroxyvitamin D3)
   d. Decreases renal tubular reabsorption of filtered calcium
   e. Inhibits the secretion of calcitonin

Answer: d

2. Which of the following describes α-Klotho?
   a. A coreceptor for parathyroid hormone and parathyroid hormone related protein (PTH/PTHrP)
   b. Essential for the regulation of renal tubular reabsorption of phosphate
   c. Necessary for the nuclear action of vitamin D
   d. A member of the intracellular adenylyl cyclase signal transduction pathway
   e. Critical for the differentiation and specificity of bone alkaline phosphatase

Answer: b

3. Which of the following describes parathyroid hormone related protein (PTHrP)?
   a. Its secretion is regulated by the serum ionized calcium concentration.
   b. It accelerates chondrocyte differentiation.
   c. It regulates placental transport of calcium.
   d. It increases the rate of cartilage ossification.
   e. It suppresses breast development in utero.

Answer: c

4. Which of the following describes calcitriol (1,25-dihydroxyvitamin D3)?
   a. Is inactivated in the kidney by hydroxylation at carbon 24
   b. Maintains a spatially fixed three-dimensional configuration
   c. Down-regulates the expression of epithelial calcium channels in the intestinal tract and kidney
   d. Suppresses osteoblast differentiation and proliferation
   e. Inhibits paracellular transport of calcium by the renal tubule

Answer: a

5. SOX9 encodes a transcription factor that does which of the following?
   a. Represses testicular differentiation
   b. Enhances osteoclast function
   c. Modulates PTH/PTHrP intracellular signal transduction
   d. Activates cellular apoptotic pathways
   e. Regulates the synthesis of cartilage matrix components

Answer: e

6. How does the WNT/β-catenin signal transduction pathway function?
   a. Enhances vitamin D synthesis
   b. Suppresses calcitonin secretion
   c. Decreases osteoclast secretion of acid
   d. Mediates osteoblast differentiation and function
   e. Modifies the fragmentation of parathyroid hormone (PTH)

Answer: d

7. What is the role of osteoprotegerin?
   a. Antagonizes function of the ligand of the receptor activator of nuclear factor κB (NFκB-ligand)
   b. Enhances osteoclastogenesis
   c. Suppresses osteoblastogenesis
   d. Moderates synthesis of parathyroid hormone related protein (PTHrP)
   e. Accelerates degradation of collagen type I

Answer: a

CHAPTER 9

Neonatal Diabetes Mellitus

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CHAPTER OUTLINE

DEFINITION

Neonatal diabetes mellitus (NDM) was strictly defined as severe hyperglycemia occurring in the first month of life, lasting at least 2 weeks, and requiring insulin therapy to control blood glucose.[1] These strict criteria have been progressively loosened, initially to include onset in the first 6 months of life, when autoimmune type 1 diabetes mellitus (T1DM) is highly unlikely, thereby implicating a genetic cause for pancreatic malformation, or faulty insulin synthesis and secretion. Increasingly, it has become recognized that, depending on the degree of expression and the inherent capacity of the genetic mutation to disrupt normal insulin output, several of the genetic forms can initially present with symptoms up to 9 months to 1 year, or even later.[2-4] Indeed, pedigree analyses conclusively demonstrate that the same defect that causes permanent or transient NDM can be present in parents or other first-degree relatives and be diagnosed as T1DM, monogenic diabetes of youth (MODY), or type 2 diabetes mellitus (T2DM) as subsequently detailed.[5,6] Herein lies the importance of understanding the genetic basis of NDM, for although considered rare, with a reported incidence varying from approximately 1 in 100,000 to 1 in 400,000 live births,[7] these entities have taught us much about the genetic pathways involved in the formation of the exocrine and endocrine pancreas.[8,9] For example, it has been shown that the specific combination of three transcription factors, *Ngn3*, *Pdx1* and *Mafa*, known to be implicated in the determination of cell lineage during pancreas formation, can reprogram adult mouse exocrine pancreatic cells into cells that closely resemble pancreatic β cells.[10] Such information is essential for the ultimate ability to generate β cells and whole islets as potential therapies for T1DM.[11] Equally important is the demonstration that activating mutations in the pore-forming $K_{ir6.2}$ and its regulatory subunit SUR1 of the K_{ATP}-regulated potassium channel, which keep the channel open and hence limit or preclude insulin secretion resulting in NDM (Figure 9-1), can be overcome by high-dose sulfonylurea therapy, which restores endogenous insulin secretion in response to feeding.[12] Due to the restoration of endogenous insulin secretion together with an incretin effect in response to feeding as opposed to intravenous glucose, this oral treatment provides better metabolic control than multiple daily injections of insulin or insulin pumps and with a better quality of life.[7,12] These findings emphasize the benefits of research for understanding pathophysiology and choosing appropriate treatment. Indeed, for those whose NDM is caused by mutations in the K_{ATP} channel that respond to sulfonylureas, these treatments border on the miraculous.

INCIDENCE

Early estimates placed the incidence at approximately 1 in 500,000,[1] and most authorities still cite figures in the 1 in 200,000 to1 in 400,000 range.[13] As increased awareness has led to these entities being recognized more frequently, the reported incidence has risen considerably. In populations with high rates of consanguinity, it has been reported that 1 in 21,000 births is associated with NDM.[14] A large representative database for pediatric diabetes reported that the incidence of NDM

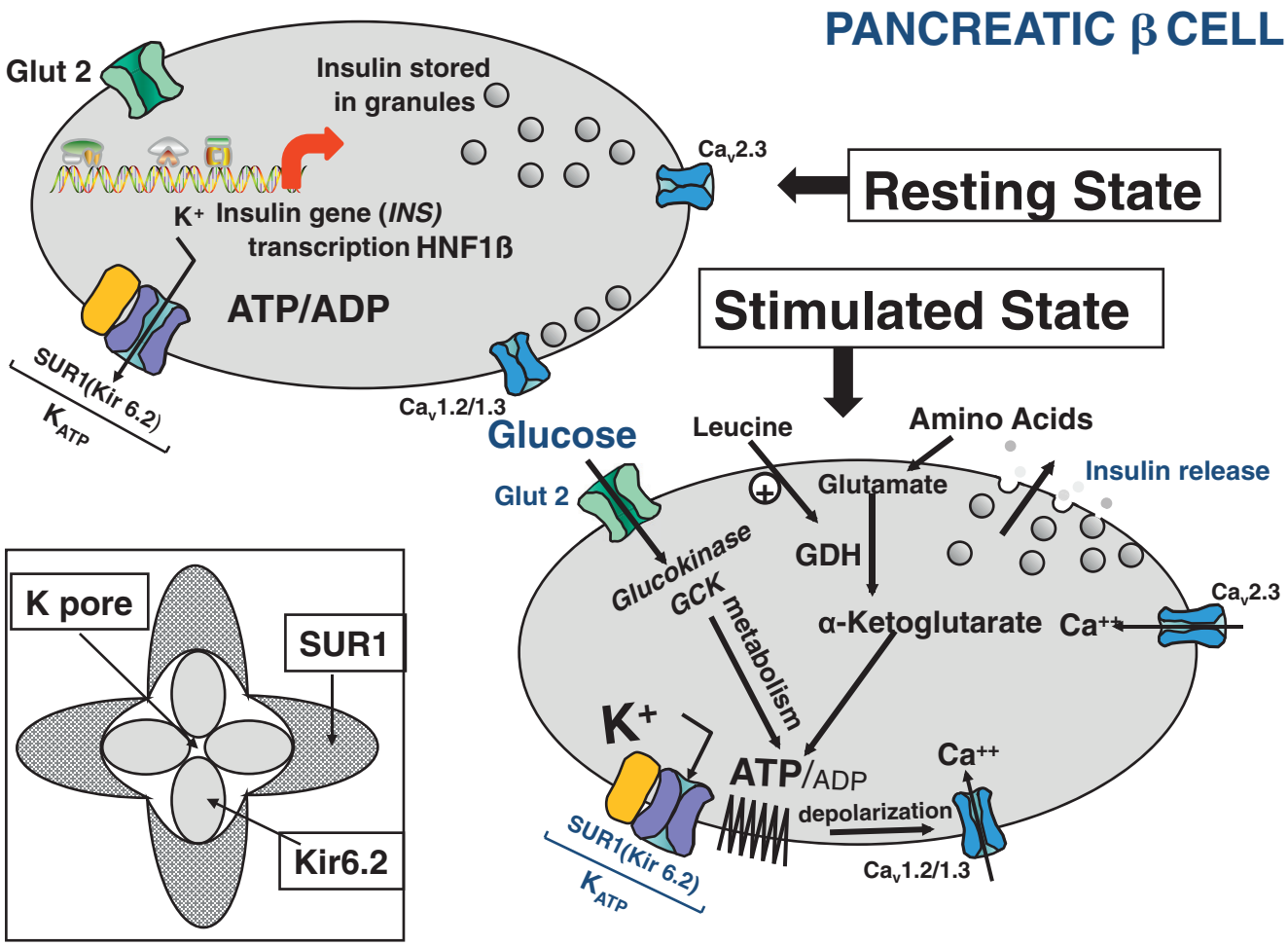

FIGURE 9-1 ■ This is a schematic representation of the role of K_{ATP} channels in nutrient regulation of insulin secretion. In the resting (nonfed) state, depicted in the upper left panel, insulin synthesis and storage are regulated by transcription from the insulin gene (INS) and by transcription factors such as hepatic nuclear factor 1β (HNF1β); mutations in INS or HNF1β as well as other genes can cause transient or permanent neonatal diabetes mellitus (NDM).The K_{ATP} channel is composed of four subunits of the inward rectifying potassium channel 6.2 (Kir 6.2) encoded by the KCNJ11 gene on chromosome #11) and four regulatory subunits of sulfonylurea receptor 1 (SUR1), encoded by the ABCC8 gene, also located on chromosome #11 (inset lower left). In the fasting nonfed state, the K_{ATP} channel remains open. However, in the stimulated (fed) state (panel lower right) glucose concentration increases and enters the β cell in a concentration-dependent, but insulin-independent manor via the Glut 2 glucose transporter encoded by the gene SLCA2A. Glucokinase (GCK) phosphorylates glucose to G6P and its metabolism generates ATP. The resultant change in ATP:ADP causes closure of the K_{ATP} channel, accumulation of intracellular potassium, membrane depolarization leading to opening of voltage-gated calcium channels and secretion of stored insulin, as depicted in the lower right panel. Metabolism of amino acids such as glutamate also generates ATP, which stimulates insulin secretion as described for glucose. The amino acid leucine acts as an allosteric stimulus to glutamate dehydrogenase (GDH), which enables metabolism and generation of ATP. Activating mutations of the KATP channel maintain it in an open state to varying degrees, in spite of ATP generation, therefore preventing insulin secretion, which leads to diabetes mellitus, including NDM. Inactivating mutations in KATP genes prevent normal channel opening, maintaining varying degrees of channel closure, and hence constant insulin secretion that causes HI (see Chapter 21). ATP, adenosine triphosphate; ADP, adenosine diphosphate; Ca++ -, calcium; GDH, glutamate dehydrogenase; K_{ATP}, ATP-regulated potassium channel; INS, insulin gene; HNF1β, hepatic nuclear factor1β; SUR1, sulfonylurea receptor1, K+, potassium; Kir6.2, potassium inward rectifying channel family6 subtype2; GCK, glucokinase, Glut2, glucose transporter 2. *(This image can be viewed in full color online at ExpertConsult)*

represented approximately 1 case in 89,000 live births in Germany, and a similar incidence occurred in Italy.[15,16] However, in three other European countries, the incidence was reported to be 1 in 260,000 live births.[17] In the SEARCH for Diabetes in Youth Study involving 15,829 subjects aged < 20 years diagnosed with diabetes during the years 2001-2008, 39 were diagnosed before the age of 6 months.[18] Among these 39 subjects with onset < 6 months of age, 35 had permanent neonatal diabetes and an additional 3 had transient NDM leaving 1 subject whose status remained unknown.[18] Hence, the total prevalence was approximately 0.246% or approximately 1 in 4000 of all children diagnosed with diabetes in that study during that time frame. The majority were classified by their primary care providers as having T1DM and treated with insulin; only seven underwent mutational analysis for three of the most common genes (*KCNJ11*, *ABCC8*, and the insulin gene *INS*) and five of these seven had mutations in one of these three genes.[18]

CLINICAL PRESENTATION

Typically, infants affected with NDM present in the first days to weeks of life with intrauterine growth retardation, reflecting the in utero deficiency of insulin and emphasizing the role of insulin as a determinant of fetal growth.[19] Their small size and low birth weight markedly contrasts with the large birth weight and size of infants with inactivating mutations in the same genes that lead to hyperinsulinemic hypoglycemia (see Chapter 21). A disproportionate number are born prematurely at < 37 weeks' gestation.[20] Hyperglycemia leads to osmotic diuresis and avid feeding from breast or bottle despite which the infants fail to thrive. Delay in diagnosis from not considering the possibility of diabetes mellitus in a newborn may lead to severe dehydration and life-threatening diabetic ketoacidosis. Some have congenital malformations including macroglossia and umbilical hernia, reminiscent of Beckwith-Wiedemann syndrome; these usually represent inheritance of genes with disordered methylation as a result of uniparental (paternal) disomy, paternal inherited duplications, or maternal hypomethylation of a differentially methylated region (DMR) at chromosome 6q24 (Figure 9-2).[20] Some of these infants have dysmorphic facies as well as renal tract anomalies such as hydronephrosis and vesicoureteral reflux, a variety of cardiac anomalies, hypothyroidism, and hand-finger anomalies.[20] A coarse facial appearance together with epilepsy and later manifestations of developmental delay constitute the *d*evelopmental delay, *e*pilepsy, *n*eonatal *d*iabetes (DEND) syndrome associated with severe mutations of the KCNJ11 gene.[12] However, clinical manifestations involving organs other than the pancreas may be part of the syndrome of a variety of genetic mutations associated with NDM as listed in Figure 9-3.

CLASSIFICATION

The entities of NDM are conveniently divided into transient and permanent, which together make up approximately 90% of the genetically recognized types of NDM; a third category constituting approximately 10% of known entities is associated with mutations that affect organs other than the pancreas and hence present as syndromes whose spectrum of clinical and radiologic features provide clues to the cause (see Figure 9-3). Transient NDM is so named because hyperglycemia is transient, and in the most common form, named TNDM1, it resolves at a median age of 3 months, although rarely it has taken up to 48 months for resolution.[20] Generally, those that do not remit by the end of the first 6 months to 1 year are considered to have permanent neonatal diabetes. Transient neonatal diabetes predominantly involves disturbances in the imprinted genes *pl*eomorphic *a*denoma *g*ene-*l*ike 1 (PLAGL-1) also known as *z*inc

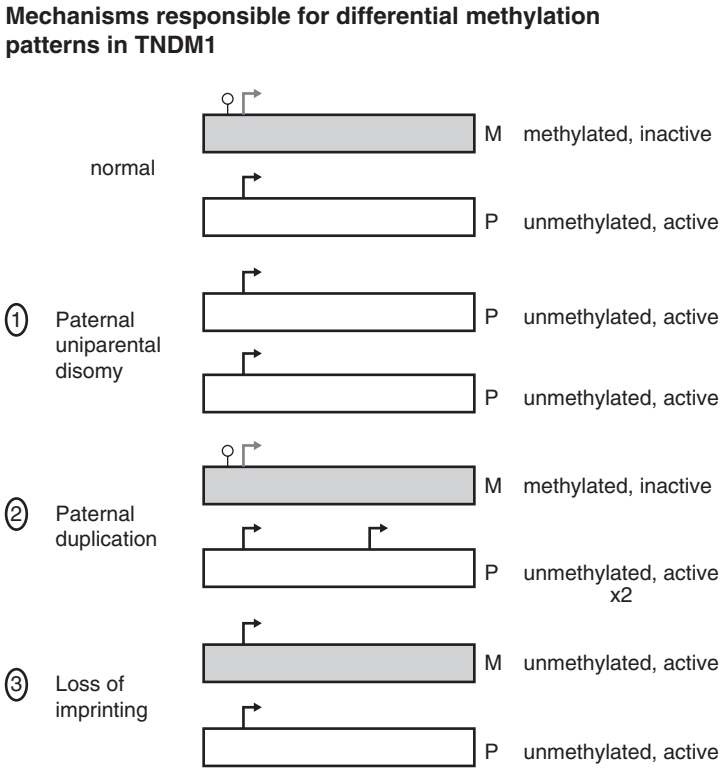

FIGURE 9-2 ■ Transient neonatal diabetes mellitus type 1 is caused by differential expression of PLAGL1 and HYMAI, genes on chromosome 6q24 that result from differential expression of the maternal and paternal alleles. Normally the maternal allele remains methylated and inactive, whereas the paternal allele is unmethylated and active. The differential expression of these genes can occur by one of three mechanisms: (1) paternal uniparental disomy, where both genes are of paternal origin; (2) paternal duplication, so that two active paternal genes are expressed; (3) loss of imprinting, also referred to as "relaxation of imprinting," on the maternal genes on 6q24 as illustrated here. See text for details.

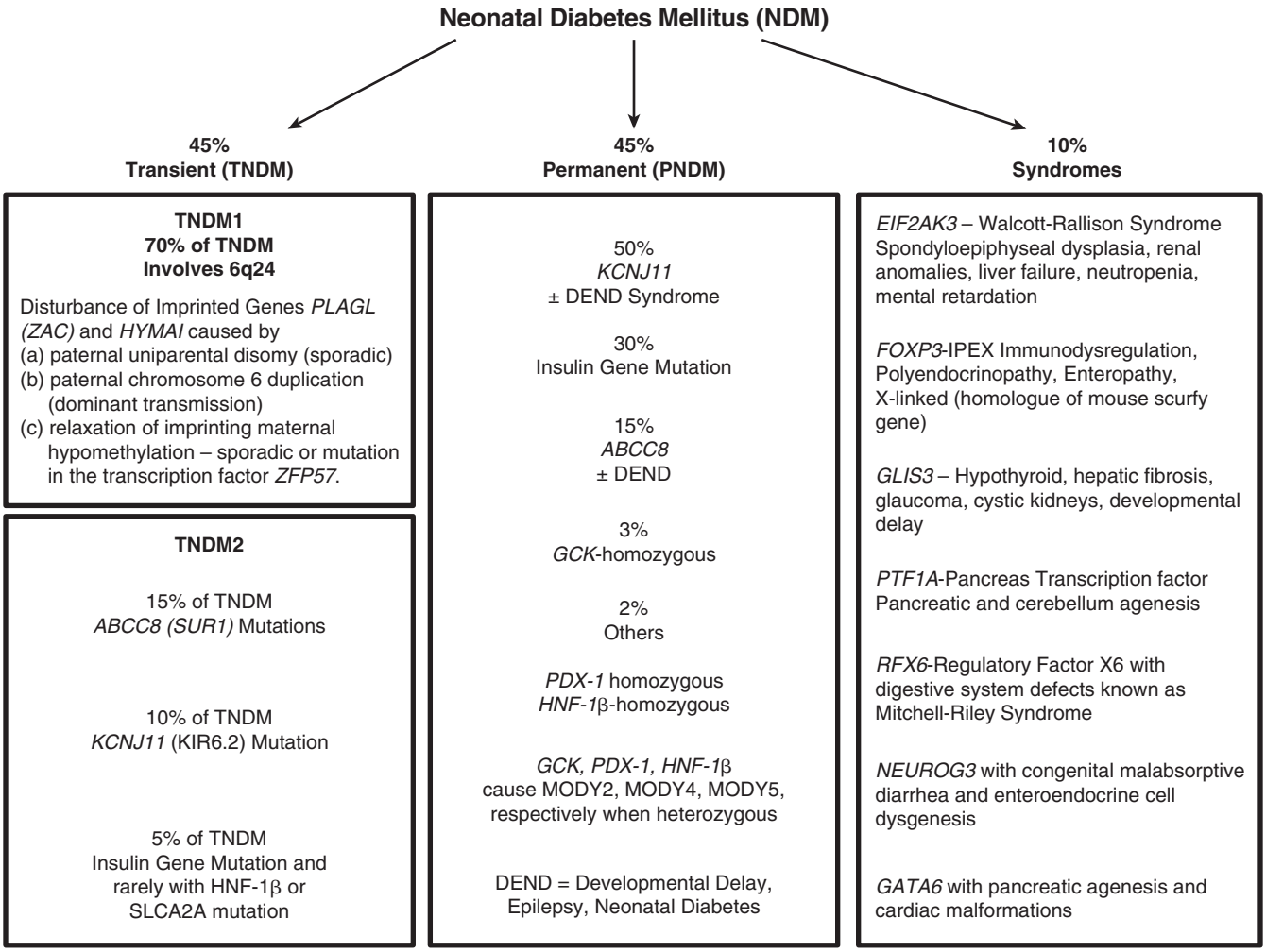

FIGURE 9-3 ■ Classification of neonatal diabetes mellitus. (Modified from Mccarthy, M. I., & Hattersley, A. T. (2008). Learning from molecular genetics: novel insights arising from the definition of genes for monogenic and type 2 diabetes. *Diabetes, 57*, 2889–2898.)

finger gene regulating *a*poptosis and the *c*ell cycle (ZAC) and *hy*datidiform *m*ole-*a*ssociated and *i*mprinted transcript (HYMAI) on the differentially methylated region (DMR) of chromosome 6q24. This entity has been named transient neonatal diabetes mellitus type 1 (TNDM1) and comprises the majority, estimated at some 70% of all forms of TNDM. A second form of TNDM, named TNDM2 to distinguish it from the defects in the 6q24 region, includes mutations in the ATP-regulated potassium channel involving predominantly mutations in ABCC8 (SUR1) and KCNJ11 (KIR6.2), with a small minority being due to recessive insulin gene mutations, mutations in the transcription factor HNF1β, and mutations in SLCA2A, the gene encoding the GLUT2 transporter, that together account for ~30% of TNDM (see Figure 9-3).

Transient Neonatal Diabetes Mellitus (TNDM)

TNDM1

TNDM1 is caused by the overexpression of the paternal PLAGL-1, which is a proapoptotic zinc finger protein, and HYMAI gene, encoding an untranslated mRNA, which arise as a result of uniparental disomy (UPD), duplication of chromosome region 6q24, or relaxation of imprinting of the maternal methylated genes on chromosome 6 (see Figures 9-2 and 9-3). It is important to emphasize that these chromosomal changes cause altered expression of genes, rather than representing mutations. For example, PLAGL-1 (ZAC) has antiproliferative properties and is thought to function as a tumor growth suppressor expressed only on the paternal allele[21]; overexpression in fetal life is believed to lead to underdevelopment of the pancreas. Although the precise mechanisms by which overexpression of PLAGL-1 lead to TNDM1 are not known, overexpression of ZAC in a clonal pancreatic beta cell line impairs glucose-stimulated insulin translation and secretion.[22] A transgenic mouse model expressing the human TNDM1 locus (6q24) is characterized by impaired glucose homeostasis with hyperglycemia in the neonatal period and impaired glucose tolerance with reduced insulin responses to IV glucose as adults.[23] The pancreata of these animals display reduced expression of endocrine differentiation factors, notably Pdx-1, Ngn3, and Pax6. There is also a reduction in the number of insulin staining cells and

reduced insulin content or insulin secretion despite normal or elevated beta cell mass at all postnatal periods.[23,24] Thus, this model recapitulates TNDM1 and suggests that altered expression of ZAC/HYMAI cause impaired development of the endocrine pancreas as well as impaired beta cell function.[23,24] This mouse model also demonstrates resolution of abnormal insulin secretion with restoration of normal glucose tolerance during the "juvenile" phase of mouse development between 1.5 and 2 months of life, during which there is an approximate doubling of beta cell number that compensates for the reduced insulin synthesis and secretion of each cell. Also, as in humans, the compensatory increase in beta cell mass is not sustained resulting in a mild diabetes mellitus characterized by normal fasting glucose but hyperglycemia after glucose challenge.[23,24] Overall, despite the recapitulation of the key features of the human disease, the mouse model displays milder features.[23,24] One possible reason for this milder phenotype in mice is that pancreatic expression of the mouse ortholog Zac-1 declines drastically during gestation and early postnatal growth in mice, whereas expression of the ZAC gene in human pancreas declines between the second trimester and adult life.[25] More important, ZAC was specifically expressed only in the islets of the human fetus, whereas Zac-1 was predominantly expressed in mesenchyme of the mouse embryo which may explain the milder features in the mouse model of TNDM1.[25]

In patients with TNDM1 due to hypomethylation of the maternal DMR of chromosome 6q24, there may be hypomethylation of other maternally imprinted loci (HIL) throughout the genome.[26] In cases that display a more generalized hypomethylation of imprinted loci (HIL), the majority have a mutation in the transcription factor zinc finger protein 57 (ZFP57).[27] HIL also occurs in the Beckwith-Wiedemann syndrome and this likely explains the macroglossia, umbilical hernia, and several congenital abnormalities described in TNDM1.[28] In a large multinational cohort involving 163 patients with TNDM1,[20] the authors describe intrauterine growth retardation with a mean birth weight of 2001 ± 417 g (mean ± SD) and adjusted Z score for birth weight of −2.5. The mean age of presentation was 8±12 days with a median of 4 days and a mode of 1 day. Mean gestation was 37.8 ± 2.4 weeks and prematurity was significantly more common than in the general population.[20] Remission occurred at a mean age of 4.5 ± 5.8 months with a median at 3 months. Age at presentation was positively correlated to gestational age, but age at remission was negatively correlated with adjusted birth weight. Thus, the higher the birth weight, the earlier the remission and vice versa. This would be consistent with the effects of insulin on intrauterine growth, so that the larger infants would have the milder defect and therefore tend to enter remission sooner. Congenital anomalies were significantly more frequent in patients with UPD of chromosome 6 or hypomethylation of multiple imprinted loci. Hypomethylation defects were overrepresented in patients born after assisted conception. Thus, babies with TNDM1 generally present with diabetes mellitus within the first days of life, are small, and may have been born prematurely. The presence of congenital anomaly suggests UPD or multiple HIL, and among the latter, almost one in seven conceived with assisted reproductive techniques. Macroglossia is present in about 50%, umbilical hernia in ~25%, and facial dysmorphism in about 20%. Cardiac and renal anomalies (~9%), hand abnormalities (~8%), and hypothyroidism (~4%) also may be present.[20] Remission, when it occurs, is usually around 3 months and about half of these patients will revert to varying degrees of hyperglycemia in the teen years or later.[20] An unusual manifestation is hypoglycemia with hyperinsulinemia, following remission of diabetes in patients with 6q24 methylation defects, most often parental unidisomy.[29] Modern molecular techniques permit diagnosis to be established, and these may influence treatment.

Ideally, these patients should be treated with an insulin pump using insulin diluted to 1:10 with appropriate diluent in order to provide the lower required amounts (0.2 to 1 u/kg/day).[30] Patients with TNDM1 are sensitive to insulin and respond with rapid and remarkable catch-up growth after several weeks of the treatment. Progressive reduction of the insulin dose required to control blood glucose while avoiding hypoglycemia heralds the onset of remission.

TNDM-2

Transient neonatal diabetes mellitus type 2 is a convenient way to classify conditions associated with a neonatal form of diabetes mellitus that remits during infancy and may recur later in life, but is due to mutations in genes regulating insulin secretion rather than expression of imprinted genes.[31,32] The majority of these entities are due to activating mutations in the ABCC8 and KCNJ11 genes, which, respectively, code for the SUR_1 and Kir6.2 subunits of the K_{ATP} channel (see Figure 9-1).[31,32] Recessive, loss of function mutations in the insulin gene itself may occasionally be responsible for TNDM2[33]; the autosomal dominant insulin gene mutations are associated with permanent NDM.[2,31,32] There are rare reports of HNF1β[34] and SLC2A2 mutations[35] also associated with TNDM.

The normal state of the K_{ATP} channel is to remain open; insulin secretion occurs when the channel closes in response to an increase in ATP generated from the metabolism of glucose or amino acids, thereby changing the ATP:ADP ratio. Closure of the channel with intracellular retention of the K+ causes depolarization of the plasma membrane, opening of voltage-gated calcium channels, influx of calcium, and secretion of insulin. Activating mutations in ABCC8 or KCNJ11 alter the ability of the channel to respond to the change in the ATP:ADP, so that the channel remains open to some extent, efflux of K+ from the β cell continues, permitting the cell membrane to remain hyperpolarized and therefore resulting in decreased insulin secretion. These same mechanisms also are responsible for the most common form of permanent neonatal diabetes mellitus as subsequently discussed.[31,32] It remains unclear how or why remission occurs, but it has been shown in vitro that mutations causing TNDM have a less pronounced effect on channel function compared with mutations that cause

PNDM.[36] The ability of ATP to close the channel in vitro correlates with the severity of neonatal diabetes mellitus, including the severe permanent form associated with the DEND syndrome, which demonstrates the greatest resistance to closure by ATP in vitro.[36,37] Both resistance to closure due to activating mutations, or resistance to opening of the channel due to inactivating mutations, segregate with certain mutations as illustrated in Figure 9-4, adapted from reference 36. As shown in the figure, near the fulcrum of this spectrum, those with minor defects may be prone to either develop milder type 2 diabetes, or be resistant to the development of diabetes by virtue of enhanced insulin secretion (see Figure 9-4).

In comparison to TNDM1, patients with TNDM2 generally have greater birth weight, present or are diagnosed with diabetes mellitus later, remit later, and recur earlier (Table 9-1). Family members of patients with these forms of TNDM2 may have diabetes that was diagnosed in adulthood as T1DM, T2DM, or MODY and yet harbor the same heterozygous mutations as the proband with NDM.[4-6] This reflects the variable penetrance of these genes or upstream factors that may modify the expression of the gene in different individuals. Confirming the presence of an ABCC8 or KCNJ11 mutation in a case of NDM is important for management because most of these K_{ATP} channel mutations respond to sulfonylurea treatment both at the time of initial diagnosis or later at the time of relapse.[12,31] During their remission phase, these patients do not require therapy.

A few patients have been reported with recessive loss of function mutation in the *INS* gene in patients with TNDM.[33] These patients appear to enter remission at a median age of 12 weeks; insulin is required before remission and after relapse, which has occasionally been reported.

Patients with mutations in the transcription factor hepatocyte nuclear factor 1 beta (HNF1β) are known to have diabetes associated with renal cysts with onset at a median of 20 years. However, two reported patients had neonatal diabetes: one was diagnosed at age 15 days and required insulin, initially intermittently and then permanently; a second patient diagnosed at the age of 17 days

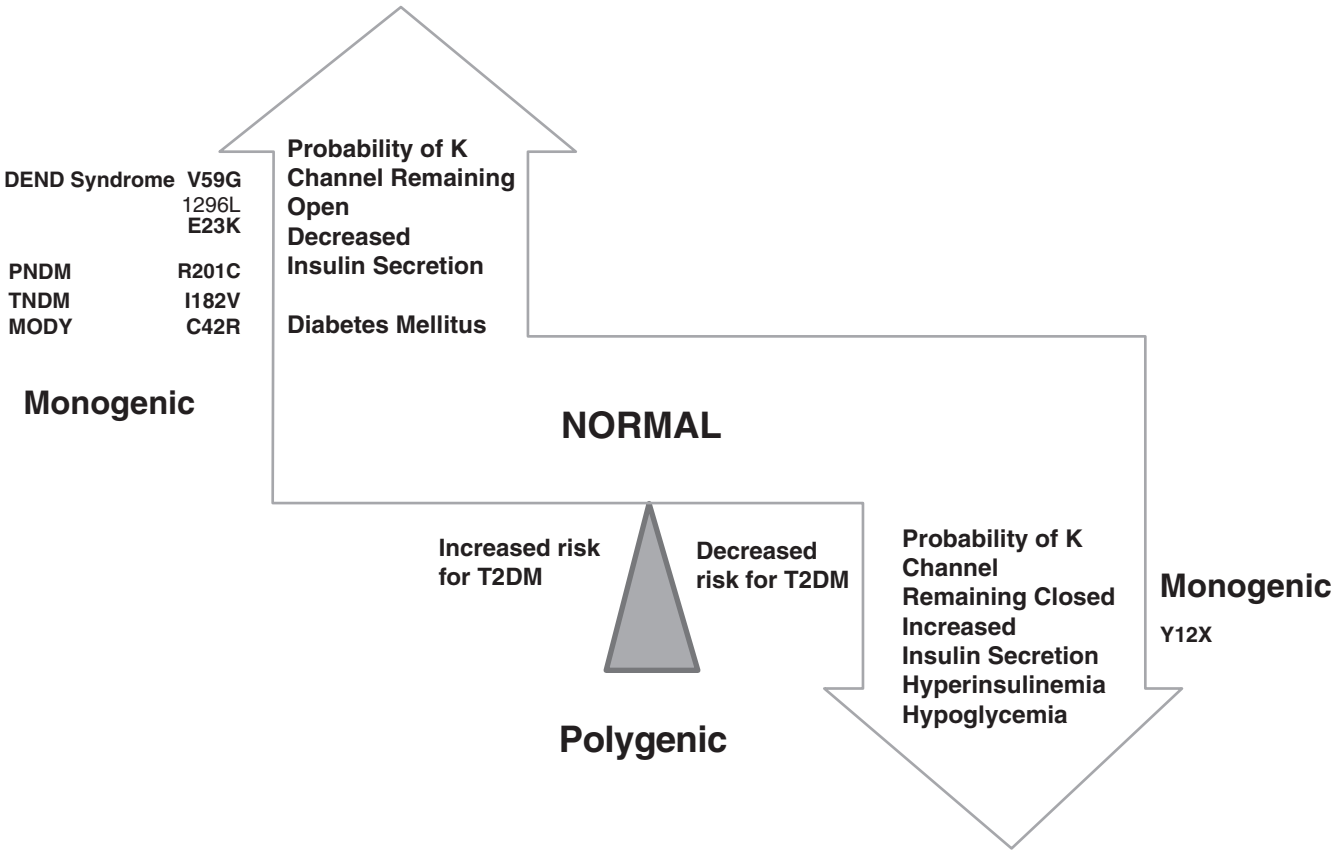

FIGURE 9-4 ■ This is a schematic representation of the relationship between K_{ATP} channel activity and insulin secretion. Activating mutations of KCNJ 11 gene maintain the channel in an open state and hence limit insulin secretion. With progressively increasing probability of the potassium channel remaining open, the severity of the diabetes increases from a mild increased risk for T2DM, to monogenic diabetes of youth (MODY), transient neonatal diabetes mellitus (TNDM), permanent neonatal diabetes mellitus (PNDM), and in the most severe state the DEND syndrome (*d*evelopmental delay, *e*pilepsy, *n*eonatal *d*iabetes) as illustrated on the left. By contrast, mutations that increase the probability that the channel remains closed also increase the likelihood of persistent insulin secretion and lead to hyperinsulinemia and hypoglycemia as illustrated on the panel on the right; in milder forms they may decrease the risk for T2DM by maintaining a higher insulin secretion. Common genetic defects are illustrated in each as examples. (Modified from Ashcroft FM [2005]. ATP-sensitive potassium channelopathies: focus on insulin secretion. J Clin Invest 115:2047-2058. Figure 6.)

TABLE 9-1 **Comparison of Clinical Characteristics of Patients with K_{ATP} Channel Mutations to Patients with 6q24 TNDM (data given in median [range])**

Characteristic	ABCC8 and KCNJ11 (n = 25)	6q24* (n = 23)	P-Value
Age at diagnosis (weeks)	4 (0-16)	0 (0-4)	< 0.001
Age at remission (weeks)	35 (2-208)	13 (5-60)	< 0.001
Age at relapse (years)	4.7 (3-15)	16 (4-25)	0.073
Birth weight (g)	2570 (1360-3570)	1950 (1600-2670)	<0.001
Percentile birth weight	12 (< 1st-89th)	<1st (< 1st-21st)	< 0.001

From Flanagan, S. (2013). Transient neonatal diabetes. Diapedia, *Rev. 21. Retrieved from www.diapedia.org/other-types-of-diabetes-mellitus/41040851198/transient-neonatal-diabetes.*

had remission 2 weeks after diagnosis but relapsed at the age of 8 years.[34] There have also been a few cases reported of mutations in the SLC2A2 gene, which encodes the glucose transporter GLUT2 (glucose transporter type 2).[35] Recessive inactivating mutations in this gene cause the Fanconi-Bickel syndrome, characterized by glucosuria, galactosuria, aminoaciduria, proteinuria, and phosphaturia as well as rickets, poor growth, and short stature with associated glucose and galactose intolerance and enlarged livers. Transient neonatal diabetes has been reported in these patients to occur in association with the classic Fanconi-Bickel syndrome.[35]

PERMANENT NEONATAL DIABETES MELLITUS (PNDM)

K_{ATP} and Insulin Gene Mutations

Almost half of newborns with NDM never enter a remission and therefore are considered to have PNDM (see Figure 9-3). Excluding the syndromic entities, mutations in three genes account for approximately 95% of affected patients with NDM; almost two thirds are mutations in the K_{ATP} channel genes, KCNJ11 coding for the pore-forming protein $K_{IR6.2}$ (50%) and ABCC8 coding for the sulfonylurea receptor protein SUR1 (15%; see Figures 9-1 and 9-3). Finally in this group, approximately 30% have autosomal dominant insulin mutations or rarely autosomal recessive insulin mutations. The remaining 5% of patients with nonsyndromic PNDM have homozygous mutations in factors that, when heterozygous, cause forms of monogenic diabetes of youth (MODY; see Figure 9-3).

Until the discovery of the genes responsible for the K_{ATP} channel, patients with PNDM were considered to have insulin-dependent diabetes mellitus; now it is known that the majority of patients with K_{ATP} mutations causing PNDM respond to oral therapy with sulfonylurea.[12,32] Patients with PNDM due to mutations in the K_{ATP} genes or the insulin gene usually present at 2 to 3 months of life, and sometimes later, and may be in severe DKA by the time of the diagnosis. Activating mutations in the $K_{IR6.2}$ subunit of the K_{ATP} channel were first reported in 2004,[37] being found in 10 out of 29 subjects with permanent NDM. Whereas the insulin secretory response to intravenous glucagon and glucose was absent, patients did respond with insulin secretion to intravenous tolbutamide, clearly hinting at possible therapy by the administration of sulfonylurea and confirmed by a landmark study published 2 years later.[12] Expression of the mutated $K_{IR6.2}$ subunit together with a normal SUR1 subunit in Xenopus laevis oocytes revealed that the ability to enable channel closure by ATP was greatly impaired. This provided a means to correlate the degree of in vitro abnormality with the clinical severity of the diabetes.[36-38] The affected patients predominantly had de novo mutations, with only 20% of the mutations inherited from a parent. It also was noted that 4 out of 10 of the patients had severe developmental delay, muscle weakness, and epilepsy, as well as dysmorphic facial features, which was termed the DEND syndrome. The degree of muscle weakness was ameliorated by treatment with sulfonylurea, raising the possibility that the developmental delay and epilepsy may also be ameliorated, or perhaps prevented, by early recognition and treatment with sulfonylurea.[32,37-39] Subsequently it was demonstrated in mice that transgenic expression of an activating mutation in Kir6.2 in mouse pancreatic β cells recapitulates neonatal diabetes[40] and that the muscle dysfunction caused by a human K_{ATP} channel mutation is neuronal and not muscular in origin.[41] K_{ATP} channels exist in other tissues and are known to modulate electric activity and neurotransmitter release at brain synapses in various regions of the brain.[36] Moreover, K_{ATP} channels in the ventromedial hypothalamic nucleus may be involved in the counter-regulatory response to hypoglycemia[42]; in the arcuate nucleus neurons, K_{ATP} channels may be involved in appetite regulation.[42,43]

A spectrum of clinical disturbances occurs with different mutations ranging from the DEND syndrome, to relapsing diabetes,[36,44,45] permanent diabetes appearing initially in childhood or later in adults.[44-46] Mutations in adjacent locations may cause either neonatal diabetes or hyperinsulinism because they increase or decrease the open state of the channel.[44-48] Likewise, mutations in the ABCC8 gene encoding SUR1 cause transient or permanent NDM, or permanent diabetes diagnosed beyond the newborn period in children, or in adults, and mutations at a similar site in the gene can result in either hyperinsulinism or neonatal diabetes.[5,49-52]

Insulin gene mutations as a cause of PNDM were first reported in 2007 and are now known to be the second most common mutations responsible for these entities.[53-55] Inheritance was autosomal dominant in the familial cases, but the majority had de novo mutations. The mutations occurred in a critical region of the preproinsulin molecule,

predicting misfolding and hence loss of normal trafficking of the proinsulin in the insulin secretory pathway. This misfolding was also proposed to induce the unfolded protein response, with degradation in the endoplasmic reticulum, leading to severe ER stress and apoptosis of the beta cells, processes known to occur in mouse models of dominant insulin gene mutations.[53] Clinically, the age at diagnosis averaged 13 weeks compared with 5 weeks for KCNJ11 and 7 weeks for ABCC8 mutations. Gestational age at birth was within the normal range of 36W to 41W, and mean birth weight was also normal at 2846 Gm. Thus, these abnormalities appear to disturb intrauterine growth less than in the 6q24 and severe K_{ATP} channel mutations. These dominant or de novo mutations are not usually associated with TNDM or remission,[53] whereas recessive mutations of the insulin gene can result in a remitting type of NDM as described earlier.[33] The initial suggestion of ER stress as a mechanism has been largely confirmed and the spectrum of disorders in the insulin gene extends to a MODY phenotype or onset in adulthood.[54-59]

Other Genetic Forms of PNDM

Three factors involved in islet regulation of insulin secretion, via an enzymatic gatekeeper function (Glucokinase-GCK), the islet formation transcription factor PDX-1, sometimes also known as IPF-1, and the transcription factor HNF1β, cause a monogenic form of diabetes respectively known as MODY2, MODY4, and MODY5 when in the heterozygous state and can cause PNDM when in the homozygous state (see Figure 9-3).

GCK

Glucokinase has been called the "glucose sensor" of the beta cell. It phosphorylates glucose to glucose-6-phosphate, permitting entry into the glycolytic pathway for metabolism and generation of ATP, which triggers insulin secretion (see Figure 9-1). GCK is also known as hexokinase IV or hexokinase D and is most active in the physiologic range of glucose at 4 to 10 mmol/L (72 to 180 mg/dL) with a K_m of ~8 mmol /L (144 mg/dL). The glucose-stimulated insulin release (GSIR) threshold occurs when glucose phosphorylating capacity reaches 30% of maximum, which generally occurs under normal circumstances at a glucose concentration of ~90 mg/dL and is maximum at glucose concentrations of 300 mg/dL or higher but not reaching more than ~80% of full (100%) glucose phosphorylating capacity.

Activating mutations of GCK exert these effects at lower glucose concentrations and hence cause hyperinsulinemic hypoglycemia (see Chapter 21). Inactivating heterozygous mutations in GCK cause a mild diabetes because the glucose phosphorylating capacity is shifted to the right, so that the 30% phosphorylating capacity needed for the GSIR occurs when glucose concentrations are ~120 mg/dL rather than 90 mg/dL and reach only a peak of 50% to 60% of the 100% phosphorylating capacity. This is enough to clear all glucose after a meal, but with periods of hyperglycemia before this occurs. In children, GCK inactivating mutations causing MODY2 are the most common form of MODY, often presenting as incidental findings discovered during blood tests for other causes and often with a positive family history for diabetes. A second common presentation of MODY2 is as "gestational diabetes" in an otherwise healthy young mother and which may persist as a mild diabetes postpartum.

Homozygous inactivating mutations in GCK were reported in two patients in 2001,[60] and the same authors reported an additional three cases in 2003.[61] The affected subjects were homozygous for the mutation or were compound heterozygous for two different mutations, being a splice site in one and a missense mutation in the other. Each of these five initial subjects was characterized by intrauterine growth retardation, permanent neonatal diabetes from day 1 of life, and hyperglycemia in parents, which was an important diagnostic clue. Apart from some other novel single case reports,[62,63] an additional four novel cases of permanent NDM due to homozygous mutations in GCK were reported in 2011.[64] Overall, these mutations are rare in NDM; in one large cohort of 54 cases of NDM, GCK mutations were found in only one case of European ancestry,[65] and the same was true for a study comparing the etiologies of PNDM in an Arab versus European population.[66]

PDX-1

PDX-1, which stands for pancreatic duodenal homeobox-1 and is also known as insulin promoter factor-1 (IPF-1), is a critical transcription factor that defines the pancreatic progenitor cell as it differentiates from the endodermal epithelium. Exocrine-duct lineage is then specified by the transcription factor PTF1 (pancreas transcription factor-1), which defines the exocrine and duct cells. However, in the presence of Ngn3, the progenitor cell differentiates into a series of endocrine pancreatic cells under the influence of other transcription factors including Pax4 and Pax6, as well as Beta2. Thus, PDX-1 is critical for the formation of both the exocrine and endocrine pancreas and hence homozygous mutations will lead to an absence of pancreas formation with manifestations of neonatal diabetes and pancreatic exocrine insufficiency. The parents may be heterozygous carriers and therefore known to have a form of diabetes known as MODY4. The first report of pancreatic agenesis attributed to homozygous deletion of a gene in the PDX-1 (IPF1) gene was reported in 1997,[67] the second case was reported in 2003,[68] and the third case was reported in 2009[69]; each was characterized by pancreatic agenesis with manifestations of both exocrine and endocrine abnormalities. In a more recent study in a large cohort of patients with permanent neonatal diabetes but without known abnormal development or clinical exocrine insufficiency of the pancreas, the possibility of PDX-1 mutations was investigated in 103 patients in whom K_{ATP} and INS mutations had been excluded. In this group, 3 additional cases of mutations in PDX1 were identified, but these patients did not have evidence of exocrine pancreatic insufficiency either clinically or biochemically. Thus, it is possible to have PNDM due to a mutation in PDX1 without necessarily having pancreatic hypoplasia or agenesis.[70] In this regard it is important to note that neonatal diabetes may be associated with

pancreatic as well as cerebellar agenesis as a result of mutations in the PTF1A gene (pancreas transcription factor 1A)[71] and that the Ngn3 gene can be associated with neonatal diabetes and congenital malabsorptive diarrhea consistent with the role of this gene in pancreas development as briefly described earlier.[72] Other genes that have been implicated in pancreatic agenesis include EIF2AK3, HNF-1B, RFX6, and GATA6, which may be associated with pancreatic agenesis and other lesions including heart defects.[73] As a diagnostic clue, if pancreatic imaging shows the absence or hypoplasia of a pancreas, the diagnostic search can be restricted to a series of genes as outlined.[74]

HNF1β

As previously noted, mutations in HNF1β can cause neonatal diabetes mellitus and neonatal polycystic dysplastic kidneys with either permanent or transient neonatal diabetes.[34]

Syndromic Neonatal Diabetes Mellitus

The syndromes associated with NDM constitute no more than 10% of all forms of NDM and hence are each rare (see Figure 9-3). But they are of interest in identifying the spectrum of genetic disorders that can result in abnormal pancreas formation, malfunction, or destruction. In a sense, even the most common forms of PNDM caused by K_{ATP} mutations may have a syndromic component as in the DEND syndrome, but this is not integral to the condition, whereas in the syndromic forms of NDM, associated features define and distinguish the entities and point to the likely etiology.

Wollcott-Rallison Syndrome: Mutations in EIF2AK3

This rare condition is actually the most common genetic cause of permanent neonatal diabetes mellitus in consanguineous families,[66,75] and it may present as isolated non-autoimmune neonatal diabetes mellitus diagnosed at 3 weeks of age or later. Typically, skeletal dysplasia and growth retardation are recognized in the first year or two of life; other manifestations include episodes of liver failure, renal dysfunction and evidence of pancreatic exocrine insufficiency, neutropenia with recurrent infections, hypothyroidism, and mental retardation.[76] The basis for this condition are mutations in the gene encoding *e*ukaryotic translation *i*nitiation *f*actor α-kinase 3 also known as PKR-like endoplasmic reticulum kinase (PERK), which participates in the unfolded protein response in the ER. Environmental factors and modification by other genes may influence the spectrum of clinical severity. Skeletal dysplasia with bone fractures and episodes of liver failure plus neonatal diabetes mellitus are pathognomonic of this entity; a review in 2010 indicated that fewer than 60 cases had been reported worldwide[76]; two novel mutations were subsequently reported.[77] The prognosis is poor for affected patients. Treatment of the diabetes mellitus by pump therapy is recommended; parents should have genetic counseling for risks of recurrence, as this is an autosomal recessive condition.

IPEX-FOXP3

The term *IPEX* is an acronym for *i*mmune dysregulation, *p*olyendocrinopathy, *e*nteropathy, *x*-linked and is due to dysfunction of T regulatory cells caused by mutations in the FOX P 3 gene, which normally is a transcriptional regulator for CD4 regulatory T cells, so that mutations result in the multiorgan autoimmunity. The clinical characteristics are early onset, insulin-dependent diabetes mellitus, or enteritis; eczema and elevated serum Ig E also occur early. Later manifestations include primary hypothyroidism, nephritis, hepatitis, enteritis, and alopecia. Bone marrow stem cell transplant offers potential for cure, but it depends on the availability of a suitable donor.[78,79] Scurfy mice serve as a model for this condition in humans.[80]

GLIS 3

GLIS 3 is a member of the GLI-similar zinc finger protein family, which can function both as a repressor and activator of transcription and is specifically involved in the development of pancreatic beta cells, the thyroid, eye, liver, and kidney. An autosomal recessive syndrome, characterized by neonatal diabetes mellitus, IUGR, congenital hypothyroidism, hepatic fibrosis with cholestasis, polycystic kidneys, and congenital glaucoma, it was first described in 2003,[81] and the responsible mutation in the GLIS 3 gene was identified in 2006.[82] As noted, this gene is expressed in the early developmental stages of the thyroid, eye, liver, kidney, and the pancreas, particularly in beta cells. Thus, mutations in GLIS 3 may have a role in other developmental anomalies and possibly in T1DM or T2DM. Novel mutations associated with the classical findings of NDM and hypothyroidism, plus bilateral sensorineural deafness, osteopenia, and pancreatic exocrine insufficiency, enlarge the clinical spectrum as reported.[83] In animal studies, it appears that GLIS 3 expression is required for pancreatic beta cell function and maintenance of beta cell mass in the adult, so that impaired function could lead to diabetes mellitus.[84,85] Genome-wide association studies identify GLIS 3 as a locus that affects risk for T1 DM,[86] and variants of GLIS 3 have been implicated in the predisposition to T2 DM.[87]

PTF1A

The gene PTF1A encodes pancreatic transcription factor 1α known to be involved in pancreatic development. Mutations in this gene were identified in two families with neonatal diabetes mellitus associated with pancreatic agenesis as well as cerebellar agenesis, implicating this factor in normal cerebellar development as well as pancreatic development, findings confirmed in knockout models of this gene in mice.[71]

Rfx6

In mice, it was shown that absence of the transcription factor Rfx 6 prevents the formation of any pancreatic endocrine cells except for pancreatic polypeptide producing

cells; mutations in the human ortholog RFX6 gene caused an autosomal recessive form of neonatal diabetes mellitus.[88] In humans, the syndrome of neonatal diabetes mellitus, intestinal atresia, and hepatobiliary abnormalities had been known as the Mitchell-Riley syndrome.[89] Clinically, patients are characterized by severe IUGR, fluctuation in glucose concentrations, stabilizing as persistent hyperglycemia and requiring insulin for stabilization, cholestatic jaundice and anatomic findings of intestinal atresia, agenesis of the gall bladder, and abnormal formation of the pancreas. Those who survive this critical neonatal period as the result of surgical and medical interventions can continue with a favorable prognosis for subsequent life apart from the likelihood of mental retardation.[90]

Neurog-3

Neurogenin 3 is a critical transcription factor for the differentiation of islet endocrine cell types from the pancreatic endoderm. Mice lacking this factor are devoid of intestinal pancreatic endocrine cells and develop diabetes mellitus as newborns. In humans, homozygous mutations in Neurog-3 cause malabsorptive diarrhea and early onset diabetes mellitus.[72,91]

GATA6

Both GATA4 and GATA6 are critical factors with differential contributions for normal pancreatic organogenesis; double Gata 4 and Gata 6 knockout mice do not develop a pancreas and hence have diabetes.[92-94] In humans, heterozygous inactivating mutations in GATA6 were found to be the most common cause of pancreatic agenesis occurring in 15 of 27 subjects with pancreatic agenesis.[95] These patients required both insulin plus pancreatic enzyme replacement.[95] In a follow-up study,[96] the same investigators sought GATA 6 mutations in 171 subjects with NDM of unknown etiology, having already identified other mutations, including 15 with GATA6 mutations, in 624 subjects out of a cohort totaling 795. In the new cohort of 171 remaining subjects, an additional 9 new cases of GATA 6 mutations were identified for a total of 24 affected out of the original cohort of 795 subjects (3%). In these 9 new cases, 2 had neonatal diabetes mellitus but did not require pancreatic enzyme replacement, and 1 had transient neonatal diabetes mellitus. In addition, four parents were found to have GATA 6 mutations, but the diabetes was diagnosed between the ages of 12 and 46 years. Several of the 9 new subjects had subclinical pancreatic enzyme deficiency. Except for one of the parents, subjects with GATA 6 mutations had extra pancreatic features, congenital heart malformations being found in 83%. Thus, these GATA 6 mutations can cause permanent neonatal diabetes mellitus or occasionally transient neonatal diabetes mellitus, as well as adult-onset diabetes with gradations in exocrine pancreatic insufficiency from complete agenesis, subclinical, or no deficiency at all; the majority manifest congenital heart defects.[96] In evaluating a patient with neonatal diabetes mellitus, pancreatic imaging is useful, because finding pancreatic hypoplasia, or aplasia, limits the possible genetic causes to GATA 6[96] as well as EIF2 AK3, PTF 1A, HN1B, PDX 1, and RFX 6.[74]

DIAGNOSIS AND TREATMENT OF NEONATAL DIABETES MELLITUS

Neonatal diabetes mellitus should be considered in babies who display IUGR in utero or at birth, and who fail to thrive despite apparent lusty suck, adequate intake, and copious urine output. A positive family history of diabetes mellitus in one or both parents, or in prior siblings, points to a likely genetic cause; most cases represent de novo mutations. The diagnosis is established by confirmation of hyperglycemia and glycosuria, both readily available in hospitals or medical clinics. Recognition of diabetes mellitus within the first week of life likely represents TNDM1, due to the differential expression of imprinted genes on chromosome 6q24, particularly if congenital defects such as macroglossia and umbilical hernia are present. Somewhat later recognition should also include consideration of defects in the K_{ATP} channel, involving KCNJ11 and ABCC8 genes as well as the INS gene; β-cell antibodies need not be determined unless features such as those found in the IPEX syndrome are present. Pancreatic antibody testing should be performed after age 6 months, however.

A molecular diagnosis should be sought; confirmation of methylation defects will raise the possibility of remission; K_{ATP} channel defects also may remit, but if confirmed, a trial of transition to oral sulfonylurea therapy may be feasible. Ascertainment of four genes, methylation defects on 6q24, KCNJ11, ABCC8, and INS, will identify the majority of the 90% of identifiable genes responsible for NDM in the first 6 months of life; defects in GCK, HNF1β, PDX-1, and SLCA2A are the result of autosomal recessive inheritance so that both parents likely have some form of diabetes mellitus (see Figure 9-3). Pending establishment of the molecular defect, patients should be treated with insulin; pumps delivering insulin should be used if available because they offer the ability to deliver small amounts via insulin diluted in appropriate diluent. Trial and error determine the amount to be given to achieve reasonable glycemic control to avoid both hyperglycemia and hypoglycemia; a starting dose of 0.5 units per kilogram per day increasing or decreasing by 0.1 unit per kilogram per day and distributed over 24 hours is a reasonable starting approach. The temptation to start these patients on sulfonylurea pending the establishment of the molecular diagnosis should be avoided; based on available data, only approximately 20% of those diagnosed in the first week of life have a high probability of responding to treatment with sulfonylurea.[97] Current methodology permits molecular diagnosis to be completed relatively quickly with results available in 1 to 2 weeks and it can be performed at established regional centers in the UK, United States, and elsewhere. Some of these centers offer molecular diagnostics to be performed without charge as a result of funding from various national agencies. In the absence of a molecular diagnosis, a trial of sulfonylurea may aggravate the hyperglycemia and lead to diabetic ketoacidosis in the patients who do not respond. Hence, we strongly urge that when a diagnosis of NDM is established in a newborn, treatment should be started with insulin and a sample sent for molecular diagnosis, the results of which will be informative regarding likely response to sulfonylurea.[31]

Presentation later than the first 2 to 4 weeks but within the first 6 months of birth is likely to yield a molecular

diagnosis in about two thirds of all patients[2]; clinical features such as specific patterns of congenital defects may offer clues to the diagnosis and prognosis as discussed earlier for syndromic NDM (see Figure 9-3).

AVAILABLE RESOURCES

The following resources offer advice in diagnostic and therapeutic services and may also perform the molecular diagnosis free of charge as part of their mandate funded by national governmental agencies:

- www.diabetesgenes.org United Kingdom
- www.kovlerdiabetescenter.org/registry Chicago (United States)
- www.mody.no Norway
- www.genetests.org CLIA certified laboratories performing the tests

TRANSITION TO ORAL THERAPY

Once the diagnosis of a mutation in the K_{ATP} channel likely to respond to sulfonylurea is established, transition to oral treatment from insulin treatment is best accomplished on an inpatient basis using tablets or suspensions of glyburide prepared in a hospital pharmacy to facilitate the swallowing of the medication. The starting dose is 0.4 milligram per kilogram per day in two divided doses. Table 9-2 outlines the sequential steps to be taken in transitioning the patient and is based on recommendations from NDM centers in both the UK and the United States.

FUTURE DIRECTIONS

The discovery of the genes responsible for various forms of NDM has been spectacular in its impact on the

TABLE 9-2 **Inpatient Transition to Oral Sulfonylurea**

Day	Glucose Monitoring	Insulin Adjustments	Glyburide Dosing
Prep	Monitor capillary blood glucose (BG) before meals, 2 hours post meals, bedtime, and 2 a.m. Monitor ketones any time BG is > 300 mg/dL (> 16 mmol/L). Have a plan for hypoglycemia.	Maintain usual insulin regimen either via pump or customary basal-bolus injections. Reduce basal insulin by 50%. (If pump is being used, can be done just before day 1 breakfast; if long-acting insulin is being used, may be reduced on previous evening.)	The experience of previous cases with the same mutation will help inform expected response. Tablets (easily halved) available: 1.25, 2.5, or 5 mg For infants, tablets can be crushed and suspended in water/formula at the bedside or by any pharmacy.
1	If BG before dinner dose is: > 126 mg/dL (7 mmol/L) → < 126 mg/dL (7 mmol/L) →	Administer rapid-acting bolus insulin as needed based on capillary BG: Give usual bolus insulin dose Give 50% of usual insulin dose	0.1 mg/kg before breakfast and dinner (total of 0.2 mg/kg/day), depending on BG: Give 0.1 mg/kg dose at dinner Dinner dose may be skipped
2-7	If BG before SU dose is: > 126 mg/dL (7 mmol/L) → < 126 mg/dL (7 mmol/L) →	Continue decreasing insulin as tolerated: Continue bolus dosing from previous day Decrease insulin bolus by 50%	Each day dose will increase by 0.2 mg/kg/day (0.1 mg/kg/dose) depending on BGs: Increase dose by 0.1 mg/kg/dose May continue dose from previous day
Last	On final day and after discharge continue checking BG at least 4-6 times per day to monitor response. Relative hypoglycemia may necessitate lowering of glyburide dose in following weeks-months.	In most SU-responsive cases, insulin can be discontinued within 5-7 days, although mild hyperglycemia may still occur. Treat with rapid-acting bolus insulin as needed, as well as low-dose basal insulin in some cases. It may be possible to discontinue insulin in the following weeks-months.	By the end of 5-7 day admission, the patient will either have clearly responded on a lower dose or will be on at least 1 mg/kg/day. The dose may continue to be increased after discharge if needed, with some patients requiring up to 2-2.5 mg/kg/day (which may be lowered eventually in the following weeks-months).
Notes	If expected response is uncertain, C-peptide levels pre and 90-120 minutes post breakfast (no insulin given) may be done on day 1 and a later day after the glyburide dose is at least 1 mg/kg/day (usually day 5). If levels pre SU are nearly undetectable but show a significant increment on glyburide, consider increasing dose as high as 2-2.5 mg/kg/day.	BG ranges and insulin adjustments are only a guideline; the physician should be guided by clinical judgment. If there is any indication that glyburide is helping to control BG levels overall, it is often better to decrease the insulin aggressively so as to avoid hypoglycemia. If little/no insulin is given with meals, corrections for high BGs (per usual sensitivity/correction scale) may be given post mealtime or at any time.	Patients with neurodevelopmental disability or those who are older at the time of transition may experience less responsiveness and require higher doses of glyburide. In such cases, the possible benefit of continuing a high dose for the long term should be carefully considered even if the patient still requires insulin.

Data from Pearson, E. R., Flechtner, I., Njolstad, P. R., et al. (2006). Switching from insulin to oral sulfonylureas in patients with diabetes due to Kir6.2 mutations. N Engl J Med, 355, *467-77. This table was created from the data by Drs. L. Philipson and S. Greeley. Contact monogenicdiabetes@uchicago.edu for more details.*

diagnosis and treatment of affected patients and its relevance to understanding the global burden of diabetes mellitus. The same genetic defects that cause NDM have been found in parents or unrelated individuals considered to have T2DM or classic T1DM non-autoimmune. Thus, these discoveries have informed potential mechanisms by which insulin secretion is impaired at the level of pancreas formation and development, insulin synthesis, and insulin secretion. In addition, this offers the possibility to overcome the defect through the pharmacogenetics of the K_{ATP} channel in which activating mutations can be overcome by sulfonylurea treatment. However, the cause in at least a third of patients with NDM in the first 6 to 9 months of life is still not known and, as yet, a genetic mutation has not been identified. Whole genome or exome sequencing[98] may reveal the basis for these undefined syndromes in the near future.

REFERENCES

1. von Muhlendahl, K. E., & Herkenhoff, H. (1995). Long-term course of neonatal diabetes. *N Engl J Med*, *333*, 704–708.
2. Stoy, J., Greeley, S. A., Paz, V. P., et al. (2008). Diagnosis and treatment of neonatal diabetes: a United States experience. *Pediatr Diabetes*, *9*, 450–459.
3. Polak, M., Dechaume, A., Cave, H., et al. (2008). Heterozygous missense mutations in the insulin gene are linked to permanent diabetes appearing in the neonatal period or in early infancy. *Diabetes*, *57*, 1115–1119.
4. Rubio-Cabezas, O., Flanagan, S. E., Damhuis, A., et al. (2012). KATP channel mutations in infants with permanent diabetes diagnosed after 6 months of life. *Pediatric Diabetes*, *13*, 322–325.
5. Babenko, A. P., Polak, M., Cave, H., et al. (2006). Activating mutations in the ABCC8 gene in neonatal diabetes mellitus. *N Engl J Med*, *355*, 456–466.
6. Bowman, P., Flanagan, S. E., Edghill, E. L., et al. (2012). Heterozygous ABCC8 mutations are a cause of MODY. *Diabetologia*, *55*, 123–127.
7. Sperling, M. A. (2006). ATP-sensitive potassium channels: neonatal diabetes mellitus and beyond. *N Engl J Med*, *355*, 507–510.
8. Rieck, S., Bankaitis, E. D., & Wright, C. V. E. (2012). Lineage determinants in early endocrine development. *Semin Cell Dev Biol*, 2912;23 (6):673–684.
9. Wilson, M. E., Scheel, D., & German, M. S. (2003). Gene expression cascades in pancreatic development. *Mech Dev*, *120*, 65–80.
10. Zhou, Q., Brown, J., Kanarek, A., et al. (2008). In vivo reprogramming of adult pancreatic exocrine cells to B-cells. *Nature*, *455*, 627–632.
11. Halban, P. A., German, M. S., Kahn, S. E., et al. (2010). Current status of islet cell replacement and regeneration therapy. *J Clin Endocrinol Metab*, *95*, 1034–1043.
12. Pearson, E. R., Flechtner, I., Njolstad, P. R., et al. (2006). Switching from insulin to oral sulfonylureas in patients with diabetes due to Kir6.2 mutations. *N Engl J Med*, *355*, 467–477.
13. Polak, M., & Cave, H. (2007). Neonatal diabetes mellitus: a disease linked to multiple mechanisms. *Orphanet J Rare Dis*, *2*, 12.
14. Habeb, A. M., Al-Magamsi, M. S., Eid, I. M., et al. (2012). Incidence, genetics, and clinical phenotype of permanent neonatal diabetes mellitus in northwest Saudi Arabia. *Pediatr Diabetes*, *13*, 499–505.
15. Grulich-Henn, J., Wagner, V., Thon, A., et al. (2010). Entities and frequency of neonatal diabetes: data from the diabetes documentation and quality management system (DPV). *Diabet Med*, *27*, 709–712.
16. Iafusco, D., Massa, O., Pasquino, B., et al. (2012). Minimal incidence of neonatal/infancy onset diabetes in Italy is 1:90,000 live births. *Acta Diabetol*, *49*, 405–408.
17. Slingerland, A. S., Shields, B. M., Flanagan, S. E., et al. (2009). Referral rates for diagnostic testing support an incidence of permanent neonatal diabetes in three European countries of at least 1 in 260,000 live births. *Diabetologia*, *52*, 1683–1685.
18. Kanakatti Shankar, R., Pihoker, C., Dolan, L. M., et al. (2013). Permanent neonatal diabetes mellitus: prevalence and genetic diagnosis in the SEARCH for Diabetes in Youth Study. *Pediatr Diabetes*, *14*, 174–180.
19. Sperling, M. A. (2005). Neonatal diabetes mellitus: from understudy to center stage. *Curr Opin Pediatr*, *17*, 512–518.
20. Docherty, L. E., Kabwama, S., Lehmann, A., et al. (2013). Clinical presentation of 6q24 transient neonatal diabetes mellitus (6q24 TNDM) and genotype-phenotype correlation in an international cohort of patients. *Diabetologia*, *56*, 758–762.
21. PLAGL1 pleiomorphic adenoma gene-like 1 [Homo sapiens (human)]. Gene ID: 5325, updated 2013.
22. Du, X., Ounissi-Benhalha, H., Loder, M. K., et al. (2012). Overexpression of ZAC impairs glucose-stimulated insulin translation and secretion in clonal pancreatic beta-cells. *Diabetes Metab Res Rev*, *28*, 645–653.
23. Ma, D., Shield, J. P., Dean, W., et al. (2004). Impaired glucose homeostasis in transgenic mice expressing the human transient neonatal diabetes mellitus locus, TNDM. *J Clin Invest*, *114*, 339–348.
24. Hattersley, A. T. (2004). Unlocking the secrets of the pancreatic B cell: man and mouse provide the key. *J of Clin Invest*, *114*, 314–316.
25. Du, X., Rousseau, M., Ounissi-Benkalha, H., et al. (2001). Differential expression pattern of ZAC in developing mouse and human pancreas. *J Mol Histol*, *42*, 129–136.
26. Mackay, D. J. G., & Temple, I. K. (2010). Transient neonatal diabetes mellitus type 1. *Am J Med Genet C Semin Med Genet*, *154C*, 335–342.
27. Mackay, D. J. G., Callaway, J. L. A., Marks, S. M., et al. (2008). Hypomethylation of multiple imprinted loci in individuals with transient neonatal diabetes is associated with mutations in ZFP57. *Nat Genet*, *40*, 949–951.
28. Bliek, J., Verde, G., Callaway, J., et al. (2009). Hypomethylation at multiple maternally methylated imprinted regions including PLAGL1 and GNAS loci in Beckwith-Wiedemann syndrome. *Eur J Hum Genet*, *17*, 611–619.
29. Flanagan, S. E., Mackay, D. J., Greeley, S. A., et al. (2013). Hypoglycaemia following diabetes remission in patients with 6q24 methylation defects: expanding the clinical phenotype. *Diabetologia*, *56*, 218–221.
30. Olinder, A. L., Kernell, A., & Smide, B. (2006). Treatment with CSII in two infants with neonatal diabetes mellitus. *Pediatr Diabetes*, *5*, 284–288.
31. Hattersley, A., Bruining, J., Shield, J., et al. (2009). The diagnosis and management of monogenic diabetes in children and adolescents. *Pediatr Diabetes*, (Suppl. 12), 33–42.
32. Murphy, R., Ellard, S., & Hattersley, A. T. (2008). Clinical implications of a molecular genetic classification of monogenic B-cell diabetes. *Nat Clin Pract Endocriol Metab*, *4*, 200–213.
33. Garin, I., Edghill, E. L., Akerman, I., et al. (2010). Recessive mutations in the INS gene result in neonatal diabetes through reduced insulin biosynthesis. *Proc Natl Acad Sci USA*, *107*, 3105–3110.
34. Yorifuji, T., Kurokawa, K., Mamada, M., et al. (2004). Neonatal diabetes mellitus and neonatal polycystic, dysplastic kidneys: phenotypically discordant recurrence of a mutation in the hepatocyte nuclear factor-1beta gene due to germline mosaicism. *J Clin Endocrinol Metab*, *89*, 2905–2908.
35. Sansbury, F. H., Flanagan, S. E., Houghton, J. A., et al. (2012). SLC2A2 mutations can cause neonatal diabetes, suggesting GLUT2 may have a role in human insulin secretion. *Diabetologia*, *55*, 2381–2385.
36. Ashcroft, F. M. (2005). ATP-sensitive potassium channelopathies: focus on insulin secretion. *J Clin Invest*, *115*, 2047–2058.
37. Gloyn, A. L., Pearson, E. R., Antcliff, J. F., et al. (2004). Activating mutations in the gene encoding the ATP-sensitive potassium-channel subunit Kir6.2 and permanent neonatal diabetes. *N Engl J Med*, *350*, 1838–1849.
38. Hattersley, A. T., & Ashcroft, F. M. (2005). Activating mutations in Kir6.2 and neonatal diabetes: new clinical syndromes, new scientific insights, and new therapy. *Diabetes*, *54*, 2503–2513.
39. Ashcroft, F. M. (2010). New uses for old drugs: neonatal diabetes and sulphonylureas. *Cell Metab*, *11*, 179–181.
40. Girard, C. A., Wunderlich, F. T., Shimomura, K., et al. (2009). Expression of an activating mutation in the gene encoding the

KATP channel subunit Kir6.2 in mouse pancreatic beta cells recapitulates neonatal diabetes. *J Clin Invest*, *119*, 80–90.
41. Clark, R. H., McTaggart, J. S., Webster, R., et al. (2010). Muscle dysfunction caused by a KATP channel mutation in neonatal diabetes is neuronal in origin. *Science*, *329*, 458–461.
42. Fan, X., Ding, Y., Cheng, H., et al. (2008). Amplified hormonal counterregulatory responses to hypoglycemia in rats after systemic delivery of a SUR-1-selective K(+) channel opener? *Diabetes*, *57*, 3327–3334.
43. Park, Y. B., Choi, Y. J., Park, S. Y., et al. (2011). ATP-sensitive potassium channel-deficient mice show hyperphagia but are resistant to obesity. *Diabetes Metab J*, *35*, 219–225.
44. Clark, R., & Proks, P. (2010). ATP-sensitive potassium channels in health and disease. *Adv Exp Med Biol*, *654*, 165–192.
45. Koster, J. C., Cadario, F., Peruzzi, C., et al. (2008). The G53D mutation in Kir6.2 (KCNJ11) is associated with neonatal diabetes and motor dysfunction in adulthood that is improved with sulfonylurea therapy. *J Clin Endocrinol Metab*, *93*, 1054–1061.
46. Gloyn, A. L., Reimann, F., Girard, C., et al. (2005). Relapsing diabetes can result from moderately activating mutations in KCNJ11. *Hum Mol Genet*, *14*, 925–934.
47. Flanagan, S. E., Patch, A. M., Mackay, D. J., et al. (2007). Mutations in ATP-sensitive K+ channel genes cause transient neonatal diabetes and permanent diabetes in childhood or adulthood. *Diabetes*, *56*, 1930–1937.
48. Shimomura, K., Flanagan, S. E., Zadek, B., et al. (2009). Adjacent mutations in the gating loop of Kir6.2 produce neonatal diabetes and hyperinsulinism. *EMBO Mol Med*, *1*, 166–77.
49. de Wet, H., Proks, P., Lafond, M., et al. (2008). A mutation (R826W) in nucleotide-binding domain 1 of ABCC8 reduces ATPase activity and causes transient neonatal diabetes. *EMBO Rep*, *9*, 648–654.
50. Proks, P., Arnold, A. L., Bruining, J., et al. (2006). A heterozygous activating mutation in the sulphonylurea receptor SUR1 (ABCC8) causes neonatal diabetes. *Hum Mol Genet*, *15*, 1793–1800.
51. Patch, A. M., Flanagan, S. E., Boustred, C., et al. (2007). Mutations in the ABCC8 gene encoding the SUR1 subunit of the K_{ATP} channel cause transient neonatal diabetes, permanent neonatal diabetes or permanent diabetes diagnosed outside the neonatal period. *Diabetes Obes Metab*, *9*, 28–39.
52. Mannikko, R., Flanagan, S. E., Sim, X., et al. (2001). Mutations of the same conserved glutamate residue in NBD2 of the sulfonylurea receptor 1 subunit of the KATP channel can result in either hyperinsulinism or neonatal diabetes. *Diabetes*, *60*, 1813–1822.
53. Stoy, J., Edghill, E. L., Flanagan, S. E., et al. (2007). Insulin gene mutations as a cause of permanent neonatal diabetes. *Proc Natl Acad Sci USA*, *104*, 15040–15044.
54. Glaser, B. (2008). Insulin mutations in diabetes: the clinical spectrum. *Diabetes*, *57*, 799–800.
55. Edghill, E. L., Flanagan, S. E., Patch, A. M., et al. (2008). Insulin mutation screening in 1,044 patients with diabetes: mutations in the INS gene are a common cause of neonatal diabetes but a rare cause of diabetes diagnosed in childhood or adulthood. *Diabetes*, *57*, 1034–1042.
56. Polak, M., Dechaume, A., Cave, H., et al. (2008). Heterozygous missense mutations in the insulin gene are linked to permanent diabetes appearing in the neonatal period or in early infancy: a report from the French ND (Neonatal Diabetes) Study Group. *Diabetes*, *57*, 1115–1119.
57. Meur, G., Simon, A., Harun, N., et al. (2010). Insulin gene mutations resulting in early-onset diabetes: marked differences in clinical presentation, metabolic status, and pathogenic effect through endoplasmic reticulum retention. *Diabetes*, *59*, 653–661.
58. Molven, A., Ringdal, M., Nordbo, A. M., et al. (2008). Mutations in the insulin gene can cause MODY and autoantibody-negative type 1 diabetes. *Diabetes*, *57*, 1131–1135.
59. Colombo, C., Porzio, O., Liu, M., et al. (2008). Seven mutations in the human insulin gene linked to permanent neonatal/infancy-onset diabetes mellitus. *J Clin Invest*, *118*, 2148–2156.
60. Njolstad, P. R., Sovik, O., Cuesta-Munoz, A., et al. (2001). Neonatal diabetes mellitus due to complete glucokinase deficiency. *N Engl J Med*, *344*, 1588–1592.
61. Njolstad, P. R., Sagen, J. V., Bjorkaug, L., et al. (2003). Permanent neonatal diabetes caused by glucokinase deficiency: inborn error of the glucose-insulin signaling pathway. *Diabetes*, *52*, 2854–2860.
62. Porter, J. R., Shaw, N. J., Barrett, T. G., et al. (2005). Permanent neonatal diabetes in an Asian infant. *J Pediatr*, *146*, 131–133.
63. Rubio-Cabezas, O., Diaz Gonzalez, F., Aragones, A., et al. (2008). Permanent neonatal diabetes caused by a homozygous nonsense mutation in the glucokinase gene. *Pediatr Diabetes*, *9*, 245–249.
64. Bennett, K., James, C., Mutair, A., et al. (2001). Four novel cases of permanent neonatal diabetes mellitus caused by homozygous mutations in the glucokinase gene. *Pediatr Diabetes*, *12*, 192–196.
65. Russo, L., Iafusco, D., Brescianini, S., et al. (2011). Permanent diabetes during the first year of life: multiple gene screening in 54 patients. *Diabetologia*, *54*, 1693–1701.
66. Habeb, A. M., Flanagan, S. E., Deeb, A., et al. (2012). Permanent neonatal diabetes: different aetiology in Arabs compared to Europeans. *Arch Dis Child*, *97*, 721–723.
67. Stoffers, D. A., Zinkin, N. T., Stanojevic, V., et al. (1997). Pancreatic agenesis attributable to a single nucleotide deletoin in the human IPF1 gene coding sequence. *Nat Genet*, *15*, 106–110.
68. Schwitzgebel, V. M., Mamin, A., Brun, T., et al. (2003). Agenesis of human pancreas due to decreased half-life of insulin promoter factor 1. *J Clin Endocrinol Metab*, *88*, 4398–4406.
69. Thomas, I. H., Saini, N. K., Adhikari, A., et al. (2009). Neonatal diabetes mellitus with pancreatic agenesis in an infant with homozygous IPF-1 Pro63fsX60 mutation. *Pediatr Diabetes*, *10*, 492–496.
70. De Franco, E., Shaw-Smith, C., Flanagan, S. E., et al. (2013). Biallelic PDX1 (insulin promoter factor 1) mutations causing neonatal diabetes without exocrine pancreatic insufficiency. *Diabet Med*, *30*, e197–e200.
71. Sellick, G. S., Barker, K. T., Stolte-Dijkstra, I., et al. (2004). Mutations in PTF1A cause pancreatic and cerebellar agenesis. *Nat Genet*, *36*, 1301–1305.
72. Pinney, S. E., Oliver-Krasinski, J., Ernst, L., et al. (2011). Neonatal diabetes and congenital malabsorptive diarrhea attributable to a novel mutation in the human neurogenin-3 gene coding sequence. *J Clin Endocrinol Metab*, *96*, 1960–1965.
73. Bonnefond, A., Sand, O., Guerin, B., et al. (2012). GATA6 inactivating mutations are associated with heart defects and, inconsistently, with pancreatic agenesis and diabetes. *Diabetologia*, *55*, 2845–2847.
74. Haldorsen, I. S., Raeder, H., Vesterhus, M., et al. (2011). The role of pancreatic imaging in monogenic diabetes mellitus. *Nat Rev Endocrinol*, *8*, 148–159.
75. Rubio-Cabezas, O., Patch, A. M., Minton, J. A., et al. (2009). Wolcott-Rallison syndrome is the most common genetic cause of permanent neonatal diabetes in consanguineous families. *J Clin Endocrinol Metab*, *94*, 4162–4170.
76. Julier, C., & Nicolino, M. (2010). Wolcott-Rallison syndrome. *Orphanet J Rare Dis*, *5*, 29.
77. Reis, A. F., Kannengiesser, C., Jennane, F., et al. (2011). Two novel mutations in the EIF2AK3 gene in children with Wolcott-Rallison syndrome. *Pediatr Diabetes*, *12*, 187–191.
78. Barzaghi, F., Passerini, L., & Bachetta, R. (2012). Immune dysregulation, polyendocrinopathy, enteropathy, x-linked syndrome: a paradigm of immunodeficiency with autoimmunity. *Front Immunol*, *3*, 211.
79. d'Hennezel, E., Bin Dhuban, K., Torgerson, T., et al. (2012). The immunogenetics of immune dysregulation, polyendocrinopathy, enteropathy, X linked (IPEX) syndrome. *J Med Genet*, *49*, 291–302.
80. Suscovich, T. J., Perdue, N. R., & Campbell, D. J. (2012). Type-1 immunity drives early lethality in scurfy mice. *Eur J Immunol*, *42*, 2305–2310.
81. Taha, D., Barbar, M., Kanaan, H., et al. (2003). Neonatal diabetes mellitus, congenital hypothyroidism, hepatic fibrosis, polycystic kidneys, and congenital glaucoma: a new autosomal recessive syndrome? *Am J Med Genet A*, *122A*, 269–273.
82. Senee, V., Chelala, C., Duchatelet, S., et al. (2006). Mutations in GLIS3 are responsible for a rare syndrome with neonatal diabetes mellitus and congenital hypothyroidism. *Nat Genet*, *38*, 682–687.
83. Dimitri, P., Warner, J. T., Minton, J. A., et al. (2011). Novel GLIS3 mutations demonstrate an extended multisystem phenotype. *Eur J Endocrinol*, *164*, 437–443.
84. Watanabe, N., Hiramatsu, K., Miyamoto, R., et al. (2009). A murine model of neonatal diabetes mellitus in Glis3-deficient mice. *FEBS Lett*, *583*, 2108–2113.

85. Yang, Y., Chang, B. H., & Chan, L. (2013). Sustained expression of the transcription factor GLIS3 is required for normal beta cell function in adults. *EMBO Mol Med*, *5*, 92–104.
86. Barrett, J. C., Clayton, D. G., Concannon, P., et al. (2009). Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes. *Nat Genet*, *41*, 703–707.
87. Rees, S. D., Hydrie, M. Z., O'Hare, J. P., et al. (2011). Effects of 16 genetic variants on fasting glucose and type 2 diabetes in South Asians: ADCY5 and GLIS3 variants may predispose to type 2 diabetes. *PLoS One*, *6*, e24710.
88. Smith, S. B., Qu, H. Q., Taleb, N., et al. (2010). Rfx6 directs islet formation and insulin production in mice and humans. *Nature*, *463*, 775–780.
89. Mitchell, J., Punthakee, Z., Lo, B., et al. (2004). Neonatal diabetes, with hypoplastic pancreas, intestinal atresia and gall bladder hypoplasia: search for the aetiology of a new autosomal recessive syndrome. *Diabetologia*, *47*, 2160–2167.
90. Spiegel, R., Dobbie, A., Hartman, C., et al. (2011). Clinical characterization of a newly described neonatal diabetes syndrome caused by RFX6 mutations. *Am J Med Genet A*, *155A*, 2821–2825.
91. Wang, J., Cortina, G., Wu, S. V., et al. (2006). Mutant neurogenin-3 in congenital malabsorptive diarrhea. *N Engl J Med*, *355*, 270–280.
92. Carrasco, M., Delgado, I., Soria, B., et al. (2012). GATA4 and GATA6 control mouse pancreas organogenesis. *J Clin Invest*, *122*, 3504–3515.
93. Xuan, S., Borok, M. J., Decker, K. J., et al. (2012). Pancreas-specific deletion of mouse Gata4 and Gata6 causes pancreatic agenesis. *J Clin Invest*, *122*, 3516–3528.
94. Rodriguez-Segui, S., Akerman, I., & Ferrer, J. (2012). GATA believe it: new essential regulators of pancreas development. *J Clin Invest*, *122*, 3469–3471.
95. Lango Allen, H., Flanagan, S. E., Shaw-Smith, C., et al. (2011). GATA6 haploinsufficiency causes pancreatic agenesis in humans. *Nat Genet*, *44*, 20–22.
96. De Franco, E., Shaw-Smith, C., Flanagan, S. E., et al. (2013). GATA6 mutations cause a broad phenotypic spectrum of diabetes from pancreatic agenesis to adult-onset diabetes without exocrine insufficiency. *Diabetes*, *62*, 993–997.
97. Chakera, A. J., Flanagan, S. E., Ellard, S., & Hattersley, A. T. (2013). Comment on: Khurana et al. The diagnosis of neonatal diabetes in a mother at 25 years of age. Diabetes Care 2012;35:e59. *Diabetes Care*, e31.
98. Johansson, S., Irgens, H., Chudasama, K. K., et al. (2012). Exome sequencing and genetic testing for MODY. *PLoS One*, 7, e38050.

QUESTIONS

1. Neonatal diabetes, hypothyroidism, and glaucoma occur in which of the following conditions?
   a. DEND syndrome
   b. Relaxation of imprinting due to mutation in ZFP57
   c. GLIS3 mutation
   d. All of the above
   e. None of the above

Answer: c

2. FOXP3 mutation is responsible for which of the following conditions?
   a. Pancreatic and cerebellum agenesis
   b. Immunodysregulation, polyendocrinopathy, and enteropathy
   c. Digestive system defects known as Mitchell-Riley syndrome
   d. Transient diabetes mellitus
   e. None of the above

Answer: b

3. A man fathers three children, all of whom develop TNDM, by three different mothers. What is the most likely mechanism?
   a. TNDM due to homozygous mutation in KCNJ11
   b. TNDM due to homozygous mutation in ABCC8
   c. Duplication of the paternal 6q24 region
   d. Relaxation of imprinting due to maternal hypomethylation of 6q24
   e. A freakish chance occurrence

Answer: c

4. Prenatal assessment via ultrasound indicates a baby with severe IUGR. At full-term birth, the baby weighs 1800 grams, has very little subcutaneous fat, and constantly cries for "the bottle" but passes copious amounts of urine so that nurses assume the baby is well hydrated. Persistent failure to thrive leads to the discovery of severe hyperglycemia. How would you advise the parents regarding this presentation?
   a. It is typical for the DEND syndrome.
   b. It is neonatal diabetes mellitus, which should respond to sulfonylureas tablets and the baby will not require insulin shots.
   c. It is TNDM for which molecular diagnosis is not necessary, as this baby's condition will most likely remit in several weeks though it may recur later in life.
   d. It is most likely TNDM1, which responds to surgical correction.
   e. None of the above

Answer: e

5. TNDM can occur in all *except* which of the following conditions?
   a. PTF1A
   b. HNF1β
   c. SLCA2A
   d. KCNJ11
   e. Recessive INS

Answer: a

SECTION **III**

ENDOCRINE DISORDERS IN CHILDREN AND ADOLESCENTS

CHAPTER 10

Disorders of Growth Hormone/ Insulin-Like Growth Factor Secretion and Action

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CHAPTER OUTLINE

NORMAL GROWTH

Normal growth is one of the fundamental characteristics of childhood and adolescence. As multifactorial and complex as the process of growth is, children normally grow in a remarkably predictable manner. Deviation from a normal pattern of growth can be the first manifestation of a wide variety of disease processes, including endocrine and nonendocrine disorders, and may involve virtually any organ system of the body. Frequent and accurate assessment of growth is, therefore, of primary importance to physicians and nurses caring for children.

Measurement

Correct assessment of statural growth requires optimizing accuracy in height determinations.[1-3] When feasible, measurement of supine length is employed in children younger than 2 years of age, and that of standing height is done in older children. Between 2 and 3 years of age, measurement of both length and height can be helpful to assess growth velocity more accurately whereas taking into account the measurement technique. In this age range the supine length averages about 1 cm greater than the standing height. However, the inherent inaccuracies involved in measuring length in infants are often obscured by their rapid skeletal growth characteristic of this age. For measurement of supine length, it is best to employ a firm box with an inflexible board (against which the head lies) and a movable footboard on which the feet are placed perpendicular to the plane of the supine infant. Alternatively, in many offices a counter-mounted or portable infantometer is used to assess infants' and toddlers' growth. To achieve reliable and accurate measurements, the technique used is of great importance. Optimally, the child needs to be relaxed—with the legs fully extended and the head positioned in the "Frankfurt plane" (meaning

the line connecting the outer canthus of the eye and the external auditory meatus is perpendicular to the long axis of the trunk). Repeating the length measurement three times and using the mean is another way to improve reliability.

When children are old enough to stand erect (and physically capable of doing so), it is best to employ a wall-mounted "Harpenden" stadiometer similar to that designed by Tanner and Whitehouse for the British Harpenden Growth Study. Freestanding stadiometers are also available but require frequent recalibration. The traditional measuring device of a flexible arm/bar mounted to a weight balance is notoriously unreliable and cannot be counted on to provide accurate serial measurements.

As with length measurements in infants, positioning of the child is critical. The patient should be fully erect, with the head in the Frankfurt plane. The back of the head, thoracic spine, and buttocks should be touching the vertical axis of the stadiometer; the heels are put together with the toes slightly separated. Every effort should be made to correct discrepancies related to lordosis (neck and lower back) or scoliosis. Ideally, serial measurements should be made at the same time of day because diurnal variation in standing height has been observed, leading to measurements in children and adolescents being up to 0.8 cm shorter at the end of the day compared with measurements obtained in the morning; this phenomenon is likely due to development of fatigue of the spine musculature throughout the day.[4]

It is critical that height determinations be performed by an individual with proper training, rather than (as is often the case) by an inexperienced member of the staff. We recommend that heights also be measured in triplicate, that variation be no more than 0.3 cm, and that the mean height be recorded. For determination of height velocity, it is obviously best to have the same individual performing the determinations. Even when every effort is made to maximize the precision of height determinations, a minimum interval of 3 months is necessary for accurate height velocity computation. Six months worth of data are preferable, although it is of note that seasonal variation in height velocity has been reported.[5]

Growth Charts

Evaluation of a child's height must be done in the context of normal standards. Such standards can be either cross-sectional or longitudinal. Most American pediatric endocrine clinics continue to use the cross-sectional data provided by the National Center for Health Statistics (NCHS), which were originally introduced in 1977 and have been updated.[6-8] Epidemiologic limitations in these growth charts have been noted, however. The data included in the original infant charts, for example, were derived from a private study of a group of subjects who were primarily white, formula-fed, middle-class infants from southwestern Ohio. Data employed for older children came from national health examination surveys conducted from 1963 to 1974.

The NCHS (now a part of the Centers for Disease Control and Prevention) has provided a set of new growth charts, representing revisions of the previous charts, and has introduced new charts for body mass index[9] (Figures 10-1 through 10-8). Interestingly, the new charts show little change in average height since the late 1980s, despite the common perception that today's children are taller than peers from 10 to 25 years ago. This is in contrast to other countries, in particular, The Netherlands, where mean height continued to rise in spite of the population being the tallest in the world. In many current and formerly developing countries, the population, which was typically shorter than in the Western world, is becoming taller.

Classic percentile-based growth charts are invaluable for plotting growth of children relative to the 3rd or 5th, 10th, 25th, 50th, 75th, 90th, and 95th or 97th percentiles of normal American children. There are, however, two major limitations of these charts. First, they do not satisfactorily define the growth rates of children below the 3rd or above the 97th percentile—the very children for whom it is most critical to accurately describe the degree to which their growth deviates from the normal growth percentiles. The NCHS data can be used to compute standard deviation (SD) scores (or Z scores), which are more helpful because a short child can be described as having a growth rate of (for example) 2 or 2.5 SD from normal. Because these SDs are defined by cross-sectional data, however, SD scores during childhood are not directly comparable with SD scores during adolescence—when great variation in growth rates can be normally observed. Second, cross-sectional data are of greater value during infancy and childhood than in adolescence—when differences in the timing of puberty can introduce considerable variability into normal growth rates. To address this issue, Tanner and colleagues[10] have developed longitudinal growth charts that accommodate the timing of puberty. These charts are of greatest value in assessing growth during adolescence and puberty and are probably superior for plotting sequential growth data on any given child.

The data from cross-sectional and longitudinal growth studies have been employed to develop height velocity standards[10] (Figures 10-9 and 10-10). It is important to emphasize that carefully documented height velocity data are invaluable in assessing the child with abnormalities of growth. Although considerable variability exists in the normal height velocity observed in children of different ages, between the age of 2 years and the onset of puberty children normally grow with remarkable fidelity relative to the normal growth curves. The physician should note any "crossing" of height percentiles during this age period, and abnormal height velocities always warrant further evaluation.

Disease-related growth curves have been developed for a number of clinical conditions associated with growth failure, such as Turner syndrome,[11] achondroplasia,[12] and Down syndrome.[13] Such growth profiles are invaluable for tracking the growth of children with these clinical conditions. Deviation of growth from the appropriate disease-related growth curve suggests the possibility of a second underlying condition.

Text continued on page 301

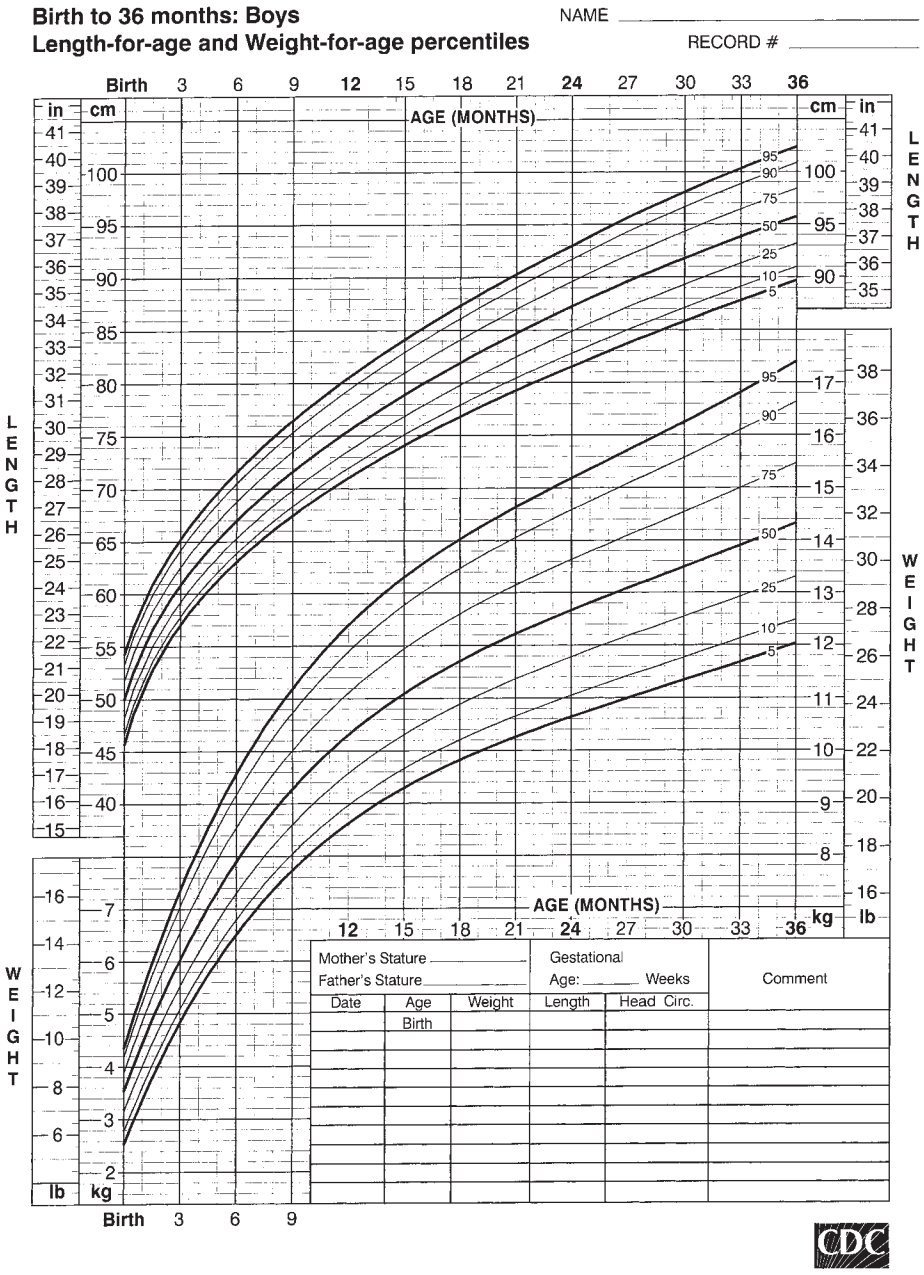


FIGURE 10-1 ■ Length-for-age and weight-for-age percentiles for boys (birth to 36 months). (Developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion (2000), www.cdc.gov/growthcharts.)

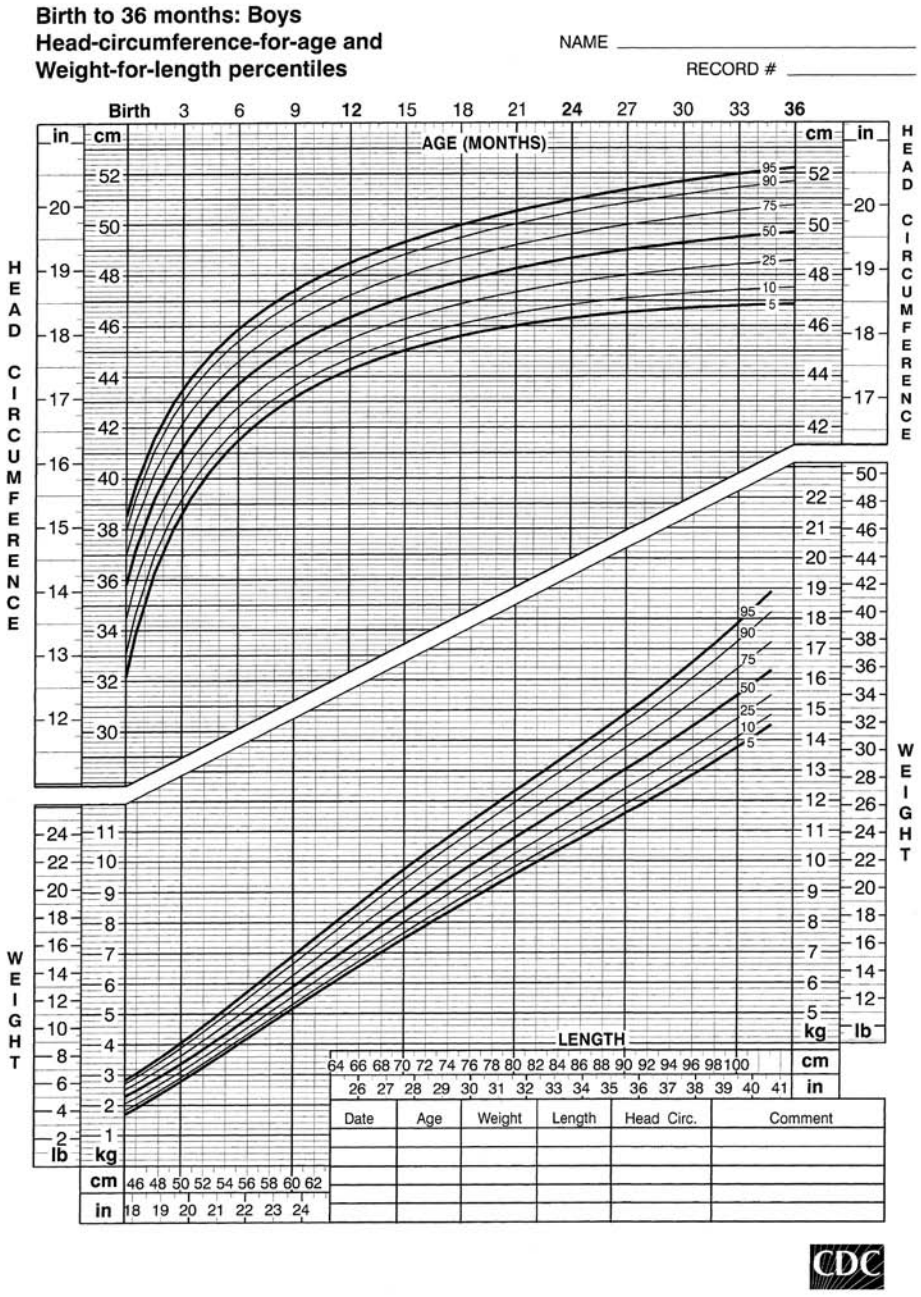


FIGURE 10-2 ■ Head-circumference-for-age and weight-for-length percentiles for boys (birth to 36 months). (Developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion (2000), www.cdc.gov/growthcharts.)

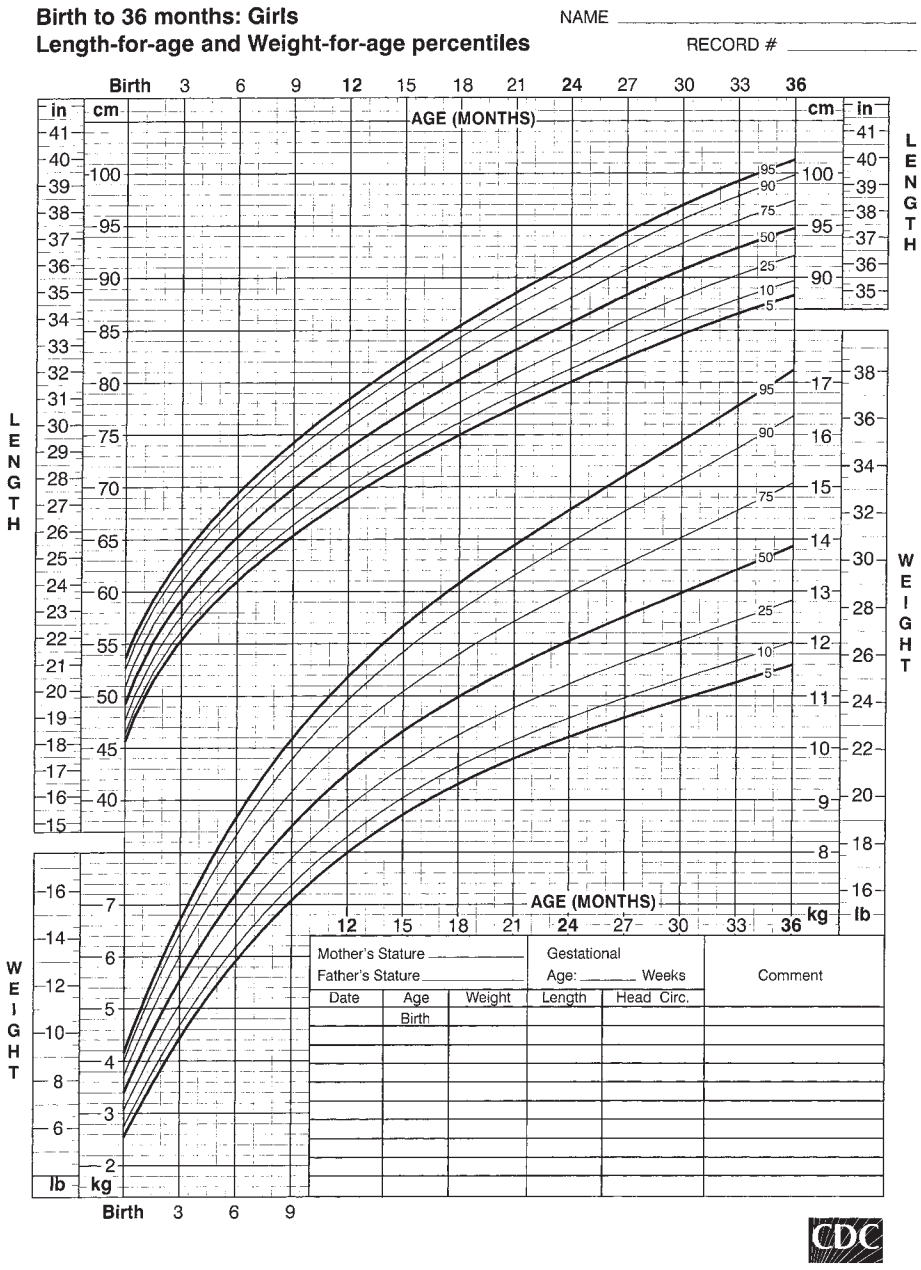


FIGURE 10-3 ■ Length-for-age and weight-for-age percentiles for girls (birth to 36 months). (Developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion (2000), www.cdc.gov/growthcharts.)

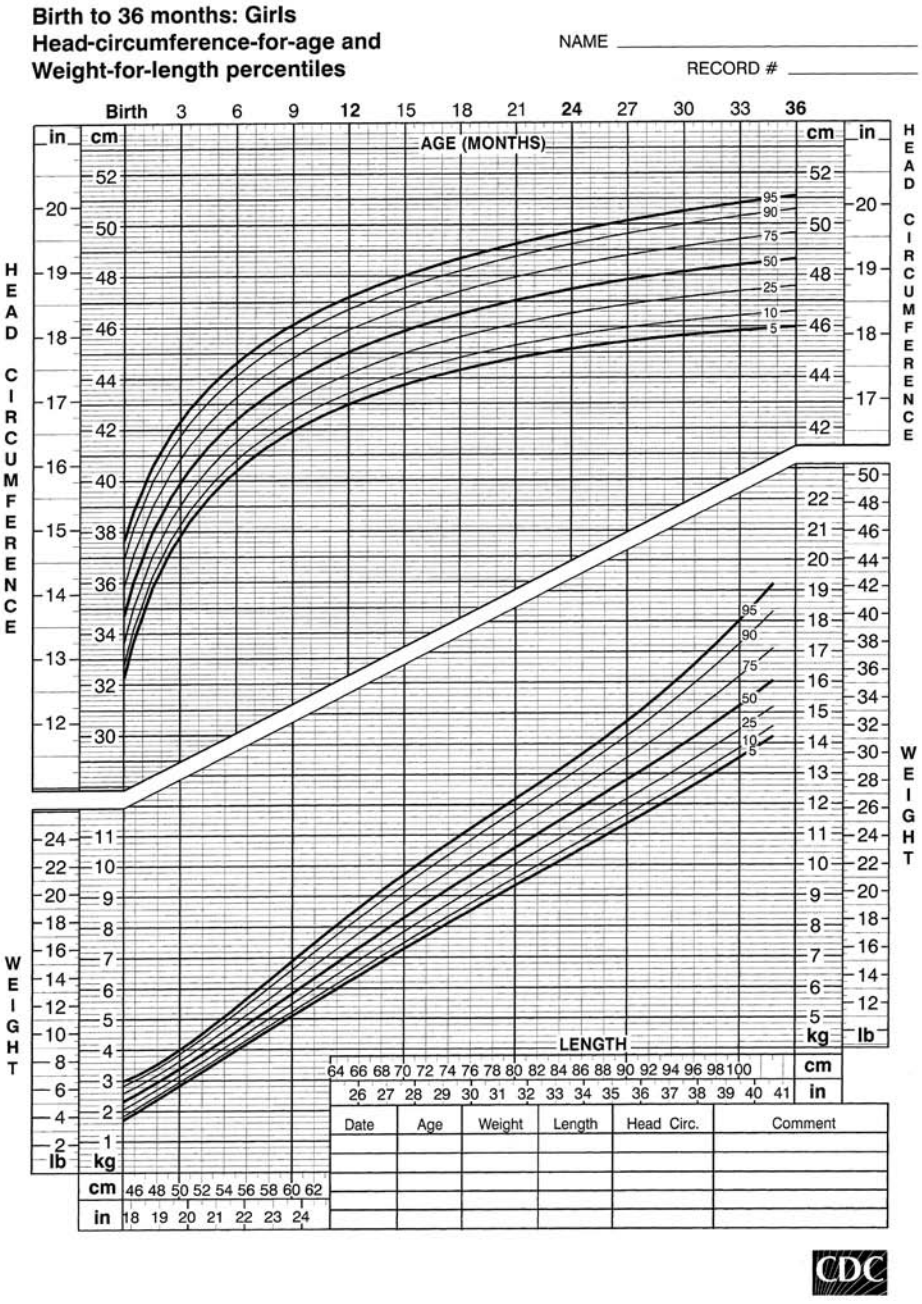


FIGURE 10-4 ■ Head-circumference-for-age and weight-for-length percentiles for girls (birth to 36 months). (Developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion (2000), www.cdc.gov/growthcharts.)

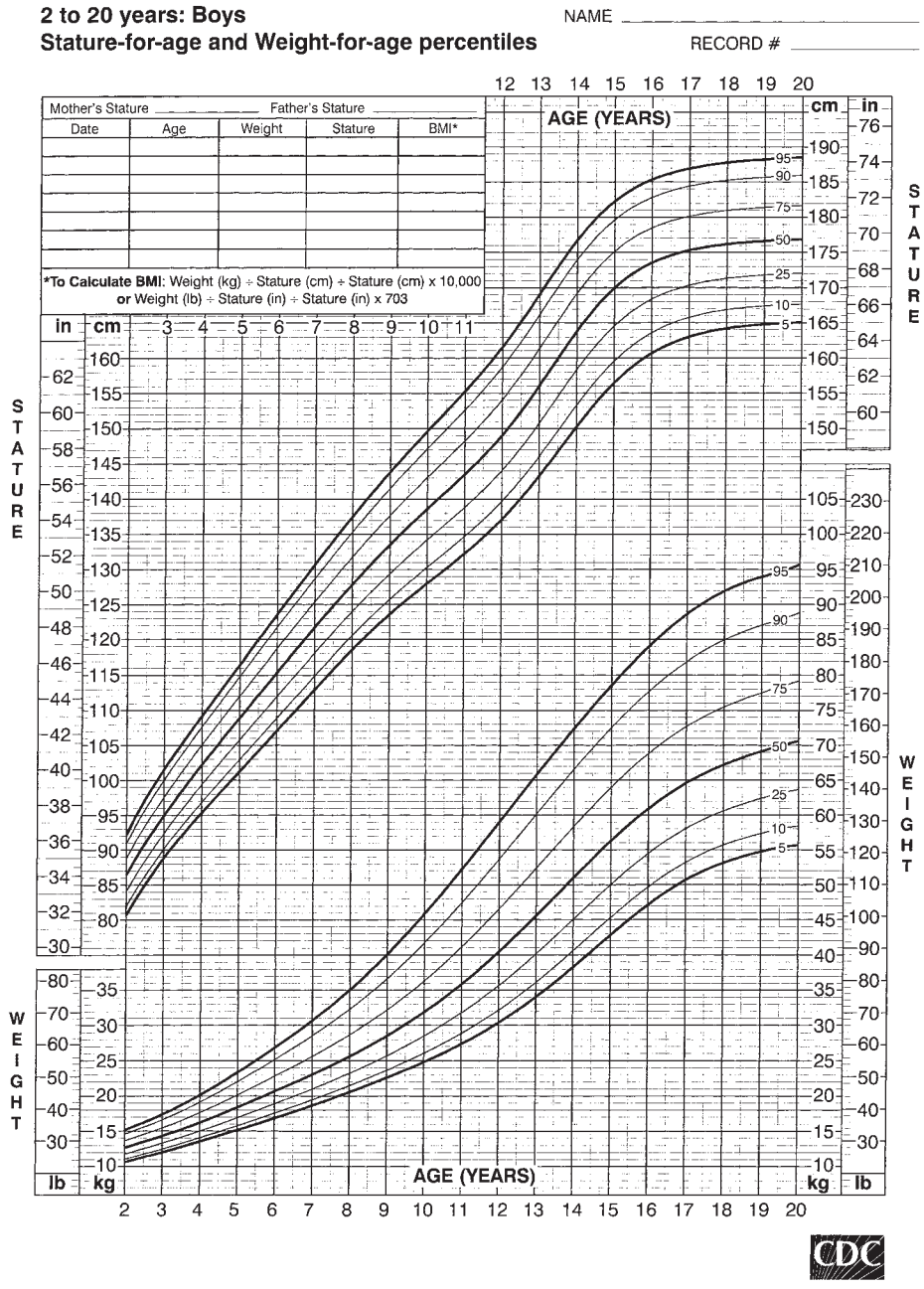


FIGURE 10-5 ■ **Stature-for-age and weight-for-age percentiles for boys (2 to 20 years).** (Developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion (2000), www.cdc.gov/growthcharts.)

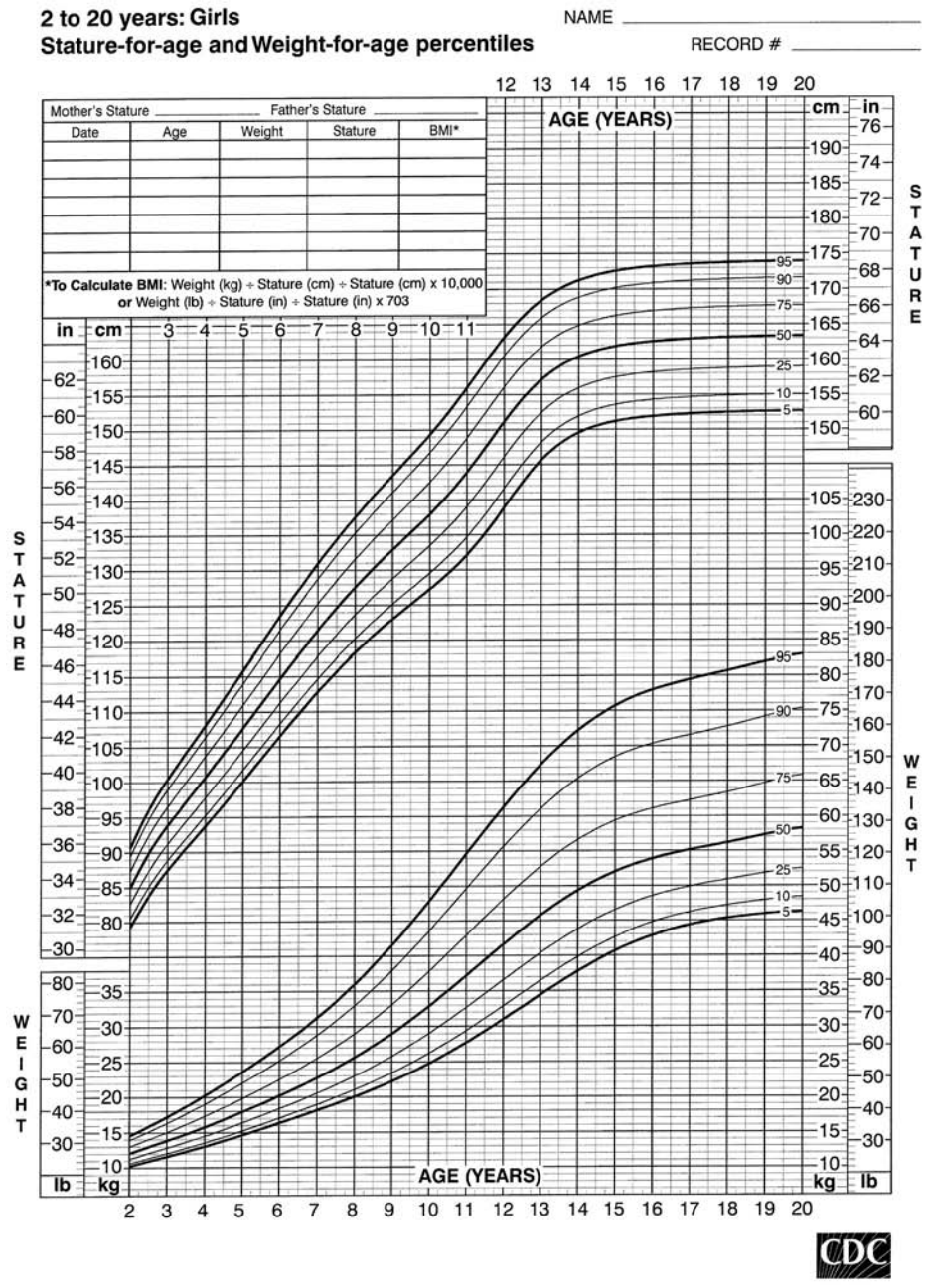


FIGURE 10-6 ■ Stature-for-age and weight-for-age percentiles for girls (2 to 20 years). (Developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion (2000), www.cdc.gov/growthcharts.)

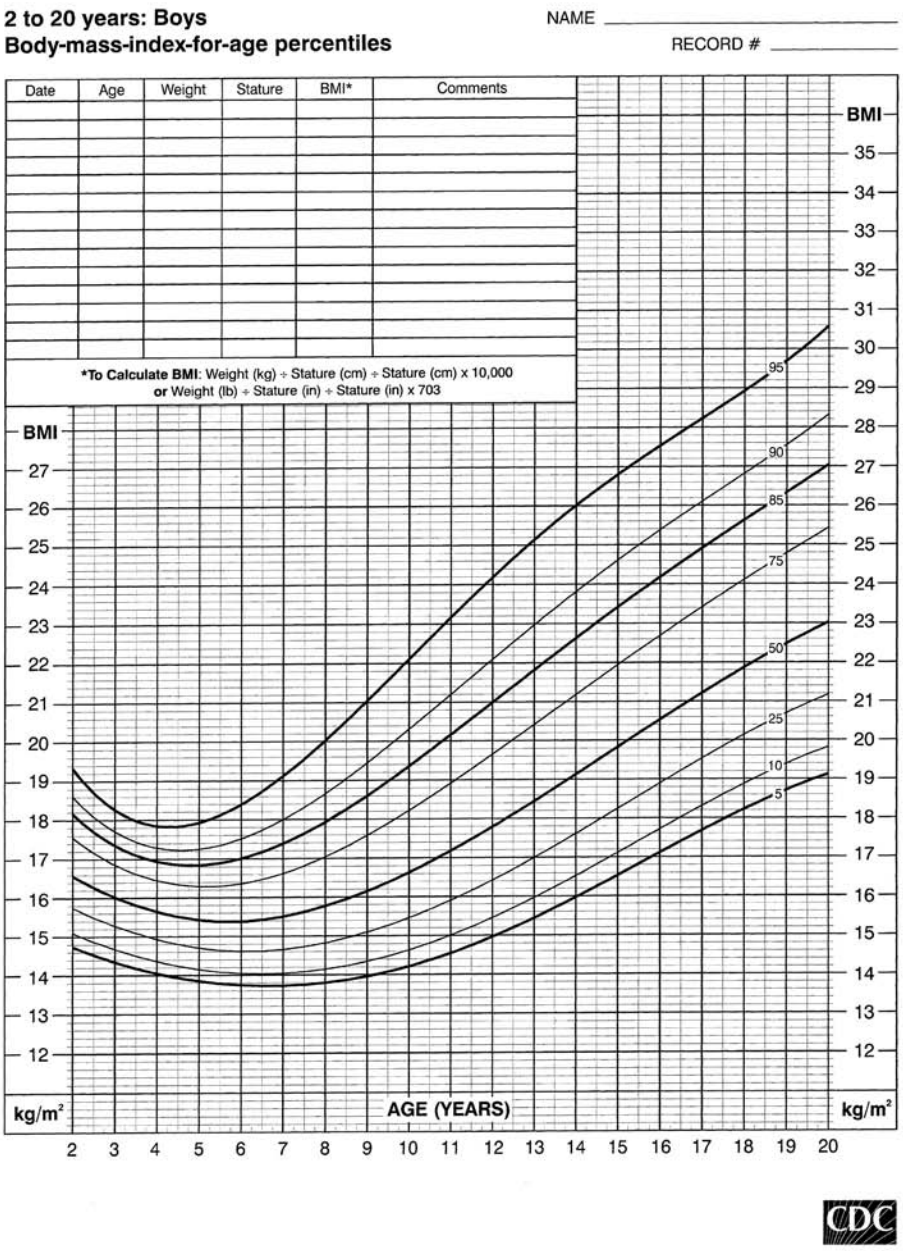


FIGURE 10-7 ■ Body-mass-index-for-age percentiles for boys (2 to 20 years). (Developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion (2000), www.cdc.gov/growthcharts.)

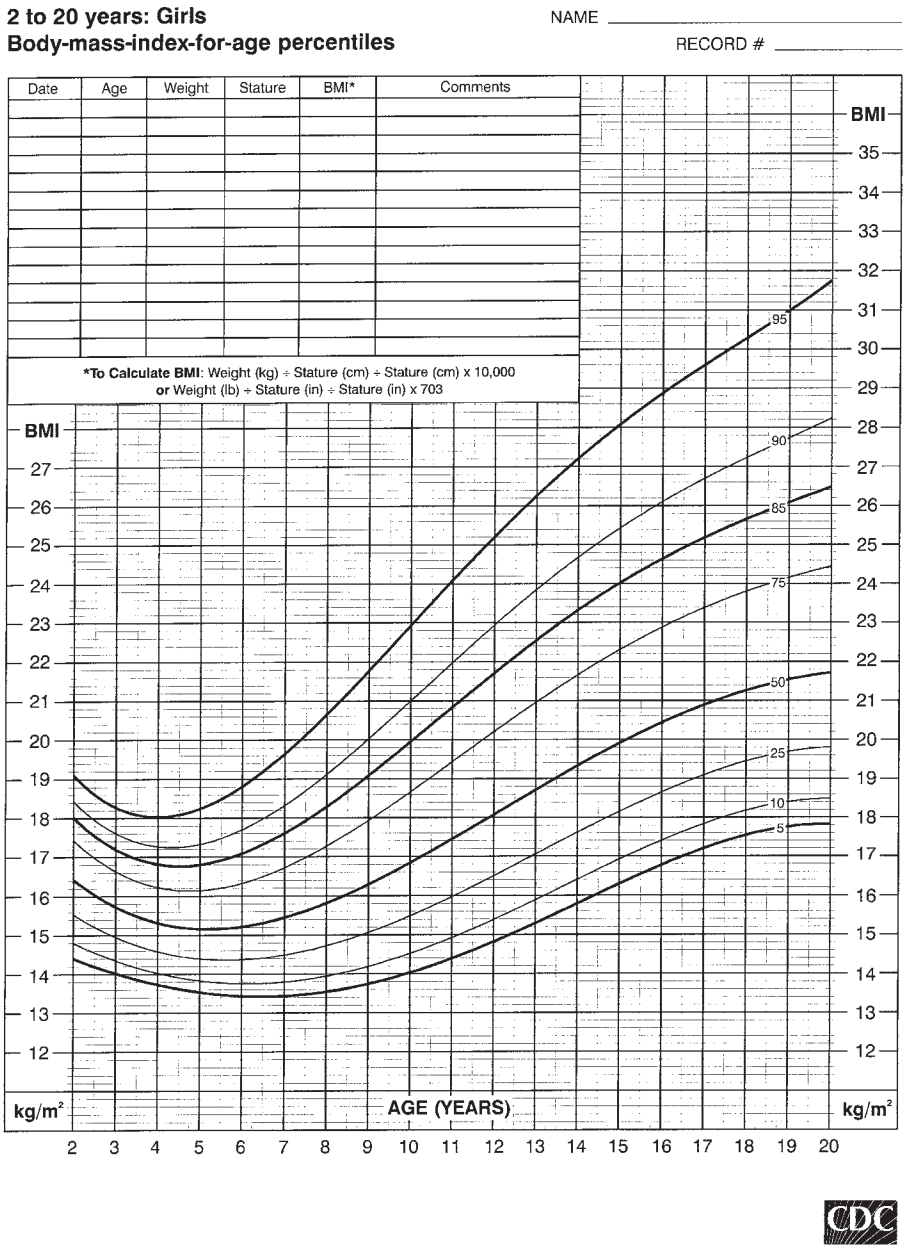


FIGURE 10-8 ■ Body-mass-index-for-age percentiles for girls (2 to 20 years). (Developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion (2000), www.cdc.gov/growthcharts.)

Body Proportions

Many growth disorders, leading to either short stature or tall stature, are associated with disproportionate growth. Some of the more common disorders leading to disproportionality include the many forms of osteochondrodysplasia and rickets. Survivors of childhood cancer who received spinal irradiation are also at increased risk for disproportionate growth.[14] To allow documentation of such disproportionate growth, the following measurements can be made:

- Occipitofrontal head circumference
- Lower body segment: distance from top of pubic symphysis to the floor
- Upper body segment: sitting height (height of stool should be subtracted from standing height)
- Arm span: measurement of the length from one end of a patient's arms (measured at the fingertip) to the other with the arms raised parallel to the ground at shoulder height

Published standards exist for these body proportion measurements, which must be evaluated relative to the patient's age.[15] The upper segment-to-lower segment ratio, for example, ranges from 1.7 in the neonate to slightly below 1 in the adult. Ethnic differences also exist: black children and adolescents have shorter sitting height and longer length of the lower extremities (about one inch for each, corrected for age and sex).[16]

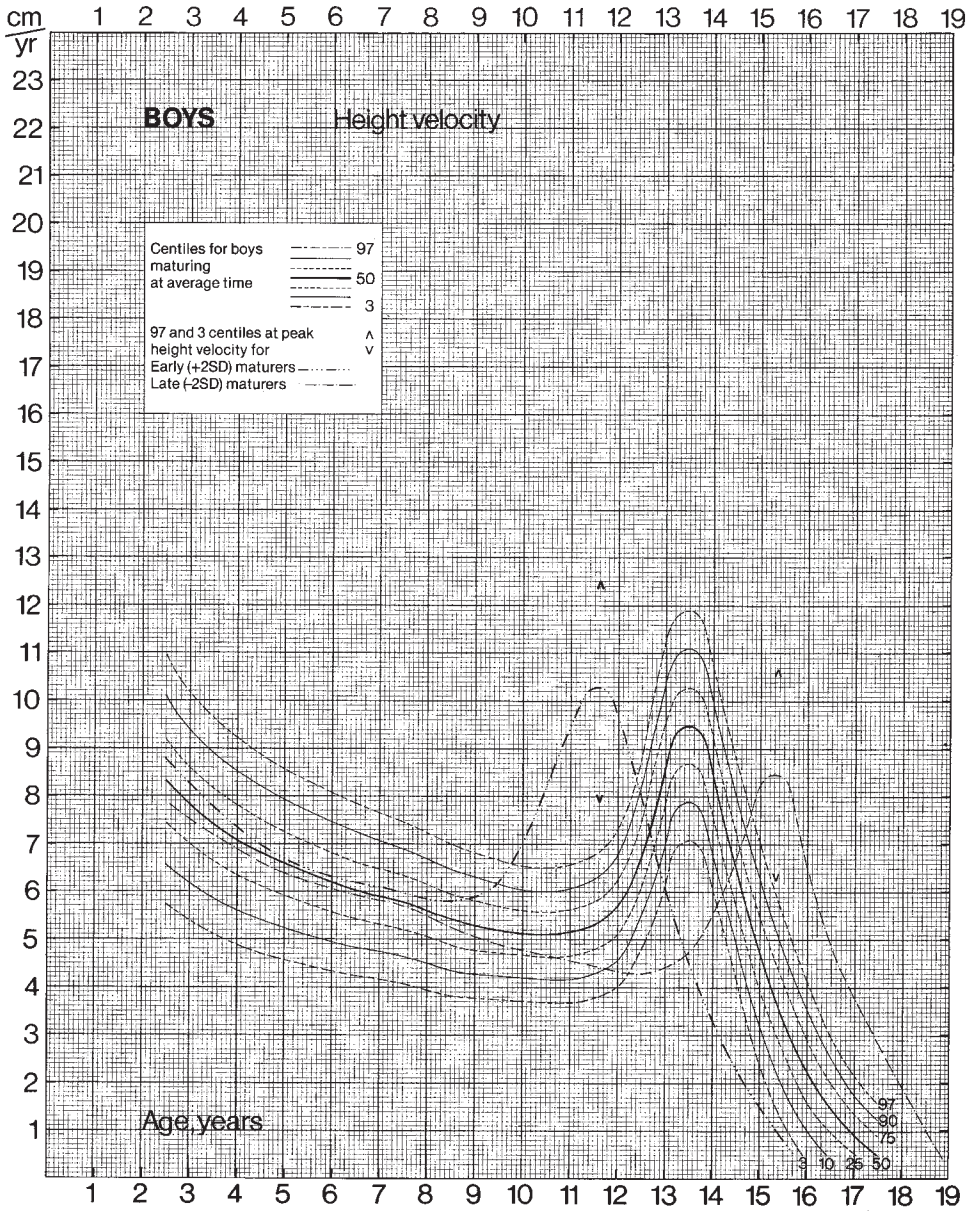


FIGURE 10-9 ■ Height velocity chart for males from 0 to 19 years. (From Tanner, J. M., & Davies, S. W. D. (1985). Clinical longitudinal standards for height and height velocity for North American children. *J Pediatr, 107*, 312.)

Skeletal Maturation

The degree to which a child's skeleton has matured over time gives the physician a window into the true physical developmental age of that child. The growth potential inherent in the tubular bones of the body can be assessed by evaluation of the progression of ossification within the epiphyses. The ossification centers of the skeleton appear and progress in a predictable sequence in normal children, and skeletal maturation can therefore be compared with normal age-related standards. This forms the basis of "bone age" or "skeletal age." It is not clear what factors determine this normal maturational pattern, but it is certain that genetic factors and multiple hormones—including thyroxine (T4), growth hormone (GH), and sex steroids—are involved in this process.

Studies in patients with mutations of the gene for the estrogen receptor[17] or for aromatase enzyme[18] have demonstrated that it is estrogen that is primarily responsible for ultimate epiphyseal fusion, although it seems unlikely that estrogen is alone responsible for all skeletal maturation. Beyond the neonatal period, and especially after age 2 years, a radiograph of the left hand and wrist is commonly used to determine "bone age"—which can be related to the published standards of Greulich and Pyle.[19] Although the authors recommend a bone-by-bone assessment approach to arrive at a mean bone age for the patient, most endocrinologists determine the bone age by finding the standard radiograph that best matches their patient's using a subjective comparative approach. This technique creates significant variability in bone age reading between different interpreters. An alternative method for assessing bone age from

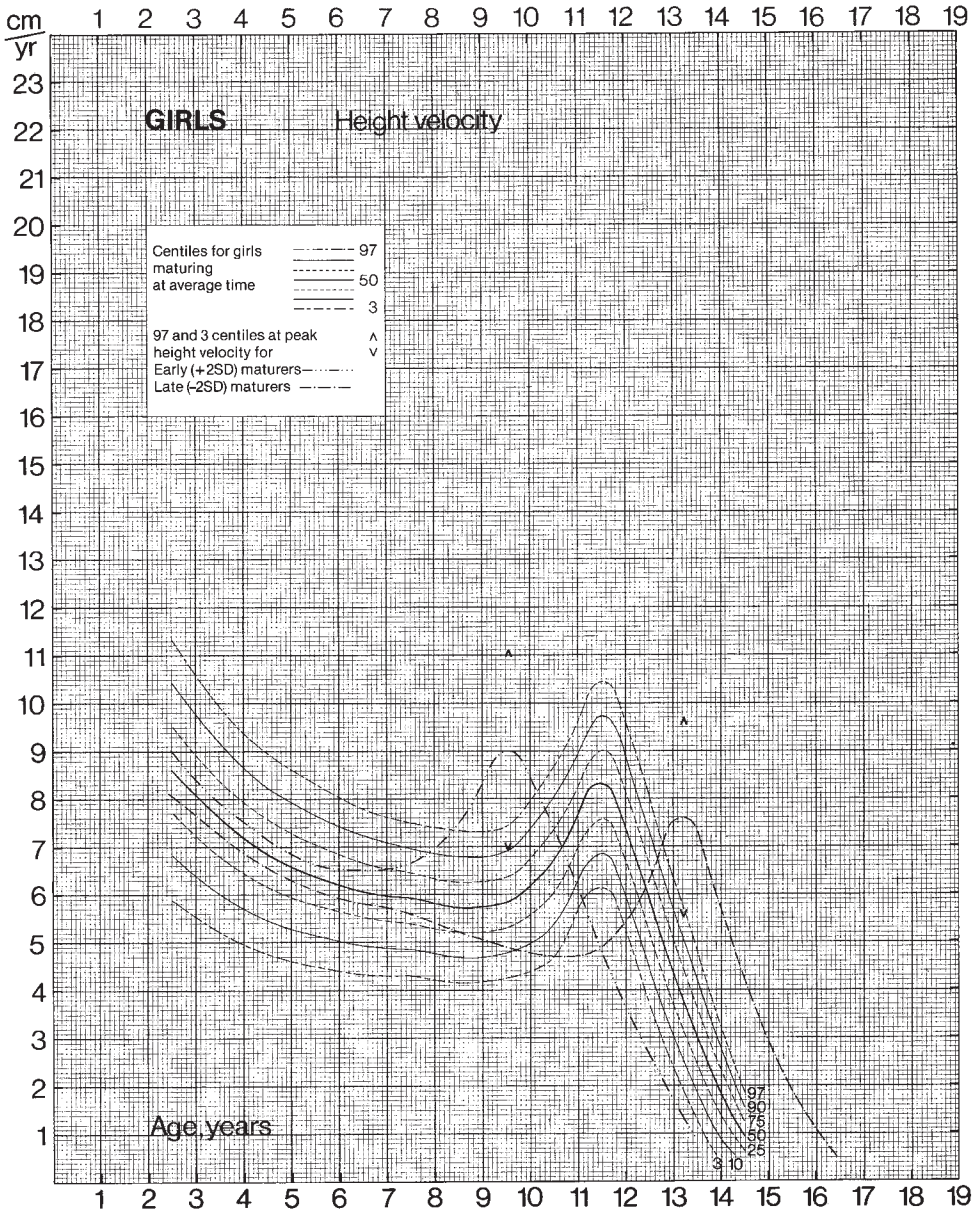


FIGURE 10-10 ■ **Height velocity chart for females from 0 to 19 years.** (From Tanner, J. M., & Davies, S. W. D. (1985). Clinical longitudinal standards for height and height velocity for North American children. *J Pediatr, 107,* 312.)

radiographs of the left hand, involving a scoring system for each individual bone, has been developed by Tanner and Whitehouse and their colleagues.[20] The left hand obviously represents a compromise, because radiographs of the entire skeleton would be tedious, expensive, and necessitate excessive radiation exposure. It is important to note, however, that the hand obviously does not contribute to the height of an individual and that accurate evaluation of growth potential might necessitate radiographs of the legs and spine.

A number of important caveats concerning bone age determination must be considered. Experience in reading bone age films is essential, and clinical studies involving bone age generally benefit from having a single reader who does all interpretations. Second, the normal rate of skeletal maturation differs between males and females (and ethnic variability exists). The standards of Greulich and Pyle are divided by sex, but they were developed in American white children. Finally, the Greulich-Pyle and the Tanner-Whitehouse standards were developed using normal children.[21] They are not necessarily applicable to children with skeletal dysplasia, endocrine abnormalities, or a variety of other causes of growth retardation.

Prediction of Adult Height

The extent of skeletal maturation observed in a patient can be used to predict the patient's height potential. The estimated adult height potential may be interpreted in function of the parental target height. Predictions of adult height are based on the observation that the more delayed the bone age (relative to the chronologic age),

the greater the length of time before epiphyseal fusion prevents further growth. The classic method for height prediction, based on Greulich and Pyle's *Radiographic Atlas of Skeletal Development*,[19] was developed by Bayley and Pinneau[22] and relies on the patient's bone age and height (Table 10-1).

Additional refinements were introduced by Tanner and colleagues[20,23] with a system that employs height, bone age, and chronologic age; and by Roche and associates,[24] who employ the combination of height, bone age, chronologic age, midparental height, and weight. All of these systems are by nature empirical and should never be used as absolute predictors. The more advanced the bone age, the greater the accuracy of the adult height prediction, but this is natural because a more advanced bone age places a patient closer to final height.

All of these methods of predicting adult height are based on data from normal children. None of these systems has been demonstrated to be accurate in children with growth abnormalities. For this type of precision, it would be necessary to develop disease-specific (e.g., Turner syndrome, Noonan syndrome, specific types of skeletal dysplasia) atlases of skeletal maturation. Retrospective analyses indicate that bone age–based adult height predictions slightly underpredict female but often overpredict male children's eventual height. Predictions are also notoriously inaccurate in children born small for gestational age.

Parental Target Height

Because genetic factors are of great importance as determinants of growth and height potential, it is always worthwhile to assess a patient's stature relative to that of siblings and parents. The parental target height can be readily ascertained by calculating the mean parental height and adding or subtracting 6.5 cm for male or female children, respectively. The standard deviation for this calculated parental target height is about 2.5 cm and the range within which it is likely to occur at least 95% of the time is approximately 7.5 to10 cm. As with predicted adult heights, calculated target heights should be taken as approximations.

Nevertheless, when a child's growth pattern clearly deviates from that of parents or siblings, one must seriously consider the possibility of an underlying pathologic process. It is important, when possible, to measure the heights of parents and siblings rather than accepting their own statural claims (mothers often overestimate the height of fathers). In addition, one should recall that it is not always possible to know the heights of the true biologic parents (or, sometimes, who the real biologic parents actually are). Finally, short parents are not an excuse to avoid working up a child who is clearly short because this may represent a treatable genetic disorder within the growth hormone signaling cascade.

ENDOCRINE REGULATION OF GROWTH

The Pituitary

The pituitary gland lies in the sella turcica, the hypophyseal fossa of the sphenoid bone, which is located in the center of the cranial base. The concept of the pituitary as a "master gland" controlling the endocrine activities of the body has become outdated and has been replaced by an appreciation of the importance of the brain, particularly of the hypothalamus, in regulating hormonal production and secretion. Nevertheless, the pituitary gland remains central to our understanding of the regulation of growth, metabolism and homeostasis, response to stress, lactation, and reproduction.

Embryologically, the pituitary gland is formed from two distinct sources.[25,26] Rathke's pouch, a diverticulum of the primitive oral cavity (stomodeal ectoderm), gives rise to the adenohypophysis. The neurohypophysis originates in the neural ectoderm of the floor of the forebrain, which also develops into the third ventricle. The adenohypophysis normally constitutes 80% of the weight of the pituitary and consists of the pars distalis (also known as the pars anterior or anterior lobe), the pars intermedia (also known as the intermediate lobe), and the pars tuberalis (also known as the pars infundibularis or pars proximalis).

Much of our knowledge of normal hypothalamopituitary development is derived from animal, particularly rodent, models. In the mouse, a thickening of the ectoderm in the midline of the anterior neural ridge, forming the hypophyseal placode, heralds the onset of pituitary development at 7.5-days postcoitum (dpc). The formation of a rudimentary Rathke's pouch follows at 9 dpc, with formation of a definitive pouch by 12 dpc and, subsequently, the anterior pituitary consisting of five different cell types secreting six different hormones (Figure 10-11). The developing Rathke's pouch is initially associated with the presumptive hypothalamic territories, and later with the developing diencephalon.

In humans, the pars distalis is the largest portion of the adenohypophysis and houses the great majority of hormone-producing cells. The pars intermedia is, typically, poorly developed and consists of several cystic cavities lined by a single layer of cuboidal epithelium. Pars distalis and intermedia are separated by a cleft, a vestigial structure of Rathke's pouch from which it develops. This structure may often develop as a cyst (Rathke's cleft cyst). In humans, in contrast to the mouse, pars intermedia is rudimentary as it largely disappears during embryogenesis. The pars tuberalis represents an upward extension of the pars distalis onto the pituitary stalk and may contain a limited number of gonadotropin-producing cells. The posterior pituitary (neurohypophysis) consists of the infundibular stem or hypophyseal stalk, the median eminence of the tuber cinereum, and the infundibular process (posterior lobe, neural lobe). The posterior pituitary contains the terminal axonal projections of magnocellular neurons from the paraventricular and supraoptic nuclei of the hypothalamus. These produce oxytocin, required during lactation and parturition, and vasopressin, required for osmotic regulation—as detailed in Chapter 11. It has no known function in the regulation of growth and will not be discussed further in this chapter.

Rathke's pouch, the origin of the adenohypophysis, can be identified in the 3-mm embryo during the third week of pregnancy in humans. Rathke's pouch then begins to

TABLE 10-1 **Fraction of Adult Height Attained at Each Bone Age**

Bone Age (Years-Months)	Girls			Boys		
	RETARDED	AVERAGE*	ADVANCED	RETARDED	AVERAGE*	ADVANCED
6-0	0.733	0.720		0.680		
6-3	0.742	0.729		0.690		
6-6	0.751	0.738		0.700		
6-9	0.763	0.751		0.709		
7-0	0.770	0.757	0.712	0.718	0.695	0.670
7-3	0.779	0.765	0.722	0.728	0.702	0.676
7-6	0.788	0.772	0.732	0.738	0.709	0.683
7-9	0.797	0.782	0.742	0.747	0.716	0.689
8-0	0.804	0.790	0.750	0.756	0.723	0.696
8-3	0.813	0.801	0.760	0.765	0.731	0.703
8-6	0.823	0.810	0.771	0.773	0.739	0.709
8-9	0.836	0.821	0.784	0.779	0.746	0.715
9-0	0.841	0.827	0.790	0.786	0.752	0.720
9-3	0.851	0.836	0.800	0.794	0.761	0.728
9-6	0.858	0.844	0.809	0.800	0.769	0.734
9-9	0.866	0.853	0.819	0.807	0.777	0.741
10-0	0.874	0.862	0.828	0.812	0.784	0.747
10-3	0.884	0.874	0.841	0.816	0.791	0.753
10-6	0.896	0.884	0.856	0.819	0.795	0.758
10-9	0.907	0.896	0.870	0.821	0.800	0.763
11-0	0.918	0.906	0.883	0.823	0.804	0.767
11-3	0.922	0.910	0.887	0.827	0.812	0.776
11-6	0.926	0.914	0.891	0.832	0.818	0.786
11-9	0.929	0.918	0.897	0.839	0.827	0.800
12-0	0.932	0.922	0.901	0.845	0.834	0.809
12-3	0.942	0.932	0.913	0.852	0.843	0.818
12-6	0.949	0.941	0.924	0.860	0.853	0.828
12-9	0.957	0.950	0.935	0.869	0.863	0.839
13-0	0.964	0.958	0.945	0.880	0.876	0.850
13-3	0.971	0.967	0.955		0.890	0.863
13-6	0.977	0.974	0.963		0.902	0.875
13-9	0.981	0.978	0.968		0.914	0.890
14-0	0.983	0.980	0.972		0.927	0.905
14-3	0.986	0.983	0.977		0.938	0.918
14-6	0.989	0.986	0.980		0.948	0.930
14-9	0.992	0.988	0.983		0.958	0.943
15-0	0.994	0.990	0.986		0.968	0.958
15-3	0.995	0.991	0.988		0.973	0.967
15-6	0.996	0.993	0.990		0.976	0.971
15-9	0.997	0.994	0.992		0.980	0.976
16-0	0.998	0.996	0.993		0.982	0.980
16-3	0.999	0.996	0.994		0.985	0.983
16-6	0.999	0.997	0.995		0.987	0.985
16-9	0.9995	0.998	0.997		0.989	0.988
17-0	1	0.999	0.998		0.991	0.990
17-3					0.993	
17-6		0.9995	0.9995		0.994	
17-9					0.995	
18-0		1			0.996	
18-3					0.998	
18-6					1	

*Average: Bone age within 1 year of chronologic age.

Data from Post, E. M., & Richman, R. A. (1981). A condensed table for predicting adult stature. J Pediatr, 98, *440, based on the data of Bayley and Pinneau.*[22] *These tables have been organized in an easy-to-use slide rule format ("Adult Height Predictor," copyright 1987, Ron G. Rosenfeld).*

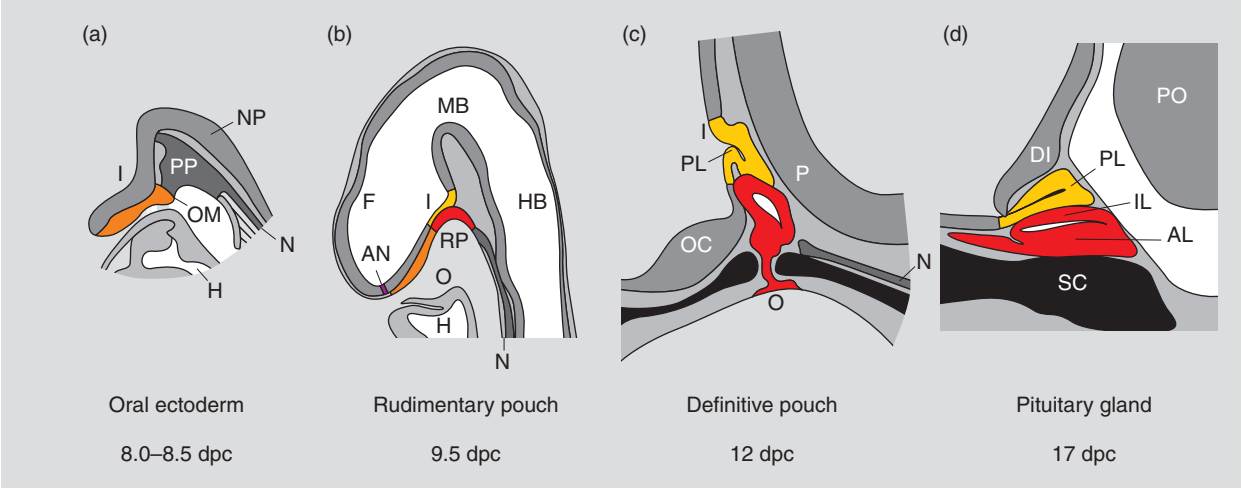


FIGURE 10-11 ■ Mouse pituitary development. Illustration showing mouse pituitary development in sagittal section: stages of development are indicated in days post coitum (dpc). AL, anterior lobe; AN anterior neural pore; DI diencephalon; F, forebrain; H, heart; HB, hindbrain; I, infundibulum; IL, intermediate lobe; MB, midbrain; N, notochord; NP, neural plate; O, oral cavity; OC, optic chiasm; OM, oral membrane; P, pontine flexure; PL, posterior lobe; PO, pons; PP, prechordal plate; RP, Rathke's pouch; SC, sphenoid cartilage. (Adapted from Sheng, H. Z., & Westphal H (1999). Early steps in pituitary organogenesis. *Trends Genet, 1*, 236–240.) *This image can be viewed in full color online at ExpertConsult.*

develop, resulting in a complete pouch disconnected from the oral ectoderm by the end of the sixth gestational week. GH-producing cells can be identified by 9 weeks of gestation.[27] It is at about this time that the vascular connections between the anterior lobe of the pituitary and the hypothalamus develop,[28,29] although it has been demonstrated that hormonal production by the pituitary can occur in the absence of connections with the hypothalamus. Somatotropic cells in the pituitary are thus frequently demonstrable in the anencephalic newborn. Nevertheless, it appears likely that the initiation of development of the anterior pituitary is dependent on responsiveness of the oral ectoderm to inducing factors from the ventral diencephalon[30-34] (Figure 10-12).

Maintained apposition and interactions between the oral ectoderm and neuroectoderm is critical for normal anterior pituitary development.[35-38]

Experimental manipulation of embryos from several species as well as Rathke's pouch explant experiments in rodents have shown that signals from the diencephalon are essential not only for the induction and maintenance of Rathke's pouch, but also for the regionalization within the pouch that allows the emergence of the different endocrine cell types. During gestation, proliferating progenitor cells are enriched around the pouch lumen, and they appear to delaminate as they exit the cell cycle and differentiate. During late mouse gestation and the postnatal period, anterior lobe progenitors reenter the cell cycle and expand the populations of specialized, hormone-producing cells. At birth, all cell types are present, and their localization appears stratified based on cell type. Current models of cell specification in the anterior lobe suggest that opposing gradients of FGF and BMP signaling pattern the progenitor cells within Rathke's pouch before they move on to the anterior lobe where they differentiate. Explant studies in the mouse have demonstrated that if Rathke's pouch is removed from the oral ectoderm on embryonic days 12 to 13 and incubated in appropriate culture medium, differentiation of each of the pituitary cell types continues, indicating that by that point organogenesis of the anterior pituitary is no longer dependent on hypothalamic signals—although such signals may remain critically involved in pituitary hormone production. These and other studies have revealed that normal pituitary development is dependent on a complex cascade of transcription factors and signaling molecules that are expressed in a spatiotemporal manner.

Signaling molecules implicated in pituitary development are either intrinsic emanating from the oral ectoderm such as sonic hedgehog (Shh) or extrinsic from the neuroectoderm such as Nkx2.1, fibroblast growth factors (Fgfs eg Fgf8), and bone morphogenetic factors (Bmps eg. Bmp4) (Figure 10-13). These molecules may activate or repress transcription factors such as Hesx1, Lhx3, and Lhx4. They may also act as morphogens creating the appropriate environment for cell differentiation, thus playing a critical role in cell fate. Such signaling molecules include members of the Shh family, Fgfs, transforming growth factors (Tgfs)/Bmps, Wingless/Wnts, and molecules in the Notch pathway, to mention a few.

One study has challenged the current dogma of pituitary cell specification, using simple yet elegant experiments.[40] The authors showed that, in mice, the pattern of cell specification that results in the rostral location of gonadotropes, the caudal location for somatotropes, and a more intermediate location for corticotropes and thyrotropes does not appear to be the result of an ordered cell cycle exit, as previously predicted. All anterior lobe cell types appear to begin the differentiation process concurrently (E11.5-E14.5), rather than in a temporally discrete manner.

To date, not many pituitary phenotypes have been reported in association with mutations in these signaling molecules. Importantly, the Wnt signaling pathway has recently been implicated in pituitary tumorigenesis. A number of microarray studies have identified altered

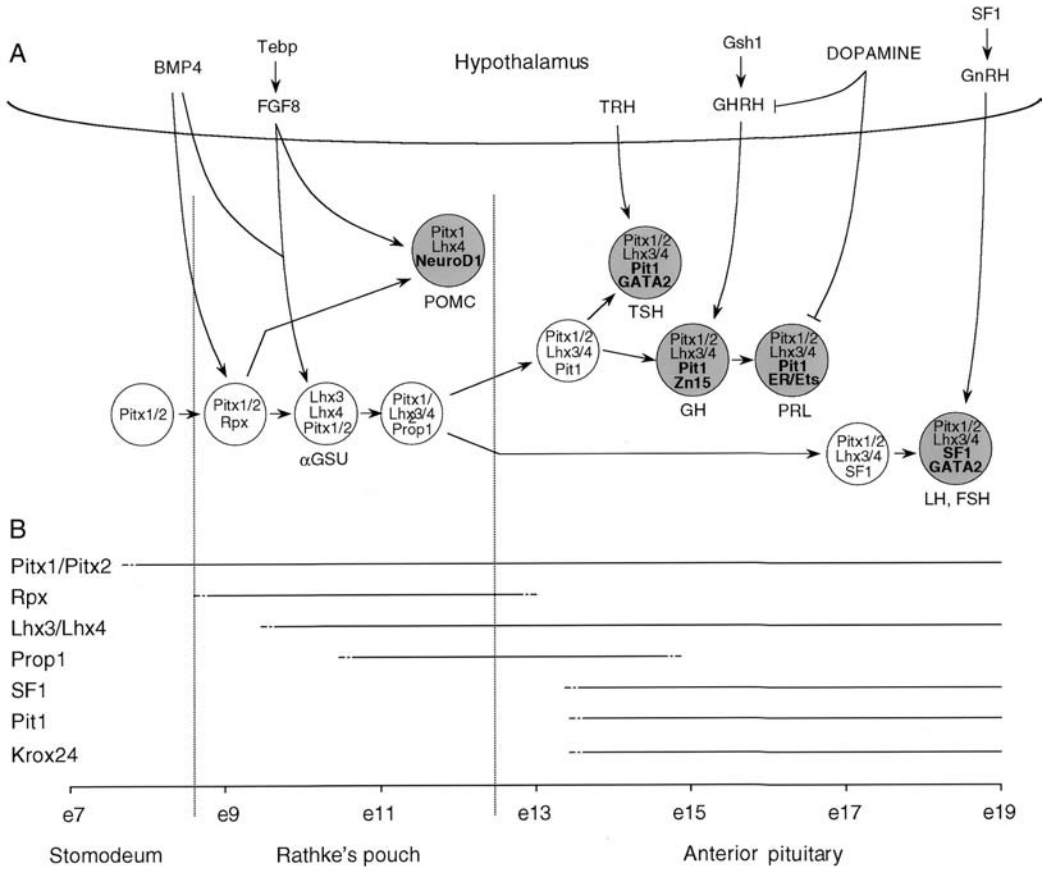


FIGURE 10-12 ■ Development of pituitary cell lineages. **A,** Schematic representation of pituitary cell precursors showing the expression of prevalent transcription factors at each stage of development. Terminally differentiated cells are shown as larger and shaded circles together with the hormones produced (lineage-specific transcription factors are highlighted in bold in these cells). The interaction with transcription factors and signaling molecules in the hypothalamus is also noted. Transcription factors are represented in lowercase (except for SFI and GATA2), whereas signaling molecules appear in uppercase. **B,** Schema showing the timing of the appearance and disappearance of pituitary transcription factors during mouse embryogenesis. BMP4, bone morphogenic protein 4; e, embryonic day; ER, estrogen receptor; FGF8, fibroblast growth factor 8; FSH, follicle-stimulating hormone; GH, growth hormone; GHRH, growth hormone-releasing hormone; GnRH, gonadotropin–releasing hormone; αGSU, α-glycoprotein subunit; LH, luteinizing hormone; POMC, pro-opiomelanocortin; PRL, prolactin; SFI, steroidogenic factor I; TRH, thyrotropin-releasing hormone; TSH, thyroid-stimulating hormone. (From Lopz-Bermejo, A., Buckway, C. K., & Rosenfeld, R. G. (2000). Genetic defects of the growth hormone–insulin-like growth factor axis. *Trends Endocrinol, 11,* 43.)

expression of Wnt inhibitors in pituitary tumors, and there is clear evidence that the Wnt/β catenin pathway is involved in the pathogenesis of craniopharyngioma, a rare tumor in the hypothalamo-pituitary region.[41,42]

Multiple pituitary-specific transcription factors are involved in the determination of pituitary cell lineages and cell-specific expression of anterior pituitary hormones.[30-34,37,38,43] To date, several homeodomain transcription factors have been shown to be involved in human anterior pituitary development and differentiation. Defects in each have now been associated with various combinations of pituitary hormone deficiencies (see Figure 10-12 and Table 10-2). Because additional gene defects have been implicated in abnormal murine hypothalamo-pituitary development, it seems likely that the number of human genetic defects will expand.

In the human adult, the mean pituitary size is 13 by 9 by 6 mm.[44] Mean weight is 600 mg, with a range of 400 to 900 mg. Pituitary weight is slightly greater in women than in men and typically increases during puberty and pregnancy.[45] In the newborn, pituitary weight averages about 100 mg. Infrequently, the craniopharyngeal canal (marking the embryonic migration of Rathke's pouch) remains patent and may contain small nests of adenohypophyseal cells—giving rise to a pharyngeal hypophysis that may be capable of hormone synthesis.[46]

Normally, however, the pituitary resides in the sella turcica immediately above and partially surrounded by the sphenoid bone. The volume of the sella turcica provides a good measure of pituitary size, which may be reduced in the child with pituitary hypoplasia.[47] It is important, however, to recognize that considerable variation in pituitary size occurs normally. The pituitary is covered superiorly by the diaphragma sellae, and the optic chiasm is directly above the diaphragma. The anatomic proximity between the optic chiasm and the pituitary is important because hypoplasia of the optic chiasm may occur together with hypothalamic/pituitary dysfunction, as in the condition of septo-optic dysplasia and because pituitary tumors may in turn impact on the optic chiasm

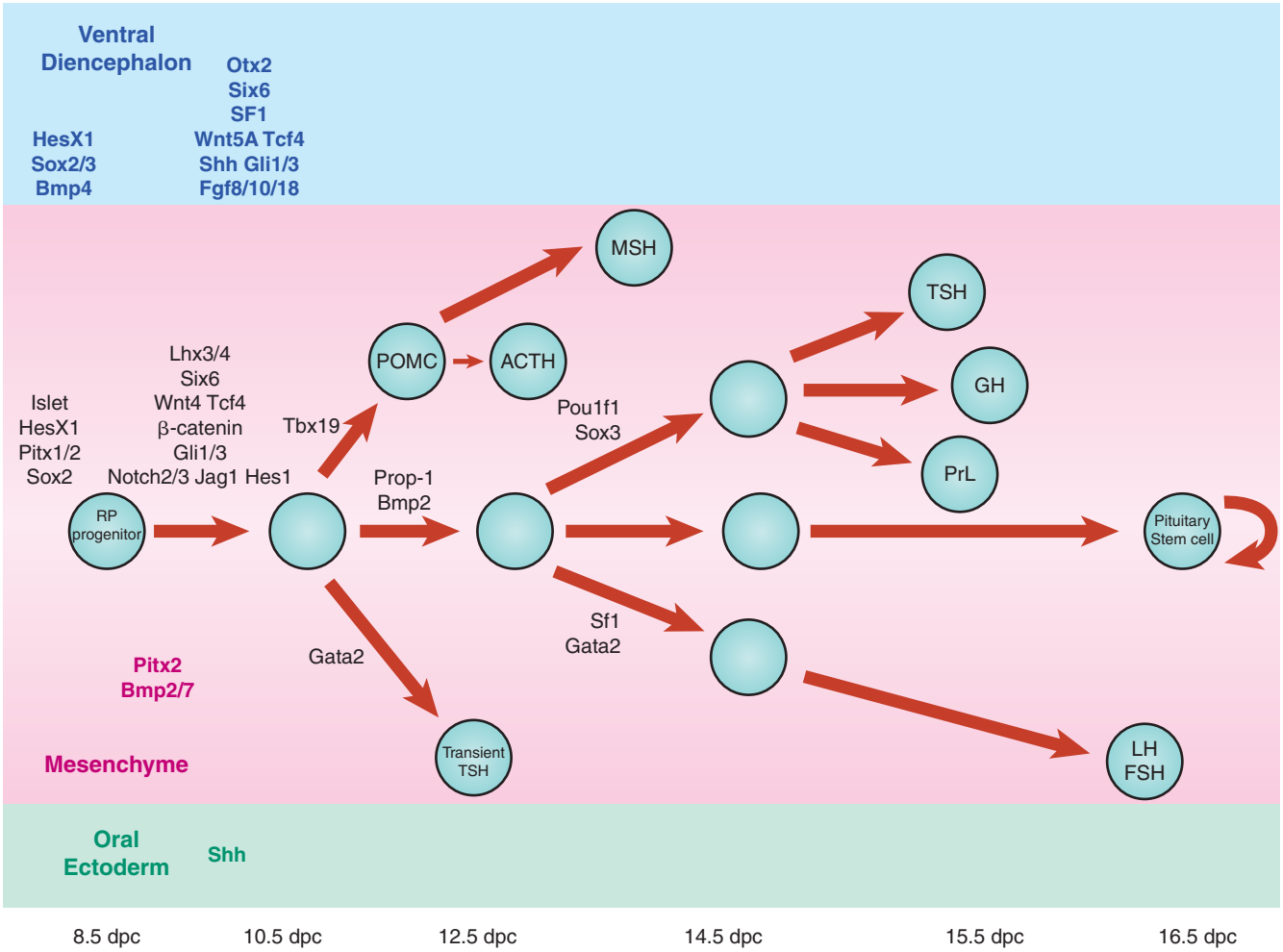


FIGURE 10-13 ■ Schematic representation of the developmental cascade of genes implicated in human pituitary development, with particular reference to pituitary cell differentiation. (From Kelberman, D., Rizzoti, K., Lovell-Badge, R., et al. (2009). Genetic regulation of pituitary gland development in human and mouse. *Endocr Rev, 30*, 790–829.) *This image can be viewed in full color online at ExpertConsult.*

TABLE 10-2 Homeodomain Transcription Factors Involved in Human Pituitary Development and Differentiation[875]

Transcription Factor	Murine Homologue
HESX1 (homeobox gene expression in embryonic stem cells)	*Hesx*1/*Rpx* (Rathke's pouch, Homeobox)
PROP1 (prophet of Pit1)	*Prop*1 (Ames mouse)
POU1F1 (POU domain/Pit1)	*Pit*1/*Ghf*1 (Snell mouse, Jackson mouse)
RIEG (Rieger syndrome)	*Rieg*/*Pitx*2
LHX3 (LIM homeodomain protein)	Lhx3

leading to visual impairment.[48] The patient with congenital blindness or nystagmus should be initially evaluated and then subsequently monitored carefully for hypopituitarism as this can evolve. In addition, suprasellar growth of a pituitary tumor may initially manifest with visual complaints or evidence of progressive impairment of peripheral vision.

The existence of a portal circulatory system within the pituitary is critical to normal pituitary function[28,29] (Figure 10-14). The blood supply of the pituitary derives from the superior and inferior hypophyseal arteries, branches of the internal carotid. The anterior and posterior branches of the superior hypophyseal artery may terminate within the infundibulum and the proximal portion of the pituitary stalk. Hypothalamic peptides, produced in neurons that terminate in the infundibulum, enter the primary plexus of the hypophyseal portal circulation and are transported by means of the hypophyseal portal veins to the capillaries of the anterior pituitary. This portal system thus provides a means of communication between the neurons of the hypothalamus and the hormone-producing cells of the anterior pituitary. The blood supply of the neurohypophysis is separate, deriving from the inferior hypophyseal artery. Regulation of the posterior lobe of the pituitary does not involve the hypophyseal portal circulation but, rather, is mediated through direct neural connections.

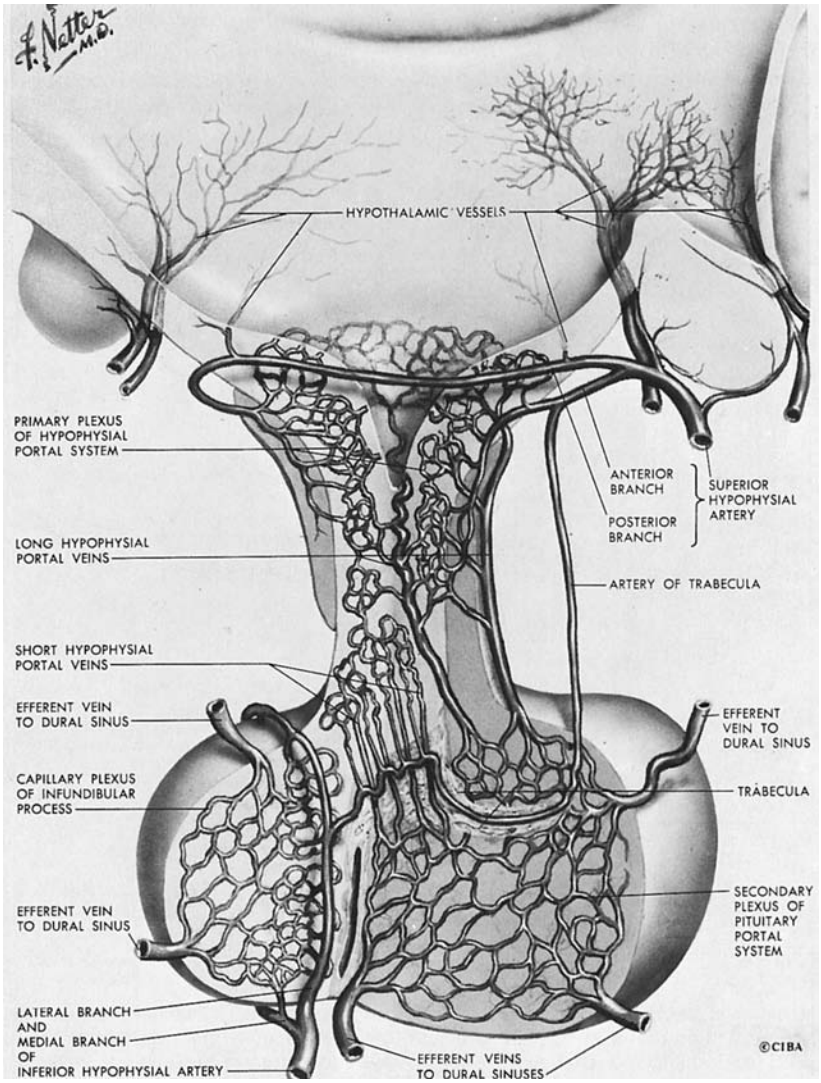


FIGURE 10-14 ■ Illustration of the main components of the hypothalamic-pituitary portal system. (NetterImages.com #4613, © Elsevier, Inc.) *See the accompanying website to view a color version of this illustration.*

The definitive Rathke's pouch comprises proliferative progenitors that will gradually relocate ventrally, away from the lumen as they differentiate. A proliferative zone containing progenitors is maintained in the embryo in a periluminal area and was found to persist in the adult. The exact nature of progenitor cells in the pituitary gland, however, remains unknown. Research has focused on the biology of these progenitor and putative stem cells within the pituitary gland.[49,50] Several reports have suggested that the pituitary stem cells take the form of a *SOX2*+ cell population, *GFRa2*+ cells, a side population of cells, *Nestin*+ cell population, or folliculostellate cells.[51] It has been hypothesized that there are two critical roles of stem cells: one is to establish the pituitary gland during development and the other is to maintain the mature pituitary gland in response to physiologic challenges and normal cell turnover. The hypothesis of two different populations of stem cells, one involved in embryogenesis and one involved in maintenance function after birth, remains highly controversial. Members of the Sox family of transcription factors are likely involved in the earliest steps of pituitary stem cell proliferation and the earliest transitions to differentiation. The transcription factor PROP1 and the NOTCH signaling pathway may then regulate the transition to differentiation. Identification of the stem cell niche is important for several reasons, and it has been proposed that the niche may be the marginal zone around the lumen of Rathke's pouch, between the anterior and intermediate lobes of mouse pituitary, because cells in this region are able to give birth to all five pituitary hormone cell lineages.

Stem cells have been shown to play a role in tumorigenesis in some tissues, and their role in pituitary hyperplasia, pituitary adenomas, and tumors is an important area for future investigation. The ability to cultivate and grow stem cells in a pituitary predifferentiation state might also be helpful for the long-term treatment of pituitary deficiencies. Indeed, a seminal study has resulted in the efficient self-formation of a three-dimensional anterior pituitary tissue in an aggregate culture of mouse embryonic stem (ES) cells.[52] ES cells were stimulated to

differentiate into non-neural head ectoderm and hypothalamic neuroectoderm in adjacent layers within the aggregate and treated with agonists of sonic hedgehog signaling. This resulted in increased expression of the Rathke's pouch marker Lhx3 followed by self-organization of Rathke's pouch–like three-dimensional structures at the interface of these two epithelia. The resulting 3D structures had a central cavity, and the resemblance to Rathke's pouch was striking, as was the topographic location between the neuroepithelium and the rostral headlike ectoderm. The juxtaposition of the two tissues, mimicking the spatial organization in embryonic development, was indeed critical as Rathke's pouch–like vesicles did not develop when neuroepithelium tissue was not present. Various endocrine cells were subsequently produced and these cells were able to respond to trophic hormones. For example, adrenocorticotropic hormone (ACTH)–expressing cells developed from the vesicle like structures, and activation of Wnt signaling led to expression of Pit1, growth hormone (GH), and prolactin. Luteinizing hormone (LH)/follicle-stimulating hormone (FSH)/thyroid-stimulating hormone (TSH) expression was also achieved, albeit after more intense manipulation of the culture conditions. When ES-derived cell aggregates were implanted under the kidney capsule in hypophysectomized mice, corticosterone was produced. These studies may therefore reflect the first step toward stem cell treatment.

Terminally differentiated secreting cells are not distributed randomly in a patchwork-like fashion throughout the pituitary gland. Instead, it is starting to emerge that these cells organize themselves in same-cell type networks. This was first shown by Bonnefont and colleagues when GH cells in murine pituitary slices were visualized using high-resolution imaging.[53] The connectivity between the cells of this network is important to deliver coordinated secretory pulses of hormones to their target tissues. Additionally, the two least abundant pituitary cell types, corticotropes and gonadotropes, are also organized in homotypic cell networks, as are pituitary lactotropes.[54,55] This distribution of cells into networks facilitates the coordinated physiologic response to stimuli.

Growth Hormone

Chemistry

Human GH is produced from somatotrope cells within the anterior pituitary as a single-chain nonglycosylated 191-amino-acid 22-kd protein (Figure 10-15)[56,57] that comprises a core of four helices in a parallel/antiparallel orientation with two disulfide bonds between cysteines 53 to 165 and 182 to 189.[58] The 217aa GH-precursor is transported in the lumen of the endoplasmic reticulum (ER) via a mechanism that involves the recognition of the signal peptide (first 26 aa). Following its cleavage, the mature protein is transported to the Golgi apparatus and secretory vesicles; the presence of zinc ions facilitates the formation of soluble GH dimer complexes within the secretory granules, as well as the storage and secretion of GH aggregates.[59]GH is homologous with several other

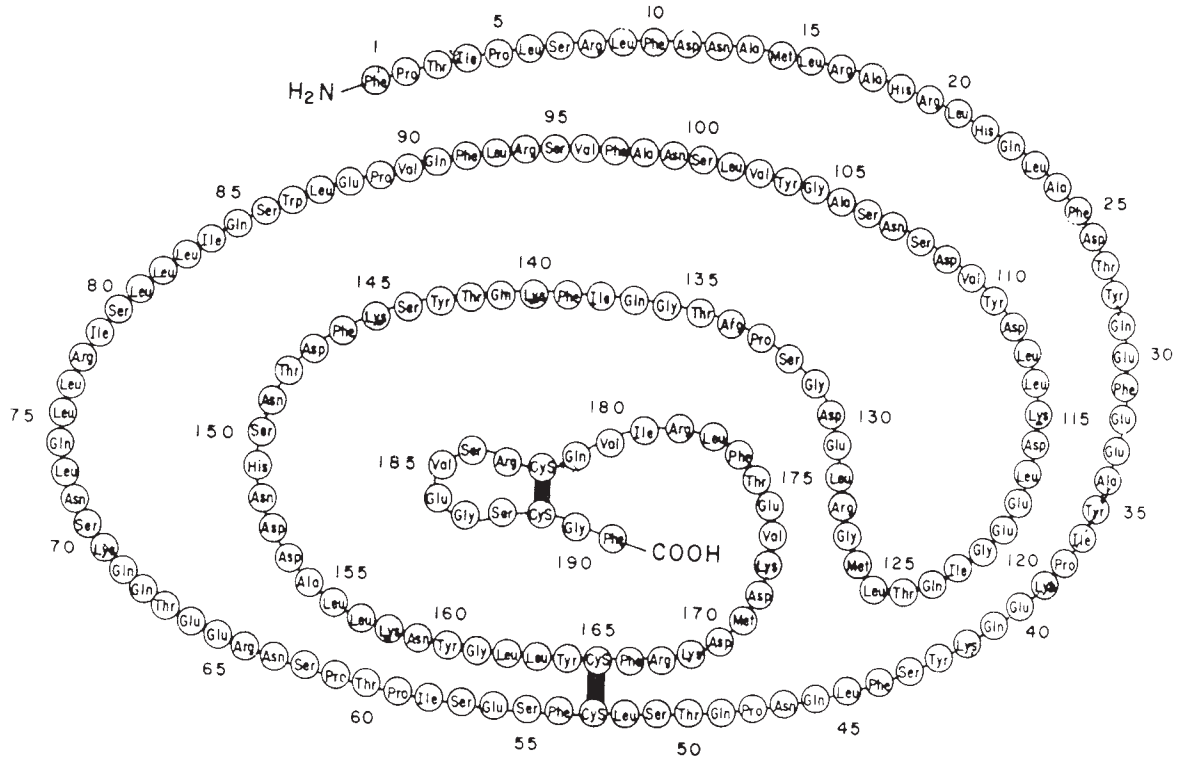


FIGURE 10-15 ■ Covalent structure of human growth hormone. (From Chawla, R. K., Parks, J. S., & Rudman, D. (1983). Structural variants of human growth hormone: biochemical, genetic and clinical aspects. *Annu Rev Med, 34*, 519.)

proteins produced by the pituitary or placenta, including prolactin, chorionic somatomammotropin (CS, placental lactogen), and a 22-kd GH variant (hGH-V) secreted only by the placenta.[60] The latter differs from pituitary GH by 13 amino acids. The genes for these proteins have probably descended from a common ancestral gene, even though the genes are now located on different chromosomes (chromosome 6 for prolactin and chromosome 17 for GH).[61]

The genes for GH, prolactin, and placental lactogen share a common structural organization—with four introns separating five exons. *GH1* is located on the long arm of chromosome 17 (17q22-24) within a cluster of five homologous genes encompassing a distance of about 65kb—*CSHP* (chorionic somatomammotropin pseudogene), *CSH-1* (chorionic somatomammotropin gene), *GH-2*, and *CSH-2*.[62] Expression of *GH1* is regulated by the highly polymorphic proximal promoter and a locus control region (LCR) 15-32kb upstream of the gene that confers the pituitary-specific and high-level expression of GH.[63] Normally, the majority of GH (75%) produced by the pituitary is of the mature 22-kd form. Alternative splicing of the second exon results in deletion of amino acids 32 through 46, yielding a 20-kd form that normally accounts for less than 10% of pituitary GH.[61,64,65] The remainder of pituitary GH includes desamidated and N-acetylated forms, as well as various GH oligomers. A 17.5-kDa variant that results from complete skipping of exon 3 and lacks amino acids 32-71 is much less abundant (1% to 5%).

Secretion

The pulsatile pattern characteristic of GH secretion largely reflects the interplay of multiple regulators, including two hypothalamic regulatory peptides: GH-releasing hormone (GHRH)[66,67] and somatostatin (somatotropin release–inhibiting factor [SRIF]).[68] The amino terminus of the 44-amino-acid protein GHRH is required for stimulation of GH secretion. GHRH activity is species specific, presumably reflecting the specificity of binding to a G protein–coupled receptor on the pituitary somatotropes.

Regulation of GH production by GHRH is largely transcriptionally mediated and is dependent on stimulation of adenylate cyclase and increases in intracellular cyclic adenosine monophosphate (AMP) concentrations. The GHRH receptor is a member of the G protein–coupled receptor family B (also called the secretin family) and has partial sequence identity with receptors for vasoactive intestinal polypeptide, secretin, calcitonin, and parathyroid hormone.[69] Solid tumors secreting GHRH are a rare cause of GH excess. GHRH has previously been approved in the United States for treatment of growth hormone deficiency but has been withdrawn from the market for therapeutic purposes. It can still be used as a diagnostic agent—if available, especially for the identification of adult growth hormone deficiency, and then it is frequently used in combination with arginine as part of a stimulation testing protocol.

The actions of the 14-amino-acid protein somatostatin appear to be related to the timing and amplitude of pulsatile GH secretion, rather than to GH synthesis. The binding of somatostatin to its specific receptor results in an inhibition of adenylate cyclase activity and a reduction in intracellular calcium concentrations.[68] Treatment of cultured somatotropic cells with GHRH and somatostatin has indicated a dominant effect of somatostatin, with a reduction of intracellular calcium concentrations and an inhibition of GH secretion. The pulsatile secretion of GH observed in vivo is believed to result from a simultaneous reduction in hypothalamic somatostatin release and increase in GHRH activity.[70] Conversely, a trough of GH secretion occurs when somatostatin release is increased in the presence of diminished GHRH activity. Somatostatin analogs are used therapeutically for the treatment of acromegaly, underscoring its role as a GH secretion inhibitor.

The regulation, on a neuronal basis, of this reciprocal secretion of GHRH and somatostatin is imperfectly understood. Multiple neurotransmitters and neuropeptides are involved in regulation of release of these hypothalamic factors, including serotonin, histamine, norepinephrine, dopamine, acetylcholine, gamma-aminobutyric acid (GABA), thyroid-releasing hormone, vasoactive intestinal peptide, gastrin, neurotensin, substance P, calcitonin, neuropeptide Y, vasopressin, corticotrophin releasing hormone,[71] and galanin.[72] These factors are clearly implicated in the alterations of GH secretion observed in a wide variety of physiologic states (such as stress, sleep, hemorrhage, fasting, hypoglycemia, and exercise) and form the basis for a number of GH-stimulatory tests employed in the evaluation of GH secretory capacity/reserve.

GH secretion is also impacted by a variety of nonpeptide hormones, including androgens,[73,74] estrogens,[75] thyroxine,[76] and glucocorticoids.[77,78] The precise mechanisms by which these hormones regulate GH secretion are complex, potentially involving actions at the hypothalamic and pituitary levels. Practically speaking, hypothyroidism and glucocorticoid excess may each blunt spontaneous and provocative GH secretion (and therefore should be corrected prior to GH testing). Sex steroids, at the onset of puberty or administered pharmacologically, appear to be responsible for the rise in GH secretion characteristic of puberty.[79]

Synthetic hexapeptides capable of stimulating GH secretion have been developed[80] and termed *GH-releasing peptides* (GHRPs). These peptides, later recognized as analogs of the gastric hormone ghrelin, are capable of directly stimulating GH release and enhancing the GH response to GHRH.[81] These agents have the potential advantage of oral administration, and in the patient with an intact pituitary they may be capable of greatly enhancing GH secretion. When these agents were administered chronically to elderly patients and to some GH-deficient children, the amplitudes of GH pulses were significantly increased. Ghrelin-mimetic ligands were used to characterize a common receptor termed the *GH secretagogue receptor* (GHS-R) for the GH-releasing substances. The GHS-R is distinct from the GHRH receptor.[82]

Subsequently, the GHS-R gene was cloned and shown to encode a unique G protein–coupled receptor with a deduced protein sequence that was 96% identical

in human and rat. Three receptor isotypes were isolated from human genomic libraries. The receptor is strongly expressed in the hypothalamus. Specific binding sites for GH releasing peptides have also been identified in other regions of the central nervous system (CNS) and peripheral endocrine and nonendocrine tissues in both humans and other organisms. Ghrelin is a 28-amino-acid peptide that has been identified as the endogenous ligand for the growth hormone secretagogue receptor (GHS-R).[83] It is expressed predominantly in the stomach, but smaller amounts are also produced within the bowel, pancreas, kidney, the immune system, placenta, pituitary, testis, ovary, and hypothalamus. Ghrelin is a unique gene product that requires octanoylation for normal function. Intravenous, intracerebroventricular, and intraperitoneal administration of ghrelin in animal models stimulates food intake and obesity[84] and raises plasma GH concentrations[85]—and to a lesser extent prolactin and adrenocorticotropic hormone (ACTH) concentrations. Additionally, it influences endocrine pancreatic function and glucose metabolism, gonadal function, and behavior. It also controls gastric motility and acid secretion and has cardiovascular and antiproliferative effects. Both ghrelin and GH-releasing peptides release GH synergistically with GHRH, but the efficacy of these compounds as growth-promoting agents is poor. Mutations in the ghrelin receptor have been identified as a possible cause of idiopathic short stature (ISS) and GH deficiency.[86] However, it is important to note that mouse models with targeted deletion of the receptor (*ghsr* $^{-/-}$) have a near normal phenotype.[87]

This suggests that ghrelin is an important stimulus for nutrient allocation for growth and metabolism and that it may represent a key component of the GH regulatory system. A second peptide encoded by the same gene as ghrelin has been identified and termed *obestatin*. This appears to regulate weight but not GH secretion.[88,89]

In addition to the complex regulatory processes described previously, the synthesis and secretion of GH are also regulated by feedback by the insulin-like growth factor (IGF) peptides.[90-95] IGF receptors have been identified in the pituitary.[96-98] Inhibition by IGF-1 of GH secretion has been demonstrated in multiple systems.[99] In addition, inhibition of spontaneous GH secretion has been demonstrated in humans treated with subcutaneous injections of recombinant IGF-1.[100,101] GH can be identified in fetal serum by the end of the first trimester. Serum concentrations are lower in term infants than in premature infants, perhaps reflecting feedback by the higher serum levels of IGF peptides characteristic of the later stages of gestation.[102] However, at birth, GH concentration in normal newborns is of the order of 40 ng/mL, declining gradually in the initial weeks of life to levels less than 10 ng/mL.

Twenty-four hour GH secretion peaks during adolescence,[79] undoubtedly contributing to the very high serum concentrations of IGF-1 characteristic of puberty. GH secretion begins to decline by late adolescence and continues to fall throughout adult life. Indeed, puberty may be considered with some justification a period of "acromegaly," whereas aging (with its characteristic decrease of GH secretion) has been termed the *somatopause*.[75,103,104]

Twenty-four-hour GH production rates for normal men range from 0.25 to 0.52 mg/m^2.[78,105] However, a wide variety of physiologic conditions (in addition to aging) affect GH secretion. These include stage of sleep,[106,107] nutritional status,[108] acute fasting, exercise,[109] stress,[109] and sex steroids.[73,74] Ho and associates[75] have reported that serum estradiol concentrations are the dominant factor affecting GH secretion. Neither age nor sex influenced the integrated serum concentrations of GH when the effects of estradiol were removed from analysis. The effects of testosterone on serum IGF-1 concentrations may be at least in part independent of GH because individuals with mutations of the GH receptor (GHR) still experience a rise in serum IGF-1 during puberty.[110]

The pulsatile nature of GH secretion is readily demonstrable by frequent serum sampling, especially when coupled with sensitive assays for GH.[108] Such assays demonstrate that under normal conditions serum GH concentrations are less than 0.2 ng/mL between bursts of GH secretion. It is consequently impractical to assess GH secretion by random serum sampling. Maximal GH secretion occurs during the night, especially at the onset of the first slow-wave sleep (stages III and IV). Rapid-eye-movement (REM) sleep is, on the other hand, associated with low GH secretion.

Normal young men generally experience 12 GH secretory bursts per 24 hours. Obesity is characterized by decreased GH secretion, reflected by a decreased number of GH secretory bursts.[111] Fasting increases the number and amplitude of GH secretory bursts, presumably reflecting decreased somatostatin secretion and possibly increased ghrelin secretion. The impact of the pulsatile secretory nature of GH secretion on its biologic actions remains uncertain.

GH Receptor/GH-Binding Protein

The GH receptor is synthesized as a 638-amino-acid peptide, which is later processed into a mature receptor of 620 amino acids with a predicted molecular weight of 70 kDa before glycosylation. The extracellular hormone-binding domain contains 246 amino acids, followed by a single membrane-spanning domain, and a cytoplasmic domain of 350 amino acids. In humans, the circulating GH-binding protein (GHBP) appears to derive from proteolytic cleavage of the extracellular domain of the receptor.

The gene for the human GHR has been localized to chromosome 5p13.1-p12, where it spans more than 87 kb.[112,113] In the mouse[114,115] and rat,[116] on the other hand, multiple transcripts for the GHR have been identified. The larger (3.4 to 4.8 kb) transcript codes for the intact receptor, whereas the 1.2- to 1.9-kb transcript codes for the soluble GHBP. The coding and 3′ untranslated regions of the human GHR are encoded by the nine exons, numbered 2 through 10.[117] Exon 2 corresponds to the secretion signal peptide, whereas exons 3 through 7 encode the extracellular domain. Exon 8 encodes the transmembrane domain. Exons 9 and 10 encode, respectively, the intracellular domain and the 3′ untranslated region. Two genomic *GHR* isoforms that exist

only in humans have arisen from ancestral homologous recombination. They differ in the retention or deletion of exon 3. Exon 3 of the GHR has been shown to be deleted in a substantial number of normal individuals. This delta-3 GHR polymorphism has been shown by some but not all investigators to determine responsiveness to GH and to be associated with birth size and postnatal growth.[118-120]

The GHR has been found to be highly homologous with the prolactin receptor and to share sequence homology with many of the receptors for interleukins, as well as receptors for erythropoietin, leptin, granulocyte-macrophage colony-stimulating factor, and interferon.[117] The GHR is a member of the class 1 hematopoietic cytokine family. A complex of the GH and the GHBP molecule has been shown to be more effective as an agonist of GH action than GH alone—indicating a physiologic and possible therapeutic role for the GHBP.[121] Examination of the crystal structure of the GH-GHR complex revealed that the complex consisted of one molecule of GH bound to two GHR molecules, indicating a GH-induced receptor dimerization—which is necessary in GH action.[58] Interestingly, as noted previously, a genetically engineered fusion complex of GH and the GHR has been shown to have a significantly improved efficacy and a dramatically longer half-life compared to growth hormone alone when tested in rodent models.[121]

The GHR, like its family group member EPO-R, is preformed as a dimer and is transported in a nonligand bound state to the cell surface.[122,123] GH then binds in a sequential manner to the GHR dimer where the first GHR binds to the stronger site 1 of the GH molecule followed by the second GHR binding to the weaker site 2. Binding of GH results in a conformational change whereby rotation of the GHRs results in repositioning of the intracellular domains and of Box1-associated Janus Kinase 2 (JAK2), a major GHR-associated tyrosine kinase. As a result, JAK2 is auto-phosphorylated and activated, leading in turn to cross phosphorylation of distal tyrosine residues of GHR that enables SH2 (*Src* homology 2) domain molecules to dock to these sites.[124,125] The GHR itself appears to have no intrinsic kinase activity. It is likely that co-localization of two JAK2 molecules by the dimerized GHR results in transphosphorylation of one JAK2 by the other, leading to JAK2 activation. Stat5a and Stat5b molecules contain SH2 domains and bind to these phosphorylated tyrosine sites. In turn they then become phosphorylated. Phosphorylated Stat5 molecules (homo- and hetero-) dimerize and translocate to the nucleus where they bind DNA, as dimers or as tetramers, and activate target genes.[126,127]

GH can activate both Stat5a and Stat5b, and they have both overlapping and distinct functions.[128-135] Gene inactivation mouse models have shown that deletion of *Stat5b*, but not of other Stat genes, even though GH also activates Stat1 and Stat3, affects growth, and that Stat5b is of greater importance for stimulation of growth than Stat5a. *Stat5b* null mice have severe postnatal growth retardation especially in males, although this is not as severe as in *Ghr* null mice. They have increased GH secretion; reduced hepatic IGF1-, IGF-binding protein (IGFBP)3-, and acid labile subunit (ALS)-expression; and increased obesity.[135] *Stat5a* null mice have normal growth but impaired mammary gland formation and lactogenesis, reflecting impaired signaling of prolactin.[130] *Stat5a/b* double null mice are more severely affected than the single null mice and display more severe growth retardation, although *Ghr* null mice are still more severely affected.[136,137] *Stat5a/b* double null mice also have a severe combined immunodeficiency, with reduced CD8 T cell number and a failure of hematopoietic stem cells to develop lymphoid lineages.[138] Transgenic Stat5 expression results in expansion of CD8 cells and lymphomagenesis[139] and also in increased proliferation and differentiation in mammary cells,[140] suggesting a critical role for Stat5 in cell proliferation, particularly in immune cells. Stat5b is phosphorylated upon stimulation by a pulse of GH, and after this rapid activation it becomes temporarily refractive to further or continuous stimulation.[141,142] GH secretion is more continuous in females and, indeed, in female rodents, Stat5b is phosphorylated to a lesser extent although phosphorylation still occurs. This gender-specific signaling plays an important role in the regulation of gender-specific proteins, especially CYP450 enzymes,[143,144] which play a role in hepatic metabolism of steroids and foreign compounds. The *Stat5b* null male mice are resistant to GH pulses,[145]and their hepatic male-specific genes are decreased to female levels, whereas female predominant genes are expressed at higher levels than in WT males. Hepatic nucleic factors (HNFs), especially HNF3, 4α, and 6, interact with Stat5b to induce these Stat5b-dependent gender-specific gene expression patterns.[134,144,146]

Phosphorylated Stat5a and Stat5b bind Stat5 response elements (Stat5 RE) as dimers or tetramers, but their binding is enhanced by the interaction of coactivators binding to adjacent DNA binding sites. Stat5 RE are located in the second and third intron of the human *IGF1* gene and 73 kb upstream of the initiation site, although the effectiveness of the distant site is much less.[147,148] Activation of JAK-STAT signaling occurs rapidly, within minutes after GH stimulation, but is transient due to the tight control of the termination of signaling. This negative regulation of signaling occurs at several levels: GHR internalization, suppressors of cytokine signaling (SOCS), protein tyrosine phosphatases (PTPs), and protein inhibitors of activated stats (PIAS). The inhibition of GH signaling by several members of the GH-inducible suppressors of the cytokine signaling (SOCS) family has been demonstrated. The SOCS family of proteins comprises cytokine inducible-Src homology 2 protein (CIS) and SOCS 1-7. GH, PRL, and many other cytokines can induce CIS, SOCS1, -2, and -3. SOCS family proteins inhibit JAK-STAT signaling by inhibiting JAK proteins, binding positive regulators of signaling or docking sites, and promoting GHR ubiquitination. The role of the several family members has become clearer from phenotypes associated with their overexpression. Mice overexpressing CIS have mild growth retardation and have also altered T-cell function and impaired mammary gland development, whereas *Socs2* null mice show gigantism similar to bovine GH overexpessing mice (30% to 40% overgrowth), are hyperresponsive to GH, and have increased extrahepatic IGF-1 production.[149]

By contrast, complete inhibition of GH activation of the signal transducer STAT5 and STAT5-dependent transcriptional activity is seen when cellular overexpression of SOCS family members is induced. SOCS also inhibit the GHR-dependent tyrosine phosphorylation of JAK2. Endotoxin and proinflammatory cytokines such as interleukin-1b (IL-1b) and tumor necrosis factor-α (TNFα) induce a state of GH resistance.

All of these agents can also induce SOCS proteins.[150] SOCS-3 induced by IL-1b and TNFα or by endotoxin in vivo may play a role in the GH resistance induced by sepsis.[151] Critically ill patients with septic shock who had been treated with GH were shown to have increased mortality, possibly related to the induction of GH resistance in specific tissues as a consequence of sepsis.[146] Thus, the role of SOCS proteins as intracellular GH signaling antagonists appears to be important in a variety of pathophysiologic states.

PTPs dephosphorylate activated phosphorylated proteins in cytokine signaling pathways but also insulin signaling pathways. Several PTPs—such as PTP-1, PTP-H1 and PTP-B1, and TC-PTP—attenuate GH signaling, and PTP-1B is involved in fasting-induced GH insensitivity.[153,154] PTPN11 mainly functions in RAS-MAPK signaling, and abnormalities result not only in Noonan syndrome but also in associated mild GH resistance.[155]

Other GH-activated pathways include mitogen-activated protein kinases (MAPKs), extracellular signal-regulated kinase (ERK)-1 and ERK2, the insulin-signaling pathway (by means of insulin receptor substrate [IRS]-1 and IRS-2), and protein kinase C (PKC).[156,157] How all of these pathways interact to mediate the various anabolic and metabolic actions of GH remains to be elucidated (Figure 10-16).

The main GH binding protein (GHBP) is the soluble extracellular domain of the GHR with an apparent molecular weight of approximately 55 kDa and has an identical affinity for GH as GHR.[112] It binds to GH with high specificity and affinity, but with relatively low capacity.[158-160] It increases its half-life in the circulation and may serve a function in the transportation of GH to target tissues and subsequent binding to the receptor. Although in rodents GHBP arises through alternative splicing, human GHBP arises from proteolytic cleavage of the cell membrane anchored GHR, for example, by TNF α converting enzyme (TACE).[161] GHBP is, like GHR, present in many tissues, but GHBP in the circulation is mostly derived from the liver. Although GHR and GHBP are regulated by and are very sensitive to GH, and GHR and GHBP often change in parallel,[162-164] measurement of plasma GHBP has not been shown to reflect GHR and GH responsiveness.[165] Initial assays for GHBP involved incubation of serum with 125-I-GH and separation of bound from free radioligand by gel filtration, high-pressure liquid chromatography, or dextran-coated charcoal. Carlsson and colleagues[166] developed a ligand-mediated immunofunctional assay (LIFA) that measures GHBP capable of binding GH. Assays of serum concentrations of GHBP have been instrumental in identifying patients with GH insensitivity (GHI) caused by genetic abnormalities of the GHR.[167,168] Patients with

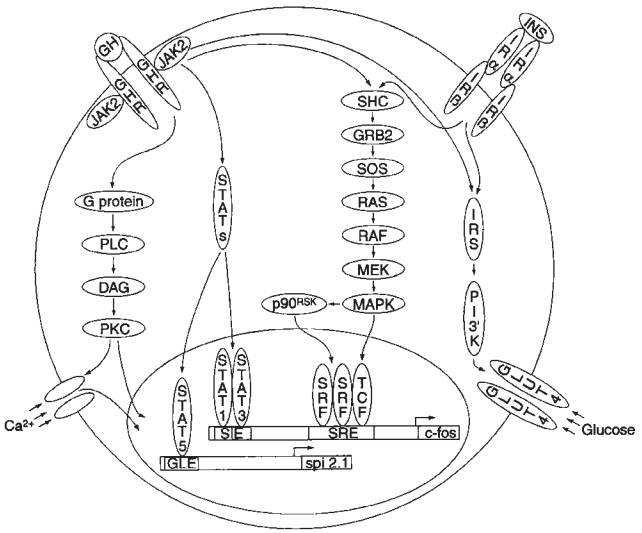


FIGURE 10-16 ■ A model depicting intracellular signaling intermediates induced by binding of growth hormone (GH) with the GH receptor (GHR). DAG, diacylglycerol; ERK, extracellular signal-regulated kinase; GLE, interferon-γ–activated sequence (GAS)–like response element; GLUT, glucose transporter; GRB, growth factor receptor–binding protein; JAK, janus kinase; INS, insulin; Irα, insulin receptor α subunit; MAPK, mitogen-activated protein kinase; MEK, MAPK-ERK kinase; PKC, protein kinase C; PLC, phospholipase C; SHC, SrC homology complex; SIE, sis-inducible element; SOS, son of sevenless; SRE, serum response element; SRF, serum response factor; STAT, signal transducer and activator of transcription; TCF, ternary complex factor. (From Kopchick, J. J., Bellush, L. L., & Coschigano, K. T. (1999). Transgenic models of growth hormone action. *Annu Rev Nutr, 19*, 437. With permission, from the Annual Review of Nutrition, Volume 19 © 1999 by Annual Reviews. www.AnnualReviews.org.)

GHI from nonreceptor abnormalities, abnormalities of the intracellular portion of the GHR, or inability of the receptor to dimerize may, however, have normal serum concentrations of GHBP.[169]

GH Actions

According to the somatomedin hypothesis, the anabolic actions of GH are mediated through the IGF peptides.[170-175] Although this hypothesis is at least in part true, it appears that GH is capable of stimulating a variety of effects that are independent of IGF activity. Indeed, the effects of GH and IGF are on occasion contradictory—as evident in the "diabetogenic" actions of GH[175,176] and the glucose-lowering activity of IGFs. Green and colleagues[177] attempted to resolve some of these differences in a "dual-effector" model in which GH stimulates precursor cells, such as prechondrocytes, to differentiate.

When differentiated cells or neighboring cells then secrete IGFs, these peptides act as mitogens and stimulate clonal expansion. This hypothesis is based on the ability of IGF peptides to work not only as classic endocrine factors that are transported through the blood but as paracrine or autocrine growth factors. GH also stimulates a variety of metabolic effects, some of which appear to occur independently of IGF production—such as lipolysis,[178] amino acid transport in diaphragm[179] and heart,[180] and production of

specific hepatic proteins. Thus, there are multiple sites of GH action—and frequently it is not entirely clear which of these actions are mediated through the IGF system and which might represent IGF-independent effects of GH.[181] The sites of action of GH include the following:

- *Epiphysis:* Stimulation of epiphyseal growth.
- *Bone:* Stimulation of osteoclast differentiation and activity, stimulation of osteoblast activity, and increase of bone mass by endochondral bone formation. Lupu and coworkers[182] have shown that mice with a complete knockout of IGF-I (making them substantially smaller than wild-type mice) become even smaller when mated into a GH receptor knockout strain, indicating a direct IGF-independent effect of GH on growth.
- *Adipose tissue:* Acute insulin-like effects, followed by increased lipolysis, inhibition of lipoprotein lipase, stimulation of hormone sensitive lipase, decreased glucose transport, and decreased lipogenesis.[183]
- *Muscle:* Increased amino acid transport, increased nitrogen retention, increased lean tissue, and increased energy expenditure.[183]

The concept of IGF-independent actions of GH is supported by in vivo studies, in which IGF-1 cannot duplicate all of the effects of GH (such as nitrogen retention and insulin resistance). The effects of GH in normal human aging[184] and in catabolic conditions[185] are subjects of active investigation.

Insulin-Like Growth Factors

Historical Background

The insulin-like growth factors (or somatomedins) constitute a family of peptides that are at least in part GH dependent and that are believed to mediate many of the anabolic and mitogenic actions of GH. Although they were originally identified in 1957 by their ability to stimulate [35S] sulfate incorporation into rat cartilage,[173] it has been established over the ensuing 45 years that they are involved in diverse metabolic activities (Figure 10-17).

In 1957, Salmon and Daughaday[173] first demonstrated that the inability of serum from hypophysectomized rats to stimulate [35S] sulfate incorporation into rat chondrocyte proteoglycans could not be restored by in vitro addition of GH. However, [35S] sulfate incorporation could be restored by the addition of serum from hypophysectomized rats that had been treated with GH, thereby demonstrating the existence of a GH-dependent "sulfation factor."

Concurrent investigations on insulin activity in rat muscle and adipose tissue indicated that only a small component of the insulin-like activity of normal serum could be blocked by the addition of anti-insulin antibodies. The remaining activity was termed *nonsuppressible insulin-like activity* (NSILA) and was subsequently demonstrated to contain two soluble 7-kd forms named *NSILA-I* and *NSILA-II*.[186,187]

A third converging line of investigation arose from studies by Dulak and Temin[188] of the mitogenic nature of bovine serum. Serum-free conditioned media from fetal Buffalo rat liver cells (BRL-3A) were found to support the growth of cultured cells. The mitogenic factor in the medium was termed *multiplication-stimulating activity* (MSA) and was found to share metabolic and mitogenic activities with sulfation factor and NSILA.

In 1972, the restrictive labels of sulfation factor and NSILA were replaced by the term *somatomedin* (SM).[189] In recognition of the broad metabolic and mitogenic actions of these factors, the following criteria were established: concentration in serum must be GH dependent, must possess insulin-like activity in extraskeletal tissues, must promote the incorporation of sulfate into cartilage, and must stimulate DNA synthesis and cell multiplication.

Purification efforts yielded two legitimate somatomedin peptides: a basic peptide (SM-C) and a neutral peptide (SM-A).[190,191] In 1978, Rinderknecht and Humbel[192,193]

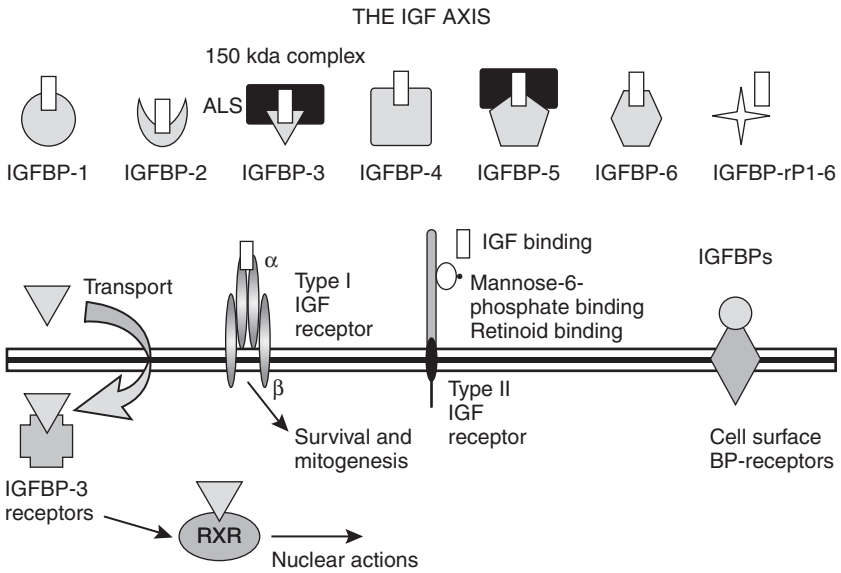


FIGURE 10-17 ■ The insulin-like growth factor (IGF) axis. BP, binding protein; RXR, retinoid X receptor.

isolated two active somatomedins from human plasma that demonstrated a striking structural resemblance to proinsulin. Accordingly, these two peptides were renamed *insulin-like growth factors* (IGFs).

IGF Structure and Molecular Biology

IGF-1, a basic peptide of 70 amino acids, correlates with SM-C—whereas IGF-2 is a slightly acidic peptide of 67 amino acids (Figure 10-18). The two peptides are structurally related, sharing 45 of 73 possible amino acid positions. They have approximately 50% amino acid homology to insulin.[174,192,193] Like insulin, both IGFs have A and B chains connected by disulfide bonds. The connecting (C peptide) region is 12 amino acids long for IGF-1 and 8 amino acids long for IGF-2 and bears no homology with the C-peptide region of proinsulin. IGF-1 and IGF-2 also differ from proinsulin in possessing carboxyl-terminal extensions (D peptides) of 8 and 6 amino acids, respectively. It is clear that this structural homology explains the ability of both IGFs to bind to the insulin receptor and for insulin to bind to the type 1 IGF receptor. On the other hand, structural differences explain the failure of insulin to bind to the IGF-binding proteins.

Two different forms of IGF-1 precursor molecules have been identified.[174] The first 134 amino acids of each are identical, comprising the signal peptide (48 amino acids), the mature IGF-1 molecule (70 amino acids), and the first 16 amino acids of the E domain of the precursor. IGF-1A has an additional 19 amino acids (total 153 residues), whereas IGF-1B has an additional 61 amino acids (total 195 residues).

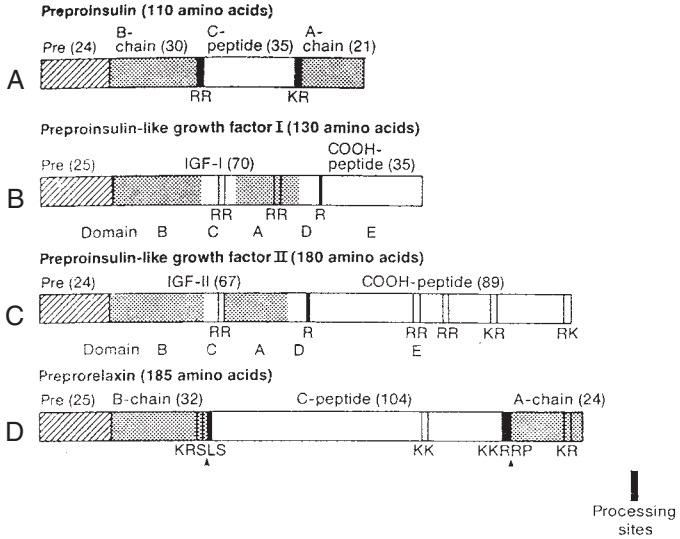


FIGURE 10-18 ■ Schematic representations of the structures of precursors to human insulin, insulin-like growth factor I (IGF-I), IGF-2, and relaxin. *Stippled segments* indicate the homologous B- and A-chain regions. Proteolytic processing sites are indicated by the following amino acid code: K, lysine; L, leucine; P, proline; R, arginines; S, serine. (From Bell, G. I., Merryweather, J. P., Sanchez-Pescado, R., et al (1984). Sequence of cDNA clone encoding human preproinsulin-like growth factor II. *Nature, 310,* 775, MacMillan Magazines Limited.)

Alternative splicing of the IGF-1 gene presumably generates the two alternative messenger RNAs (mRNAs). The primary IGF-2 translation product in human, rat, and mouse contains 180 amino acids—including a 24-residue signal peptide, the 67-amino-acid mature IGF-2 sequence, and a carboxyl-terminal E-peptide of 89 amino acids.

Control of IGF gene expression appears to be complex, perhaps explaining variability in tissue expression as well as differential expression in the embryo, fetus, child, and adult.[174,194-196] IGF-1 and IGF-2 are encoded by single large genes, spanning approximately 95 and 35 kb of genomic DNA, respectively. The human IGF-1 gene contains at least six exons. Exons 1 and 2 encode alternative signal peptides, probably each containing several transcription start sites. Exons 3 and 4 encode the remaining signal peptide, the remainder of the mature IGF-1 molecule, and part of the trailer peptide. Exons 5 and 6 encode alternatively used segments of the trailer peptide (resulting in the IGF-1A and IGF-1B forms), as well as 39 untranslated sequences, with multiple different polyadenylation sites. The human IGF-1 gene is located on the long arm of chromosome 12.[197,198]

The human IGF-2 gene is located on the short arm of chromosome 11,[197-199] adjacent to the insulin gene, and spans 35 kb of genomic DNA—containing nine exons. Exons 1 through 6 encode 59 untranslated RNA, including multiple promoter sites. Exon 7 encodes the signal peptide and most of the mature protein, whereas exon 8 encodes the carboxyl-terminal portion of the protein plus the trailer peptide (whose coding is completed in exon 9). The result is that multiple mRNA species exist for both IGF-1 and IGF-2. This permits remarkable complexity in the regulation of gene expression, allowing for tissue-specific expression of specific transcripts as well as ontogenic and hormonal regulation.

Assay Methodologies for the IGF Peptides

Since their first identification in 1957, the IGF peptides have proven to be remarkably difficult to permit accurate measurement. Bioassay methods were often influenced by a variety of other serum factors capable of mimicking or inhibiting IGF action. More important, virtually all assays were influenced by the presence of IGF-binding proteins (IGFBPs)—which have been found in all biologic fluids tested to date.

Bioassay methods included stimulation of [35S] sulfate incorporation, using various modifications of the original method described by Salmon and Daughaday.[173,200,201] A wide variety of several bioassays included stimulation of DNA synthesis,[202] RNA synthesis, protein synthesis,[203] glucose uptake,[204] and others. In general, however, such assays were cumbersome, subject to interference by IGFBPs, and unable to distinguish between IGF-1 and IGF-2. When SM-C (and later, IGF-1 and IGF-2) were purified, it became possible to radiolabel the pure proteins and employ them in a variety of radioreceptor assays[205,206] and competitive protein-binding assays.[207,208] It was not until the development of specific antibodies that it became possible to accurately distinguish between IGF-1 and IGF-2 and measure each peptide

accurately.[209-212] Current frequently used assays for IGF-I are primarily "double antibody sandwich assays" such as ELISA and have a reasonable accuracy and reproducibility.[213]

Nevertheless, the issue of IGFBPs must be dealt with in any IGF assay.[214] Powell and coworkers,[215] for example, have demonstrated that the discrepant results found in uremic sera assayed for IGF by bioassay, radioreceptor assay, and radioimmunoassay can be entirely attributed to the interference of IGFBPs. Even antibodies with high affinity and specificity will still exhibit interference by IGFBPs. This is particularly true in conditions in which there is a relatively high IGFBP/IGF peptide ratio, or at the clinical extremes of the assay—that is, GH deficiency (GHD) or acromegaly.

In general, the most effective and reliable way to deal with IGFBPs is their separation from IGF peptides by sizing chromatography under acidic conditions.[216] IGF-1 and IGF-2, each with a molecular weight of approximately 7 kd, can be readily separated from the IGFBPs—whose molecular weights range from 25 to 45 kd (glycosylated form of IGFBP-3). This is, however, a labor-intensive procedure and has been occasionally replaced by the more rapid acid ethanol extraction.[217] Although this method may be reasonably effective for most serum samples, it is problematic in conditions of high IGFBP/IGF peptide ratios—such as conditioned media from cell lines and sera from newborns, GHD, and uremia.

Alternative methodologies include the use of antibodies generated against synthetic peptides, such as the C-peptide region of IGF-1 or IGF-2. In general, such antibodies have high specificity but relatively low affinity. Nevertheless, the radiolabeled peptide does not bind to endogenous IGFBPs—offering an important advantage. An alternative approach, developed by Blum and colleagues,[218] has been the use of an antibody with high specificity for IGF-2—which permits the addition of excess unlabeled IGF-1 to saturate endogenous IGFBPs. Bang and coworkers[219] have bypassed the interference of IGFBPs by employing truncated IGF-1, which has decreased the affinity for IGFBPs, as a radioligand. Another way to perform more accurate IGF assays with minimal interference by IGFBPs is through the use of the so-called sandwich assay.[209] Such an assay (either an enzyme-linked immunosorbent assay or an immunoradiometric assay) does not employ a radiolabeled IGF molecule, as compared to the conventional radioimmunoassay.[210] However, at present, the state-of-the-art method for measuring IGFs is liquid chromatography followed by tandem mass spectrometry (LC/MS-MS).[220-222]

Serum Levels of IGF Peptides

In human fetal serum, IGF-1 levels are relatively low and are positively correlated with gestational age (Figure 10-19).[223,224] A correlation between fetal cord serum IGF-1 levels with birth weight has been reported by some groups,[223-225] although others have reported no correlation.[226] IGF-1 levels in human newborn serum are generally 30% to 50% of adult levels. There is a slow, gradual rise in serum concentrations during childhood, with attainment of adult levels at the onset of sexual maturation.[227]

During the process of puberty, IGF-1 concentrations rise to be two to three times the concentrations seen in adults.[228] Thus, concentrations during adolescence correlate better with Tanner stage (bone age) than with chronologic age. Girls with gonadal dysgenesis show no adolescent increase in serum IGF-1, clearly establishing the association of the pubertal rise in IGF-1 with the production of sex steroids.[229-231] It has been suggested that the pubertal rise in sex steroids stimulates IGF-1 production indirectly by first leading to a rise in GH secretion. It is of note, however, that patients with GH insensitivity (GHI) resulting from GHR mutations show a pubertal rise in serum IGF-1, which could not have been produced by GH action inducing IGF-1, thereby implicating a direct effect of sex steroids on IGF-1.[232] After adolescence, or at least after 20 to 30 years of age, serum IGF-1 concentrations demonstrate a gradual and

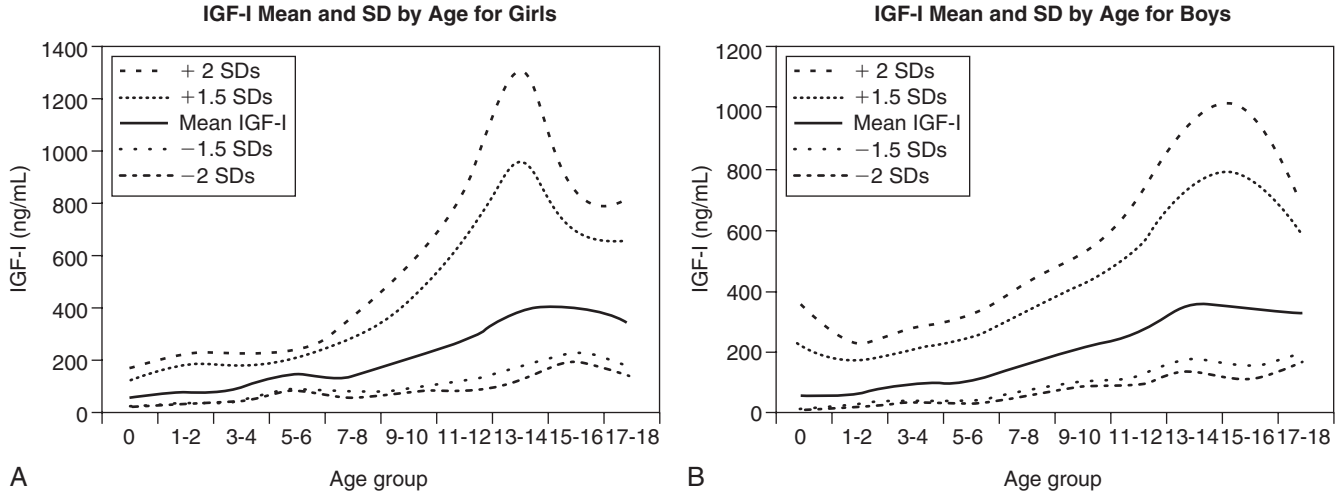


FIGURE 10-19 ■ Serum insulin-like growth factor I (IGF-I) values by age for females (**A**) and males (**B**). SD, standard deviation.

progressive age-associated decline.[184,233] It has been suggested that this decline may contribute to the negative nitrogen balance, decrease in body musculature, and osteoporosis characteristic of aging.[184] This provocative hypothesis remains unproven at this time, although it has generated considerable interest in the potential use of GH or IGF-1 therapy in normal aging.

Human newborn levels of IGF-2 are generally 50% of adult levels. By 1 year of age, however, adult concentrations are attained with little if any subsequent decline—even out to the seventh or eighth decade of life. It is of interest that this pattern of IGF-2 concentrations is distinctly different from that in the rat or mouse (in which serum IGF-2 levels are highest in the fetus and rapidly decline postnatally to essentially undetectable levels in the adult).[234,235]

Measurement of IGF Levels in Growth Disorders

The GH dependency of the IGFs was established by the initial report from Salmon and Daughaday.[173] Following the development of sensitive and specific radioimmunoassays that could distinguish between IGF-1 and IGF-2, the relationship of serum IGF levels to GH status has been established.[213] Measurement of each IGF peptide offers its own particular advantages. IGF-1 concentrations are far more GH dependent than are IGF-2 concentrations and are useful in identifying changes in GH secretory patterns. Serum IGF-1 concentrations, however, are greatly influenced by chronologic age, degree of sexual maturation, and nutritional status. As a result, construction of age-defined normative values is critical. IGF-1 levels in normal children younger than 5 years of age may be so low that extensive overlap exists between the normal range and values in GH-deficient children.

Ranke and coworkers [232] performed GH stimulation tests in 400 children with heights below the 5th percentile and assessed the value of IGF-I as a surrogate of the diagnosis of GHD. The children subsequently diagnosed as GH deficient on the basis of standard provocative tests had very low levels of IGF-I. However, significant overlap of serum IGF-1 levels existed between GH-deficient patients and children with other forms of short stature and normal provocative GH levels. It was only in children with bone ages greater than 12 years that serum IGF-1 levels permitted complete discrimination between GHD and normal short children.

Similarly, Rose and colleagues[236] found that in children with low provocative GH or overnight GH concentrations serum IGF-1 concentrations were lower but not dramatically lower compared to children with normal GH levels. Rosenfeld and associates[213] evaluated the efficacy of IGF-1 and IGF-2 radioimmunoassays in 68 children with GHD, 197 children of normal stature, and 44 normal short children. Eighteen percent of the GH-deficient children had serum IGF-1 levels within the normal range for age, whereas 32% of normal short children had low IGF-1 concentrations. Low IGF-2 levels were found in 52% of GH-deficient children but also in 35% of normal short children. The use of combined IGF-1/IGF-2 assays provided better discrimination, however, but pediatric endocrinologists do not commonly practice this method.

The observation that many "normal but short" children have low serum concentrations of IGF-1 or IGF-2 (or both) calls into question the criteria by which the diagnosis of GHD is currently made. Given that provocative GH testing is both arbitrary and nonphysiologic and that inherent variability in GH radioimmunoassays exists, it is not surprising that the correlation between IGF-1 levels and provocative GH levels is imperfect. These points are further supported by observations with radioimmunoassays for IGFBP-3.

IGF Receptors

In the early 1970s, it became apparent that the IGFs could bind (although generally with low affinity) to insulin receptors, thus providing an explanation for their insulin-like activity.[237] Shortly thereafter, Megyesi and associates[238] identified distinct receptors for insulin and IGF in rat hepatic membranes. Specificity studies, employing radiolabeled IGF preparations, demonstrated that at least two classes of IGF receptors existed. Insulin, at high concentrations, could compete for occupancy of one form of IGF receptor but had essentially no affinity for the second form of receptor.

The development of methodologies for structural characterization of these receptors enabled the clear discrimination of two receptor forms[239-242] (see Figure 10-17). The type 1 IGF receptor is closely related to the insulin receptor. Both are heterotetramers composed of two membrane-spanning alpha subunits of apparent molecular weight of 130 kd and two intracellular beta subunits of apparent molecular weight of 90 kd. The alpha subunits contain the binding sites for IGF-1 and are linked by disulfide bonds. The beta subunits contain a transmembrane domain, an adenosine triphosphate (ATP)-binding site, and a tyrosine kinase domain, which constitutes the presumed signal transduction mechanism for the receptor. Whereas each alpha-beta heterodimer appears capable of binding one molecule of ligand, it appears that 1 mole of the full heterotetrameric receptor binds only 1 mole of ligand.

Although the type 1 IGF receptor has been commonly referred to as the "IGF-1 receptor," studies indicate that the receptor is capable of binding IGF-1 and IGF-2 with high affinity—and both IGF peptides appear capable of activating tyrosine kinase by binding to this receptor. Affinity of the type 1 receptor for insulin is generally 100-fold less, thereby providing a mechanism for the relatively weak mitogenic effect commonly observed with insulin. Ullrich and coworkers[243] deduced the complete primary structure of the human type 1 IGF receptor from cDNA cloned from a human placental library. The mature peptide constitutes 1,337 amino acids, with a predicted molecular mass of 152 kd.

The translated alpha-beta heterodimer is subsequently cleaved at an Arg-Lys-Arg-Arg sequence at positions 707 to 710. As is the case with the insulin receptor, the beta subunit has the expected hydrophobic transmembrane domain and the intracellular tyrosine kinase domain and ATP binding site. Although it appears reasonable to presume

that insulin and IGF-1 receptors have both evolved from a common ancestor protein, they are encoded by genes on separate chromosomes (chromosome 15 q26.3 for the type 1 IGF receptor and chromosome 19 for the insulin receptor).

IGF-1 acts primarily through IGF1R but can bind with lower affinity to the highly homologous insulin receptor and to IGF1R/IR heterodimers. Vice versa, insulin is able to signal through the IGF1R.[244,245] Signs of such alternative signaling can become apparent in pathologic states of IGF-1 or insulin secretion.

The type 1 IGF receptor mediates IGF actions on all cell types, and these actions are diverse and tissue specific. In general, it is believed that all of the effects of IGF receptor activation are mediated by tyrosine kinase activation and phosphorylation of substrates—which activate specific cellular pathways, leading to various biologic actions. Among these effects is induction of cell growth through activation of the cell cycle machinery, maintenance of cell survival (prevention of apoptosis) mediated by effects on the bcl family members, and induction of cellular differentiation, which occurs by as yet incompletely characterized mechanisms.

The substrates, which are phosphorylated by the IGF receptor, include the members of the insulin receptor substrate family (particularly IRS-1 and IRS-2) because both of the knockout mice models for these genes result in poor growth (as well as insulin resistance).[246] Other IRS molecules may have a negative feedback role in regulating IGF action.[247] In addition, several other signaling molecules respond to IGF receptor activation. Blockade of the IGF-I receptor has been proposed as a cancer therapy, and clinical studies show promise in early trials.[248]

It is of particular note that a prototypic molecule for the IGF-1/insulin receptor in nematodes (termed Daf2) is related to longevity, such that mutations of this gene extend the life expectancy of these organisms. This extension of life expectancy in nematodes has also been shown for other components of the GH-IGF system, as well as being demonstrated in several other species including flies and mice.[249] It is unclear, however, what relevance the IGF-1/insulin receptor has for human longevity. Data have suggested that genetic alterations in the human IGF1R that result in altered IGF signaling confer an increase in proclivity for human longevity, suggesting a role of this pathway in modulating human life span.[250] The type 2 IGF receptor, however, bears no structural homology with either the insulin or the type 1 IGF receptors. On sodium dodecyl sulfate–polyacrylamide gel electrophoresis, the type 2 IGF-R has been demonstrated to migrate at an apparent molecular weight of 220 kd under nonreducing conditions and 250 kd after reduction—indicating that it is a monomeric protein.

The cloned human type 2 receptor has a predicted molecular mass of 271 kd and is characterized by a lengthy extracellular domain containing 15 repeat sequences of 147 residues each,[251] followed by a 23-residue transmembrane domain and a small cytoplasmic domain consisting of only 164 residues. The receptor does not contain an intrinsic tyrosine kinase domain or any other recognizable signal transduction mechanism. Surprisingly, the type 2 IGF receptor has been found to be identical to the cation-independent mannose-6-phosphate (CIM6P) receptor—a protein involved in the intracellular lysosomal targeting of a variety of acid hydrolases and other mannosylated proteins.[252,253] The majority of these receptors are located on intracellular membranes, where they are in equilibrium with receptors on the plasma membrane.[254]

Why this receptor binds IGF-2 and mannose-6-phosphate–containing lysosomal enzymes remains unresolved. Unlike the type 1 IGF receptor, which binds both IGF peptides with high affinity and insulin with 100-fold lower affinity, the type 2 receptor only binds IGF-2 with high affinity. IGF-1 binds with substantially lower affinity, and insulin not at all.[254] One mole of IGF-2 binds per mole of receptor. The binding sites for IGF-2 and mannose-6-phosphate appear to reside in different portions of the receptor. Nevertheless, the two classes of ligand do show some reciprocal inhibitory effects on receptor binding—suggesting a potential effect of IGF-2 on the sorting of lysosomal enzymes.

Most studies have indicated that the classic mitogenic and metabolic actions of IGF-1 and IGF-2 are mediated through the type 1 IGF receptor, with its tyrosine kinase signal transduction mechanism. Conover and coworkers[255] and Furlanetto and associates[256] have demonstrated that monoclonal antibodies directed against the IGF-1 binding site on the type 1 IGF receptor inhibit the ability of IGF-1 and IGF-2 to stimulate thymidine incorporation and cell replication. Similarly, several groups have shown that polyclonal antibodies capable of blocking IGF-2 binding to the type 2 IGF/mannose-6-phosphate receptor do not block IGF-2 actions.[257-259]

More direct evidence for the role of the type 1 IGF receptor in mediating classic IGF actions of IGF-2 comes from the use of IGF-2 analogues as probes of receptor function. IGF-2 analogs with decreased affinity for the type 1 receptor but preserved affinity for the type 2 receptor were markedly less potent than IGF-2 in stimulating DNA synthesis.[260] In further support of the concept that the type 2 IGF receptor does not mediate the mitogenic actions of IGF-2, it has been shown that the mannose-6-phosphate receptor in hepatic tissues from chicken[261] or frogs[262] does not bind IGF-2. Presumably, IGF-2 mitogenic actions in these species are mediated solely through the type 1 IGF receptor.

Nevertheless, a number of observations are consistent with the possibility of an IGF-2 action mediated via the type 2 IGF receptor. Rogers and Hammerman[263] have suggested that the type 2 receptor is involved in production of inositol triphosphate and diacylglycerol in proximal tubule preparations and canine kidney membranes. Tally and coworkers[264] have reported that IGF-2 stimulates the growth of a subclone of the K562 human erythroleukemia cell line, an action not duplicated by either IGF-1 or insulin. Minniti and coworkers[265] reported that IGF-2 appears capable of acting as an autocrine growth factor and cell motility factor for human rhabdomyosarcoma cells, actions apparently mediated through the type 2 receptor. It has been suggested that IGF-2 can activate a calcium-permeable cation channel by means of the type 2 IGF receptor,

perhaps through coupling to a pertussis toxin–sensitive guanine nucleotide–binding protein (Gi protein).[266]

It now appears that the type 2 IGF receptor binds several other molecules in addition to IGF-2. The ability of this receptor to bind mannose-6-phosphate–containing enzymes (such as cathepsin and urokinase) is well recognized and may be important in its ability to remove these enzymes from the cellular environment, thus modulating tissue remodeling.[267] In addition, reports indicate that the type 2 IGF receptor binds retinoic acid and may mediate some of the growth inhibitory effects of retinoids.[268] Knocking out the gene for type 2 IGF receptor results in excessive growth, as subsequently detailed. It appears that this receptor acts as a growth inhibitory component of the IGF system responding to and mediating multiple antimitogenic systems.[269]

The occasional observation of seemingly anomalous competitive binding results[270] has led to the suggestion that variant or atypical insulin and IGF receptors might exist.[271] One possible explanation for such findings is the existence of hybrid receptors composed of 1 alpha-beta dimer of the insulin receptor and 1 alpha-beta dimer of the type 1 IGF receptor.[272] Ligand-dependent formation of hybrid IGF/insulin receptors has been reported by Treadway and associates,[273] and studies with monoclonal antibodies specific for the insulin or type 1 IGF receptor have suggested that such receptors may develop spontaneously in cells with abundant native receptors.[274] The physiologic significance of such hybrid receptors, however, is entirely speculative.

IGF-Binding Protein Superfamily

Although insulin and the IGFs share significant structural homology, and despite the structural-functional similarity of the insulin and type 1 IGF receptors, the IGFs differ from insulin in one important respect. In contrast to insulin, the IGFs circulate in plasma complexed to a family of binding proteins.[275-277] These carrier proteins extend the serum half-life of the IGF peptides, transport the IGFs to target cells, and modulate the interaction of the IGFs with their surface membrane receptors.

The existence of IGFBPs was initially inferred from chromatographic studies of size distribution of IGF peptides in serum,[278] but the complexity of the interactions among the IGFs, IGFBPs, and IGF receptors has been fully appreciated only recently. The identification and characterization of IGFBPs in body fluids and in conditioned media from cultured cells have been facilitated by the development of a number of biochemical and assay techniques, including gel chromatography, radioreceptor assays, affinity cross-linking, Western ligand blotting,[279] immunoblotting, and specific radioimmunoassay and ELISA assays. It has been the study of the molecular biology of the IGFBPs that has provided the most information concerning their structural interrelationship, however.

Six distinct human and rodent IGFBPs have been cloned and sequenced.[276,277,280] Their structural characteristics are summarized in Figure 10-20. The determination of the primary amino acid sequences from the cloned cDNAs of the IGFBPs has revealed important structural relationships among the IGFBPs. Probably the most impressive similarity in structure is the conservation of the number and placement of the cysteine residues. The total number of cysteines varies from 16 to 20, and each of the IGFBPs has cysteine-rich regions at the amino and carboxyl termini of the protein. Conservation of the spatial order of the cysteines presumably indicates that the secondary structure of the IGFBPs, which is dependent on disulfide bonding, must also be well maintained. Disulfide bonding establishes the IGF-binding site of each IGFBP. Reduction of the binding proteins results in loss of IGF binding.

Analysis of the amino acid sequences of the IGFBPs also reveals the presence of an arginine-glycine-aspartic acid (RGD) positioned near the carboxyl terminus of

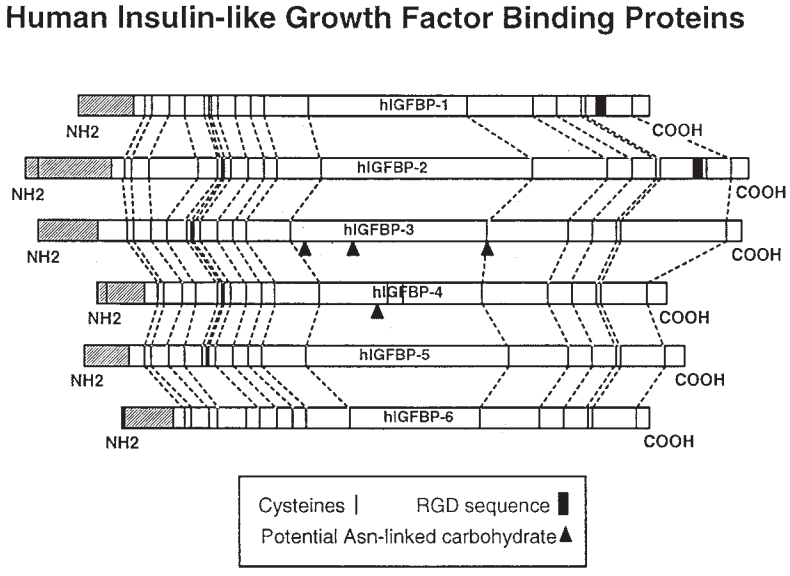


FIGURE 10-20 ■ Schematic representation of the insulin-like growth factor (IGF)–binding proteins. (Adapted from Lamson, G., Giudice, L., & Rosenfeld, R. G. (1991). Insulin-like growth factor binding protein and molecular relationship. *Growth Factors, 5,* 19.)

IGFBP-1 and IGFBP-2.[281] This sequence has been demonstrated to be the minimum required in many extracellular matrix proteins for their binding by membrane receptors of the integrin protein family. It has been suggested that IGFBPs may associate with the cell surface through such amino acid sequences. On the other hand, IGFBP-3 (which lacks an RGD sequence) also appears capable of specific binding to cell membrane receptors.[282,283] Membrane proteins capable of specifically binding IGFBP-3 have been proposed.[284]

Under most conditions, the IGFBPs appear to inhibit IGF action—presumably by competing with IGF receptors for IGF peptides.[285] This conclusion is supported by the observation that IGF analogues with decreased affinity for IGFBPs generally appear to have increased biologic potency.[286-288] In many studies involving transfection of the hIGFBP-3 gene into cells, expression of IGFBP-3 resulted in an inhibition of cell growth even in the absence of added IGF—suggesting a direct inhibitory role of the binding protein.[289] Under specific conditions, however, several of the IGFBPs apparently are capable of enhancing IGF action—perhaps by facilitating IGF delivery to target receptors.[290]

Of interest is the discovery of several groups of cysteine-rich proteins that contain domains strikingly similar to the amino-terminal domain of the IGFBPs. This has led to the proposal of an IGFBP superfamily,[291] which includes the family of six high-affinity IGFBPs—as well as a number of families of IGFBP-related proteins (IGFBP-rPs). Three of the IGFBP-rPs (Mac25/IGFBP-rP1; connective tissue growth factor, CTGF/IGFBP-rP2; NovH/IGFBP-rP3) have been shown to bind IGFs, although with considerably lower affinity than in the case of IGFBPs. Like the IGFBPs, the IGFBP-rPs are modular proteins—and the highly preserved aminoterminal domain appears to represent the consequence of exon shuffling of an ancestral gene. It is not clear what role, if any, the IGFBP-rPs play in normal IGF physiology. However, it is likely that they can influence cell growth by IGF-independent (and perhaps IGF-dependent) mechanisms.

Evidence indicates that IGFBPs are essentially bioactive molecules that in addition to binding IGF have a variety of IGF-independent functions. These clearly include growth inhibition in some cell types,[292] growth stimulation in other tissues,[293] direct induction of apoptosis,[294] and modulation of the effects of other non-IGF growth factors. These effects of IGFBPs are mediated, undoubtedly, by binding to their own receptors. These IGFBP-signaling pathways are being unraveled and involve the interaction of IGFBPs with nuclear retinoid receptors as well as with other molecules on the cell surface and in the cytoplasm.[295] Because IGFBP-3 is regulated by GH, it is intriguing that in vivo IGFBP-3 enhances IGF-I action when given to hypophysectomized rats (rather than inhibiting it).[296] The mechanisms involved in this effect have not been elucidated but may explain the limited effects of IGF-1 therapy on the growth of Laron patients (discussed later in the chapter).

Analysis of IGFBPs is further complicated by the presence of IGFBP proteases, capable of various levels of IGFBP degradation.[297,298] Initially reported in the serum of pregnant women,[297,298] proteases for IGFBP-3, -4, and -5 have already been demonstrated in a variety of biologic fluids—including serum, seminal plasma,[67] cerebrospinal fluid,[299] and urine.[300] Proteolysis of IGFBPs complicates their assay by both Western ligand blotting and radioimmunoassay methodologies and must be taken into consideration when concentrations of the various IGFBPs in biologic fluids are reported.[301] The physiologic significance of limited proteolysis of IGFBPs remains to be determined, although evidence suggests that protease activity results in decreased affinity of the IGFBP for IGF peptides.

The relative amounts of each of the IGFBPs vary among biologic fluids. IGFBP-1 is the major IGFBP in human amniotic fluid.[302] IGFBP-2 is prominent in cerebrospinal fluid[303] and seminal plasma.[304] IGFBP-3 is the major IGFBP in normal human serum and demonstrates clear GH dependence.[305] Among the IGFBPs, IGFBP-3 and IGFBP-5 are unique in that they normally circulates in adult serum as part of a ternary complex consisting of IGFBP-3 or IGFBP-5, an IGF peptide, and an acid-labile subunit.[306]

Specific immunoassays have been developed for the IGFBPs, including IGFBP-1,[302,307,308] IGFBP-2,[309] and IGFBP-3.[301,310,311] Currently, measurement of IGFBP-3 appears to have the greatest potential clinical value because this IGFBP appears to be directly GH dependent (Figure 10-21). Blum and associates[45] have suggested that radioimmunoassay determination of serum concentrations of IGFBP-3 might be more specific (but less sensitive) than IGF-1 assays in the diagnosis of GHD because normal concentrations of IGF-1 are so low in young children and many "normal" short children have low concentrations of IGF-1. Because IGFBP-3 determinations reflect not only IGF-1 levels but IGF-2 concentrations, their age dependency is not nearly as striking as that of IGF-1. Even in young children, normal concentrations are at least above 500 ng/mL. The use of IGFBP assays in the evaluation of IGF deficiency and GHD is discussed later in this chapter.

Targeted Disruption of Components of the IGF System

The critical role of the IGF system in fetal and postnatal growth was demonstrated in a series of elegant gene knockout studies in mice.[312] Unlike GH and GHR knockouts,[213] which are near normal size at birth, Igf1 null mice have a birth weight 60% of normal.[110] Postnatal growth is abnormal, and the surviving mice are only 30% of normal size by 2 months of age. A similar prenatal and postnatal growth phenotype has been observed in a reported human case of an IGF-1 gene deletion.[313]

Igf2 null mice (or heterozygous mice carrying a paternally derived mutated Igf2 gene) also have birth weights 60% of normal and remain about 60% of normal size throughout life.[314] When the gene for the IGF-1 receptor is knocked out (Igf1r null mice), the mice are severely growth retarded with a birth weight only 45% of normal

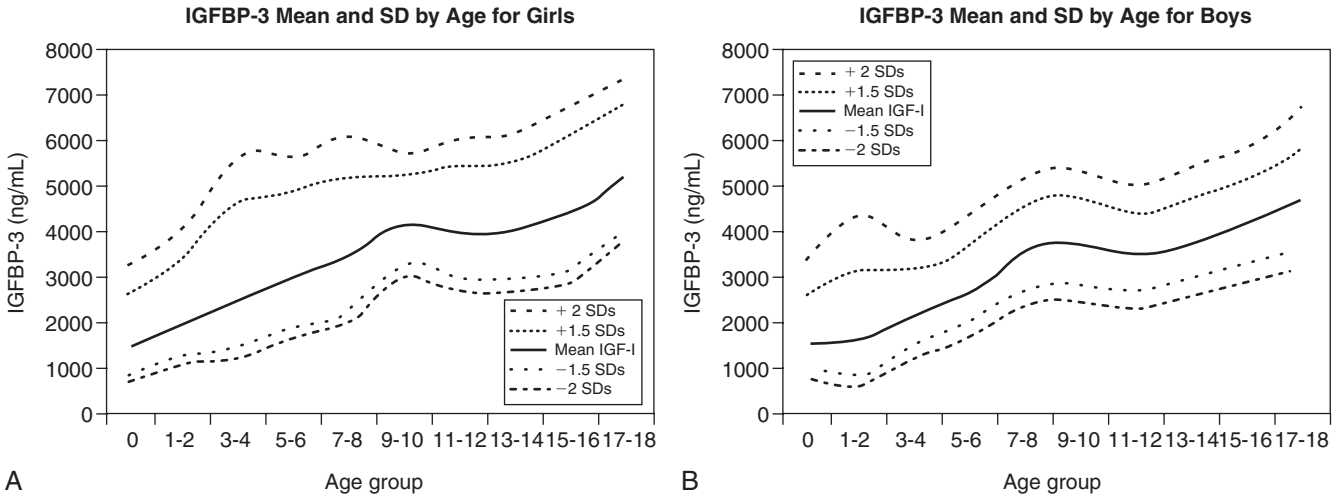


FIGURE 10-21 ■ Insulin-like growth factor–binding protein 3 (IGFBP-3) values by age for females (**A**) and males (**B**). SD, standard deviation.

and die soon after birth—apparently of respiratory failure.[315] The addition of an Igf1 knockout to the Igf1r knockout does not change the growth characteristics significantly, consistent with the hypothesis that IGF-1 is signaling exclusively (at least from the perspective of growth) through the IGF-1 receptor. On the other hand, the combination of Igf2 and Igf1r knockouts results in further growth retardation (birth weight 30% of normal)—indicating that IGF-2 is signaling through the IGF-1 receptor and a second receptor (probably the insulin receptor).

The relationship between GH and IGF-1 in controlling postnatal growth was analyzed in mouse mutants lacking GHR, IGF-1, or both.[182] This demonstrated that GH and IGF-1 promote postnatal growth by independent and common functions because the growth retardation of double GHR/IGF-1 null mice is more severe than that observed with either class of single mutant. In fact, the body weight of these double-mutant mice is only approximately 17% of normal—indicating IGF-independent GH actions. Thus, the growth control pathway in which the components of the GH/IGF-1 signaling systems participate constitutes the major determinant of body size.

The Igf2 gene has been shown to be maternally imprinted in mice and humans (only the paternal gene is expressed). On the other hand, the gene for the IGF-2 receptor (Igf2r) is paternally imprinted (although only in mice).[316] The phenotypes of Igf2r null mice or of heterozygous mice inheriting a mutated maternal gene are indistinguishable and demonstrate overgrowth, with birth weights 140% of normal. Because this receptor normally degrades IGF-2, increased growth reflects excess IGF-2 acting through the IGF-1 receptor. As mentioned previously, mice with deletions in GH and GH-receptor-related genes (as well as IGF-1R and downstream signaling genes) display increased life span and reduced oxidative stress. The human counterparts of these mutations in mice are now known to occur in humans and are discussed in detail later.

Other Growth Factors

The FGF Family of Peptides and Receptors

Fibroblast growth factors (FGFs) constitute an expanding family of peptide cytokine growth factors, which are important in the physiologic regulation of many tissues. In the early 2000s, a large amount of data from both animal and human studies confirmed that these pluripotent FGFs are involved in angiogenesis, wound healing, and proliferation and differentiation of a wide variety of cells and tissues.[317] Initially, it was suggested that FGFs may be specific for cells of stromal lineage. However, it appears that many other cell types respond to FGFs; their multifunctional nature includes both morphologic and endocrine/autocrine/paracrine effects, and they have a mitogenic role as well. There are 22 different members of the FGF family identified in humans so far.[318] FGF1 through FGF10 are all structurally related and bind to one of four fibroblast growth factor receptors (FGFRs). This group includes the better characterized FGF1 (acidic FGF oraFGF), FGF2 (basic FGF or bFGF), and keratinocyte growth factor (KGF or FGF-7).[318] In addition to these FGFs, FGF11 to FGF14 are also known as FGF homologous factors 1 to 4 (FHF1 to FHF4). Although these peptides are structurally homologous to the other FGFs, they do not bind to the FGFRs, which results in their different intracellular function. FGF15 is only found in mice and is the orthologue of human FGF19—both play a role in the intestinal-to-liver signaling of bile acid regulation.[319] FGF16 to FGF23 have been discovered and studied more recently, and some of these peptides appear to have more systemic endocrine-like effects.

The FGFs mediate their actions by binding to one of four receptors[320] (FGFR1, FGFR2, FGFR3, and FGFR4), which have distinct tissue distributions.[321] FGFRs have three extracellular domains, one transmembrane domain, and an intracellular tyrosine kinase domain. Alternative FGFR mRNA splicing leads to several FGFR variants on the cell surface, which determines both specific

ligand-receptor interaction and function for the different FGFs. For the most part, FGFs appear to be autocrine-paracrine growth factors that participate in organ growth and differentiation (as well as in carcinogenesis) but not in somatic growth. In contrast, FGF23, a phosphatonin, is a crucial factor—in an endocrine manner—in the pathophysiology of hypophosphatemic disorders leading to osteomalacia and rickets.[322] Another exception to this rule is the observation that several genetic forms of skeletal dysplasia (achondroplasia, hypochondroplasia, thanatophoric dysplasia) are caused by activating mutations in the FGFR3 gene, suggesting that normal FGFR3 signaling is essential to the normal growth of long bones.[323,324] A human mutation in the FGFR2 gene causes craniosynostosis, a condition characterized by abnormal closure of the bones in the skull, and FGFR2 appears to be a major regulator of bone formation during embryologic development.[325] Other craniosynostosis syndromes have now been associated with mutations that enhance FGFR2 function, and mutations leading to diminished FGFR2 function have also been documented, including distinct forms of perinatal lethal skeletal dysplasia.[326] These clinical conditions in humans together with targeted disruptions of several murine FGF and FGFR genes underscore the importance of the FGFs on a multitude of developmental systems and processes, including, but not limited to, angiogenesis, neurogenesis, limb development, and many other functions.

The EGF System

Epidermal growth factors (EGFs) and their receptors are ubiquitous in many tissues and participate in developmental processes in mice, such as precocious eyelid opening and tooth eruption. EGF itself it the best studied polypeptide in the larger family of EGF proteins, which further include transforming growth factor alpha (TGF-α), epiregulin, neuregulin, and betacellulin among several other proteins. The mitogenic actions of EGF have been extensively explored in cell culture systems, and the receptor for EGF (EGFR) was characterized as a prototype model for signal transduction involving tyrosine kinases. EGFR, a transmembrane glycoprotein, is one of four members of the erbB family of tyrosine kinase receptors.

The extensive in vitro data indicate multiple cellular functions of EGF. EGF has been identified in most body fluids of several mammalian species. EGF-EGFR interaction promotes cellular proliferation, differentiation, and survival. However, neither EGF antibody administration to newborn animals nor gene targeting of EGF has caused major deleterious effects—as might have been expected from the in vitro studies.[327]

The EGF family of growth factors appears to be important in mammalian development and function, although the precise roles and significance are not entirely clear. Members of the EGF family may have a role in embryogenesis and fetal growth because receptors have been identified in fetal tissues. It has been proposed that abnormal EGF-EGF receptor interactions may be instrumental in the development of cancer, but it appears that they are not involved in somatic growth. It has long been documented that EGFR is overexpressed in a number of tumor cell lines. This may worsen tumor prognosis and patient survival, because EGFR may affect the tumors' resistance to chemo- and radiation therapies. For this reason, newer anticancer therapies have been developed to interfere with EGFR signaling, either by blocking ligand-EGFR binding (monoclonal antibodies) or by inhibiting the intracellular signal transduction cascade (such as with small molecule inhibitors). These treatments have allowed tumor arrest and even regression, as well as strengthened the effect of standard anticancer treatment modalities.[328]

Other Growth-Promoting Peptides

An ever-growing number of growth factors are being recognized, and multiple hormones and peptides are being characterized as having growth-promoting activities in certain cell types. In general, these molecules appear to lack somatic growth-promoting effects but play important autocrine-paracrine and endocrine roles. Notable among these molecules are groups of growth factors that have tissue-specific effects. Endothelin, platelet-derived growth factor (PDGF), and vascular-epithelial growth factor (VEGF) all regulate angiogenesis and other vascular processes in addition to modulating the function of numerous cultured cells. A variety of hematopoietic growth factors—such as the granulocyte and macrophage colony-stimulating factors (GCSF, MCSF), erythropoietin, and thrombopoietin—promote the growth of the different lineages of the hematopoietic cells.

The growth of various cells of the immune system is stimulated by an array of cytokines, including interleukins (ILs) and interferons (IFNs). Up to 35 different human interleukins, small cell-signaling molecules that organize communication between immune system cells, have been described, each with a variety of target receptors. Their functions are highly variable and involve differentiation of immune response and modulation of inflammation, proliferation of myeloid progenitor cells, and they serve a regulatory role in the differentiation and proliferation of multiple other cell types (osteoclasts, keratinocytes, etc.). ILs are being used in cancer immunotherapy and immunomodulation of transplant patients.[329] About 10 different IFNs have been identified in mammals; 7 of these are believed to be important in humans. Based on which receptor they signal through, the IFNs are further subdivided in three major subclasses (IFNs types I to III). The major function of IFNs relates to (1) their antiviral effect and (2) their tumor fighting ability (most often for hematologic malignancies). This is attributed to their antiproliferative, apoptotic, and antiangiogenic action, as well as their ability to modulate immune responsiveness.[330] In 2012, more than 10 pharmaceutical formulations of IFNs were available for variable therapeutic usage.

The complex array of cells that constitute the nervous system is under the regulatory influence of specific growth factors, such as nerve growth factor (NGF), the neurotrophins, and brain- and glial-derived neurotrophic factors (BDNF, GDNF). Other growth factors that have been attributed to specific tissues (e.g., hepatocyte growth factor [HGF]) are being recognized as

having a general growth-promoting effect in numerous tissues, and additional organ-specific growth regulatory processes have been described in the gastrointestinal tract and kidneys.

Growth Inhibitory Peptides

Of particular interest is a class of cytokines that can negatively modulate cellular growth. Transforming growth factor-beta (TGF-β) is a protein that comes in at least three isoforms and is part of the superfamily of proteins known as the transforming growth factor beta superfamily. Other members include inhibin, activin, and anti-Mullerian hormone. TGF-β mediates cellular growth and malignant transformation and can function as a growth inhibitory substance with the potential of arresting the growth of both normal and neoplastic cells. Abnormal TGF-β signaling also appears to play a role in disorders such as Marfan syndrome (possibly through abnormal TGF-β sequestration) and Loeys-Dietz syndrome (TGF-β receptor mutation). Tumor necrosis factors (TNFs) and other compounds have been reported to have similar effects to TGF-β.

All these molecules may regulate the entry of cells into programmed cell death (apoptosis). The growth inhibitory processes of TGF- β and other cytokines may prove to be of great importance in the development of cancer treatments. A family of genes/proteins, of which the most important is p53, is also critical to growth and tumor suppression.[331] These genes/proteins that function as tumor suppressors may also be involved in fetal growth.

Sex Steroids

Whereas androgens and estrogens do not contribute substantially to normal growth before the onset of puberty, the rise in serum sex steroid concentrations during adolescence is an important part of the pubertal growth spurt. States of androgen or estrogen excess before epiphyseal fusion are invariably characterized by rapid linear growth and skeletal maturation. Thus, just as growth deceleration requires further evaluation, growth acceleration can be just as abnormal and may be a sign of increased sex steroid production or action, as observed in precocious puberty and with the virilizing effects of congenital adrenal hyperplasia.

A GH-replete state is obligatory for a normal growth response to sex steroids. Children with GHD do not have a normal growth response to either endogenous or exogenous androgens. Although androgens work at least in part by enhancing GH secretion, they must also have a direct effect on IGF-1 production—as observed with the rise in serum IGF-1 concentrations and pubertal growth spurt characteristic of children with mutations of the GHR.[110]

Androgens and estrogens have been shown to increase skeletal maturation. Advancement of skeletal age and epiphyseal fusion appear to be estrogen-mediated, as indicated by the report of tall stature with open epiphyses in a patient with a mutation of the estrogen receptor.[17] Similar findings are noted in those with inactivating mutations of the gene regulating the aromatase enzyme that normally converts androgens to estrogens.[332] Whichever the mechanism, however, clinical states of androgen or estrogen excess are characterized by disproportionate skeletal maturation and premature epiphyseal fusion. Interestingly, a condition of aromatase excess has been described, resulting from mutations in the promoter region of the gene and leading to early epiphysial closure and gynecomastia.[333]

Thyroid Hormone

Thyroid hormone is also a major contributor to postnatal growth, although, like GH, it is of relatively little importance to growth of the fetus. Hypothyroidism occurring postnatally can, however, result in profound growth failure and virtual arrest of skeletal maturation. In addition to a direct effect on epiphyseal cartilage, thyroid hormone appears to have a permissive effect on GH secretion. Patients with hypothyroidism have decreased spontaneous GH secretion and have a blunted response to GH provocative tests. Treatment with thyroid hormone results in rapid "catch-up" growth, which is typically accompanied by marked skeletal maturation—potentially resulting in overly rapid epiphyseal fusion and compromise of adult height.

GROWTH RETARDATION

Systems for classification of growth disorders are problematic because diagnostic categories have not always been sharply defined and frequently overlap. Genetic short stature, for example, may often be associated with constitutional delay of growth and maturation—and both disorders fall under the umbrella of idiopathic short stature (ISS). Because diagnostic criteria for GHD have been problematic, overlap has frequently existed among genetic short stature, constitutional delay, and the vague category of "partial" GHD. In addition, the cause of growth failure in intrauterine growth retardation (IUGR) and various syndromes associated with poor growth has generally remained obscure.

Box 10-1 represents an effort at classification of growth retardation. Growth disorders have been subdivided into primary growth abnormalities, in which the defect(s) appears to be intrinsic to the growth plate, genetic short stature, and secondary growth disorders (i.e., growth failure resulting from chronic disease or endocrine disorders). The category of "IGF deficiency (IGFD)" has become accepted as an overall category encompassing disorders that can result from various causes of GH deficiency (sometimes called secondary IGFD) or GH insensitivity (sometimes referred to as primary IGFD). The category of "IGF deficiency" takes on special meaning in light of recommendations for reevaluation of the diagnosis of GHD and primary IGFD.

Primary Growth Abnormalities

Osteochondrodysplasias

The osteochondrodysplasias represent a heterogeneous group of disorders characterized by intrinsic abnormalities of cartilage or bone.[334] The conditions share the following

BOX 10-1 Classification of Growth Retardation

I. Primary Growth Abnormalities

A. Osteochondrodysplasias
B. Chromosomal abnormalities

II. Secondary Growth Disorders

A. Malnutrition
B. Chronic disease
C. Intrauterine growth retardation
D. Endocrine disorders
- Hypothyroidism
- Cushing syndrome
- Pseudohypoparathyroidism
- Vitamin D deficient or resistant rickets

III. IGF Deficiency

A. Secondary IGFD
- GH deficiency due to hypothalamic dysfunction
- GH deficiency due to pituitary GH deficiency

B. Primary IGFD (GH insensitivity)
- Primary GH insensitivity-GH receptor defects
- Secondary GH insensitivity (Stat-5b)-GHR signal transduction defects
- Primary defects of IGF synthesis
- Primary defects of IGF transport/clearance (ALS)

C. IGF resistance
- Defects of the IGF-1 receptor
- Postreceptor defects

IV. Idiopathic Short Stature (ISS)

A. Constitutional delay of growth and puberty with normal height prediction
B. ISS with delayed bone age and tempo of puberty
C. ISS with normal bone age and tempo of puberty
D. ISS with a familial component
E. ISS without a familial component

GH, growth hormone; IGF, insulin-like growth factor.

BOX 10-2 Classification of Osteochondrodysplasias[876]

- Defects of tubular (and flat) bones or axial skeleton
- Achondroplasia group
  - Achondrogenesis
  - Spondylodysplastic group (perinatally lethal)
  - Metatropic dysplasia group
  - Short rib dysplasia group (with/without polydactyly)
  - Atelosteogenesis/diastrophic dysplasia group
  - Kniest-Stickler dysplasia group
  - Spondyloepiphyseal dysplasia group
  - Other spondyloepi(meta)physeal dysplasias
  - Dysostosis multiplex group
  - Spondylometaphyseal dysplasias
  - Epiphyseal dysplasias
  - Chondrodysplasia punctata (stippled epiphyses) group
  - Metaphyseal dysplasias
  - Brachrachia (short spine dysplasia)
  - Mesomelic dysplasias
  - Acromelic/acromesomelic dysplasias
  - Dysplasias with significant (but not exclusive) membranous bone involvement
  - Bent bone dysplasia group
  - Multiple dislocations with dysplasias
  - Osteodysplastic primordial dwarfism group
  - Dysplasias with increased bone density
  - Dysplasias with defective mineralization
  - Dysplasias with increased bone density
  - Disorganized development of cartilaginous and fibrous components of the skeleton
  - Idiopathic osteolyses

features: genetic transmission; abnormalities in the size or shape of bones of the limbs, spine, or skull; and radiologic abnormalities of the bones (generally). More than 100 osteochondrodysplastic conditions have been identified, based on physical features and radiologic characteristics. The ongoing characterization of biochemical and molecular abnormalities in these conditions will undoubtedly lead to an increase in the number of these disorders.

An international classification for the osteochondrodysplasias was developed.[335] Box 10-2 provides a brief summary of this classification. Of note, the category of dysostoses has been dropped from the classification—which now focuses on developmental disorders of chondro-osseous tissues. Diagnosis of osteochondrodysplasias can be problematic and often relies on careful radiologic evaluation. Although progress has been made in identifying the underlying molecular and biochemical defects in many of these conditions, clinical and radiologic evaluation remains central to the diagnosis at this time. Frequently, the clinical features are obvious—and the diagnosis can then be made at birth (or even prenatally) by ultrasound.

The family history is obviously critical. However, many cases represent fresh mutations—as is generally the case in the classic autosomal-dominant achondroplasia and hypochondroplasia. Careful measurement of body proportions is necessary, including arm span, sitting height, upper/lower body segments, and head circumference. Clinical and radiologic evaluation should be used to determine whether involvement is of the long bones, skull, or vertebrae—and whether abnormalities are primarily at the epiphyses, metaphyses, or diaphyses. Two of the more common forms (achondroplasia and hypochondroplasia) of the more than 100 defined osteochondrodysplasias are discussed in the following sections.

Achondroplasia. This is the most common of the osteochondrodysplasias, with a frequency of approximately 1:26,000 births. Although transmitted as an autosomal-dominant disorder, 90% of cases apparently represent new mutations. Studies have indicated that achondroplasia is caused by a mutation of the gene for fibroblast growth factor receptor 3 (FGFR3), located on the short arm of chromosome 4.[336] The overwhelming majority of cases identified represent mutations at a "hot spot" at nucleotide 1138 of FGFR3, and because these mutations create new recognition sites for restriction enzymes, it is easy to test for the mutation.

Infants homozygous for this condition have severe disease, typically dying in infancy of respiratory insufficiency

resulting from the small thorax. Short stature may not be evident until after 2 years of age, although the deviation from the normal growth curve is progressive. Mean adult height in males and females is 131 and 124 cm, respectively. Growth curves for achondroplasia have been developed and are of great value in following patients.[12]

With increasing age, the diagnosis of achondroplasia becomes easier because in addition to short stature these patients have other abnormalities of the skeleton—including megalocephaly, low nasal bridge, lumbar lordosis, short trident hand, and rhizomelia (shortness of the proximal legs and arms). Radiologic abnormalities include small cuboid-shaped vertebral bodies with short pedicles and progressive narrowing of the lumbar interpedicular distance. The iliac wings are small, with narrow sciatic notches. The small foramen magnum may lead to hydrocephalus, and spinal cord or root compression may result from kyphosis, stenosis of the spinal canal, or disk lesions. Acanthosis nigricans may be present.[337]

Hypochondroplasia. Hypochondroplasia has been described as a "milder form" of achondroplasia. However, although the two disorders are both transmitted as autosomal-dominant traits due to mutations in the same gene (*FGFR3*), they have not been reported to occur in the same family. Hypochondroplasia, however, has been shown to result from a different mutation (Asn540Lys) of the FGFR3 gene. The facial features characteristic of achondroplasia are absent, and the short stature and rhizomelia are less pronounced.

Adult heights are typically in the 120- to 150-cm range. As in the case of achondroplasia, short stature may not be evident until after 2 years of age—but stature then deviates progressively from normal. Outward bowing of the legs accompanied by genu varum is frequently observed. Lumbar interpedicular distances diminish between L1 and L5, and as with achondroplasia there may be flaring of the pelvis and narrow sciatic notches.

Chromosomal Abnormalities

Abnormalities of the autosomes and sex chromosomes may be characterized by growth retardation. In general, these disorders are also associated with somatic abnormalities and mental retardation—as in deletion of chromosome 5 or trisomy 18 or 13. Such abnormalities, however, may be subtle—and, for example, the diagnosis of Turner syndrome must be considered in any girl with unexplained short stature. In many cases, the precise cause of growth failure in these chromosomal abnormalities is unclear because the genetic defects do not appear to affect known components of the GH-IGF system. It is presumed, then, that the chromosomal defect influences normal cell proliferation and tissue growth or development or indirectly affects the responsiveness to IGF or to other growth factors yet to be identified.

Down Syndrome. Trisomy 21, or Down syndrome, is probably the most common chromosomal disorder associated with growth retardation, affecting approximately 1 in 600 live births. On average, newborns with Down syndrome have birth weights 500 g below normal and are 2 to 3 cm shorter. Growth failure continues postnatally and is typically associated with delayed skeletal maturation and a poor delayed pubertal growth spurt. Adult heights range from 135 to 170 cm in males and 127 to 158 cm in females.[338] The cause of growth failure in Down syndrome and in other autosomal defects is unknown. Attempts to find underlying hormonal explanations for growth retardation have been unsuccessful, even though hypothyroidism is more common than normal in Down syndrome and should be excluded. It is likely that growth failure in such conditions reflects a generalized biochemical abnormality of the epiphyseal growth plate.

Turner Syndrome. Short stature is the single most common feature of Turner syndrome, occurring more frequently than delayed puberty, cubitus valgus, and webbing of the neck.[339-341] Reviews of large series of Turner syndrome individuals have indicated that short stature occurs in 95% to 100% of girls with a 45,X karyotype. Ranke and colleagues identified several distinct phases of growth in girls with Turner syndrome,[342] including mild IUGR, with mean birth weights and heights of 2,800 g and 48.3 cm, respectively; normal height gain from birth until 3 years of age; progressive decline in height velocity from 3 years of age until approximately 14 years of age, resulting in a gradual and progressive deviation from normal height percentiles; and a prolonged adolescent growth phase characterized by a partial return toward normal height, followed by delayed epiphyseal fusion.

More recent detailed analyses of longitudinal and cross-sectional growth data from several centers have indicated that growth in girls with Turner syndrome is frequently abnormal in infancy and early childhood and that most cases of Turner syndrome diagnosed before adult life have fallen off the normal growth curve by 2 to 3 years of age.[343,344] Investigations of Turner syndrome from the United States and Europe have indicated mean adult heights ranging from 142 to 146.8 cm (lower in Asia). Parental height can influence final height significantly. (see Chapter 16).

The cause of growth failure in Turner syndrome is multifactorial, although the loss of one copy of the homeobox gene—SHOX (short stature homeobox-containing gene)—is the major contributor.[345] The SHOX gene covers a 40-kb region of the pseudoautosomal region of the X chromosome, escapes X inactivation, and is highly expressed in osteogenic tissue. Haploinsufficiency for SHOX has been implicated in the short stature of Turner syndrome, as well as several other somatic features. In addition, SHOX mutations appear responsible for the mesomelic growth retardation and Madelung deformity characteristic of Leri-Weil dyschondrosteosis—and complete absence of SHOX is associated with Langer mesomelic dysplasia. Girls with Turner syndrome are more likely to manifest autoimmune hypothyroidism and inflammatory bowel disease, both of which can also impact on growth.

The majority of patients have normal GH concentrations during childhood. Reports of low GH concentrations in adolescents with Turner syndrome are likely ascribable to low serum concentrations of sex steroids.[346] Nevertheless, GH therapy is capable of accelerating

short-term growth and increasing adult height in both Turner syndrome and SHOX haplo-insufficiency.[230,347-349] Indeed, early initiation of GH therapy at appropriate dosages appears to enable most girls with Turner syndrome to attain heights within the normal adult range.[348,349] Turner syndrome is described in greater detail in Chapter 16. The point is worth repeating, however, that the diagnosis of Turner syndrome should be considered in any phenotypic female with unexplained growth failure.

18q Deletions. Deletion of the long arm of chromosome 18 has an estimated prevalence of 1 in 40,000 live births. In one review of 50 cases, 64% of children (mean age 5.8 years) had heights greater than 2 SD below the mean.[350] Fifteen percent had serum IGF-1 concentrations below 2 SD, and 9% had IGFBP-3 concentrations below 2 SD. Seventy-two percent of children had reduced GH responses to provocative testing, although such testing was not always rigorous.

Intrauterine Growth Retardation

Despite the critical importance of the endocrine system in postnatal growth, normal intrauterine growth is largely independent of fetal pituitary hormones.[351,352] Athyreotic and agonadal infants are of normal length and weight at birth. Based on the normal size of anencephalic fetuses, it has been proposed that even the pituitary is unnecessary for fetal growth.[353,354] Careful documentation of birth size of rats with congenital GHD[355] and of human newborns with mutations of the GH or GHR gene[110] has indicated, however, that fetal pituitary-derived GH makes a small but statistically significant contribution to birth size.

These observations should not be interpreted to mean that the IGF axis is irrelevant in fetal growth. Gene knockout studies have shown that elimination of paracrine/autocrine production of IGF-1 has a major impact on fetal and postnatal growth.[356] A human with an IGF-1 gene deletion had the same growth characteristics as observed in the murine IGF-1 knockout, namely IUGR and postnatal growth failure that was unresponsive to GH administration.[357] Similarly, several reports of IGF-1 receptor defects associated with IUGR and postnatal growth retardation have appeared, and a family with bioinactive IGF-1 has also been reported.[351,352]

Thus, although the fetus may be largely GH independent, the production and activity of IGF-1 are clearly critical for normal intrauterine (as well as postnatal) growth. Human cord lymphocytes have been demonstrated to have increased numbers of IGF receptors[358] and mRNAs because IGF-1 and IGF-2 are abundant in fetal tissues.[359,360] Similarly, the IGFBPs are identifiable in serum and other biologic fluids, although it is of note that the relative serum concentrations of various IGFBPs are different in the fetus and newborn than in the older child or adult.[361] In particular, serum concentrations of IGFBP-3 and acid-labile subunit (ALS)—which together comprise the major serum carriers of IGF peptides in the adult—are much lower in the fetus and newborn.

IUGR is defined on the basis of a birth weight or birth length more than 2 SD below the mean for gestational age. Although the majority of such infants show good catch-up growth during the first few years of life, approximately 15% of such children fail to have sufficient catch-up growth to bring them into the normal height range by 4 years of age.[351,352,362] Whereas several studies have demonstrated that infants with IUGR tend to have low serum concentrations of IGF-1 and IGFBP-3 at birth,[361] it is not entirely clear whether failure of catch-up growth represents the effects of subtle persistent abnormalities of the GH-IGF axis. Barker and colleagues have proposed that in the process of adapting to a limited supply of nutrients in utero IUGR fetuses permanently alter their physiology and metabolism, a reprogramming process that results in physiologic consequences in later life—such as increased risk of coronary artery disease, stroke, hypertension, and type 2 diabetes mellitus.[363,364]

IUGR can arise from intrinsic abnormalities in the fetus, placental insufficiency, or maternal disorders (Box 10-3). Although it is understandable why uterine constraint or twin pregnancies might result in limited fetal growth, the biochemical and cellular basis for abnormal fetal growth in most cases of IUGR is unclear.

BOX 10-3 Etiology of Intrauterine Growth Retardation

Intrinsic Fetal Abnormalities

- Chromosomal disorders
- Syndromes associated with primary growth failure
  - Russell-Silver syndrome
  - Seckel syndrome
  - Noonan syndrome
  - Progeria
  - Cockayne syndrome
  - Bloom syndrome
  - Prader-Willi syndrome
  - Rubinstein-Taybi syndrome
- Congenital infections
- Congenital anomalies
- Primary abnormalities of insulin-like growth factor axis

Placental Abnormalities

- Abnormal implantation of the placenta
- Placental vascular insufficiency; infarction
- Vascular malformations

Maternal Disorders

- Malnutrition
- Constraints on uterine growth
- Vascular disorders
  - Hypertension
  - Toxemia
  - Severe diabetes mellitus
- Uterine malformations
- Drug ingestion
  - Tobacco
  - Alcohol
  - Narcotics

Adapted from Underwood, L. E., & Van Wyk, J. J. (1992). Normal and aberrant growth. In J. D. Wilson, & D. W. Foster (Eds.), Williams textbook of endocrinology *(8th ed.) (p.1110). Philadelphia: WB Saunders.*

It has been proposed that such cases result from reduced cell number or cell size, but the mechanisms for such abnormalities remain to be elucidated. Intrinsic fetal endocrine abnormalities are unlikely explanations for IUGR in most cases.

Congenital thyroid or GH deficiencies are typically characterized by near-normal birth size. Recently recognized human mutations in IGF-1 and IGF-1 receptors are associated with IUGR, but serum IGF-1 concentrations in IUGR children are highly variable—indicating that there is a large clinical diversity in this condition. Infants with IUGR frequently exhibit poor postnatal growth, particularly when the abnormalities are intrinsic to the fetus. Such conditions have frequently been categorized as "primordial growth failure."

Russell-Silver Syndrome (RSS). This condition was independently described by Russell,[365] and later by Silver and associates, in the early 1950s. Although this syndrome probably represents a heterogeneous group of patients, the "common" findings include IUGR, postnatal growth failure, congenital hemihypertrophy, and small triangular facies. Because no genetic or biochemical basis for this disorder has been identified until recently, Russell-Silver syndrome was often used improperly as a designation for IUGR of unknown cause. Other common nonspecific findings include clinodactyly, precocious puberty, delayed closure of the fontanelles, and delayed bone age. A genetic basis to RSS has now been described. Maternal uniparental disomy for chromosome 7 (mUPD7) is found in 5% to 10% of patients with RSS. More commonly, epigenetic changes in the form of DNA hypomethylation at the telomeric imprinting control region (ICR1) on chromosome 11p15, involving the *H19* and *IGF2* genes, have been identified in up to 60% of cases of RSS. These changes result in relaxation of imprinting and biallelic expression of *H19* and down-regulation of *IGF-2*.[366] Attempts to relate genotype to phenotype suggest that prominent forehead, relative macrocephaly, body asymmetry, and a low body mass index (BMI) were significantly associated with ICR hypomethylation.[367] Bruce and colleagues[368] identified hypomethylation of H19 ICR in 62% of a cohort of RSS patients and found that these patients manifested a more severe phenotype than those with maternal UPD7. Additionally, the degree of hypomethylation correlated with the phenotype; the most severe degree of hypomethylation was associated with the asymmetry, skeletal, and urogenital abnormalities. In another study by Binder and coworkers, patients with hypomethylation of 11p15, but not other causes of RSS or SGA, showed increased IGF-1- and IGFBP-3 concentrations, suggesting that there may be counterregulation by IGF-1 or resistance to some actions of IGF-1.[369]

Maternally inherited mutations in *CDKN1C* (also known as *P57KIP2*) have been implicated in the etiology of IMAGe syndrome, characterized by IUGR, metaphyseal dysplasia, adrenal hypoplasia congenita, and genital abnormalities. *CDKN1C* is an imprinted gene that is maternally expressed, and the protein inhibits cell cycle progression. Loss of function alterations in the gene have previously been associated with Beckwith-Wiedemann syndrome. In contrast, mutations associated with IMAGe syndrome cause gain in function.[370]

Seckel Syndrome. Although originally described by Mann and Russell in 1959,[371] this condition is most commonly termed *Seckel syndrome* or *Seckel birdheaded dwarfism*.[372] A genetically heterogeneous autosomal-recessive condition, this syndrome is caused by mutations in *ATR*, *RBBP8*, *CENPJ*, *CEP152*, and *CEP63*, Seckel syndrome is characterized by IUGR and severe postnatal growth failure combined with microcephaly, prominent nose, and micrognathia. Final height is typically 91.4 to 106.7 cm, with moderate to severe mental retardation.

Noonan Syndrome. Although this condition shares certain phenotypic features with Turner syndrome, the two disorders are clearly distinct.[373] In Noonan syndrome, several genes have been implicated—including KRAS (Kirsten rat sarcoma 2 viral oncogene homolog), *SOS1 (Son of Sevenless 1)*, *RAF1*, *NRAS*, and *BRAF* —but the majority of cases are caused by PTP11 (protein tyrosine phosphatase nonreceptor type 11) mutations. Both males and females may be affected, explaining the misleading names "Turner-like syndrome" and "male Turner syndrome." As in Turner syndrome, patients typically have webbing of the neck, a low posterior hairline, ptosis, cubitus valgus, and malformed ears.

Cardiac abnormalities are, however, primarily right-sided (pulmonary valve)—rather than the left-sided lesions (aorta, aortic valve) characteristic of Turner syndrome. Microphallus and cryptorchidism are common, and puberty is frequently delayed or incomplete. There is an increased risk of malignancies such as leukemia. Mental retardation is observed in approximately 25% to 50% of patients. In a manner similar to Turner syndrome, Noonan syndrome responds to GH therapy—and this treatment has been approved by the U.S. Food and Drug Administration (FDA).

Progeria. The senile appearance characteristic of progeria (Hutchinson-Gilford syndrome) typically appears by 2 years of age.[374] There is a progressive loss of subcutaneous fat, accompanied by alopecia, hypoplasia of the nails, joint limitation, and early onset of atherosclerosis—typically followed by angina, myocardial infarction, hypertension, and congestive heart failure. Skeletal hypoplasia results in severe growth retardation, which typically becomes evident by 6 to 18 months of age. The molecular basis of this syndrome, as well as that of the Cockayne syndrome, is described at www.ncbi.nlm.nih.gov/omim.

Cockayne Syndrome. Cockayne syndrome, like progeria, is characterized by a premature senile appearance.[375] Patients also have retinal degeneration, photosensitivity of the skin, and impaired hearing. Growth failure typically appears at 2 to 4 years of age. Transmission is as an autosomal-recessive disorder.

Microcephalic Osteodysplastic Primordial Dwarfism. This is characterized by severe growth failure, microcephaly,

facial dwarfism, and skeletal abnormalities. The condition is genetically heterogeneous and mutations in genes, including *NU4ATAC*, encoding a small nuclear RNA (snRNA) component of the U12-dependent (minor) spliceosome, and PCNT, encoding pericentrin, have been identified in a proportion of cases.

Prader-Willi Syndrome. Growth failure may be evident at birth but is generally more impressive postnatally. The neonatal period is characterized by hypotonia, and in the male by cryptorchidism and microphallus. With advancing age, hyperphagia and obesity become prominent. Hypogonadism may persist into adult life. The cause of growth failure is unclear. Low serum GH concentrations may reflect the impact of obesity and are not necessarily etiologic. On the other hand, low GH secretion and hypogonadism may reflect subtle defects of hypothalamic-pituitary function—and these patients respond well to GH therapy.[376-378] Blunted cortisol responses to CRH have been reported in some patients.[379] Patients with Prader-Willi syndrome have been found to have deletions of the paternal short arm of chromosome 15, or uniparental disomy of the maternal imprinted region of chromosome 15—a situation equivalent to paternal deletion of that region.

Other Genetic Causes of Short Stature

A variety of other syndromes may be associated with moderate-profound growth failure. These include Bloom syndrome, de Lange syndrome, leprechaunism (mutations of the insulin receptor gene), Ellis-van Creveld syndrome, Aarskog syndrome, Rubinstein-Taybi syndrome, Mulibrey nanism (Perheentupa syndrome), Dubowitz syndrome, and Johanson-Blizzard syndrome. The syndromes discussed in this section are described in greater detail in Online Mendelian Inheritance in Man (OMIM), a National Institutes of Health–supported regularly updated website that publishes information on genetic conditions (www.ncbi.nim.nih.gov/omim).

Placental insufficiency and maternal factors may also contribute to poor fetal growth. Whereas such infants have a better growth potential than do cases of "primordial growth failure," postnatal growth is not always normal. Maternal nutrition is an important contributor to fetal growth, impacting not only the size of the fetus but growth during the first year of life.[380] Fetal growth retardation may also result from alcohol consumption during pregnancy,[381-383] as well as from use of cocaine,[384,385] marijuana,[385] and tobacco.[386] The mechanisms for such drug-induced fetal growth retardation are unclear but probably include uterine vasoconstriction and vascular insufficiency, as well as placental abruption and premature rupture of membranes. Although maternal tobacco use is statistically a major contributor to reduced fetal size, it is unlikely by itself to result in severe IUGR, although it has been shown to impact on the GH-IGF-1 axis.[387]

The implications of IUGR may extend beyond decreased fetal size. In a retrospective study of 47 children evaluated before puberty for growth failure secondary to IUGR, 23 boys had an adult height of 162 cm and 24 girls an adult height of only 148 cm[388] in the United States and Europe. Being SGA and failing to catch up are indications for GH therapy. More recent studies have indicated that small-for-gestational-age infants have an increased risk of hypertension, maturity-onset diabetes mellitus, and cardiovascular disease.[389] It remains unclear, however, whether IUGR is causally related to these disorders or is a symptom of an underlying inborn metabolic disorder.

Regulation of skeletal growth involves multiple factors, including hormones and growth factors, nutrition, general health, and a wide variety of other environmental factors. Even when evaluating hereditary aspects of skeletal growth, it is clear that control of growth in childhood (as well as final height) is polygenic by nature. Numerous genome-wide association studies have been done in an attempt to explain the genetic basis of growth, but to date they explain only 2.9% to 3.7% of the variance in adult height. Nevertheless, a direct impact of familial height on an individual subject's growth is normally evident, and evaluation of a child's growth pattern must be placed in the context of familial growth and stature. As described previously, formulas have been developed for determination of a person's target height based on parental heights—and growth curves that relate a child's height to parental height are available.[390] As a general rule, a child who is growing at a rate that is clearly inconsistent with that of siblings or parents warrants further evaluation.

Many organic diseases characterized by growth retardation are genetically transmitted. This list includes many endocrine causes, such as GHI resulting from mutations of the GHR gene, GH-1 gene mutations and deletions, mutations of the *PROP1* and POU1F1 gene, pseudohypoparathyroidism, and familial thyroid hormone deficiency. Many other nonendocrine diseases characterized by short stature may be genetically transmitted, such as osteochondrodysplasias, dysmorphic syndromes associated with IUGR, diabetes mellitus, metabolic disorders, renal disease, thalassemia, and others. Identifying short stature as genetically transmitted thus does not by itself relieve the physician of responsibility for determining the underlying cause of growth failure.

Secondary Growth Disorders

Malnutrition

Given the worldwide presence of undernutrition, it is not surprising that inadequate caloric or protein intake represents by far the most common cause of growth failure.[391] Marasmus refers to cases with a global deficiency of calories, although often accompanied by protein insufficiency. Kwashiorkor, on the other hand, refers to inadequate protein intake—although it may also be characterized by caloric undernutrition. Frequently, the two conditions overlap.

The impaired growth characteristic of protein-energy malnutrition is frequently characterized by elevated basal or stimulated serum GH concentrations.[392] In generalized malnutrition (marasmus), however, GH concentrations may be normal or even low.[393] In both

conditions, nevertheless, serum IGF-1 concentrations are typically reduced.[394,395] Malnutrition may, consequently, be considered a form of GHI in cases in which serum IGF-1 concentrations are reduced in the presence of normal or elevated GH levels. It has been suggested that elevated serum GH concentrations represent an adaptive response whereby protein is spared by the lipolytic and anti-insulin actions of GH. Reduced serum IGF-1 concentrations may represent a mechanism by which precious calories are shifted from use in growth to survival requirements of the organism. These adaptive mechanisms may be further enacted by changes in serum IGFBPs during periods of malnutrition.[396]

Inadequate caloric or protein intake may also complicate many chronic diseases characterized by growth failure. Anorexia is a common feature of renal failure and inflammatory bowel disease but may also be associated with cyanotic heart disease, congestive heart failure, central nervous system (CNS) disease, and other illnesses. Some of these conditions may, furthermore, be characterized by deficiencies of specific dietary components—such as zinc, iron, and various vitamins necessary for normal growth and development.

Undernutrition may also be voluntary, as is the case with dieting and food fads.[397] Caloric restriction is especially common in girls (such as among gymnasts and ballet dancers) during adolescence, when it may be associated with anxiety concerning obesity. Anorexia nervosa and bulimia represent extremes of "voluntary" caloric deprivation and are commonly associated with impaired growth if undernutrition occurs before epiphyseal fusion. Even later in adolescence, these conditions may be characterized by delayed puberty or menarche and a variety of metabolic alterations.

Chronic Diseases

Malabsorption. Intestinal disorders associated with inadequate absorption of calories or protein are typically associated with growth failure for many of the reasons cited previously.[398-401] It is not uncommon for growth retardation to predate many of the other manifestations of malabsorption or chronic inflammatory bowel disease. Accordingly, such conditions—especially gluten-induced enteropathy (celiac disease) and regional enteritis (Crohn disease)—must be in the differential diagnosis of unexplained growth failure. Serum concentrations of IGF-1 may be reduced,[402] reflecting the malnutrition. It is therefore all the more critical to discriminate these conditions from GHD or related disorders.

Documentation of malabsorption requires demonstration of fecal wasting of calories, especially fecal fat. The diagnosis of celiac disease ultimately requires a biopsy of the small intestine and demonstration of the characteristic flattening of the mucosa. Normalization of jejunal mucosa after gluten withdrawal and reappearance of abnormalities on gluten challenge are necessary to confirm the diagnosis. The use of antigliadin autoantibodies has been disappointing in the diagnosis of celiac disease because of its low specificity, but tissue transglutaminase antibodies are very useful.

On the other hand, whereas some have recommended jejunal biopsy to rule out celiac disease in all cases of unexplained growth failure during the first 5 years of life, this aggressive an approach is not usually necessary.[403] Generally, an alternative would be to reserve biopsies for children with a history of diarrhea or steatorrhea in the first 2 years of life, abnormal D-xylose absorption tests, and positive transglutaminase antibodies.[404] The growth failure characteristic of Crohn's disease probably represents a combination of malabsorption, anorexia, chronic inflammation, inadequacy of trace minerals, and use of glucocorticoids.[405] As stated previously, growth retardation may precede other clinical manifestations such as fever, abdominal pain, and diarrhea. An elevated erythrocyte sedimentation rate is a useful clue, although diagnosis ultimately requires endoscopy and biopsy.

Cardiovascular Disease. Cyanotic heart disease and congestive heart failure may be associated with growth failure.[406,407] Because cardiac defects are usually congenital, many infants have syndromes associated with dysmorphic features and IUGR. Postnatal growth failure is usually attributable to hypoxia and the increased energy demands of a failing heart. These conditions are often accompanied by feeding difficulties that exacerbate the poor growth.

Corrective surgery often results in restoration of normal growth, frequently with a phase of "catch-up" growth. Unfortunately, surgery must on occasion be delayed until the infant has reached an appropriate size—resulting in the conundrum that surgery corrects growth failure but cannot be performed because the infant is too small. In these situations, meticulous attention to caloric support and alleviation of hypoxia and heart failure is necessary to maximize growth before surgery.

Renal Disease. A wide variety of clinical conditions that affect renal function can result in significant growth retardation.[408,409] Uremia, Fanconi syndrome, and renal tubular acidosis can all lead to growth failure before other clinical manifestations become evident. It is probable that renal disease leads to growth retardation through multiple mechanisms, including decreased caloric intake, loss of electrolytes necessary for normal growth, metabolic acidosis, protein wasting, inadequate formation of 1,25-dihydroxycholecalciferol, insulin resistance, chronic anemia, and compromised cardiac function.

Although earlier studies suggested a decrease in serum IGF concentrations in uremia, it is now evident that these erroneous determinations reflected inadequate separation of IGF peptides from IGFBPs before assay.[215] Serum IGF-1 and IGF-2 concentrations are in general within normal limits, but increases in serum IGFBPs (especially IGFBP-1) may lead to inhibition of IGF action. The use of chronic glucocorticoid therapy in the treatment of a variety of nephritic and nephrotic conditions can exacerbate the growth retardation characteristic of renal disease.

The age at onset of renal dysfunction is a factor in the resultant growth failure. Impairment of renal function at an early age typically results in a greater degree of growth

failure, probably owing at least in part to the cumulative effects of growth retardation over many years. Subsequent correction of renal failure does not always allow for full catch-up growth. In a study in which renal transplantation was performed before 15 years of age (with a mean age at onset of hemodialysis of 10.6 years and at initial transplantation of 11.8 years), height SD scores did not significantly improve.[410] In patients with renal disorders, approximately 75% of subjects had adult heights below the third percentile.

Although GH or IGF deficiency does not cause the growth failure of renal disease, GH therapy has proven useful in accelerating skeletal growth. It is likely that such treatment increases the molar ratio of IGF peptides to IGFBPs, potentially overriding the inhibitory actions of IGFBPs.

Hematologic Disorders. Chronic anemias, such as sickle cell disease, are characterized by growth failure.[411-413] In these disorders, the causes of growth retardation probably include impaired oxygen delivery to tissues, increased work of the cardiovascular system, energy demands of increased hematopoiesis, and impaired nutrition. Thalassemia, in addition to the consequences of chronic anemia, can also be characterized by endocrine deficiencies resulting from chronic transfusions and accompanying hemosiderosis.[414,415] Despite vigorous efforts to maintain hemoglobin near normal and to employ chelation therapy, growth failure has remained a common feature of thalassemia—especially in adolescents. It is likely that impaired IGF-1 synthesis,[416,417] hypothyroidism, gonadal failure, and hypogonadotropic hypogonadism—combined with chronic anemia—all contribute to growth failure.

Diabetes Mellitus. Growth failure can be observed in children whose diabetes is under chronically poor control.[94] The so-called Mauriac syndrome[418] describes children with diabetes mellitus, severe growth failure, and hepatomegaly resulting from excess hepatic glycogen deposition. This type of striking growth retardation is unusual in diabetes, and in general growth failure is modest. As with other chronic diseases, growth retardation probably represents a combination of pathophysiologic processes—such as calorie wasting from hyperglycemia or malabsorption secondary to celiac sprue, chronic acidosis, increased glucocorticoid production, hypothyroidism, and delayed puberty. Modern treatment modalities, when available and applied, have markedly reduced the prevalence of delayed puberty and poor growth in children with diabetes mellitus.

Because IGFBP-1 is normally suppressed by insulin, chronic hypoinsulinemia results in elevated serum IGFBP-1 concentrations—which may inhibit IGF action. In addition, insulin regulates the expression of the GHR—and hypoinsulinemia commonly leads to low IGF-1 levels through this mechanism.[419] Nevertheless, the correlation between glycemic control and skeletal growth is surprisingly unreliable—and many children with apparently marginal control appear to grow well.[420] One can only surmise that such patients are able to attain normal intracellular nutrition despite seeming hypoinsulinemia. It is likely, however, that progress in improving glycemic control in diabetes will improve growth in these children.

Inborn Errors of Metabolism. Inborn errors of protein, carbohydrate, and lipid metabolism are often accompanied by growth failure—which can be pronounced. Glycogen storage disease, the mucopolysaccharidoses, glycoproteinoses, and mucolipidoses may all be characterized by poor growth. Many inborn metabolic disorders are also associated with significant skeletal dysplasia.

Pulmonary Disease. Cystic fibrosis is the classic example of growth failure associated with pulmonary disease, although poor growth undoubtedly represents the combined effects of pulmonary and pancreatic dysfunction.[238] The Cystic Fibrosis Foundation reports that 18% of patients with cystic fibrosis fall below the 5th percentile in height, and 23% fall below the 5th percentile in weight. In addition, the appearance of diabetes, the use of steroids, and the presence of frequent infections all contribute to the poor growth cystic fibrosis. Any condition associated with chronic hypoxemia may result in growth retardation. In children with chronic asthma, the long-term use of glucocorticoids undoubtedly contributes significantly to growth failure. Children with asthma often have associated growth delay with catch-up during puberty.

Chronic Infection. In many developing countries, chronic infestation with intestinal and systemic parasites (such as schistosomiasis, hookworm, and roundworm) contributes to nutritional debilitation and growth failure.[421]

Endocrine Disorders

Hypothyroidism. Many of the clinical features characteristic of adult myxedema are lacking in pediatric patients with acquired hypothyroidism. The most common and prominent manifestation of chronic acquired hypothyroidism is growth failure, which may be profound.[422] Postnatal growth retardation may also be observed in the infant with congenital hypothyroidism, but the development of newborn screening programs for hypothyroidism have generally resulted in prompt diagnosis and treatment of such patients. In acquired hypothyroidism, growth retardation may take several years to become clinically evident. However, once present growth failure is typically severe and progressive.

Rivkees and associates[422] have reported a mean 4.2-year delay between documentation of growth deceleration and the diagnosis of hypothyroidism. At diagnosis, girls were 4.04 ± 0.5 SD and boys 3.15 ± 0.4S D below the mean heights for age, respectively. (This is one of several situations in which the diagnosis of short stature is more delayed in girls than in boys.) Although chronic hypothyroidism is generally characterized by delayed puberty, precocious puberty and even premature menarche can occur in hypothyroid children—an entity called Van Wyk–Grumbach syndrome. However, in males with this clinical presentation, the testes may be enlarged with relatively minimal virilization, possibly due

to a predominant effect on the FSH receptor. In some females with severe primary hypothyroidism, large recurrent ovarian cysts may manifest.[423] Skeletal age is usually markedly delayed in both sexes. High serum TSH (with prepubertal LH concentrations) is consistent with Van Wyk–Grumbach syndrome, in which the high TSH may act directly on the FSH receptor to mediate the precocious puberty.

Confirmation of the diagnosis of primary hypothyroidism is usually straightforward. Serum concentrations of T4 are reduced, and TSH concentrations are elevated. The presence of antithyroid antibodies is consistent with a diagnosis of Hashimoto thyroiditis, the most common cause of acquired hypothyroidism in the United States. Isolated secondary and tertiary hypothyroidism, caused by TSH and TRH deficiency (respectively), are very rare causes of acquired hypothyroidism. Mutations in the genes encoding TSHβ or the TRH receptor have been identified in a proportion of cases.

Replacement therapy with levothyroxine is associated with a period of rapid catch-up growth. Despite this gratifying response, however, accelerated growth often does not result in restoration of full growth potential—largely because of the rapid increase in skeletal age during the first 18 months of treatment, often with rapid progression through puberty. In the study of Rivkees and associates,[422] children treated at an initial mean chronologic age of 11 years had adult heights approximately 2 SD below the means for sex. These final heights were significantly lower than midparental heights or initial predicted adult heights based on data of Bayley and Pinneau. The deficit in adult stature correlated significantly with the duration of hypothyroidism before initiation of treatment. Accordingly, it may be appropriate to use lower than usual replacement dosages of levothyroxine or to consider delaying puberty and epiphyseal fusion pharmacologically.

Cushing Syndrome. Glucocorticoid excess has a profound effect on skeletal growth[423,424] whether the cause of Cushing syndrome is hypersecretion of ACTH, a primary adrenal tumor, or glucocorticoid therapy. It is assumed that the effects of glucocorticoids are directly at the epiphysis because GH secretion is typically normal and serum concentrations of IGF peptides and IGFBPs are not generally affected. This is supported by the observation that treatment with GH cannot completely overcome the growth inhibiting effects of excess glucocorticoids. The "toxic" actions of glucocorticoids on the epiphysis often persist at least in part after termination of chronic glucocorticoid excess, and patients frequently do not attain their target heights.[425]

The longer the duration and the greater the intensity of glucocorticoid excess, the less likely the patient will experience complete catch-up growth. It is therefore important to limit exposure to excess glucocorticoids as much as the underlying condition being treated will allow. This may in part be accomplished by the use of alternate-day steroid therapy. The characteristic signs of Cushing syndrome (such as truncal obesity, decreased muscle mass, striae, easy bruising, thin skin, hypertension, and osteoporosis) are well known. Adrenal tumors secreting large amounts of glucocorticoids frequently also produce excess androgens, which may mask the growth inhibitory effects of glucocorticoids, but also advance epiphyseal fusion.

It is also important to note that Cushing syndrome in children may be lacking many of the clinical signs and symptoms associated with the disorder in adults and may present exclusively with growth arrest. On the other hand, Cushing syndrome is an unlikely diagnosis in children presenting with obesity because exogenous obesity is associated with normal or even accelerated skeletal growth—whereas in Cushing syndrome growth deceleration is generally evident by the time other signs appear.

Pseudohypoparathyroidism. This condition is discussed in more detail elsewhere, but it is included here because at time of presentation growth failure is a frequent feature.[426] In its classic form, this condition combines growth failure and characteristic dysmorphic features with hypocalcemia and hyperphosphatemia secondary to end organ resistance to parathyroid hormone (PTH). Children with pseudohypoparathyroidism are short and truncally obese, with short metacarpals, subcutaneous calcifications, round facies, and mental retardation. Resistance to TSH with ensuing mild hypothyroidism may compound the suboptimal growth.

Rickets. Hypovitaminosis D is historically a major cause of abnormal skeletal growth and short stature. Often it is associated with other causes of growth failure, such as malnutrition, prematurity, malabsorption, hepatic disease, and chronic renal failure. When vitamin D deficiency occurs by itself, typically when infants have poor exposure to sunlight and are not being nutritionally supplemented with vitamin D, the characteristic skeletal manifestations of rickets are evident: frontal bossing, craniotabes, rachitic rosary, and bowing of the legs.

Vitamin D–Resistant (Hypophosphatemic) Rickets. This condition results from decreased renal tubular reabsorption of phosphate. Clinical features are generally more severe in males and include short stature and prominent bowing of the legs, but other rachitic signs may be present.[427] The metabolic and skeletal abnormalities cannot be overcome by vitamin D therapy alone, thus the name "vitamin D–resistant rickets." Treatment requires oral phosphate replacement, but such therapy often results in poor calcium absorption from the intestine. The addition of vitamin D, especially 1,25 (OH)2 VitD (calcitriol), to oral phosphate increases intestinal phosphate absorption and prevents hypocalcemia. Preliminary studies with GH therapy have indicated (at least in the short term) an enhancement of skeletal growth.

IGF Deficiency. Thyroid hormone and IGF-1 appear to be the major mediators of skeletal growth. Studies involving targeted disruption of genes for various components of the IGF system have established the critical role of the IGF axis in prenatal and postnatal growth.[428] Given the critical role of the IGFs in both intrauterine and postnatal growth, in 1996 Rosenfeld proposed that a critical factor in the diagnostic evaluation for a child with

growth failure was the identification of "IGF deficiency." Deficiency of IGF-1 could result from hypothalamic dysfunction, pituitary GHD, or primary or secondary GHI. It is not always possible to completely discriminate between hypothalamic and pituitary dysfunction because both organs may be involved in the same pathologic process. In addition, embryonic development of the hypothalamus and pituitary appears to be codependent.[30-34]

A number of factors produced in the developing ventral diencephalon function as molecular signals for initial formation and development of Rathke's pouch. Subsequent differentiation of each of the various anterior pituitary cell types appears to be primarily regulated by a strict temporal and spatial pattern of pituitary transcription factors.

It thus becomes somewhat of a semantic issue as to whether to label some of the molecular defects "hypothalamic" or "pituitary." Nevertheless, some arbitrary classification decisions have been made. Table 10-3 presents our current classification of molecular defects of the GH-IGF axis, and sites of established and hypothetical defects are shown in Figure 10-22. Interestingly, many of the "hypothetical" molecular defects proposed in 1996 have subsequently been identified, such as abnormalities of GH signal transduction, IGF synthesis, IGF transport and IGF receptors. Figure 10-23 provides a "decision tree" for investigation of genetic defects in patients with IGF deficiency. Significant developments are likely in our understanding of these defects so that this classification will require updating and modification.

Hypothalamic Dysfunction. Hypothalamic dysfunction can arise from congenital malformations of the brain or hypothalamus, trauma, infections, sarcoid, tumors, or cranial irradiation. Anencephaly results in a pituitary gland that is small or abnormally formed and frequently ectopic. Holoprosencephaly, resulting from abnormal midline development of the embryonic brain, is also typically associated with hypothalamic insufficiency.[429,430] The clinical spectrum of holoprosencephaly can range from cyclopia to hypertelorism, accompanied by absence of the philtrum or nasal septum and midline clefts of the palate or lip. In these situations, the classic endocrine feature is diabetes insipidus, often accompanied by GH, TSH and ACTH deficiencies.

Debate continues as to whether the incidence of GHD is increased in cases of simple clefts of the lip or palate

TABLE 10-3 **Established Genetic Defects of the GH-IGF Axis Resulting in IGF Deficiency**

Mutant Gene	Inheritance	Phenotype
HPA		Developmental abnormalities
HESX1	AR	Septo-optic dysplasia; variable involvement of pituitary hormones
PROP1	AR	GH, PRL, TSH, LH, FSH deficiencies; variable ACTH deficiency
POU1F1 (Pit1)	AR, AD	GH, PRL deficiency; variable degree of TSH deficiency
RIEG	AD	Rieger syndrome
LHX3	AR	GH, TSH, LH, FSH, prolactin deficiencies
LHX4	AD	GH, TSH, ACTH deficiencies
SOX3	XL	GH deficiency, mental retardation
GLI2	AD	Holoprosencephaly, hypopituitarism
GLI3	AD	Pallister-Hall syndrome, hypopituitarism
Isolated GHD		
GHRHR	AR	IGHD, type IB form of IGHD
GHS-R	AD	GHD and ISS
GH1	AR	Type IA form of IGHD
	AR	Type IB form IGHD
	AD	Type II form of IGHD
	X-linked	Type III form of IGHD; hypogammaglobulinemia
	AD	Bioinactive GH molecule
GHI		
GHR		
Extracellular domain	AR, AD	IGF-I deficiency; decreased or normal GHBP
Transmembrane	AR	IGF-I deficiency; normal or increased GHBP
Intracellular domain	AD	IGF-I deficiency; normal or increased GHBP
IGF		
IGF1 (see also Box 10-4)	AR	IGF-I deficiency; IUGR and postnatal growth failure
STAT 5 b (see also Table 10-5)	AR	IGF-I deficiency, variable immune defect, hyperprolactinemia, chronic pulmonary infections, recurrent eczema
ALS (see also Table 10-6)	AR	IGF-I deficiency; variable postnatal growth failure, delayed puberty

HPA, hypothalamic pituitary; ACTH, adrenocorticotropic hormone (corticotropin); AD, autosomal dominant; AR, autosomal recessive; FSH, follicle-stimulating hormone; GH, growth hormone; GHBP, GH-binding protein; GHRHR, GH-releasing hormone receptor; IGF, insulin-like growth factor; IGHD, isolated GHD; IUGR, intrauterine growth retardation; LH, luteinizing hormone; PRL, prolactin; TSH, thyroid-stimulating hormone; and ALS, acid labile subunit.

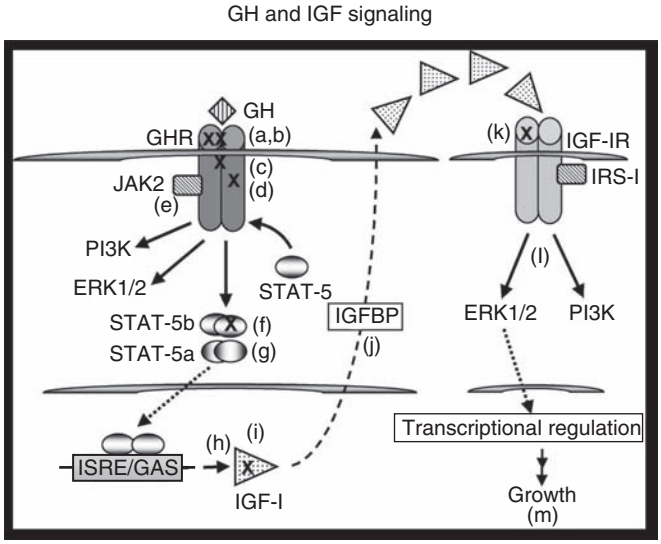


FIGURE 10-22 ■ Schematic diagram of the GH–IGF axis, showing identified and theoretical defects: (*a*) defects of the extracellular domain of the GHR, affecting binding of GH; (*b*) defects in GHR dimerization; (*c*) defects of the transmembrane domain of the GHR; (*d*) defects of the intracellular domain of the GHR; (*e*) defects of JAK2 (theoretical at this time); (*f*) defects of STAT5b; (*g*) defects of STAT5a (theoretical at this time); (*h*) defects of transcriptional regulation of IGF-I (theoretical at this time); (*i*) defects of the IGF-I gene; (*j*) defects of IGFBPs, affecting IGF availability (theoretical at this time); (*k*) defects of the IGF receptor; (*l*) defects of IGF receptor signal transduction (theoretical at this time); and (*m*) defects at the epiphyseal growth plate, potentially affecting IGF action. GH, growth hormone; GHR, growth hormone receptor; JAK2, janus-family tyrosine kinase 2; PI3K, phosphatidylinositol-3 kinase; ERK, extracellular signal-regulated kinase; STAT, signal transducer and activator of transcription; ISRE, interferon-stimulated response element; GAS, interferon-gamma-activated sequences; IGF, insulin-like growth factor; IGFBP, IGF binding proteins; IGF-IR, IGF-I receptor; and IRS, insulin receptor substrate. (Used with permission from Rosenfeld, R. G., & Hwa, V. (2004). New molecular mechanisms of GH resistance. *Eur J Endocrinol, 151*, S11–S15.)

alone.[431,432] Clearly, children with clefts who are growing abnormally certainly require further evaluation, and both GHD and combined pituitary hormone deficiencies (CPHD) are more frequent in this cohort. Septo-optic dysplasia (SOD) is a rare congenital heterogeneous anomaly with a prevalence ranging from 6.3 to 10.9 per 100,000.[433,434] The condition is defined by presence of any two of three features: midline forebrain defects such as absence of the septum pellucidum or corpus callosum, optic nerve hypoplasia (ONH) and hypopituitarism due to hypothalamo-pituitary maldevelopment.[48,435] De Morsier, in 1956, described the postmortem findings of optic nerve hypoplasia (ONH) and agenesis of the septum pellucidum and coined the term "septo-optic dysplasia," also known as De Morsier's syndrome. Approximately 30% of patients with SOD manifest the complete clinical triad, 62% of patients have some degree of hypopituitarism, and 60% have an absent septum pellucidum.[436,437] The condition is equally prevalent in males and females. ONH may be unilateral or bilateral and may be the first presenting feature, with the later onset of endocrine dysfunction. Bilateral ONH is more common (88% as compared with 12% unilateral cases). Additionally, there appears to be little correlation between the size of the optic nerve and its visual function. Neuroradiologic abnormalities are present in from 75% to 80% of patients with ONH.[438,439] Pituitary hypoplasia may manifest as endocrine deficits varying from isolated GH deficiency to panhypopituitarism. There has been some suggestion that abnormalities of the septum pellucidum and hypothalamo-pituitary axis on neuroimaging can predict the severity of endocrine dysfunction.[439] A decrease in growth rate due to GH deficiency is the most common feature, with hypoglycemia and polyuria and polydipsia being less common. Either sexual precocity or failure to develop in puberty may occur. Abnormal hypothalamic neuroanatomy or function and diabetes insipidus may be features. The endocrinopathy may be evolving with a progressive loss of endocrine function over time. The most common endocrinopathy is GH deficiency followed by TSH and ACTH deficiency. Gonadotropin secretion may be retained in the face of other pituitary hormone deficiencies.[440] Neurologic deficit is common, but not invariably so, and in one study it was documented in 15 of 24 children with a severe degree of optic nerve hypoplasia. The deficit ranged from global retardation to focal deficits such as epilepsy or hemiparesis. Other neuroanatomic abnormalities include cavum septum pellucidum, cerebellar hypoplasia, schizencephaly, and aplasia of the fornix. An association between SOD and other congenital anomalies such as digital abnormalities is not uncommon.[441]

The etiology of SOD has remained largely unknown until recently. Both genetic and environmental factors have been implicated in the etiology of the condition.[442,443] Environmental agents such as viral infections, vascular or degenerative changes, and exposure to alcohol or drugs have been implicated in the etiology of SOD. The condition presents more commonly in children born to younger mothers and clusters in geographic areas with a high frequency of teenage pregnancies.[433,443] As forebrain and pituitary development occur as early as 3 to 6 weeks gestation in the human embryo, and are closely linked, any insult at this critical stage of development could account for the features of SOD. First indications of a genetic etiology came from the targeted mutagenesis of the transcription factor Hesx1 in mice. Hesx1 is a paired-like homeodomain transcriptional repressor that is expressed in the prospective cephalic region earlier during gastrulation. Later, it can be found throughout the prosencephalon—but it is restricted ultimately to Rathke's pouch. Its expression is switched off at E13.5 in the mouse.[444,445] The phenotype of these mice included a reduction in the prospective forebrain tissue, absence of developing optic vesicles, markedly decreased head size, and severe microphthalmia reminiscent of the syndrome of SOD in humans. Other abnormalities included absence of the optic cups, the olfactory placodes and Rathke's pouch, reduced telencephalic vesicles, hypothalamic abnormalities, and aberrant morphogenesis of Rathke's pouch. In 5% of null mutants, the phenotype was characterized by complete lack of the pituitary gland. In the majority of mutant mice, they were characterized by formation of multiple oral ectodermal invaginations and hence multiple pituitary glands. In the light of the phenotype demonstrated in *Hesx1* null mutant mice, the human homologue of the gene was screened for mutations in patients with

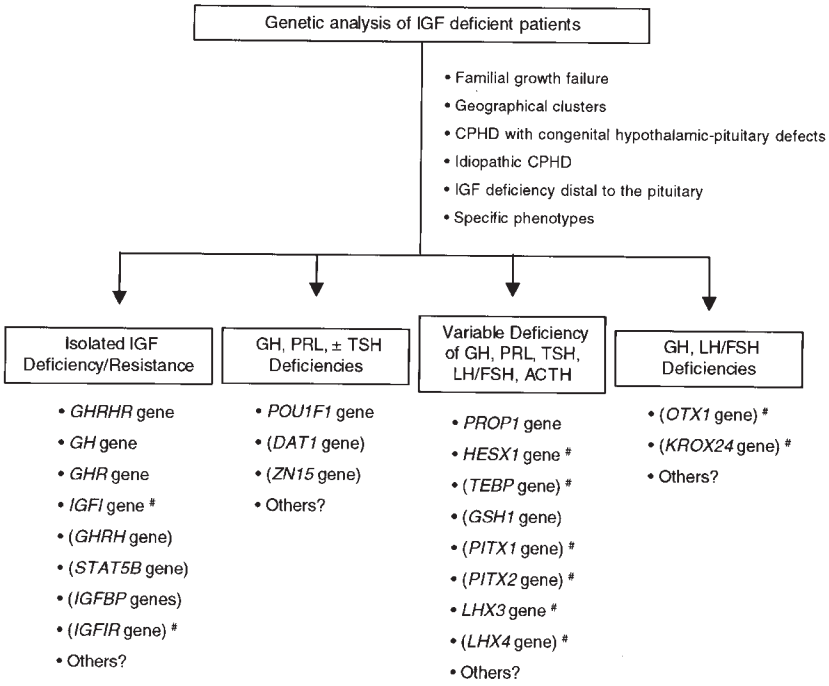


FIGURE 10-23 ■ Decision tree for investigation of genetic defects in patients with insulin-like growth factor (IGF) deficiency. Hypothetical genetic defects are presented in parentheses. Abnormalities in other organs and structures besides the hypothalamus-pituitary-IGF axis are expected to occur as a result of these genetic defects. ACTH, adrenocorticotropic hormone; CPHD, combined pituitary hormone deficiencies; FSH, follicle-stimulating hormone; GH, growth hormone; GHR, growth hormone receptor; GHRHR, GH-releasing hormone receptor; IGFIR,IGF-1 receptor; LH, luteinizing hormone; PRL, prolactin; STAT5, signal transducer and activator of transcription 5; TSH, thyroid-stimulating hormone. (From Lopez-Bermejo, A., Buckway, C. K., & Rosenfeld, R. G. (2000). Genetic defects of the growth hormone–insulin-like growth factor axis. *Trends Endocrinol, 11*, 43.)

SOD. A homozygous missense mutation (Arg160Cys) was found in the homeobox of *HESX1* in two siblings within a highly consanguineous family in which the affected siblings presented with optic nerve hypoplasia, absence of the corpus callosum, and hypoplasia of the anterior pituitary gland with panhypopituitarism.[361] The parents were heterozygous for the mutation and phenotypically normal. Screening of extended members of the family revealed another nine phenotypically normal heterozygotes within this highly consanguineous pedigree, consistent with an autosomal recessive inheritance. The mutation led to a complete loss of DNA binding. Subsequently, *HESX1* mutations have been identified in association with isolated growth hormone deficiency (IGHD) or combined pituitary hormone deficiency (CPHD), in addition to SOD. These patients exhibit variable magnetic resonance imaging (MRI) abnormalities ranging from a structurally normal pituitary to a more severe radiologic phenotype characterized by anterior pituitary hypoplasia or aplasia, an undescended or ectopic posterior pituitary, optic nerve hypoplasia, and agenesis of the corpus callosum.[446] Mutations in *HESX1* can result in hypopituitarism without midline defects or optic nerve anomalies, as the pituitary is believed to be more sensitive to Hesx1 dosage than the developing brain.[447] In addition to recessive mutations, dominant mutations in *HESX1* have been described. Haplotype analysis using markers that closely flanked *HESX1* revealed that such heterozygous *HESX1* mutations are associated with a dominant inheritance that is incompletely penetrant, although a de novo heterozygous insertion mutation has also been described. The phenotypes associated with heterozygous mutations are, on the whole, milder, classically characterized by isolated GHD with an ectopic/undescended posterior pituitary. The overall frequency of *HESX1* mutations in SOD has, however, been low, suggesting that mutations in other known or unknown genes may contribute to this complex disorder.[448,449]

Mutations in orthodenticle homeobox 2 (OTX2) have been identified in patients with SOD. Otx2 is a transcription factor whose role in hypothalamo-pituitary development remains largely unclear. Expression in mice is restricted to developing neural and sensory structures such as the brain, eyes, nose, and ears. Studies show that *Otx2* is expressed in the ventral diencephalon (VD) by E10.5 where it may affect *Fgf8* or *Bmp* induction of RP formation.[450] Its expression at the same time in RP suggests an intrinsic role in RP development as well, which is consistent with its proposed ability to activate *Hesx1* expression. By E12.5, *Otx2* mRNA expression is undetectable in RP (however, it is still detectable in the VD), although the protein persists through to E14.5. By E16.5, Otx2/*Otx2* is absent from both RP and the VD. Homozygous-null mutant mice, which die midgestation, exhibit severe malformations of the forebrain structures as well as the eyes due to impaired gastrulation. Heterozygous mice present with variable eye phenotypes ranging from normal to severe (anophthalmia/microphthalmia) and brain structural abnormalities (holoprosencephaly or anencephaly). Its potential role in pituitary development

was suggested in a report looking at *Prop1*-mutant mice.[450] In these animals, *Otx2* expression in RP persists through to E16.5, which is 4 days after peak expression of *Prop1*. This suggested that *Prop1* may regulate genetic expression of factors that may suppress *Otx2* expression and implies a role for *Otx2* in murine pituitary development. Further evidence of a role in hypothalamo-pituitary development was shown in GnRH-neuron-*Otx2* knockout mice that exhibited gonadotropic hypogonadism.[451] These data are consistent with human *OTX2* phenotypes, which can encompass highly variable hypopituitary phenotypes (ranging from isolated growth hormone deficiency to panhypopituitarism) and hypogonadotrophic hypogonadism, both of which are most commonly detected in association with severe eye abnormalities including anophthalmia or microphthalmia. To date, *OTX2* mutations, of which 20 have been described, account for 2% to 3% of severe eye abnormalities. Two complete deletions and 12 heterozygous intragenic mutations (of which 6 have been shown to be associated with functional compromise) have been associated with severe ocular and CNS phenotypes including developmental delay and seizures. The association of deletions of 14q22-23 (which also includes the candidate genes *BMP4*, *RTN1*, *SIX6*, *SIX1*, and *SIX4*) with anophthalmia, hypopituitarism, and ear abnormalities[452] originally led to the investigation of the role of OTX2 in hypothalamo-pituitary development. Subsequently, mutations were identified in association with eye defects (e.g., bilateral anophthalmia), CNS defects such as developmental delay and seizures, and variable hypopituitarism associated with a small anterior pituitary gland, an ectopic/undescended posterior pituitary, and an absent infundibulum.[453-455] Because Otx2 appears to regulate the expression of *Hesx1*, it is possible that mutations in the protein compromise the development of the forebrain and ventral diencephalon, in addition to any direct effect within RP.

The sonic hedgehog (SHH) signaling pathway has been implicated in more complex disorders of pituitary development. Mutations within SHH are associated with holoprosencephaly.[456] Three members of the Gli gene family of transcription factors have been implicated in the mediation of SHH signals, and heterozygous loss-of-function mutations within *GLI2* have been identified in patients with holoprosencephaly.[457] Phenotypic penetrance was variable, with the disorder transmitted through a parent carrying the mutation but showing no obvious phenotype in one family. In all affected individuals with *GLI2* mutations, pituitary gland function was abnormal, accompanied by variable craniofacial abnormalities. Other features included postaxial polydactyly, single nares, single central incisor, and partial agenesis of the corpus callosum. Mutations in *GLI2* have been associated with hypopituitarism in the absence of any midline defects.[458]

SOX2 and SOX3 are members of the SOX (SRY-related high mobility group [HMG] box) family of transcription factors and are early markers of progenitor cells; their expression is down-regulated as cells differentiate. Strong *SOX2* expression is detected within RP in human embryos (4.5 to 9 weeks of development), which is maintained throughout anterior pituitary development as well as in the overlying diencephalon. However, there is no *SOX2* expression in the infundibulum or posterior pituitary. Initially *SOX2* mutations had been associated with bilateral anophthalmia, severe microphthalmia, learning difficulties, esophageal atresia, and genital abnormalities. However, further phenotypic characterization has revealed the presence of anterior pituitary hypoplasia, hypogonadotrophic hypogonadism, and variable GHD, often with accompanying phenotypes such as hippocampal abnormalities, corpus callosum agenesis, esophageal atresia, hypothalamic hamartoma, and sensorineural hearing loss.[459,460] Although in the majority of patients the MRI reveals a small anterior pituitary, in occasional cases, the pituitary is enlarged and remains so for years.[461]

The definitive Rathke's pouch comprises proliferative progenitors that will gradually relocate ventrally, away from the lumen as they differentiate. A proliferative zone containing progenitors is maintained in the embryo in a periluminal area and was found to persist in the adult.[49] During pituitary development, SOX2 is expressed in the early ectoderm and maintained throughout the pouch. Its expression is down-regulated as endocrine cell differentiation proceeds. Expression is maintained in the prospective progenitor proliferative zone, around the Rathke's pouch lumen, during embryogenesis but also in the mature gland.[49] It is also expressed in the VD.

Normal hypothalamo-pituitary development is critically dependent on the dosage of SOX3; over- or underdosage can lead to hypopituitarism or infundibular hypoplasia. *Sox3* null mutant mice have a variable phenotype showing poor growth, craniofacial defects, and variable endocrine deficits. In humans, *SOX3* duplications or mutations are associated with variable phenotypes including either IGHD or panhypopituitarism. There is variable developmental delay, and MRI usually reveals a small anterior pituitary, an ectopic/undescended posterior pituitary, and dysgenesis of the corpus callosum.[462,463] A *SOX3* variant that leads to a deletion within a polyalanine tract has been associated with hypopituitarism in a heterozygous female patient; the variant has been shown to be associated with a gain in function as opposed to the previously described polyalanine expansions that led to loss of function.[464]

Molecular Defects of GHRH or the GHRH Receptor. No cases of mutations of the GHRH gene in humans have been identified,[465] a somewhat surprising observation, and the GHRH gene must still be considered a candidate gene for familial forms of isolated GHD. Abnormalities of the gene for the GHRH receptor (GHRHR), on the other hand, have been found in number of pedigrees.[466-469] The GHRH receptor consists of a 423-amino-acid peptide, which contains an N-terminal extracellular domain, seven transmembrane domains, and an intracellular C-terminal domain. *GHRHR* is a 13-exon gene located on chromosome 7p14, and its expression requires the presence of the pituitary-specific transcription factor 1 (Pou1F1). The implication of growth-hormone-releasing hormone receptor in the etiology of isolated growth hormone deficiency stemmed from observations in a spontaneously occurring dwarf mouse model (termed the "*little* mouse") that has a homozygous missense mutation (Asp60Gly) in

the extracellular domain of the receptor gene. Although the development of the anterior pituitary is not affected in the *little* mouse, it shows severe anterior pituitary hypoplasia, with an almost 10-fold decrease in the number of somatotrophs and pituitary growth hormone content. In 1996, Wajnrajch and colleagues reported on two patients of a consanguineous pedigree who had severe isolated growth hormone deficiency resulting from a homozygous mutation in the *GHRHR* that caused premature termination of translation (Glu72X, where X represents a stop codon after amino acid 72); this would result in a truncated protein without transmembrane and intracellular domains.[466] An apparently identical mutation has been identified in a family from Sri Lanka[468] and in 17 members of an inbred kindred in Sindh (Pakistan).[467] Since then, homozygous or compound heterozygous mutations have been reported in *GHRHR* (missense, nonsense, splice site, deletions or regulatory mutations), leading to type IB isolated growth hormone deficiency. The largest kindred with a mutation of GHRHR has been identified in Brazil.[469] A donor splice mutation in position 1 of exon 1 results in a severely truncated GHRHR protein.

Missense mutations seem to predominantly affect ligand binding, but not cell surface expression of the receptor[470] whereas nonsense and splice site mutations result in truncated, nonfunctioning receptor products.

GHRHR mutations are identified in about 10% of patients with familial recessive isolated growth hormone deficiency,[471] and in about 3% of a selected cohort of patients with isolated growth hormone deficiency.[472] Patients are of consanguineous pedigrees or from certain ethnic backgrounds, such as from the Indian subcontinent and Brazil. Nevertheless, compound heterozygous *GHRHR* mutations have been described in patients from nonconsanguineous families. Children with mutations in *GHRHR* have severe growth hormone deficiency and short stature, but midfacial hypoplasia, neonatal hypoglycemia, and microphallus, as found in those with recessive *GH1* mutations, are rare. The anterior pituitary may rarely be normal; in the majority, it is hypoplastic. The posterior pituitary and the pituitary stalk are within the normal range.

Trauma of the Brain or Hypothalamus. Traumatic brain injury (TBI) has been recognized as a cause of acquired hypopituitarism in a number of adult studies. Data on pediatric patients are still sporadic, but there is a growing awareness that hypopituitarism after TBI is underdiagnosed with possible negative effects on growth and development.[473] Although the pituitary gland is protected within the sella turcica, the rich vascular network of the hypothalamus and pituitary and the structure of the pituitary stalk make it vulnerable to the effects of traumatic brain injury. The hypothalamus and pituitary have a complex vascular supply consisting of long hypophyseal vessels and a rich network of portal capillaries that surround the pituitary and infundibulum. The pathophysiology of hypopituitarism related to TBI is not clearly defined, but it is thought that it is the result of direct trauma or of vascular injury resulting in ischemia and infarction.[474,475] This is supported by the anatomic findings of autopsies following head trauma, which include anterior lobe necrosis, pituitary fibrosis, hemorrhage, infarction, or necrosis of the pituitary stalk.[476]

It is of note that the peripheral layer of anterior pituitary cells, under the capsule, receives arterial blood from the capsule and not from the system of portal veins, and this may explain why these cells and those in a small area adjacent to the posterior lobe are the only surviving cells in cases of pure anterior lobe necrosis.[477] Somatotrope cells are located in the wings of the pituitary gland, their vascular supply comes from portal vessels, and they are vulnerable to the disruption of blood supply after head injury. On the other hand, ACTH and TSH secreting cells are located in the medial portion of the pituitary and receive blood supply from portal vessels and the anterior pituitary artery. This may explain why GHD is the most common deficiency seen after TBI.[478] Hormone deficiencies may be identified in the first days to weeks post trauma (acute phase) or may develop over time (late effect). As there is overlap between the symptoms and signs of hypopituitarism and those of neurologic-psychological sequelae of TBI, it is possible that late evolving or partial deficiencies can remain undiagnosed for a long period (in different studies the time to diagnosis ranges from 1 to 40 years).

In the acute phase alterations in endocrine function may reflect an adaptive response to acute illness. The clinically significant alterations involve mainly the regulation of fluid and electrolyte balance (diabetes insipidus, syndrome of inappropriate antidiuretic hormone secretion [SIADH], cerebral salt wasting) and the hypothalamo-pituitary adrenal axis. Most of the pituitary hormone changes observed in the acute phase are transient, and their development cannot predict the development of permanent hypopituitarism.[479] It has been suggested that trauma severity may be the only predictor of permanent hypopituitarism,[480] although not all studies have arrived at this conclusion. It would appear that the majority of patients show a degree of pituitary hormone dysfunction in the first days after TBI (53% to 76%); there is, however, wide variation in the reported hormone responses, which reflects the differences in patient selection and time of testing.[481] All anterior pituitary hormones can be affected. Pituitary hormone deficiencies present in the acute phase are usually transient, but they may persist or appear and evolve over time. In adults, the incidence of permanent hypopituitarism ranges between 23% and 69% depending on the study. The growth hormone axis is the most frequently affected (10% to 33%), followed by the gonadal (8% to 23%), adrenal (5% to 23%), and thyroid (2% to 22%) axes. The prevalence of permanent DI varies between 0% and 6%.[482-485]

There have been only sporadic reports of hypopituitarism following TBI in children, but prospective studies designed to address the problem in the pediatric and adolescent population are in progress. The incidence of hypopituitarism is reported to range from 10% to 60%, and although this is lower in children as compared with adults, it is not uncommon.[486-488] In general, the long-term outcome of TBI seems to be more favorable in children. Growth hormone deficiency appears to be the

main endocrine manifestation, followed by gonadotropin deficiency. GHD can present as growth failure, whereas delayed or arrested puberty and secondary amenorrhea may present in adolescents and in patients in the transition phase. Hypopituitarism may contribute to the lack of energy, fatigability, and reduced bone mineral density that can occur after severe head trauma.[489] In a number of case reports, central precocious puberty has also been described in association with head injury presenting 0.4 to 1.6 years after the event.[490] Patients with hypopituitarism after head injury may have no clinical signs and symptoms suggestive of this disorder, and prompt diagnosis requires a high degree of suspicion. A consensus guideline on the screening of patients post TBI suggests that all patients who had TBI, regardless of its severity, should undergo baseline endocrine evaluation 3 and 12 months after the event or discharge from hospital.[491] For children and adolescents, an algorithm for endocrine assessment and follow-up has also been suggested.[473] However, one study reported that permanent hypopituitarism is uncommon after severe brain injury in young children when stringent clinical criteria for pituitary insufficiency are used. Routine testing of pituitary function, as suggested for adults, may not be necessary in children and will lead to a high number of abnormal test results.

Perinatal trauma to the brain, hypothalamus, pituitary stalk, or anterior pituitary may result in isolated GHD or in multiple deficiencies of the anterior pituitary, as may child abuse.[492,493] Many published series of patients with GHD indicate an increased incidence of birth trauma, such as breech deliveries, extensive use of forceps, prolonged labor, or abrupt delivery.[493] Debate continues as to whether GHD is the consequence of difficult delivery or merely reflects the perinatal consequences of fetal pituitary insufficiency.

Inflammation of the Brain or Hypothalamus. Inflammation of the brain (resulting from bacterial, viral, or fungal infections) may result in hypothalamic/pituitary insufficiency.[494,495] Hypothalamo-pituitary dysfunction has been reported after infectious diseases of the central nervous system (i.e., meningitis or encephalitis), with most cases being reported as isolated case reports. Despite the bias in the selection of cases, one can assume that the incidence of endocrine deficiencies depends on the virulence of the infectious organism, the severity and localization of the disease, and the immune status of the host. In a study of 19 adult patients who were investigated 10 to 56 months following CNS infection, 21% had ACTH deficiency and 11% had gonadotropin deficiency, whereas no GHD or DI was reported.[496] Hypopituitarism has been reported following infection by a variety of agents, including group B streptococcus, *Haemophilus influenza*, and *Mycobacterium tuberculosis*. The hypothalamus or pituitary may also be involved in sarcoidosis.[497] Sarcoidosis is a multisystem granulomatous disease of unknown etiology that clinically affects the central nervous system in 5% to 10% of cases.[498] The effects of sarcoidosis on the hypothalamo-pituitary axis are the result of infiltration by granulomatous tissue: on MR imaging the lesion may infiltrate the hypothalamus and pituitary, enhances with gadolinium, and there is thickening of the pituitary stalk. The most frequently reported endocrine abnormality is diabetes insipidus, reported in 25% to 50% of patients with neurosarcoidosis.[499,500] This is followed by hyperprolactinemia, although anterior pituitary dysfunction with hypogonadism has also been reported.[501] Sarcoidosis of the nervous system has a poor prognosis but long-term remissions have been reported with high-dose intravenous pulse methyl-prednisolone therapy. Hormonal defects of less than 1-year duration may respond to steroid treatment, but longer-standing deficits usually persist.[502]

Hypophysitis is an inflammation of the pituitary gland that can be either primary or secondary resulting from infection, systemic disease, or irritation from adjacent lesions. This inflammatory process mimics, clinically and radiologically, tumors of the pituitary area. There are three histologic types of primary hypophysitis: lymphocytic, granulomatous, and xanthomatous. Lymphocytic hypophysitis is the most common type; it involves the anterior pituitary and may infiltrate the infundibulum and posterior lobe. Lymphocytic hypophysitis occurs mainly in young women and is associated with pregnancy or the presence of autoimmune diseases including Hashimoto's thyroiditis, Graves' disease, type I diabetes, and SLE.[503] There are rare case reports of hypophysitis in children and adolescents, and in most cases diagnosis has been made only after biopsy and histologic examination. In many cases, hypophysitis presented with DI and hypopituitarism and preceded the diagnosis of an intracranial tumor, such as germinoma.[504-506] In other reports, it presented with diabetes insipidus and hypogonadotropic hypogonadism,[507] or in association with common variable immunodeficiency.[508] Once the diagnosis is established, management is generally conservative, unless there are signs of increased intracranial pressure or optic nerve compression.

Langerhans cell histiocytosis (LCH) is characterized by clonal proliferation and an accumulation of abnormal dendritic cells that can affect either a single site or many systems causing multiorgan dysfunction.[509] In children, the median age of diagnosis ranges between 1.8 and 3.4 years.[510,511] LCH infiltrates the hypothalamo-pituitary area in 15% to 35% of patients with subsequent development of at least one pituitary hormone deficiency.[512-514] In a multicenter French national study of 589 pediatric patients with LCH, 145 patients (25%) had pituitary dysfunction. In 60 patients, pituitary involvement was already present at the time of diagnosis and in 20 of them it was the first manifestation of the disease. Patients at high risk of pituitary involvement seem to be those with multisystem disease involving skull and facial bones, mastoid, sinuses, and mucous membranes (i.e. gums, ear, nose, and throat region). Furthermore, compared to patients without pituitary involvement, patients with pituitary involvement have a higher rate of relapse (10% at 5 years versus 4.8% at 5 years) and a higher incidence of neurodegenerative LCH.[515]

Diabetes insipidus is the most frequently reported permanent consequence of LCH and the most common endocrinopathy; almost all patients with pituitary involvement have DI. Diabetes insipidus usually presents

early in the course of the disease, within the first 3 to 5 years, and occasionally may precede the diagnosis of LCH. Children with LCH and DI may also have anterior pituitary hormone deficiencies, with most deficits developing in the 6 years following the diagnosis of DI. The second most common endocrinopathy is growth hormone deficiency, which occurs in 14% of all patients with LCH and in more than 40% of patients who have pituitary involvement.[513,514] In the majority of patients, GHD is associated with DI, with a median interval of 2.9 to 3.5 years between the diagnosis of DI and development of GHD.[516,517] Isolated GHD, or the association of GHD with other anterior pituitary hormone deficiencies, occurs less commonly.

Pituitary MRI findings in patients with LCH include thickening of the pituitary stalk, suggestive of the infiltrative process, enhancing changes in the pituitary gland and hypothalamus, and absence of the bright signal of the posterior pituitary in T1-weighted mages, caused by the loss of the phospholipid rich ADH secretory granules. The latter is an invariable feature of patients who develop DI.[518,519] Although at the time of diagnosis of DI, 75% show a thickened pituitary stalk, only 24% have persistent stalk thickening after 5 years. These changes are variable and do not correlate with treatment or with clinical recovery as DI persists in all cases.[520] The role of MRI in predicting the development of anterior hormone deficiencies is uncertain. It has been reported that patients who will become GHD are more likely to have a smaller anterior pituitary, whereas the size of the stalk and posterior pituitary are not significantly different.

Tumors of the Brain or Hypothalamus. Tumors of the CNS are a major cause of hypothalamic insufficiency.[521] This is especially true for midline brain tumors, such as germinomas, meningiomas, gliomas, colloid cysts of the third ventricle, ependymomas, and gliomas of the optic nerve. Although metastasis from extracranial carcinomas is rarely found in children, hypothalamic insufficiency may result from local extension of craniopharyngeal carcinoma or Hodgkin's disease of the nasopharynx. Pituitary adenomas represent less than 3% of supratentorial tumors in childhood and about 3.5% to 6% of all surgically treated pediatric pituitary tumors. In most cases they are hormonally active, arising from any of the five cell types of the anterior pituitary, and may produce prolactin (prolactinomas, 52%), ACTH leading to Cushing's disease (corticotropinomas 33.3%), GH (somatotropinomas 8%), or, rarely, TSH (thyrotropinomas). Nonfunctioning pituitary adenomas are rare in children (2.7%) compared to adults, where they represent almost 20% of pituitary adenomas. Although the majority of childhood pituitary adenomas are prolactinomas presenting in adolescence, corticotropinomas are the most common tumors in pre pubertal children.[522,523] Pituitary adenomas occur in isolation or may be part of a genetic syndrome such as multiple endocrine neoplasia type 1 (MEN1), McCune-Albright syndrome, or Carney complex. Their pathogenesis is not clear, but there is increasing evidence that dysregulation in hormone receptor signaling, changes in molecules that regulate cell cycle or are important for adhesion to extracellular matrix, as well as changes in growth factors may be implicated.[524] Their clinical presentation results from pituitary hormone hypersecretion or deficiencies, disruption of growth and sexual maturation, and pressure effects. On MRI, pituitary adenomas show slow uptake of gadolinium and appear as hypoenhancing lesions that may displace the pituitary stalk.

Optic Gliomas

Tumors of the optic pathway represent 4% to 6% of all pediatric intracranial tumors and among these the most common are optic gliomas (65%). Most optic gliomas are low-grade lesions with favorable prognosis if treated optimally.[525] Gliomas confined to the optic nerve have a predilection for females (60% to 70%) and are associated with neurofibromatosis type 1 (NF-1) in more than half of cases, whereas 38% are sporadic. Children with sporadic gliomas were more likely to manifest increased intracranial pressure, decreased visual activity and more commonly documented endocrine complications.[526] The most frequent symptoms at presentation are visual defects (diminished vision, optic atrophy, strabismus, nystagmus, proptosis), ataxia and precocious puberty.[527]

Because of their close anatomic relation to the hypothalamus and pituitary, dysregulation of the HP axis is common and is due either to the tumor itself or secondary to treatment. Premature sexual maturation (PSM) is a frequently presenting symptom, whereas the most common defect of postcranial irradiation is GHD.[528]

Cystic Lesions

Cystic lesions in the pituitary area include Rathke's cleft cysts, arachnoidal cysts, and cystic adenomas and craniopharyngiomas. Rathke's cleft cysts are benign cystic remnants of the Rathke's pouch. They are usually small (less than 5 mm), asymptomatic, and are found in almost 20% of routine autopsies.[529] Rathke's cleft cysts consist of well-differentiated columnar or cuboidal epithelial cells, and the content of the cysts varies. Rathke's cleft cysts can grow gradually and become symptomatic, especially if they have suprasellar extension. Symptoms include headache, visual defects, and pituitary dysfunction ranging from increased prolactin to pituitary hormone deficiencies. Differential diagnosis from other cystic lesions in the area is not always easy, as on MRI the cyst fluid shows variable signal intensities and therefore cysts can appear as hypodense or hyperdense. Almost 50% of Rathke's cleft cysts show rim enhancement. Recurrence of the cyst after treatment is rare (2 of 14 patients in one series), and it is recommended that treatment should include both fluid drainage and cyst wall removal in order to avoid relapses.[530]

Arachnoid cysts consist of a collection of CSF-like fluid surrounded by a wall made of arachnoid structures. They are mainly suprasellar, with only rare cases being intrasellar. Suprasellar cysts are usually diagnosed following nonendocrine symptoms such as neurologic deficits, macrocephaly, and visual symptoms. Due to the proximity of the lesion to the hypothalamo-pituitary area, arachnoid cysts may cause central precocious puberty, amenorrhea, and hyperprolactinemia, in addition to thyrotropin,

ACTH, or GH deficiency. Craniopharyngiomas may result in hypothalamic dysfunction and are discussed in greater detail under "Pituitary GH Deficiency."

Irradiation of the Brain or Hypothalamus. Cranial irradiation continues to emerge as an increasing cause of hypothalamic/pituitary dysfunction.[531-534] Irradiation may directly impair hypothalamic and pituitary function, and it is not always easy to discriminate between damage at each of these levels. In addition, thyroidal and gonadal function may also be directly affected if total body irradiation or craniospinal irradiation is administered. The degree of pituitary impairment is related to the dose of radiation received. Low doses typically result in isolated GHD. With higher doses, multiple pituitary hormone deficiencies are observed. Two to 5 years after irradiation, 100% of children receiving more than 3000 cGy over 3 weeks to the hypothalamic-pituitary axis showed subnormal GH responses to insulin-provocative tests.[535]

The degree of pituitary deficiency observed is also directly correlated to the length of time since irradiation. Children who test normally at 1 year post irradiation may still develop pituitary deficiencies at later times. Even when serum GH concentrations after provocative testing are normal, measures of spontaneous GH secretion may indicate impairment. As little as 1800 cGy has been shown to affect spontaneous GH secretion in pubertal children.

Decreased GH secretion may be further complicated by the impact of irradiation on spinal growth, which can result in short stature and skeletal disproportion. Surprisingly, cranial irradiation can also result in precocious puberty, now believed to be the result of inhibition of the normal restraint of puberty imparted by the product of the MKRN3 gene—the gene encoding makorin ring-finger protein 3, which is a paternally expressed, imprinted gene located in the Prader-Willi syndrome critical region (chromosome 15q11-q13).[536] This earlier occurrence of puberty has the consequence that epiphyseal fusion occurs at an earlier age than is ideal from the perspective of maximizing growth. Gonadotropin-releasing hormone (GnRH) analogs may be necessary to delay pubertal progression. The endocrinologist observing the child who has received craniospinal irradiation must weigh these three factors: evolving hypopituitarism, decreased spinal growth potential, and early puberty and premature epiphyseal fusion. Care must be taken to maximize the growth potential of the patient without causing skeletal disproportion and without delaying puberty excessively or allowing growth to terminate too early.

Pituitary GH Deficiency. As discussed previously, many of the disease processes that affect hypothalamic regulation of GH secretion also impact pituitary function. Given current diagnostic limitations, it is not in fact always possible to completely discriminate between hypothalamic and pituitary dysfunction. Furthermore, it is likely that many cases of so-called idiopathic GHD will be found to have a molecular basis for the disorder. Indeed, there has been an explosion of information concerning genes critically involved in pituitary somatotrope differentiation and function (Tables 10-2 and 10-3).

At this time, however, a clear cause for pituitary GHD is often not identified—hence the term *idiopathic*. An incidence of pituitary GHD of 1:60,000 live births was reported from the United Kingdom.[537] A more recent survey of 48,000 Scottish schoolchildren has indicated an incidence as high as 1:4000,[538] whereas the best estimate available in the United States population is at least 1:3480.[539]

It is likely, however, that childhood GHD is an overdiagnosed condition. In particular, the diagnosis of acquired idiopathic isolated GHD should always be suspect. Although one may argue that destructive or inflammatory lesions of the hypothalamus or pituitary may only affect GH secretion, that isolated GHD caused by a mild mutation/deletion of the GHRH receptor gene or GH gene may appear late, or that combined pituitary hormone deficiency (CPHD) may first present with what appears to be isolated GHD, such circumstances appear to be rare. In the absence of anatomic abnormalities evident on imaging studies or biochemical evidence of CPHD, the diagnosis of acquired isolated idiopathic GHD demands careful and thorough documentation.

Genetic Abnormalities Resulting in Combined Pituitary Hormone Deficiency. Septo-optic dysplasia and its relationship to HESX1 was discussed previously. Recessive mutations in *LHX3* have been identified in 13 patients from eight unrelated consanguineous families, in addition to a single patient who was found to be compound heterozygous for two missense mutations within the gene. These patients usually present with a multiple anterior pituitary hormone deficit, with sparing of ACTH in the majority of cases, although patients with ACTH deficiency have also been described.[540] Pituitary morphology is variable between patients with *LHX3* mutations: most patients have a hypoplastic anterior pituitary with a normal posterior pituitary and midline structures[540-542]; conversely, an enlarged anterior pituitary has also been reported in a patient that was not evident in a previous MRI scan performed 10 years earlier.[541] Additionally, a patient with a hypointense lesion in the anterior pituitary consistent with a microadenoma has also been described.[543]

The majority of patients with *LHX3* mutations reported to date have also exhibited a short rigid cervical spine with limited neck rotation and trunk movement. Again, the skeletal phenotypes can vary, and a single patient with normal neck rotation and no other syndromic features has been reported.[542] Analysis of *LHX3* expression during human development shows a pattern of expression in the developing embryonic pituitary highly similar to that observed in the mouse. Expression is detected within Rathke's pouch at 5 weeks of development and later in the anterior and intermediate region of the pituitary, but not in the posterior lobe. Expression of *LHX3* is also observed in specific regions of the spinal cord corresponding to interneuron and motor neuron populations.[544] The underlying mechanism of the vertebral and skeletal defects in patients with *LHX3* mutations is unclear because expression is not detected in the sclerotome or myotome, the tissues giving rise to the vertebrae and skeletal muscle.[544]

More recently, an additional phenotype of sensorineural deafness has been reported in association with homozygous loss of LHX3.[540] The severity of hearing loss is also highly variable and can range from profound to very mild and may be missed in some cases. A direct role may be implicated here because LHX3 is expressed in specific regions of the inner ear in a pattern highly conserved between humans and mice, and it is likely to have a role in cochlea hair cell development.[540]

To date, four separate reports have described six heterozygous mutations within LHX4, with all patients exhibiting GH deficiency and associated short stature on presentation, again with variable additional endocrine deficits and extrapituitary abnormalities. A heterozygous intronic mutation that abolishes normal splicing of LHX4 was initially reported by Machinis and colleagues[545] in a three-generation family segregating in a dominant and fully penetrant manner. The probands presented with short stature and were found to be GH, TSH, and ACTH deficient, with anterior pituitary hypoplasia, an undescended posterior pituitary, and absent pituitary stalk on MRI. Other affected family members presented with short stature associated with IGHD and a normal posterior pituitary. Additional manifestations included a poorly formed sella turcica and pointed cerebellar tonsils. A second, de novo mutation, producing a p.P366T substitution, was associated with a more severe panhypopituitary phenotype. MRI demonstrated a hypoplastic anterior pituitary, an undescended posterior lobe, a poorly developed sella turcica, and a Chiari malformation. In a screen of 253 patients, Pfaeffle and colleagues[546] identified an additional three heterozygous missense mutations—one occurring between the two LIM domains of the protein (p.R84C) and two within the homeodomain (p.L190R, p.A210P). The p.A210P mutation was identified in two female siblings presenting with short stature and GH deficiency; MRI showed that both had a hypoplastic anterior lobe with cystic lesions but a eutopic posterior pituitary. One sister had a more severe hypopituitary phenotype with additional TSH, ACTH, and gonadotropin deficiencies, whereas the other had only partial TSH deficiency. The mutation was inherited from their father who had short stature and low GH but no evidence of other hormone deficiencies. The p.R84C mutation was identified in a single male patient presenting with short stature and was found to be GH and TSH deficient, later developing gonadotropin deficiency with pubertal failure. The p.L190R mutation was associated with GH, TSH, and ACTH deficiency. Patients with both of the latter mutations had a small anterior pituitary and an undescended posterior pituitary on imaging, with no abnormalities in other regions of the brain. More recently, two brothers have been described with a single base insertion in exon 3 (c.293_294insC) resulting in a frameshift after codon 99. Both siblings presented with GH and TSH deficiencies with pituitary hypoplasia and a poorly developed sella turcica. The youngest brother also had a hypoplastic corpus callosum and an undescended posterior pituitary. Their father, who also harbored the mutation, was GH deficient and had experienced delayed puberty.[547]

The gene PROP1 (standing for prophet of Pit1) is involved in the early determination and differentiation of multiple anterior pituitary cell lineages.[548,549] Abnormalities of PROP1 result in CPHD, characterized by variable degrees of deficiency of GH, prolactin, TSH, follicle-stimulating hormone (FSH), luteinizing hormone, and occasionally ACTH.[549-553]

As a result of the identification of Prop1 as the gene underlying the Ames dwarf phenotype, the first mutations in *PROP1* were identified in human patients with hypopituitarism characterized by GH, TSH, and PRL deficiencies in addition to reduced gonadotrophins and failure to enter spontaneous puberty.[551] Subsequently, more than 26 distinct mutations have been identified in more than 180 patients from over 21 different countries, implicating PROP1 mutations as the most common genetic cause of CPHD accounting for approximately 50% of familial cases,[549-553] although the incidence in sporadic cases is much lower.[554,555] All affected individuals exhibit recessive inheritance, and the majority of mutations identified involve the DNA binding homeodomain, which is highly conserved between human and mouse, showing 91% identity at the nucleotide level.[551,556] The mutations in *PROP1* identified to date include nonsense, missense, frameshift, intronic, and deletion mutations. The majority of the mutations are predicted to result in complete loss of function by ablating DNA binding and transcriptional activation, although some missense mutations retain partial activity.[557,558] By far the most common mutation, accounting for 50% to 72% of all familial PROP1 mutations in multiple unrelated families,[549] is a 2-bp deletion within exon 2 resulting in a frameshift at codon 101 and the introduction of a termination codon at position 109 (often referred to as p.S109X). The deletion occurs within three tandem GA repeats, so the 2 bp deleted cannot be defined; consequently this mutation has been referred to as c.296delGA and c.301_302delAG in different reports. This mutation is likely to represent a mutational hot spot within the gene, rather than a common founder mutation, and combined with the incidence of the c.150delA mutation it accounts for more than 90% of all known PROP1 mutations.

Homozygosity for mutations in *PROP1* is typically associated with a deficit of GH, TSH, PRL, and gonadotrophins, although the time of onset and severity of hormone deficiency varies. Most patients present with early-onset GH deficiency and growth retardation; however, normal growth in early childhood has been reported in a patient who attained normal final height without GH replacement therapy.[559,560] Patients may present with gonadotropin deficiency with the evolution of other hormone deficiencies later in life. TSH deficiency is also highly variable and has been reported as the initial presenting symptom in some cases, whereas other patients show delayed onset. Onset of ACTH deficiency is significantly correlated with increasing age, and most patients exhibit normal ACTH and cortisol levels in early life[561-564]; however, patients as young as 6 years have been described with cortisol deficiency emphasizing the necessity for complete and continued clinical assessment of patients with PROP1 mutations.[565,566]

Although PROP1 has been shown to play a critical role in mouse gonadotrope differentiation, the spectrum of human gonadotropin deficiency is extremely variable in patients with *PROP1* mutations, ranging from early hypogonadism with a micropenis and undescended testes and complete lack of pubertal development to spontaneous, albeit often delayed, onset of puberty with subsequent deficiency of gonadotropins, requiring hormone replacement therapy. Variation in the timing and severity of gonadotropin deficiency could mean that hypogonadism in patients with *PROP1* mutations is acquired and late evolving rather than early congenital, consistent with a role for PROP1 in maintenance or terminal differentiation of gonadotropes rather than initial specification. However, a number of individuals with mutations may have congenital gonadotropin deficiency, given the presence of a micropenis and bilaterally undescended testes at birth.

The pituitary morphology in patients with PROP1 mutations is unpredictable; most cases show a hypoplastic or normal-sized anterior pituitary gland on imaging, with a normal pituitary stalk and posterior lobe, although some reports have documented an enlarged anterior gland.[551,561] Longitudinal analyses of anterior pituitary size over time have revealed that several patients with an enlarged anterior gland at initial scanning in childhood show spontaneous regression and involution, so that MRI in older patients often demonstrates anterior pituitary hypoplasia, although the size of the pituitary can wax and wane during this time.[555] The pituitary enlargement consists of a mass lesion interposed between the anterior and posterior lobes, possibly originating from the intermediate lobe[567] or Rathke's pouch remnant in the cleft, although the underlying mechanism for the mass remains unknown. Evidence from the mouse (discussed previously) suggests that PROP1 regulates the migration of progenitor cells from Rathke's pouch into the developing anterior pituitary, and in the absence of functional PROP1, undifferentiated cells are trapped in the periluminal area resulting in enlargement of the anterior pituitary followed by apoptosis.[568] Such a mechanism would be an attractive explanation for the human imaging findings, but, of course, would be difficult to establish and cannot account for the waxing and waning of the mass. Earlier biopsies of such a mass failed to reveal any definitive histopathology, with no cell types identified,[569] and such material is now likely to prove elusive, as these masses no longer require surgical removal in patients with identified *PROP1* mutations.

The evolving nature of hormone insufficiencies in patients with *PROP1* mutations suggests a progressive decline in the anterior pituitary axis, so such patients require regular monitoring for the development of hormone deficits that may not be apparent on initial presentation. The highly variable nature of the phenotype associated with PROP1 mutations, even between siblings within the same family carrying identical mutations, together with the observation of phenotypic differences in Prop1 mutant mice on different genetic backgrounds again implicate unidentified genetic modifiers playing a role in the severity and onset of disease pathogenesis.

POU1F1 is the human homologue of the mouse gene pit1 and encodes a transcription factor involved in activation of GH and prolactin genes, regulation of the TSH-b promoter, and specification, proliferation, and survival of the corresponding cell lineages.[92,570] Mutations within *POU1F1* (PIT1) were first reported in 1992 by four independent groups[571-575] and are generally associated with GH, PRL, and TSH deficiencies with variable anterior pituitary hypoplasia, consistent with the phenotype of Snell and Jackson dwarf mice, which harbor a point mutation and a genetic rearrangement of *Pit1* respectively. Deficiencies of GH and PRL are generally complete and present early in life, whereas TSH deficiency can be highly variable. The majority of cases present with early TSH deficiency; however, in some cases hypothyroidism occurs later in childhood.[571,576] MRI of patients with *POU1F1* mutations demonstrates a small or normal-sized anterior pituitary gland with a normal posterior pituitary and infundibulum, but with no extrapituitary abnormalities. More than 28 mutations in *POU1F1* have been described to date; 23 of these show recessive inheritance, including a complete gene deletion as well as splice site mutations, whereas 5 are dominant mutations, found in more than 60 patients from several countries. Of these, the amino acid substitution p.R271W is the most frequent having been identified in several unrelated patients from a variety of ethnic backgrounds. The R271W substitution results in production of a protein that remains capable of binding to DNA but acts as a dominant negative inhibitor of transcription.[572]

Studies of *POU1F1* in CPHD patient cohorts suggest that the incidence of mutations in cases of sporadic CPHD is quite low (approximately 3% to 6%), whereas the incidence among familial patients with hypopituitarism is much greater (25%).[576] Overall, screening studies suggest that abnormalities of POU1F1 are less common causes of CPHD than are abnormalities of the PROP1 gene.[549,570]

Haploinsufficiency of the homeobox gene RIEG results in Rieger syndrome, an autosomal-dominant disorder that involves abnormal development of the anterior chamber of the eye, teeth, and umbilicus—with an occasional association with GHD.[577,578] The rieg/pitx2 null mouse has been shown to be characterized by multiple pituitary hormone deficiency (MPHD).

Genetic Abnormalities of GH Production or Secretion Resulting in isolated GHD

It has been reported that up to 30% of patients with isolated GHD have an affected parent or sibling. In addition to the abnormalities of the PROP1 and POU1F1 genes described previously, in which abnormalities of GH secretion are associated with decreased secretion of other anterior pituitary hormones, four Mendelian forms of isolated GHD have been reported[579,580] (see Table 10-3).

Isolated GHD IA results from deletions (6.7, 7, 7.6, and 45 kb deletions have been reported) or mutations of the GH1 gene that totally block GH synthesis or secretion.[581-583] Transmission of isolated GHDIA is autosomal recessive, and patients have profound congenital GHD.

Because GH has never been produced by the patient, even in fetal life, patients are immunologically intolerant of GH and typically develop anti-GH antibodies when treated with pituitary-derived or recombinant DNA–derived GH, although development of growth-attenuating antibodies appears to be less frequent with the newer synthetic GH preparations.

When antibodies prevent a patient from responding to GH, GHDIA can be viewed as a form of GHI and the patient is a candidate for IGF-1 therapy. The less severe form of autosomal-recessive GHD (isolated GHDIB) is likely to also be the result of mutations or rearrangements of the GH1 gene, presumably resulting in a GH molecule that retains some function but is perhaps unstable.[584] Additionally, mutations in the GHRHR also lead to GHD1B. To date, however, most patients with presumed isolated GHDIB have not demonstrated an alteration of the GH1 and GHRHR genes and the cause of their GHD remains unclear. These patients generally respond to GH therapy, and development of clinically significant anti-GH antibodies is unusual.

Isolated GHDII is transmitted as an autosomal dominant. Such patients usually have abnormalities of the GH1 gene that function in a dominant negative manner. The most common causes of this disorder appear to be splice site and intronic mutations that inactivate the 5′-splice donor site of intron 3, resulting in skipping of exon 3 with generation of the 17.5-kDa isoform of growth hormone that exerts a dominant-negative effect on the secretion of the 22-kDa molecule. McGuiness and colleagues demonstrated that transgenic mice that overexpress the 17.5-kDa isoform have a defect in the maturation of the growth hormone-containing secretory vesicles and display anterior pituitary hypoplasia.[572] More important, genetically modified mouse strains that express multiple copies of the exon 3-deleted allele had a more severe phenotype, almost undetectable growth hormone levels and profound pituitary hypoplasia, with loss of somatotrophs and macrophage invasion, compared with mice expressing fewer copies of the allele. Unexpectedly, the most severely affected animals also developed deficiencies in TSH, prolactin, and luteinizing hormone. Thus, the activated macrophages possibly accelerate the loss of somatotrophs, and other cell types may be destroyed as a consequence of a "bystander" effect.[585]

At the cellular level, the 17.5-kDa isoform lacks the protein linker domain and a cysteine residue (Cys53) involved in the formation of a disulfide bond between the first two helices of growth hormone and is, therefore, retained in the endoplasmic reticulum. This retention triggers a misfolded protein response and disrupts the secretory pathway and trafficking of growth hormone and other hormones, including adrenocorticotropic hormone (ACTH). The amount of the 17.5-kDa product has to reach a critical threshold to exert its dose-dependent effects, with increasing amounts leading to reduced cell proliferation and apoptosis of somatotrophs.[586-588]

Exon 3 skipping and the generation of the 17-kDa isoform can also result from mutations other than in the conserved consensus splice sites. The first seven bases of exon 3 are crucial for correct *GH1* splicing as they contain an exonic splice enhancer motif (eSe1, which has the sequence GAAGAAG) that strengthens the use of the weak 3′-splice site upstream of exon 3 and suppresses a downstream cryptic splice site.[589] The first mutation in eSe1 was reported by Moseley and colleagues[590] in the fifth nucleotide of exon 3 (e3 + 5A > G); although this mutation results in an amino acid change from glutamate to glycine (Glu33Gly), its mechanism of action has been shown to occur via its effect on splicing. In vitro transient expression assays demonstrated that, in addition to complete exon skipping, the mutation resulted in the activation of the downstream cryptic splice site at nucleotide 45 of the exon (e3 + 45) causing loss of amino acids 32 to 46 of the growth hormone molecule, which leads to the production of a 20-kDa isoform. The 22-kDa isoform represented only 11% of transcripts, whereas the majority comprised the 17.5-kDa (62%) and 20-kDa (27%) products. In fact, mutations in any of the bases of eSe1 lead to either complete or partial skipping of exon 3, resulting in the generation of both the 17.5-kDa and 20-kDa isoforms at various concentrations (35% to 68% and 20% to 37%, respectively). Exon 3 skipping can also result from disruption of sequences in IvS3 downstream of the consensus splice sites that affect intronic splice enhancers or the branching point site. These findings support the notion that the size of intron 3 is important for the integrity of the splicing mechanism.

Missense mutations that affect growth hormone secretion or action can also cause autosomal dominant isolated growth hormone deficiency. Patients with the Arg183His mutation have impaired release of growth hormone as, at the cellular level, the secretory granules that contain the mutant Arg183His protein are not exocytosed as effectively as those containing the wild-type hormone.[591] Other mutations (for example, Pro89leu) can cause more profound and early disturbances in the secretory pathway by alteration of the orientation of the growth hormone helices and an effect on the correct folding of the molecule.[592]

Patients with autosomal dominant isolated growth hormone deficiency have substantial variation in the severity of growth hormone deficiency. They present with low but detectable serum growth hormone levels, variable height deficit, and can show anterior pituitary hypoplasia on MRI (38% to 50%).[593,594] Data on pedigrees with the Arg183His or e3+1G>A mutations highlight the fact that patients with the same mutation can vary considerably in height (≤ –4 SDS to normal) and even attain normal adult height without treatment. Patients with splice site mutations are thought to be more severely affected than those with missense mutations[595]; however, patients with the IvS3+1 or IvS3+2 splice site or the Pro89leu mutations can develop additional pituitary hormone deficiencies, including ACTH, prolactin, TSH, or gonadotropin deficiency.[596,597] This evolving phenotype is unpredictable and dictates the need for lifelong follow-up of affected individuals. Another intriguing observation is that, even in patients with a genetic cause (for example, Glu32Ala), growth hormone deficiency seems to reverse when they are retested at the end of growth, in the transition period before transfer from pediatric to adult services. However, this effect is

temporary, observed in patients tested at the time of transition who should not be discharged from follow-up.[598]

Isolated GHDIII is transmitted in an X-linked manner. Little is known about the etiology of this condition, although rare mutations in *SOX3* can be associated with IGHD with or without learning difficulties.

Congenital Abnormalities of the Pituitary

A number of reports have described the association of "idiopathic" GHD with an ectopic neurohypophysis.[599-601] Magnetic resonance imaging (MRI) findings have been described in several series of patients with idiopathic dwarfism. Abrahams and associates[600] studied 35 patients with idiopathic GHD and found that those with MRI findings could be divided into two groups: 43% had an ectopic neurohypophysis (neurohypophysis located near the median eminence), absent infundibulum, and an absence of the normal posterior pituitary bright spot, and 43% had a small anterior pituitary as an isolated finding or combined with an ectopic neurohypophysis.

All in all, an ectopic neurohypophysis was found in 87% of cases with multiple pituitary hormone deficiencies but in only 10% of cases with isolated GHD. Kuroiwa and coworkers[601] suggested that the ectopic neurohypophysis, typically visualized as a bright spot at the median eminence, might be the consequence of perinatal asphyxia.

In other studies, however, high-resolution MRI findings of one or more of the following have been suggested to be sensitive or specific indicators of hypopituitarism: a small anterior pituitary, attenuated pituitary stalk, and ectopic posterior pituitary. In one study,[602] pituitary abnormalities were found in 80% with isolated GHD and 93% with MPHD. In patients whose peak growth hormone level was less than 3 ng/mL, 90% had MRI findings—compared with 39% of those with growth hormone levels 3 ng/mL or greater. In another study,[603] the stalk was abnormal in 90% of patients with IGHD and was absent in 96% of patients with MPHD. Thus, MRI abnormalities are common in children with isolated GHD and MPHD and are closely associated with the severity of GHD. Patients with structural abnormalities will need lifelong follow-up as adults due to the risk of developing other pituitary hormone deficiencies. Patients with an ectopic posterior pituitary, however, can also show reversal of their growth hormone deficiency on retesting at the time of transition from pediatric to adult services or in adulthood.[604,605]

Tumors Involving the Pituitary. Many of the tumors that affect hypothalamic function also directly impact pituitary secretion of GH.[521] In particular, craniopharyngiomas comprise a major cause of pituitary insufficiency.[606,607] Some consider this tumor a congenital malformation because it is believed to be present at birth, gradually growing over the ensuing years and decades. Craniopharyngiomas are rare epithelial tumors of embryonal origin, derived from remnants of Rathke's pouch.[608] The tumor arises from rests of squamous cells at the junction of the adenohypophysis and neurohypophysis. As it enlarges, it forms a cyst that contains degenerated cells and that may calcify but does not undergo malignant degeneration. In children, craniopharyngiomas represent 5% to 15% of intracranial tumors and are the most common neoplasm of the hypothalamo-pituitary area, accounting for up to 80% of tumors in this location.[609,610] Their incidence in the United States is 1.3 per million per year, and almost 28% affect children younger than 14 years.[611] Data from the UK registry show that there are 15 new cases per year in children under 15 years of age. There is a bimodal peak in incidence: the first peak occurs in children between 5 to 14 years and the second peak in adults older than 50 years. Patients can, however, be diagnosed at any age and have even been reported in the neonatal period.

At presentation, most craniopharyngiomas have a combined intra and suprasellar location (74.2%) and almost half have hypothalamic involvement (51.6%). A smaller percentage is exclusively suprasellar (22.6%) or confined within the sella turcica (6% to 3%). Almost one third invade the floor of the third ventricle and may cause obstructive hydrocephalus.[612,613] In pediatric patients, craniopharyngiomas are predominantly cystic (56.7%), multicystic (16.7%), predominantly solid (13.3%), purely solid (10%), or purely cystic (3.3%). The cystic fluid is viscous and rich in cholesterol and the incidence of calcification is much higher in children (83.3%) compared to adults.[614]

There are two main histologic types: the most common is the adamantinomatous type and consists of epithelial neoplastic cells that resemble those found in lesions of the jaw; the papillary type is much more rare and is found almost exclusively in adults. Although craniopharyngiomas are histologically benign, they can extend from their initial site, develop papillae, and invade vital surrounding tissues including the hypothalamus and optic chiasm. This attachment makes their complete excision difficult, if not impossible, and contributes to tumor recurrence and morbidity.

Craniopharyngiomas are typically sporadic tumors. There have been, however, rare case reports of affected family members suggesting recessive inheritance. Genetic studies of craniopharyngiomas have suggested a role for the Wnt signaling pathway; there is evidence that the activation of beta-catenin may have a role in the pathogenesis of adamantinomatous craniopharyngiomas. Beta-catenin is a cytoplasmic protein important for cell-cell adhesion and association with cadherins. It is also a downstream component of the *Wnt* signaling pathway that regulates many developmental processes such as cell proliferation, axis orientation, and organ development. Sekine and colleagues found mutations in beta-catenin in all adamantinomatous craniopharyngiomas they examined.[615] Although further studies confirmed that it is the adamantinomatous, and not papillary, craniopharyngiomas that have heterozygous missense mutations in beta-catenin, the reported rate was lower (16%, 7 of the 43 examined).[616] Overactivation of the Wnt signaling pathway in the pituitary in a mouse model has led to the development of tumors that are highly reminiscent of craniopharyngioma and offers further hope for possible medical therapy.[41]

Clinical signs and symptoms of craniopharyngiomas can arise at any age, from infancy to adulthood, but most typically occur in mid-childhood. The most common presentation is with symptoms of increased intracranial pressure (up to 75%), such as headaches, vomiting, or oculomotor abnormalities. Impaired vision is common. Visual field defects may result from compression of the optic chiasm, and papilledema or optic atrophy may be observed. Visual and olfactory hallucinations have been reported, as have seizures and dementia.

It is estimated that 70% to 80% of children have evidence of endocrine deficiencies at presentation and that growth failure is observed in 32% to 52% of children prior to diagnosis. Low concentrations of IGF-1 have been reported in 80% of children at time of diagnosis. GH deficiency is the most common hormone deficiency, documented in 75% to 100% of those tested before treatment. It is followed by ACTH (20% to 70%) and TSH (3% to 30%) deficiencies. Compression of the pituitary stalk or damage of hypothalamic dopaminergic neurons results in elevated PRL concentrations, observed in 8% to 20% of children at diagnosis. The incidence of diabetes insipidus at presentation varies between 10% and 29% of patients depending on the study. However, in one study almost half of patients had DI at presentation. The authors suggested that the incidence of DI is either underestimated or may be masked by the simultaneous presence of ACTH deficiency.[617] In adolescence in particular, craniopharyngiomas present with delayed puberty or pubertal arrest: in a series of 56 patients, all adolescents complained of delayed puberty.[617] Rare presentations include precocious puberty[39] and syndrome of inappropriate antidiuretic hormone secretion.

Lateral skull films often demonstrate enlargement or distortion of the sella turcica, frequently accompanied by suprasellar calcification(s). Nevertheless, some children with craniopharyngiomas will have normal plain films (and alternative radiologic techniques are recommended). Computed tomography is a sensitive technique for identification of small amounts of calcification or cystic abnormalities. MRI is probably the most sensitive technique.

Craniopharyngiomas appear as mass lesions in the sellar or suprasellar area that may extend to the hypothalamus and invade the third ventricle. Adamantinomatous craniopharyngiomas are predominantly cystic and the cystic portion of the lesion appears hyperintense in T1 and T2 images. The solid part of the tumor shows areas of high and low signal intensity that represent areas of calcification and hemosiderin deposits. In the majority of cases (58% to 76%), the size of craniopharyngiomas, as estimated by MRI or computed tomography (CT), has been reported to be 2 to 4 cm, whereas it is smaller than 2 cm in 4% to 28% cases and more than 4 cm in 14% to 20%.[610,614]

The management of craniopharyngiomas is complex, controversial, and it is best achieved by a multidisciplinary approach. Aims of the treatment are to relieve acute signs and symptoms of compression (raised intracranial pressure, threatening visual failure), to preserve hypothalamic function, thus reducing later morbidity and mortality, and to provide long-term control and prevent recurrence. What has been evident from different studies is that the extent of surgical resection is probably the most important factor that influences the recurrence of craniopharyngioma.[618] In patients who had surgery only, the 10-year recurrence free survival rate was 83% after total removal, 50.5% after subtotal removal, and 15.6% after partial removal. Tumor recurrence usually occurs in the first 5 years and is relatively rare thereafter. However, even after complete resection confirmed radiologically, relapses occur in up to 15% to 25% of patients. In older series, however, when patients were treated with radical and repeated surgical resections, mortality was high (25% to 50%) and hypothalamic, visual, and cognitive morbidity occurred in the majority (75%), especially in craniopharyngiomas with suprasellar or retro-chiasmatic extension.[612]

Guidelines for the multidisciplinary management of children with craniopharyngioma have been published.[619,620] It is now suggested that patients can be categorized in two risk groups with respect to management and prognosis. The good risk group includes older children with small tumors (2 to 4 cm) and no hypothalamic syndrome or hydrocephalus. Younger children, with larger tumors (> 2 to 4 cm) and hypothalamic syndrome or hydrocephalus are in the poor risk group. Complete radical resection, with or without adjuvant radiotherapy, is suggested for the good risk group, whereas limited surgery and immediate or delayed radiotherapy is the treatment of choice for the poor risk group.

Psychosocial Dwarfism. An extreme form of "failure to thrive" is observed in a condition labeled "psychosocial dwarfism" or "emotional deprivation dwarfism."[621-623] Most cases of failure to thrive can be traced back to a poor home environment and inadequate parenting, with improved weight gain and growth observed on removal of the infant from the dysfunctional home. In 1967, Powell and coworkers[622] described a group of children with dramatic behavioral manifestations beyond those observed in the typical infant with failure to thrive. Behavior was characterized by bizarre eating and drinking habits (such as drinking from toilets), social withdrawal, and primitive speech. GH secretion was abnormally low after provocative testing but returned to normal on removal from the home. Concomitantly, when eating and behavioral habits normalized children experienced a period of catch-up growth.

The neuroendocrinologic mechanisms involved in psychosocial dwarfism remain to be elucidated; however, GH secretion is abnormal, and ACTH and TSH activity may also be reduced—although some patients have been reported to be hypercortisolemic. Even though GH secretion is reduced, treatment with GH is not usually of benefit until the psychosocial situation is improved. It is our experience that whereas failure to thrive is a common cause of poor growth in infancy, the constellation of findings described in psychosocial dwarfism is, fortunately, rare.

GH Neurosecretory Dysfunction. Because of concerns that tests of GH secretion after pharmacologic provocation do not accurately reflect normal GH secretion, it has

been argued that there exists a group of children with "GH neurosecretory dysfunction"—identified by frequent serum sampling over a 12- to 24-hour period or by continuous serum withdrawal over a similar period of time.[624,625] GH neurosecretory dysfunction is characterized by short stature and poor growth, normal provocative serum GH concentrations, reduced 24-hour serum GH concentrations, and low serum IGF-1 concentrations. There appears to be little doubt that some children should be considered GH deficient, even if they pass provocative GH testing, although whether such patients should be identified by 24-hour GH sampling or by measures of the IGF axis remains controversial. This subject is discussed later in the chapter.

Acquired Idiopathic Isolated GHD. In most pediatric endocrine centers, many children receiving GH carry a diagnosis of acquired idiopathic isolated GHD. As stated previously, this diagnosis should always be considered somewhat suspect—although it is clear that some of these patients may actually have undiagnosed gene defects in GH production/secretion or have first manifestations of combined pituitary hormone deficiencies. Tauber and colleagues[626] performed GH stimulation tests on 131 young adults who carried the diagnosis of childhood-onset GHD. Of 10 subjects with organic GHD, 90% had peak GH concentration less than 5 ng/mL. On the other hand, 67% of 121 subjects carrying a diagnosis of idiopathic GHD had a peak GH concentration that was greater than 10 ng/mL and only 17% had a peak concentration that was less than 5 ng/mL.

This study also compared retesting results when subjects were divided into complete GHD (defined by an initial peak), stimulated GH level less than 5 ng/mL, and partial GHD (defined by a peak GH level after two stimulation tests of between 5 and 10 ng/mL or one test below 10 ng/mL and a 24-hour GH level below 2.5 ng/mL). Subjects with partial GHD were twice as likely to have normal GH responses on retesting than were those with complete GHD (71% versus 36%, respectively).

In a similar study, Maghnie and coworkers[627] reinvestigated 35 young adults with childhood-onset GHD divided into four groups according to their first pituitary MRI: isolated GHD and normal pituitary volume; isolated GHD and small pituitary volume; isolated GHD or MPHD, together with hypothalamic-pituitary abnormalities on MRI such as pituitary hypoplasia, pituitary stalk agenesis, and posterior pituitary ectopia; and MPHDs secondary to craniopharyngioma. On retesting, all subjects in the first and second groups had normal GH responses to provocative testing, regardless of pituitary size. On the other hand, all subjects in the third and fourth groups had peak GH responses less than 3 ng/mL. These findings and those of a number of similar studies[628] have indicated that the likelihood of sustained GHD is much greater in cases of MPHDs or structural abnormalities of the hypothalamus/pituitary.

Bio-inactive GH. Serum GH exists in multiple molecular forms, representing alternative posttranscriptional or posttranslational processing of the mRNA or protein, respectively. It is conceivable that different molecular forms of GH may have varying potency in stimulating skeletal growth, although this remains to be rigorously demonstrated. It has been suggested that some cases of short stature may be characterized by serum GH forms that have normal immunopotency but reduced biopotency.[629] No completely convincing cases of "bio-inactive GH" had been demonstrated until 1996, when Takahashi and colleagues[630,631] reported two cases that were heterozygous for point mutations in GH1. One patient was characterized by a R77C mutant GH molecule that bound with high affinity to the GHBP but abnormally to the GHR.

The mutant molecule appeared to behave in a dominant negative fashion and could inhibit tyrosine phosphorylation through the GHR. The affected patient had a partial clinical response to GH, and the father (who was heterozygous for the same mutation) was phenotypically normal—thereby raising some questions about the physiologic significance of this mutation. A second patient, with a D112G mutation, produced a GH molecule that apparently inhibits GHR dimerization. This patient, however, was able to respond to exogenous GH by increasing serum IGF-1 and accelerating growth. Although searches will continue for other examples of GH variants with decreased activity, it seems likely that true "bio-inactive GH" is a rare cause of growth failure.

Primary IGFD and GH Insensitivity(GHI). It is important to consider growth in the context of both GH secretion and GH sensitivity, each of which can operate alone or in combination in a variety of patients—including those with ISS (Figure 10-24). GHI describes patients with the phenotype of GHD but with normal or elevated

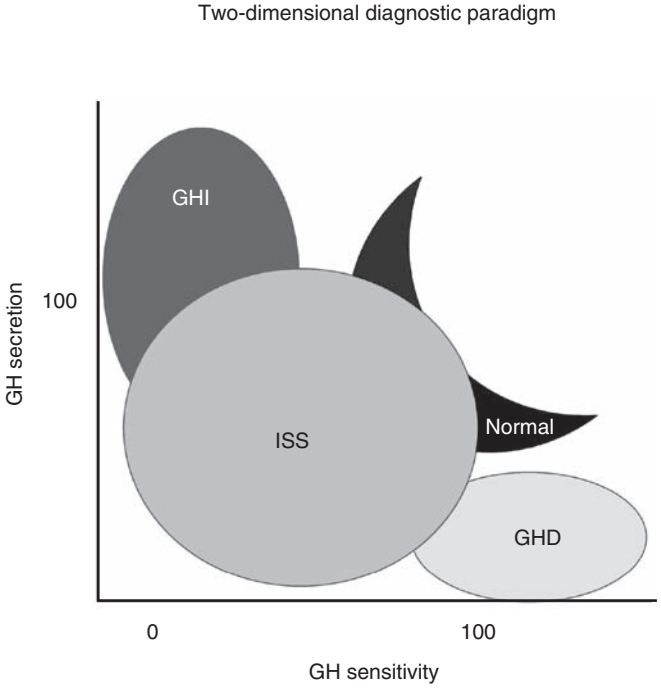


FIGURE 10-24 ■ Dual contributions of GH secretion and action to growth. (From Cohen, P. (2006). Controversy in clinical endocrinology: problems with reclassification of IGF-1 production and action disorders. *J Clin Endocrinol Metab, 91*, 4235–4236.)

BOX 10-4 Proposed Classification of Growth Hormone (GH) Insensitivity[877]

Primary GH Insensitivity (Hereditary Defects)

1. GH receptor defect (may be positive or negative for GH-binding protein)
   - Extracellular mutation (e.g. Laron syndrome)
   - Cytoplasmic mutation
   - Intracellular mutation
2. GH signal transduction defects (distal to cytoplasmic domain of GH receptor)
   - Stat-5b mutations
3. Insulin-like growth factor-1 defects
   - IGF-1 gene deletion
   - IGF-1 transport defect (ALS mutation)
   - IGF-1 receptor defect
4. Bioinactive GH molecule (responds to exogenous GH)

Secondary GH Insensitivity (Acquired Defects)

- Circulating antibodies to GH that inhibit GH action
- Antibodies to the GH receptor
- GH insensitivity caused by malnutrition, liver disease, catabolic states, diabetes mellitus
- Other conditions that cause GH insensitivity

GH insensitivity: Clinical and biochemical features of IGF-1 deficiency and insensitivity to exogenous GH, associated with GH secretion that would not be considered abnormally low.
GH insensitivity syndrome: GH insensitivity associated with the recognizable dysmorphic features described by Laron.
Partial GH insensitivity: GH insensitivity in the absence of dysmorphic features described by Laron.

serum GH concentrations[110,632] (Box 10-4). Primary GHI implies abnormalities of the GHR, including the extracellular GH binding site, the extracellular GHR dimerization site, the transmembrane domain, or the intracellular site; post-receptor abnormalities of GH signal transduction; and primary defects of IGF-1 biosynthesis. The term "Laron syndrome" is commonly applied to these entities. Secondary GHI is an acquired condition and includes circulating antibodies to GH, antibodies to the GHR, malnutrition, and hepatic disease. As Boxes 10-1 and 10-4 and Table 10-3 show, a growing number of genes have been identified in the distal portion of the GH-IGF axis, and defects of each one have specific phenotypic and biochemical characteristics (Table 10-4). Additionally, different mutations of the same gene may result in significant variability in the phenotype. This has led to the concept of a continuum of GH insensitivity, ranging from severe (with heights as low as −10 SD and undetectable serum IGF-I concentrations) to quite mild (heights and serum IGF-I concentrations at the lower part of the normal range). Details concerning each of the molecular defects involved in GHI, IGFD, and IGF resistance are available at www.growthgenetics.com.

The initial report of what proved to be a defect of the GH receptor, by Laron and colleagues, described "three siblings with hypoglycemia and other clinical and laboratory signs of growth hormone deficiency, but with abnormally high concentrations of immunoreactive serum growth hormone.[632] To date, several hundred such cases have been identified worldwide.[110] The majority of reported cases come from the Mediterranean region or from Ecuador (in presumed descendants of Spanish "conversos": that is, Jews who converted to Christianity during the Inquisition).[632] Such patients have been demonstrated to be unresponsive to exogenous GH in terms of growth, metabolic changes, and alterations in serum concentrations of IGF-1 and IGFBP-3.[633]

Cellular unresponsiveness to GH was demonstrated in vitro by the failure of GH to stimulate erythroid progenitor cells from the peripheral blood of patients.[634] Direct evidence of receptor failure was provided by the demonstration that hepatic microsomes obtained by liver biopsy failed to bind radiolabeled GH.[635] Absence of detectable GHBP activity in the sera of patients with this familial form of GHI was subsequently demonstrated by the reduced ability of serum to bind radiolabeled GH.[167,168]

This latter observation was rapidly followed by the purification, cloning, and sequencing of the serum GHBP and the demonstration that it was in essence identical to the extracellular domain of the GHR.[112] Initial studies of the GHR gene in Israeli patients indicated that some contained gene deletions.[636] A wide variety of point mutations (missense, nonsense, and abnormal splicing) were identified subsequently.[110,637-639] The majority of reported point mutations are in the extracellular domain of the GHR, although there are some at the extracellular domain that did not affect GH binding but prevented dimerization of the receptor[640]; such cases may be characterized by normal or even increased serum concentrations of GHBP because the GHBP binding site is intact. There are, additionally, reports of patients with separate amino acid substitutions in the intracellular domain of the GHR.[641]

Woods and associates[642] reported two cousins with severe GHI resulting from a mutation in the 59-splice donor site of intron 8, resulting in a truncated GHR lacking the transmembrane and intracellular domains and leading to increased concentrations of serum GHBP—presumably owing to accelerated release from the cell membrane. A similar defect has been described in a Druse girl with a mutation of the 39-acceptor site of intron 7.[643] Defects directly affecting the intracellular domain have been reported to result in dominantly inherited GHI. In one, a girl and her mother (both with short stature and biochemical evidence of GHI) were found to be heterozygous for a single G-to-C transversion in the 39-splice acceptor site of intron 8—resulting in a truncated GHR 1–277 lacking most of the intracellular domain.[644]

A second report described high serum GHBP concentrations in two Japanese siblings and their mother, who were characterized by partial GHI. The patients and their mother had a point mutation that disrupted the 59-splice donor site of intron 9, causing skipping of exon 9 and the appearance of a premature stop codon in exon 10—resulting in the same GHR 1–277 molecule described by Ayling and associates.[644] Experiments under in vitro conditions showed that the Japanese mutation

TABLE 10-4 **Clinical and Biochemical Features of Molecular Defects of the GH-IGF-I Axis**

Gene Defect/Phenotype	*GHR*	*STAT5b*	*PTPN11*	*IGF1*	*IGFALS*	*IGFIR*	Bioinactive GH	*GH1* with Anti-GH Antibodies
Severe growth failure	+/−	+	−	+	−	−	−	+
Mild growth failure	−/+	−	+	−	+	+	+	−
Midface hypoplasia	+/−	+/−	−	−	−	−	−	+
Other facial dysmorphism	−	−	+	+	−	+	−	−
Deafness	−	−	−	+/−	−	−	−	−
Microcephaly	−	−	−	+	−	+	−	−
Intellectual delay	−	−	−/+	+	−	+/−	−	−
Puberty delay	+/−	+/−	+/−	−	+	−	−	−
Immune deficiency	−	+	−	−	−	−	−	−
Hypoglycemia	+	−/+	−	−	−	−	−	−
Hyperinsulinemia	−	−	−	+/−	+	−	−	−
IGF-I deficiency	+	+	−/+	+/−	+	−	+	+
IGFBP-3 deficiency	+	+	−/+	−	+	−	+	+
ALS deficiency	+	+	−/+	−	+	−	+	+
GH excess	+	+	−	+/−	+	−	−	−
GHBP deficiency	+/−	−	−	−	−	−	−	−
Homozygous or compound heterozygous mutations	+	+	−	+	+	−	−/+	+
Heterozygous mutations	−	−	+	−/+	−	+	+/−	−

+, Positive; −, negative; +/−, predominantly positive; −/+, predominantly negative.

From David, A., Hwa, V., Metherell, L., et al. (2011). Evidence for a continuum of genetic, phenotypic, and biochemical abnormalities in children with growth hormone insensitivity. Endocr Rev*, 32, 472–497, Table 2.*

results in a GHR molecule that behaves in a dominant negative manner—inhibiting GH-induced tyrosine phosphorylation of STAT5.

The clinical features of GHI due to GHR deficiency (GHRD) are identical to those of other forms of severe IGF deficiency, such as congenital GHD.[110] Basal serum GH concentrations are typically elevated in children but may be normal in adults (Figure 10-25). Most patients have decreased serum GHBP concentrations, at least as measured by functional assays. A normal (or even elevated) serum GHBP concentration does not, however, exclude the diagnosis of GHRD because mutations at the GHR dimerization site have been already described, as well as mutations of the transmembrane or intracellular domain of the receptor. Serum IGF-1, IGF-2, and IGFBP-3 concentrations are profoundly reduced in classic GHI (Figure 10-26)—but partial clinical and biochemical phenotypes have been described, typically related to milder mutations of the GHR gene and resulting in only a modest reduction in binding activity or receptor action.

Unlike the cases with "classic" GHR defects, as described in Israel and Ecuador, such cases may have heights and serum IGF-I concentrations that border on the lower limits of normal, again supporting the concept of a continuum of GHI defects.[639] Molecular defects that have been associated with milder phenotypes include an intronic base change resulting in activation of a pseudoexon sequence and insertion of 36 amino acids into the extracellular domain of the GHR,[645,646] some compound heterozygous mutations,[647] and some unequivocal dominant negative mutations.[648,649] Given that the GHR must dimerize to elicit a signal, molecular defects involving the intracellular domain of the GHR are particularly likely to result in such dominant negative expression.[649]

Sequencing of the *GHR* gene should not be limited, consequently, to coding exons, as intronic defects and splice site defects may result in GH insensitivity, often with atypical phenotypes and milder clinical expression. By the same token, it has become clear that not all mutations result in physiologically and clinically significant defects and that functional studies of mutations should be performed before attributing a phenotype to a specific mutation.

GHR Signaling Defects. Although some cases of primary IGFD with normal serum GHBP have proven to have defects of the transmembrane or intracellular domain of the GH receptor, patients have been reported with similar clinical and biochemical phenotypes but with normal sequencing of the GH receptor gene. Until recently, such cases (even when characterized by apparently abnormal activation of the STAT or MAPK pathways) have had no demonstrable molecular basis. Kofoed and colleagues,[650] however, reported a 16-year-old girl with a height of –7.5 SD and markedly low serum concentrations of IGF-I, IGFBP-3, and ALS despite normal serum concentrations of GHBP and a normal GHR gene sequence.

The patient, born to consanguineous parents, proved to be homozygous for a point mutation resulting in a substitution of proline for alanine at position 630 of the STAT5b gene—with resulting marked decrease in phosphorylation of tyrosine, a critical step in the pathway to STAT activation of IGF-I gene transcription. Subsequent investigations indicated that the mutant STAT5b

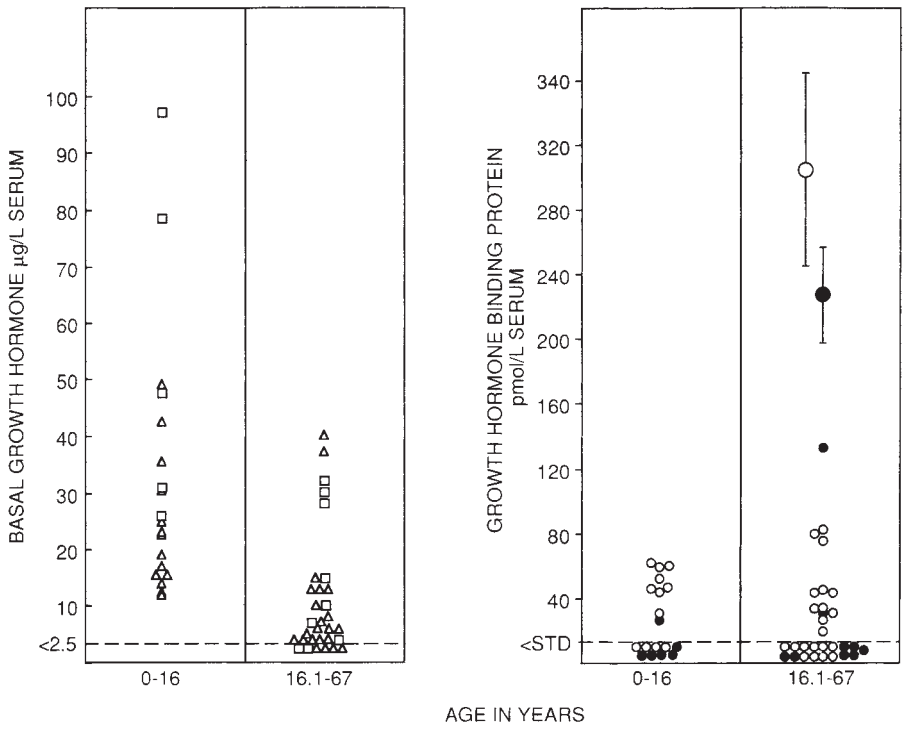


FIGURE 10-25 ■ Growth hormone and growth hormone-binding protein concentrations in sera of patients from Ecuador with GH receptor deficiency. (From Rosenfeld, R. G., Rosenbloom, A. L., & Guevara-Aguirre, J. (1994). Growth hormone [GH] insensitivity due to primary GH receptor deficiency. *Endocr Rev*, *15*, 369.)

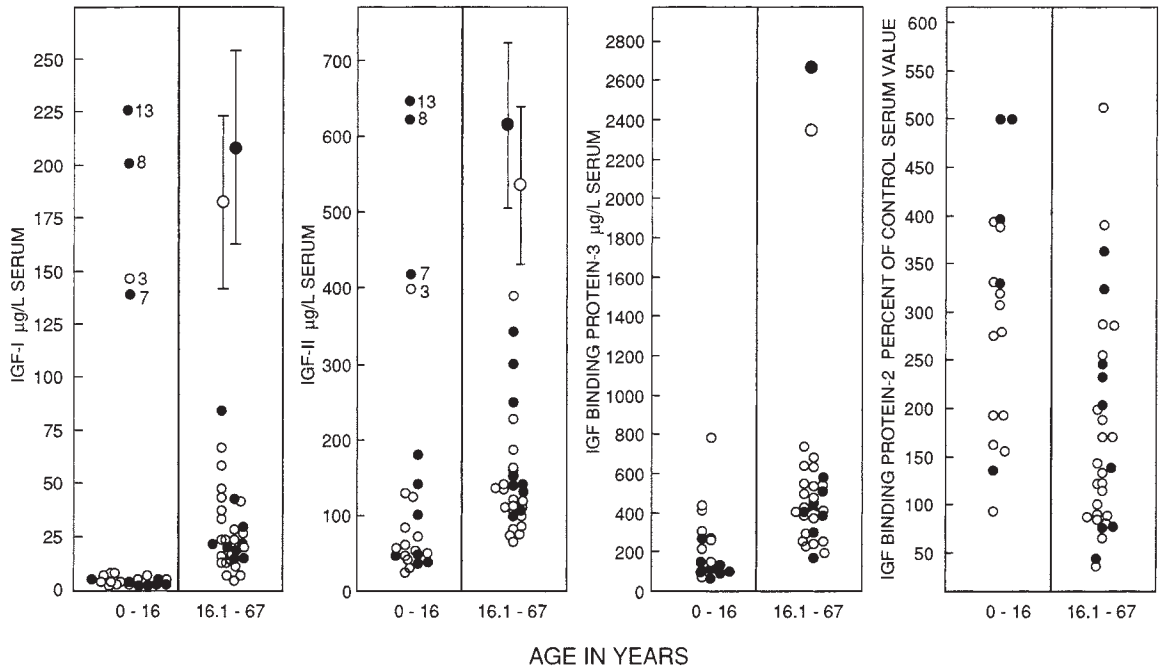


FIGURE 10-26 ■ Serum concentrations of insulin-like growth factor-1 (IGF-1), IGF-binding protein-3, and IGF-binding protein-2 in patients from Ecuador with growth hormone receptor deficiency. (From Rosenfeld, R. G., Rosenbloom, A. L., & Guevara-Aguirre, J. (1994). Growth hormone [GH] insensitivity due to primary GH receptor deficiency. *Endocr Rev*, *15*, 369.)

could not function as a signal transducer or transcription factor, presumably because of an inability to dock with phosphotyrosines on GH-activated receptors as well as an inability to form a stable interaction with DNA. The A630P STAT5b was shown to be characterized by aberrant folding and diminished solubility, resulting in aggregation and formation of cytoplasmic inclusion bodies.

A second case of severe primary IGFD and GH insensitivity resulting from a novel mutation of STAT5b has

been reported. The patient was shown to be homozygous for a single nucleotide insertion in exon 10 of the STAT5b gene, leading to early protein termination. Because STAT5b is involved in the signaling pathway for multiple cytokines, it is of note that both patients had evidence of immune dysfunction and recurrent pulmonary infections. The growth and clinical characteristics of these two patients strongly support the hypothesis that STAT5b mediates the overwhelming majority, if not all, of GH's effects on IGF-I gene transcription. To date, no convincing mutations of the genes for JAK2 or MAPK have been implicated in primary IGFD and GH insensitivity. It is possible that severe mutations of JAK2 (involved in signaling for multiple growth factors and cytokines) are incompatible with life.

Table 10-5 provides details on all of the cases of homozygous *STAT5b* defects reported to date. A total of 10 cases, involving 7 different mutations, have been described. Size at birth was generally within normal limits, as observed with *GHR* defects, but postnatal growth was severely impaired, with heights ranging from −3 to −9.9 SD. The biochemical data were consistent with GHI, with generally elevated serum GH concentrations and low serum IGF-I, IGFBP-3, and ALS concentrations. Serum prolactin levels, when measured, were elevated. Unlike defects of *GHR*, however, clinical evidence of immune compromise was evident in all but one patient, often accompanied by severe and life-threatening pulmonary disease. Given that STAT5b is involved in the signaling of multiple cytokines, this combination of growth failure, GHI, and immunodeficiency is not surprising.[651]

ALS Mutations. Markedly reduced serum concentrations of IGF-I and IGFBP-3 have also been observed in cases involving mutations of the ALS gene.[652,653] Of note is that even though serum concentrations of IGF-I and IGFBP-3 were as low as in patients with mutations of the GHR or STAT5b genes, growth was only modestly affected (Table 10-6). Indeed, the index case actually attained an adult height within the normal range. Whether the relatively normal growth reflects the greater importance of locally produced IGF-I or reflects altered kinetics of serum IGF-I in the face of reduced concentrations of binding proteins remains uncertain.

At least 16 different mutations have been identified to date, involving a minimum of 21 cases. Classical cases were either homozygous or compound heterozygotes, consistent with an autosomal recessive transmission. Heights ranged from −0.5 to −4.2 SD, but even this modest growth failure could reflect some degree of ascertainment bias. The possibility of heterozygous expression of *IGFALS* mutations has also been considered.[654] Analysis of heights within individual families has suggested that having two affected alleles results in about 2 standard deviations of height loss compared to wild-type relatives, whereas having one affected allele results in a loss of almost 1 SD of height. Given the remarkably low serum concentrations of both IGF-I and IGFBP-3 in these patients, however, the degree of growth failure is actually modest, especially when compared with other molecular defects of the GH-IGF axis.

IGF1 Gene Defects. Growth failure associated with a primary defect of IGF-1 synthesis has also been reported[357] (Table 10-7). The first case was a 15-year-old boy who had many of the growth characteristics predicted from mouse knockout models,[356,428,655] including intrauterine and postnatal growth failure. Additional features were mental retardation and sensorineural deafness. These features serve to distinguish IGF1 defects from GHI resulting from disorders of the GHR and STAT5b genes, where intrauterine growth is normal, as are head growth and intellectual function. These phenotypic features serve to underscore the observation that, although IGF-I is critical for both intrauterine and postnatal growth, GH, itself, has little role in prenatal growth and development. The patient was shown to be homozygous for deletions of exons 4 and 5 of the human IGF-1 gene, with both parents being heterozygous carriers and perhaps mildly affected. Although unresponsive to GH therapy, the patient was able to accelerate growth velocity on treatment with IGF-1. Of note is that the patient with IGF-1 gene deletion had in addition to severe growth failure and mental retardation substantial insulin resistance, and that IGF-1 therapy resulted in a marked improvement of insulin sensitivity, reduction of serum insulin, and overall improvement in various aspects of carbohydrate metabolism.[656]

Two cases of homozygous mutations have been reported where missense mutations (V44M[657] and R36Q)[658] resulted in an immunologically active, but bioinactive IGF-I molecule. Both patients had prenatal and postnatal growth attenuation, microcephaly, and one of the two had sensorineural deafness. Bioinactivity in these cases reflects a decrease in the affinity of the altered IGF-I for the IGF-I receptor.

IGF-I deficiency resulting from a homozygous T. A transversion in exon 6 has also been reported.[659] Although the phenotype of this case resembles those of the cases described earlier, this defect, which affects the E domain of the IGF-I precursor, may prove to be a polymorphism.

IGF1 Receptor Mutations. Cases of IGF-1 receptor mutations have also been described (Table 10-8).[660,661] In knockout models created in mice, homozygous mutations of the IGF-I receptor result in profound growth failure and neonatal mortality. Heterozygous mutations are phenotypically similar to wild-type mice.

More than a dozen patients with IUGR and postnatal growth failure associated with defects of the IGF1R have been reported.[661] Clinical findings have included microcephaly and mild mental retardation. The combination of prenatal and postnatal growth failure, in association, at times, with microcephaly and developmental delay, is similar (although milder) to the observations noted in molecular defects of *IGF1*, again emphasizing the importance of IGF-I and its receptor in both intrauterine and childhood growth. In general, serum IGF-I and IGFBP-3 concentrations were normal to elevated, possibly reflecting decreased IGF-I feedback on GH production. Functional studies typically demonstrate decreased affinity for IGF-I and decreased downstream phosphorylation in response to IGF-I stimulation. Other mechanisms whereby mutations of IGF1R result in IGF-I resistance

TABLE 10-5 **Phenotypes of Patients with STAT5b Deficiency**

Observation	Kofoed, 2003[878]	Hwa, 2005[879]	Bernasconi, 2006[880]	Boyanovsky, 2009[881]	Vidarsdottir, 2006[882]	Hwa, 2007[883]		Martinez, 2007[884]	Pugliese-Pires, 2010[885]	
						SIBLING 1	SIBLING 2		SIBLING 1	SIBLING 2
Consanguinity	Yes	Yes	NA	Adopted	NA	Yes	Yes	Adopted	NA	NA
Paternal height SDS	−0.3	−0.9	−2.2	NA	−0.8	−1.28	−1.28	NA	−1.5	−1.5
Maternal height SDS	−1.2	−0.6	−3.3	NA	−2.8	−0.6	−0.6	NA	−1	−1
Sex of proband	F	F	F	F	M	F	F	F	M	M
Age (in years)	16.5	16.4	15.3	12	31	2	4	14.8	6	2
Height SDS	−7.5	−7.8	−9.9	−5.3	−5.9	−5.8	−5.6	−5.95	−5.6	−3
Puberty	Delayed	Delayed	Delayed	Delayed	Delayed	NA	NA	Normal	Delayed	NA
CPD	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes
Atopy	Eczema	Eczema	Eczema	Eczema	Ichthyosis			Eczema	Atopic	Atopic
Other		Bleeding diathesis		Thyroiditis	Sickle cell anemia	JIA		Thyroiditis		Thrombocytopenic purpura
Hormonal evaluation										
GH basal (ng/mL)	9.4	14.2	6.6	1.8	0.13	17.7	5.7	NA	1.7	1
GH stimulated (ng/mL)	53.8	NA	NA	12.5	14.2	NA	NA	27.1	20.6	14
GF-I (ng/mL)*	38	7	< 10	0.8	14-17	< 5	< 5	< 5	34	< 25
GF-I, GH-stimulated (ng/mL)*	55	NA	NA	0.8	78†	NA	NA	12	48	< 25
GFBP-3 (mg/liter)*	0.87	0.54	NA	0.5	0.18	0.7	0.8	0.84	0.52	0.75
ALS (mg/liter)*	2.9	1.2	NA	0.7	0.7	0.4	0.8	NA	NA	NA
Prolactin (μg/liter)	> 102 (H)	NA	169 (H)	13-15	110 (H)	NA	NA	83 (H)	61.1 (H)	76.6 (H)
Molecular defect	p.A630P	*c.1191insG*	p.R152X	p.R152X	*c.1102insC*	*c.1680delG*	*c.1680del G*	p.F646S	*c.424_427del*	*c.424_427del*

Homozygous *STAT5B* mutations were identified in 10 case reports. Birth weight and length (data not shown) were normal for gestation. Height SDS (of probands) is at first observation or as reported. Molecular defects encompass missense and nonsense mutations (nomenclature, protein designation) and frameshifts due to insertion or deletion of exonic nucleotides (nomenclature, in *italics*, based on cDNA).

*All reported values are significantly below the normal range (methodology varied from site to site).

†Value determined after 7 days of daily GH injections (at 50 μg/kg body weight).

c, Coding DNA sequence where nucleotide 1 is the A of the ATG-translation initiation codon; F, female; M, male; H, high, above normal of 20 μg/liter; CPD, chronic pulmonary disease; JIA, juvenile idiopathic arthritis; NA, not available; p, protein sequence; X, stop codon.

From David, A., Hwa, V., Metherell, L., et al. (2011). Evidence for a continuum of genetic, phenotypic, and biochemical abnormalities in children with growth hormone insensitivity. Endocr Rev, 32, *472–497, Table 6.*

TABLE 10-6 **Phenotypes of Patients with Homozygous or Compound Heterozygous *IGFALS* Mutations**

Observation	Domené, 2004[886]	Hwa, 2006[652]	Domené, 2007 ([887])			Heath, 2008[888]		
			SIB 1	SIB 2	SIB 3			
Consanguinity	NA	Yes	No	No	No	No	No	No
Paternal height SDS	NA	−2	+1.5	+1.5	+1.5	−0.1	−2	−0.4
Maternal height SDS	NA	−2.8	−0.9	−0.9	−0.9	+0.7	−0.6	−0.4
Sex of proband	M	M	M	M	F	M	M	M
Age (in years)	14.6	13.9	15.3	19.6	15.4	4.5	4.6	15
Height SDS	−2.1	−2	−2	−0.5	−1	−2.4	−3.9	−2.6
Puberty (in years)	15.7	13	16	16.9	13	12.4	13.5	14.5
Adult height SDS	−1.6	NA	−0.5	−0.5	NA	−2.5	NA	−1.3
Hormonal evaluation								
GH basal (ng/mL)	4.5	1.2	0.2	6.8	NA	3.2	3.9	NA
GH stimulated (ng/mL)	31	23.4	10	NA	NA	20.7	27	6.7
IGF-I (ng/mL) *	31	25	8	10	14	15	11	40
IGF-I, GH-stimulated (ng/mL) *	39	NA	10	NA	NA	NA	NA	NA
IGFBP-3 (mg/liter) *	0.22	0.49	0.38	0.39	0.43	0.7	0.75	0.5
ALS (mg/liter) *	< 0.5	< 0.4	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Fasting insulin (μIU/mL) (NR for age)	NA	NA	NA	NA	NA	26.5 (4–11)	40.3 (4–11)	18.7 (4–16)
HOMA (NR <2.6)	NA	NA	NA	NA	NA	4.4	6.2	NA
Mutation	c.103delG	p.D440N	p.C540R/ p.S195_197Rdup	p.C540R/ p.S195_197Rdup	p.C540R/ p.S195_197Rdup	p.N276S	p.N276S	p.Q320X

Height SDS (of probands) is at first observation or as reported. Molecular defects encompass missense and nonsense mutations (nomenclature, protein designation) and frameshifts due to insertion or deletion of exonic nucleotides (nomenclature, in *italics*, is based on coding cDNA).

*All reported values are significantly below the normal range (methodology varied from site to site).

F, female; M, male; SGA, small for gestational age; Sib, sibling; NA, not available; p, protein sequence; X, stop codon; HOMA, homeostasis model of assessment; NR, normal range. Puberty is expressed as onset in males or menarche in females.

From David, A., Hwa, V., Metherell, L., et al. (2011). Evidence for a continuum of genetic, phenotypic, and biochemical abnormalities in children with growth hormone insensitivity. Endocr Rev, 32, *472–497, Table 8*.

TABLE 10-7 **Characteristics of Six Cases with *IGF1* Defects**

Observation	Woods, 1996[357]	Bonapace, 2003[659]	Walenkamp, 2005[657]	Netchine, 2009[658]	van Duyvenvoorde, 2010[889]	
Sex	M	M	M	M	F	M
Consanguinity	Yes	Yes	Yes	Yes	No	No
Birth weight (SDS/g)	−3.9/1400	−4/1480	−2.5/1420	−2.5/2350	−2.9/2300	−1.2/3300
Birth length (SDS/cm)	−5.4/37.8	−6.5/41	−3/39	−3.7/44	−3.8/44	−1/50
Cranial circumference (SDS/cm)	−4.9/27	−7.5/26.5	−8/44.2	−2.5/32	−2.4/47.8	−1.6/49
Growth (SDS)	−6.9 at 16 years	−6.2 at 1.6 years	−9 at 55 years	−4.5 at 3 years	−4.1 at 8.2 years	−4.6 at 6.2 years
Microcephaly	Yes	Yes	Yes	Yes	Yes	Mild
Development delay	Yes	Yes	Yes	Mild	Yes	No
Deafness	Yes	Yes	Yes	No	No	No
Adiposity	Yes	No	Yes	No	No	No
Hormonal evaluation						
IGF-I levels	Undetectable	1 ng/mL	+7.3 SDS	Variable	−2.3 SDS	−2.6 SDS
IGFBP-3 levels	3.3 mg/liter	3.6 mg/liter	1.98 mg/liter (+0.1 SDS)	4.3 mg/liter	+1.2 SDS	+0.1 SDS
Molecular defect	Hom p.? (Del ex 4–5)	Hom p.?*	Hom p.V44M	Hom p.R36Q	Het *c.243-246dupCAGC*	Het *c.243-246dupCAGC*
IGF1R affinity	Zero	Not studied	Extremely low	Partially reduced	Not studied	Not studied

*This mutation is localized within the polyadenylation site and alters mRNA splicing. The 3′ end of the resulting aberrant IGF-I transcript contains a partial sequence from the downstream gene *KIAA0537*.

F, female; M, male; Het, single heterozygous defect; Hom, homozygous defect; p.0? or p.?, the consequences of the mutation at protein level are unknown.

From David, A., Hwa, V., Metherell, L., et al. (2011). Evidence for a continuum of genetic, phenotypic, and biochemical abnormalities in children with growth hormone insensitivity. Endocr Rev, 32, *472–497, Table 7.*

TABLE 10-8 **Phenotypes of Patients with Heterozygous or Compound Heterozygous *IGFIR* Mutations**

Observation	Abuzzahab, 2003[664]				Kawashima, 2005[890]		Walenkamp, 2006[891]	
	INDEX CASE	MOTHER	STEP-BROTHER		INDEX CASE	MOTHER	DAUGHTER	MOTHER
Consanguinity	No	No	No	No	No	No	No	No
Paternal height SDS	0.1	−2.4	−0.3	−2.8	−2.2	NA	NA	−1
Maternal height SDS	−2.6	−0.8	−2.6	−1.6	−2.9	NA	−4	−1.3
Sex of proband	M	F	M	F	F	F	F	F
Age (in years)	5.3	NA	NA	4.5	6	35	1	31
Birth weight (SD)	2000 g (−3.5)	2400 g (−2.4)	2100 g (−2.7)	1400 g (−3.5)	2686 g (−1.5)	2500 g (−1.6)	2100 g (−3.3)	2600 g (−2.1)
Height SDS	−2.6	−2.6	NA	<−4	−2.1	−2.9	<−2	−4
Adult height SDS	NA	−2.6	NA	−4.8	NA	−2.9	NA	−4
Puberty	NA	NA	NA	NA	NA	Normal	NA	Delayed
Microcephaly	Yes	NA	NA	NA	No	NA	Yes	Yes
Intellectual delay	Yes	NA	NA	NA	Yes	NA	NA	No
Other	Facial and body dysmorphisms	NA	NA	NA			Facial dysmorphism, failure to thrive in infancy	Failure to thrive in infancy
Hormonal evaluation								
GH basal (ng/mL)	NA	NA	NA	NA	NA	NA	NA	NA
GH stimulated (ng/mL)	5.7	NA	NA	51	NA	NA	70.4	20.67
IGF-I (NR or SD) (ng/mL)	121 to 222 (+1.1 to +2.3 SD)	NA	NA	1130 (191 to 462)	208 (+1.5 SD)	255 (+0.5 SD)	145 (+2.9 SD)	239 (+1.6 SD)
IGF-I, GH-stimulated (ng/mL)	NA	NA	NA	NA	NA	NA	NA	NA
IGFBP-3 (NR/SD) (mg/liter)	2.1 to 3.7 (−0.7 to +1.8 SD)	NA	NA	5.6 (2.1 to 6.2)	2.22 (−1 SD)	2.05 (−0.4 SD)	1.9 (+1)	2.63 (+0.1 SD)
ALS (NR) (mg/liter)	NA	NA	NA	28 (5.6 to 16)	NA	NA	NA	NA
Molecular defect	Het p.R59X	Het p.R59X	Het p.R59X	p.R108Q/p.K115N	Het p.R709Q	Het p.R709Q	Het p.E1050K	Het p.E1050K

Height SDS (of proband) is at first observation or as recorded.
F, female; M, male; NA, not available; NR, normal range; p.0? or p.?, the consequences of the mutation at protein level are unknown.
From David, A., Hwa, V., Metherell, L., et al. (2011). Evidence for a continuum of genetic, phenotypic, and biochemical abnormalities in children with growth hormone insensitivity. Endocr Rev, 32, *472–497, Table 9.*

include defects that result in degradation of IGF1R mRNA through the nonsense-mediated mRNA decay pathway[662] and defective pro-receptor processing and plasma membrane localization.[663]

The first reported case of *IGF1R* defect was a compound heterozygote (*p.*R108Q; *p.*K115N).[664] Only one additional compound heterozygote has been reported, with both defects in exon 3 (*p.* E121K; *p.*E234K).[665] In the latter case, two siblings were affected and the growth defect was more severe than that generally described in heterozygous cases. All other reported cases have been simple heterozygotes. The absence of homozygous cases may reflect the observation in mice that *IGF1R* knockouts were generally lethal.

In leprechaunism, a syndrome of growth failure and insulin receptor dysfunction, there is variable IGF-I insensitivity. The profound abnormality of the insulin receptor suggests that heterodimeric insulin- and IGF-I receptor combinations could possible lead to failed activation of the IGF-I signaling cascade. As the IGF-I receptor gene resides at 15q26.3, deletions of the distal long arm of chromosome 15 or ring chromosome 15 may lead to hemizygosity for the IGF-I receptor. Although such patients may have intrauterine growth retardation and striking postnatal growth failure, lack of a biologic response to IGF-I has not been conclusively demonstrated. Whether growth failure in such patients is due to altered levels of IGF-I receptor or represents the net effect of the loss of other genes located on 15q remains to be determined.[660]

Clinical Features. Cases of IGF-1 deficiency resulting from hypothalamic dysfunction, decreased pituitary GH secretion, or GHI share a common phenotype, although specific features characteristic of each molecular disorder may help distinguish the various etiologies (Table10-4). The striking clinical similarity among patients with GHD caused by a GH gene deletion and patients with GHI secondary to mutations of the GHR gene emphasizes the role of IGF-1 in mediating most, but not all, of the anabolic and growth-promoting actions of GH. This point is further supported by the ability of IGF-1 therapy to partially normalize growth in children with mutations of the GHR gene. Children with less severe IGF deficiency will generally have milder clinical characteristics. If GH or IGF deficiency is acquired, clinical signs and symptoms will obviously appear at a later age (Box 10-5).

As stated previously, birth size is remarkably normal even in severe forms of congenital GHD or GHI due to defects of *GHR*, *STAT5b* or *IGFALS*. Birth length and weight are typically within 10% of normal, and severe IUGR is not part of the classic phenotype. Neonatal signs can exist, however, including hypoglycemia and prolonged jaundice.[666] When GHD is combined with ACTH and TSH deficiency, hypoglycemia may be severe. On the other hand, when GHD is combined with gonadotropin deficiency, microphallus, cryptorchidism, and hypoplasia of the scrotum may be observed.[667] GHD (or GHI) must therefore be considered in the differential diagnosis of neonatal hypoglycemia or microphallus/cryptorchidism.

BOX 10-5 Clinical Features of Growth Hormone Insensitivity

Growth and Development

- Birth weight: near-normal
- Birth length: may be slightly decreased
- Postnatal growth: severe growth failure
- Bone age: delayed, but may be advanced relative to height age
- Genitalia: micropenis in childhood; normal for body size in adults
- Puberty: delayed 3 to 7 years
- Sexual function and fertility: normal

Craniofacies

- Hair: sparse before the age of 7 years
- Forehead: prominent; frontal bossing
- Skull: normal head circumference; craniofacial disproportion due to small facies
- Facies: small
- Nasal bridge: hypoplastic
- Orbits: shallow
- Dentition: delayed eruption
- Sclerae: blue
- Voice: high-pitched

Musculoskeletal/Metabolic/Miscellaneous

- Hypoglycemia: in infants and children; fasting symptoms in some adults
- Walking and motor milestones: delayed
- Hips: dysplasia; avascular necrosis of femoral head
- Elbow: limited extensibility
- Skin: thin, prematurely aged
- Osteopenia

When intrauterine growth retardation is noted, in addition to postnatal growth failure, the possibility of GHI resulting from molecular defects of *IGF1* or *IGF1R* should be considered. This is particularly true if any of the following additional signs are observed: microcephaly, developmental delay, or sensorineural deafness.

Postnatal growth is strikingly abnormal in severe congenital IGF deficiency (Figure 10-27). Although earlier reports suggested that growth in such cases was relatively normal during the first 6 months of life, more recent surveys of GHD and GHI have indicated that growth failure may be observed during the first months of life. By 6 to 12 months of age, the child is clearly growing at an abnormally slow rate and has usually deviated away from the normal growth curve. It is important to emphasize that the single most important clinical manifestation of IGF deficiency is growth failure, and careful documentation of growth rates is critical to making the correct diagnosis. Deviation away from the normal growth curve should always be a cause of concern, and between the ages of 2 years and the onset of puberty growth deceleration (or acceleration) is always pathologic.

Skeletal proportions tend to be relatively normal, although they often correlate better with bone age than with chronologic age. Skeletal age is delayed, often to

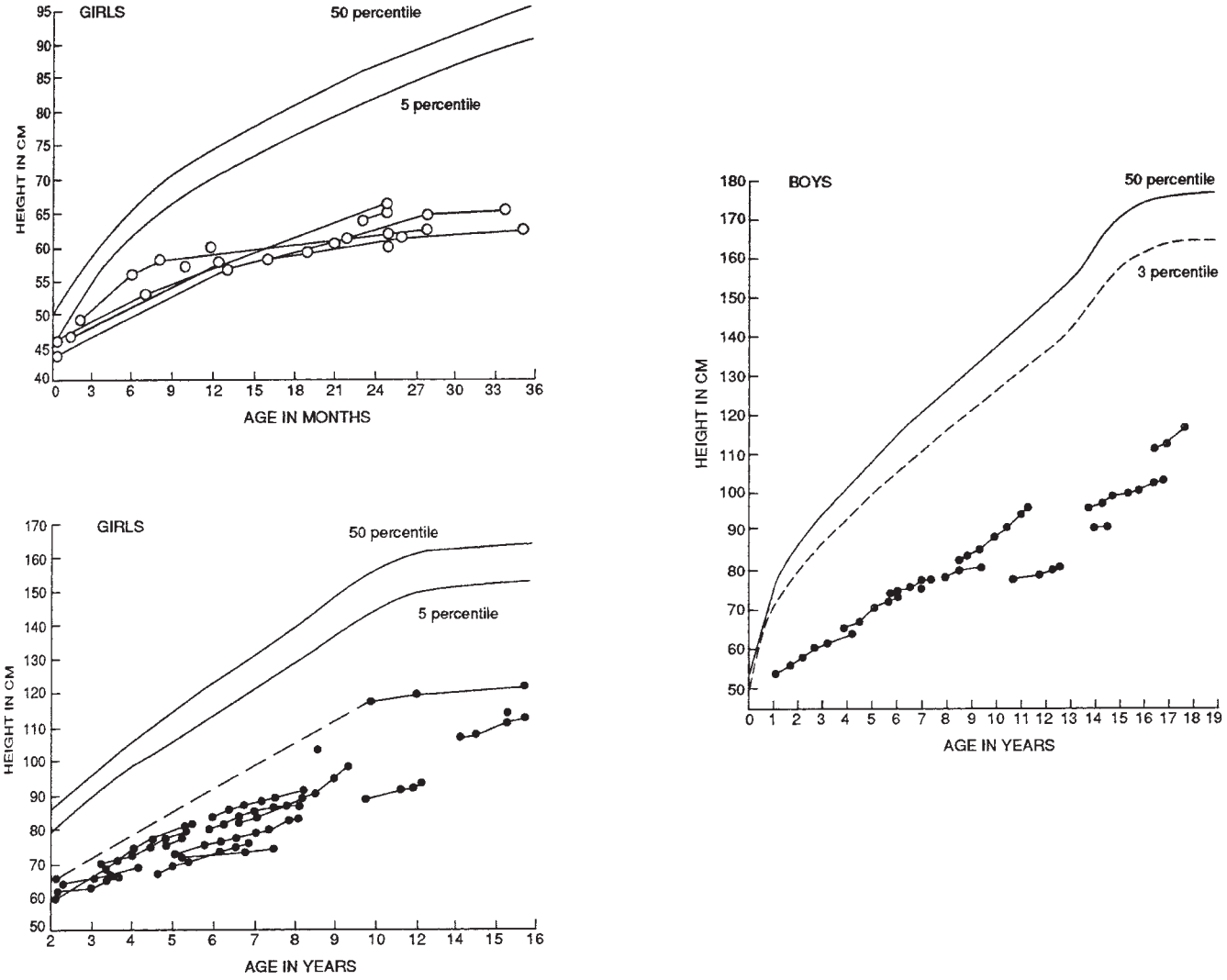


FIGURE 10-27 ■ Height measurements for children from Ecuador with growth hormone receptor deficiency. (From Rosenfeld, R. G., Rosenbloom, A. L., & Guevara-Aguirre, J. (1994). Growth hormone [GH] insensitivity due to primary GH receptor deficiency. *Endocr Rev, 15,* 369.)

less than 60% of the chronologic age. In acquired GHD, as from a tumor of the CNS that presents as symptoms resulting from increased intracranial pressure, the skeletal age may approximate the chronologic age and should therefore not be considered a requirement for the diagnosis of GHD. Weight/height ratios tend to be increased, and fat distribution is often "infantile" in pattern.

Musculature is poor, especially in infancy, which may result in significant delays in gross motor development—leading to the erroneous impression of mental retardation. Facial bone growth is particularly retarded, and the nasal bridge may appear underdeveloped (Figure 10-28). Fontanelle closure is often delayed, but the overall growth of the skull is normal—leading to cephalofacial disproportion and the appearance of hydrocephalus. The voice remains infantile, owing to hypoplasia of the larynx. Hair growth is sparse and the hair itself is thin, especially during the early years of life. Nail growth is also frequently slow. Even with normal gonadotropin production, the penis is small and puberty is generally delayed.

Diagnosis of IGF Deficiency. The proper means of establishing a diagnosis of GHD continues to be highly controversial.[314] With the availability of highly specific assays for the IGF peptides and binding proteins, and with increasing understanding of the GH-IGF axis, we believe that evaluation of patients with growth failure should rest on a combination of careful auxologic assessment and appropriate measures of the GH-IGF system. Establishment of deficiency of IGF peptides and concomitant alterations in serum concentrations of IGFBPs then necessitates a thorough evaluation of hypothalamic-pituitary-IGF function.

The foundation for the diagnosis of IGF deficiency must be auxology, with careful documentation of serial heights and determination of height velocity. In the absence of other evidence of pituitary dysfunction, it is generally unnecessary to perform tests of GH secretion. Thus, even in children below the 5th percentile in height (which obviously applies to 5% of the population), documentation of a normal height velocity

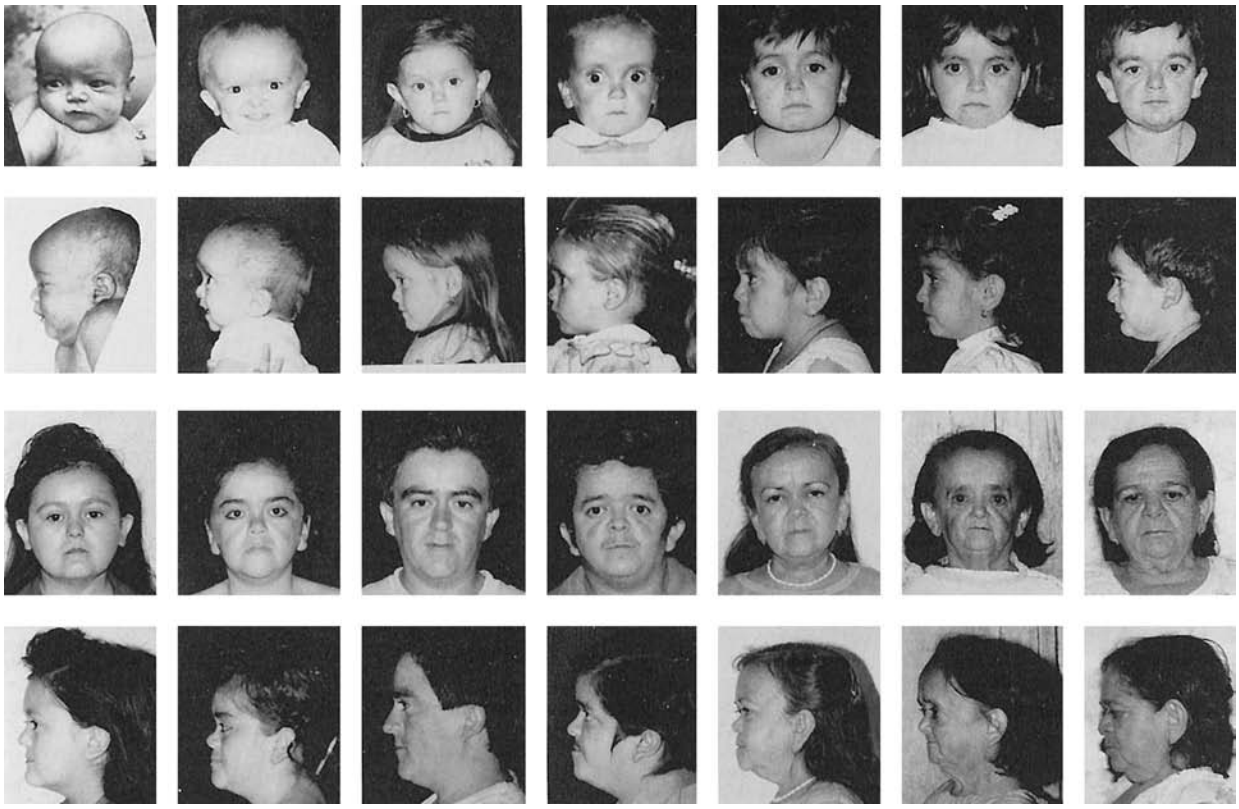


FIGURE 10-28 ■ Facial appearance of patients from Ecuador with growth hormone receptor deficiency. (From Rosenfeld, R. G., Rosenbloom, A. L., & Guevara-Aguirre, J. (1994). Growth hormone [GH] insensitivity due to primary GH receptor deficiency. *Endocr Rev, 15*, 369. Photography by Arlan L. Rosenbloom, MD.)

makes the diagnosis of IGF deficiency and GHD highly unlikely.

Assessment of pituitary GH production is problematic because GH secretion is pulsatile throughout the day and night, with the most consistent surges occurring at times of slow-wave electroencephalographic rhythms during phases 3 and 4 of sleep. Regulation of GH secretion is complex, involving two hypothalamic proteins (GHRH and somatostatin) as well as multiple other peptides and neurotransmitters. Spontaneous GH secretion varies significantly with gender, age, and pubertal status—all of which must be factored into any evaluation of GH production.

Between normally occurring pulses of GH secretion, serum GH concentrations are normally low—below the limits of sensitivity of most conventional assays. Accordingly, measurement of random serum GH concentrations is virtually useless in establishing a diagnosis of GHD. Measurement of GH "reserve" has therefore relied on the use of physiologic or pharmacologic stimuli, and these "provocative tests" have been the basis for diagnosing GHD since the 1980s.[314,668]

"Physiologic" stimuli have included fasting, sleep,[669] and exercise,[670,671] whereas pharmacologic stimuli have included levodopa,[672] clonidine,[673] glucagon,[674] propranolol, arginine,[675] and insulin[676,677] (Table 10-9). Stimulation tests have often been divided into "screening tests" (exercise, fasting, levodopa, clonidine)—characterized by ease of administration, low toxicity, low risk, and low specificity—and "definitive tests" (arginine, insulin, glucagon). To improve specificity, provocative tests are customarily combined or given sequentially.[678-680] It has been generally accepted that a child must "fail" provocative tests with at least two separate stimuli to be considered as having GHD. Standard provocative GH tests are summarized in Table 10-9.

Although provocative GH testing has been the foundation for the diagnosis of GHD since GH radioimmunoassays first became available, its use as a determiner of GH status has come under criticism for a number of reasons.[314,681] These issues, as follows, have been summarized in a consensus report on the diagnosis of childhood GHD.[667]

Provocative GH Testing Is Nonphysiologic. None of the standard pharmacologic provocative tests satisfactorily mimics the normal secretory pattern of pituitary GH. Even when naturally occurring regulatory peptides are used for stimulation, their dosage, route of administration, and interactions with other regulatory factors are artificial. In addition, because most endocrine centers use several different stimulation tests, there is no validated means of resolving conflicting data from two or more provocative tests.[682] In a report of 6373 GH stimulation tests performed on 3233 short French children, 11 different pharmacologic tests were employed, 62 of the possible 66 pairs were employed at least once, and the most frequent combination of tests was used in only 12.7% of the patients.[683]

TABLE 10-9 **Growth Hormone Stimulation Tests***

Stimulus	Dosage	Samples (Min)	Comments/Side Effects
Levodopa (PO)	< 15 kg: 125mg 15-30 kg: 250 mg > 30 kg: 500 mg	0, 60, 90	Nausea
Clonidine (PO)	0.15 mg/m^2	0, 30, 60, 90	Tiredness, postural hypotension
Arginine HCl (IV)	0.5 g/kg (max 30 g) 10% arginine HCl in 0.9% NaCl over 30 min	0, 15, 30, 45, 60	
Insulin (IV)	0.05-0.1 IU/kg	0, 15, 30, 45, 60, 75, 90, 120	Hypoglycemia; requires supervision†
Glucagon (IM)	0.03 mg/kg (max 1 mg)	0, 30, 60, 90, 120, 150, 180	Nausea

*Tests should be performed after an overnight fast. Some authorities generally recommend that prepubertal children be "primed" with sex steroids (e.g., Premarin, 5 mg PO, the night before and the morning of the test, or ethinyl estradiol, 50 to 100 μg/day for 3 consecutive days before testing; or depot testosterone, 100 mg 3 days before testing). Patients should be euthyroid at time of testing.

†Insulin-induced hypoglycemia is a potential risk of this procedure, which is designed to lower the blood glucose by at least 50%. Documentation of appropriate lowering of blood glucose is recommended. If there is growth hormone deficiency, the lower dosage of insulin may be advisable, especially in infants. $D_{50}W$ solutions and glucagon should be available.

Arbitrary Definitions of "Subnormal" Response to Provocative Tests. Endocrine centers vary in their definition of a "normal" response to stimulation tests. Whereas early reports generally employed a cutoff level of 5 ng/mL, this was gradually increased to 7 ng/mL. With the availability of recombinant DNA–derived hGH, this level was increased to 10 ng/mL—although no data exist for validating any of these arbitrary cutoff levels. The lack of objective confirmation of any defined normal response can be seen in the use of language such as "lack of adequate endogenous growth hormone secretion"[684] and "inadequate secretion of normal endogenous growth hormone."[684] As reported by Guyda, many of the newer GH assays measure GH concentrations twofold to threefold lower than older assays and yet there has been no systematic reevaluation of the "normal" GH cutoff level.[670]

Age Dependency and Use of Sex Steroids. Serum GH concentrations typically rise during puberty, manifested as an increase in pulse-amplitude but not pulse frequency.[79,685] Immediately before puberty and during the earliest phases of puberty, GH secretion may normally be so low as to blur the distinction between GHD and constitutional delay of growth and maturation.[79,685] There are multiple reports of children who "failed" provocative testing before the onset of puberty but proved to have "normal" GH secretion after puberty or after administration of exogenous sex steroids.[686-689] A study of provocative GH testing in children of normal stature has clearly demonstrated the inherent problems with provocative GH testing and the need for standardization of sex steroid administration during stimulation tests.[690]

When exercise and arginine/insulin stimulation tests were administered to these normal children, the lower limit of normal (2 SD) for peak serum GH concentration in prepubertal children was only 1.9 ng/mL—whereas in children of Tanner stage 5 puberty this level was 9.3 ng/mL. When estrogen was administered before provocative testing, the lower 95% confidence limit for the normal serum GH range rose to 7.2 ng/mL. All in all, when estrogen was not administered 61% of the normal prepubertal children failed to raise their serum GH concentration above 7 ng/mL after three provocative tests. These children could have been erroneously labeled as having GHD.

GH Assays of Limited Accuracy. Several studies have demonstrated as much as threefold variability in the measurement of serum GH concentrations by established laboratories.[691,692] This is explained at least in part by the existence of several molecular forms of GH in serum and by the use of monoclonal versus polyclonal antibodies. The inevitable result has been that children labeled as having GHD by one assay would be considered normal by another.

A highly sensitive immunofunctional GH assay has been developed that measures concentrations of GH capable of binding to GHBP. It is not clear, however, that such assays necessarily have any advantages over standard radioimmunoassays for routine GH measurements.[693] When arginine/levodopa or arginine/insulin stimulation tests were used, approximately 50% of normal children had peak GH concentrations less than 7 ng/mL and 30% were less than 5 ng/mL whether GH was measured by immunofunctional assay or enzyme-linked immunosorbent assay.

Expense, Discomfort, and Risks of Provocative GH Testing. Provocative testing typically requires multiple timed blood samples and frequently necessitates parenteral administration of drugs. The resulting discomfort to the patient and expense are self-evident. In addition, insulin administration carries the risk of hypoglycemia and seizures and should be performed by experienced medical personnel under appropriate supervision. Death has been reported from insulin-induced hypoglycemia and from its overly vigorous correction with parenteral glucose.[694]

Poor Reproducibility of Provocative Tests. The reproducibility of provocative GH tests has never been adequately demonstrated, even when GH concentrations are determined with the same assay.[694] An alternative approach is the measurement of spontaneous GH secretion. This can be done either by multiple sampling (every 5 to

30 minutes) over a 12- to 24-hour period or by continuous blood withdrawal over 12 to 24 hours.[78,624,695,696] The former method allows one to evaluate and characterize GH pulsatility, whereas the latter only permits determination of mean GH concentration. Either approach, however, is subject to many of the same criticisms as provocative GH testing. The potential expense and discomfort of such testing are obvious. Furthermore, although it has been claimed that this technique is more reproducible than are provocative GH tests, variability remains problematic.[418,420,697,698] The ability of such tests to discriminate between GHD and normal short children is also an issue. Rose and colleagues[699] reported that spontaneous GH determinations identified only 57% of children with GHD, defined by provocative testing. In this report, no case of "neurosecretory dysfunction" could be identified in the group of normal short children. Similarly, Lanes and coworkers[700] reported that one fourth of normally growing children had decreased overnight GH concentrations.

Given the problems with GH testing in general, it is not surprising that provocative tests and 24-hour GH profiles do not correlate perfectly. It is in fact likely that 12- to 24-hour GH profiles can correctly identify the majority of children with GHD and may be superior in sensitivity and specificity to provocative GH testing. With the advent of more routine treatment of ISS with GH, GH testing is no longer the controversial topic it once was—and many experts recognize that a large overlap exists between GHD and ISS as they are commonly defined.

"Neurosecretory dysfunction" probably does exist in children who have had cranial irradiation and likely does describe a subgroup of children with GHD and IGF deficiency. However, the expense and discomfort of frequent overnight or 24-hour sampling combined with many of the problems intrinsic to GH determinations preclude this form of testing for GH deficiency from being the test of choice in establishing the diagnosis of GHD.

The measurement of GH concentrations in urine has provided an alternative means of assessing "integrated" GH secretion (or at least excretion).[701-703] This technique requires anti-GH antibodies of high affinity because urinary GH concentrations are normally low. It also requires timed urine collections. Adequate age- and sex-related standards have not yet been fully developed, and the diagnostic use of urinary GH determinations remains to be adequately evaluated.

Whereas some have interpreted these difficulties in measuring GH as a reason to stop performing GH testing altogether, we strongly recommend continuing use of this testing modality that is nevertheless critical to differentiating GHD from ISS, and secondary from primary IGFD. These conditions are vastly different in a variety of ways. Rather, we believe that the results of the GH testing should not serve as an absolute determinant of the decision to treat with GH (or other drugs, including IGF-I). A complementary approach to the diagnosis of GHD is the use of IGF-related assays.[209,210,213,236] GHD then becomes part of the differential diagnosis of IGF deficiency, which includes hypothalamic dysfunction, pituitary insufficiency, and GHI.

With the development of sensitive and specific assays for IGFs, as well as for the IGFBPs, it has become apparent that these peptides can reflect the GH status of the patient. Furthermore, they have the advantage that they normally circulate in serum at high concentrations—and thus assay sensitivity is less of an issue. Serum levels of these peptides remain relatively constant during the day, and provocative testing or multiple sampling is not necessary. It is important, however, to recognize the following potential limitations of assays for IGF-1.

- The IGFBPs potentially interfere with radioimmunoassays, radioreceptor assays, and bioassays.[215-217] These binding proteins must be completely removed, as with acid gel chromatography (which is labor intensive)[215-217] or blocked by the addition of excess IGF-2 (which requires a high-affinity antibody with a high degree of specificity for IGF-1).[218] An alternative approach is to employ a radiolabeled IGF-1 analog with reduced affinity for IGFBPs.[219]
- Serum IGF-1 concentrations are highly age dependent.[211,227,228] They are lowest in young children (< 5 years of age), the age at which one most wishes to have a simple diagnostic test.
- Serum IGF-1 concentrations may be reduced in a variety of conditions other than GHD. These include primary and secondary forms of GHI. Malnutrition or any cause of diminished insulin concentration (such as poor food intake, dieting, type 1 diabetes) and malabsorption (such as with celiac sprue) will result in low circulating IGF-1 levels. Patients whose weights are less than the 25th percentile for age are likely to have low IGF-1 levels in the range of classic GH deficiency, but their primary problem is malnutrition and not GH deficiency.
- Serum concentrations of IGF-1 (and IGFBP-3) are frequently normal in adult-onset GHD and in children with GHD resulting from brain tumors or cranial irradiation.

Even when these caveats are considered, the correlation between serum IGF-1 concentrations and provocative or spontaneous GH measurements is imperfect. Juul and coworkers[704] have reported that in children younger than 10 years of age, IGF-1 levels were below 2 SD in only 8 of 15 children with a diagnosis of GHD based on provocative testing (53.3% sensitivity) and were normal in 47 of 48 children with a normal GH response (97.9% specificity).

In one study, 18% of patients with abnormally low provocative GH concentrations had IGF-1 concentrations in the normal range but only 4% of "GHD" patients had normal serum concentrations of IGF-1 and IGF-2. Serum levels of IGF-1 and IGF-2 were reduced in only 0.5% of normal children and in 11% of normal short children.[213] The development of specific immunoassays for IGFBP-3, normally the major serum carrier of IGF peptides, has provided a supplementary means of establishing a diagnosis of IGF deficiency and GHD (Figures 10-21 and 10-29).[301,310,311] Because molar concentrations of IGFBP-3 correlate with the sum of the molar concentrations of IGF-1 and IGF-2, IGFBP-3 determinations offer the following advantages over assays of IGF peptides and other IGFBPs.

- IGFBP-3 immunoassays are technically simple and do not require any separation of the binding protein from IGF peptides.

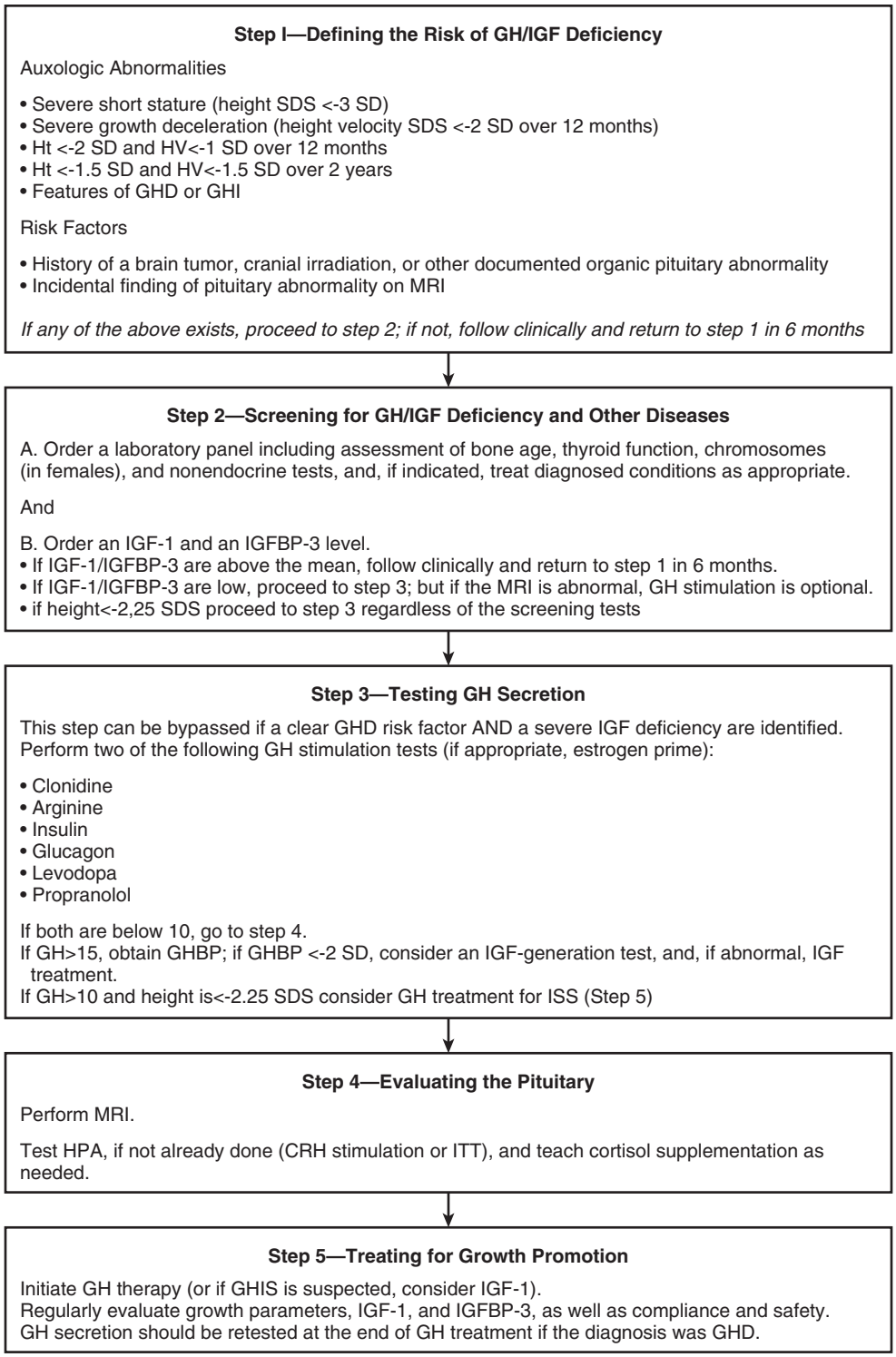


FIGURE 10-29 ■ Algorithm of biochemical evaluation of growth failure. CRH, corticotropin-releasing hormone; GH, growth hormone; GHBP, GH-binding protein; HPA, hypothalamic-pituitary axis; HV, height velocity; IGF, insulin-like growth factor; IGFBP-3, IGF-binding protein-3; ITT, insulin tolerance test; MRI, magnetic resonance imaging; SD, standard deviation; and SDS, standard deviation score.

- Normal serum concentrations of IGFBP-3 are quite high, typically in the 1- to 5-mg/mL range, and thus assay sensitivity is not an issue.
- Although age dependency exists, serum IGFBP-3 concentrations vary with age to a lesser degree than is the case for IGF-1. Even in infants, serum IGFBP-3 concentrations are normally sufficiently high to allow discrimination of pathologically low values from the normal range.
- Serum IGFBP-3 concentrations are less nutritionally dependent than concentrations of IGF-1, reflecting the "stabilizing" effect of IGF-2 concentrations.
- IGFBP-3 concentrations are clearly GH dependent.

The utility of IGFBP-3 assays in the diagnosis of GHD was evaluated by Blum and colleagues,[311] who found that serum IGFBP-3 concentrations were below the 5th percentile for age in 128 of 132 (97%) of children diagnosed as having GHD by conventional criteria (height < 3rd percentile, height velocity < 10th percentile, and peak serum GH < 10 ng/mL). At the same time, 124 of 130 (95%) of non-GHD short children had normal IGFBP-3 concentrations.

It is likely that Blum's patients largely consisted of children with severe GHD because this degree of correlation between provocative GH testing and serum IGFBP-3 concentrations has not been consistently identified. For example, Hasegawa and associates[705] reported that the sensitivity of the IGFBP-3 radioimmunoassay in complete GHD (peak GH < 5 ng/mL) was 93% but was only 43% in cases of partial GHD (peak GH 5 to 10 ng/mL). Smith and coworkers[706] found that 100% of children with severe GHD (peak GH < 1 ng/mL) and low serum IGF-1 also had decreased serum IGFBP-3. Four of 8 children with GHD and normal serum IGF-1 levels had reduced IGFBP-3 concentrations, and 10 of 23 (43%) of normal short children had decreased serum IGFBP-3. The addition of a radioimmunoassay for IGFBP-2 further enhanced the ability of IGF axis measures to identify children who had GHD by conventional criteria.

The correlation between IGF-axis determinations and measures of spontaneous GH secretion nevertheless remains imperfect. Even in healthy normal children the correlation between 24-hour GH secretion and serum IGF-1 and IGFBP-3 concentrations is modest ($r = 0.78$ and $r = 0.62$, respectively).[706] In the Smith study,[707] 18% of patients were found to have discordant measures of IGFBP-3 and provocative GH response. It is impossible at this time to resolve conflicts between assays of the IGF axis and measurements of GH secretion because there is no way to definitively diagnose GHD. Experience in patients with GHRD, however, has provided further evidence in support of the utility of IGF-related determinations.[110,708,709]

Although such patients may have normal or elevated serum GH concentrations, mutations or deletions of the GHR gene render them unresponsive to GH—and such patients may be considered "functionally GH deficient." In the experience in Ecuador, of approximately 70 documented cases of GHR gene mutations, all patients were found to have markedly reduced serum concentrations of both IGF-I and IGFBP-3. Even so, IGF-1 and IGFBP-3 correlated significantly with height. Measures of serum concentrations of IGF-1 and IGFBP-3 have been used in other studies of GHI to establish diagnostic criteria.

Ultimately, the diagnosis of GHD (or IGF deficiency) should be made on the basis of combined clinical and laboratory criteria. Short children who have well-documented normal height velocities do not generally require evaluation of GH secretion, and normal (> 25%) serum IGF-1 and IGFBP-3 concentrations can be reassuring. Children with Turner syndrome and short stature that is consistent with the expected growth pattern for this syndrome should not be required to undergo GH testing to qualify for GH therapy because such treatment is not predicated on abnormal GH secretion.

On the other hand, the child with documented growth deceleration requires further evaluation—even if tests of GH secretion appear normal. Documentation of decreased serum IGF-1 or IGFBP-3 concentrations would then constitute a diagnosis of IGF deficiency, and the diagnoses of GHD and GHI would need to be considered. The child with a history of cranial irradiation, decreased height velocity, and reduced serum concentrations of IGF-1 and IGFBP-3 should be considered to have GHD—even in the presence of normal provocative tests.

This approach still requires measurements of GH secretion. Such determinations are critical to distinguishing between GHD and GHI as causes of IGF deficiency. Documentation of abnormal pituitary GH secretion alerts the physician to the possibility of intracranial tumors and to the potential for other pituitary hormone deficiencies. Evaluation for GHD allows for concomitant assessment of ACTH secretion, and TSH and gonadotropin determinations can be added where appropriate. Ultimately, however, one must conclude that the single most important parameter in assessment of children with growth failure is careful clinical evaluation—including accurate serial measurements of height and determinations of height velocity.

The possibility of hypothalamic-pituitary dysfunction should always be considered in children with documented growth deceleration, particularly in the presence of a known intracranial pathologic process (e.g., tumors, irradiation, malformations, infection, trauma, blindness, nystagmus). Similarly, the neonate with hypoglycemia or microphallus warrants evaluation of pituitary function—and patients with documented TSH, ACTH, ADH, or gonadotropin deficiency are candidates for GHD. For children with proportional short stature and documented growth deceleration, assessment of serum concentrations of IGF-1 and IGFBP-3 is clearly warranted—and based on those results the possibilities of hypothalamic dysfunction, pituitary insufficiency, and GHI can be investigated.

Recommendations by the Growth Hormone Research Society (GRS) for the diagnosis of GHD recognize that there is no "gold standard" for the diagnosis of GHD and suggest that in a child with slow growth whose history and auxology suggest GHD, testing for GH/IGF-1 deficiency requires the measurement of IGF-1 and IGFBP-3 levels as well as GH provocation tests (after hypothyroidism has been excluded). In suspected isolated GHD, two GH provocation tests (sequential or on separate days) are required. In those with a defined CNS pathologic process, history of irradiation, MPHD, or genetic defect, one GH test will suffice. In addition, an evaluation of other pituitary function is required. In patients who have had cranial irradiation or malformations of the hypothalamic-pituitary unit, GHD may evolve over years and its diagnosis may require repeat testing of the GH-IGF axis.

It is recognized, however, that some patients with auxology suggestive of GHD may have IGF-1 or IGFBP-3 levels below the normal range on repeated tests but GH responses in provocation tests above the cutoff level.

These children are not classically GH deficient but may have an abnormality of the GH/IGF axis, and after the exclusion of systemic disorders affecting the synthesis or action of IGF-I could be considered for GH treatment. An MRI (or computed tomographic scan) of the brain with particular attention to the hypothalamic-pituitary region should be carried out in any child diagnosed as having GHD. These GRS recommendations underscore the importance of good clinical judgment rather than specific tests as the key to the diagnosis of GHD.

Testing in the Neonate. The diagnosis of GHD in a newborn is particularly challenging and important. The presence of a micropenis in a male newborn should always be addressed by an evaluation of the GH axis. A GH level should always be measured in the presence of neonatal hypoglycemia in the absence of a metabolic disorder. A GH level in a polyclonal radioimmunoassay of less than 20 mg/L would suggest GHD in the newborn. The use of standard GH stimulation tests is not recommended in newborns, with the exception of the glucagon test (which is safe). In the newborn, although normative data are not available for stimulated serum GH, a cutoff value of 25 ng/mL is probably appropriate and certainly stimulated values under 20 ng/mL should raise suspicion.

An MRI is essential when the diagnosis is suspected, and results may be available sooner than with serum assays. An IGFBP-3 level is of great value to the diagnosis of GHD in infancy, but IGF-1 levels are rarely helpful.[711] In fact, serum IGFBP-3 should be performed as the test of choice in suspected neonatal GHD. Figure 10-29 provides an algorithm for the biochemical evaluation of growth failure. The diagnosis of IGF or GHD should be considered in any child who meets one or more of the criteria listed in Box 10-6.

A child should be considered a candidate for GH therapy if he or she meets one of these criteria, supported by biochemical evidence of GHD based on sex-steroid–primed provocative tests or evidence of IGF deficiency based on measurement of IGF-1 and IGFBP-3 concentrations. Such cases need to also have MRI of the hypothalamus-pituitary and assessment of other pituitary hormone deficiencies. It is understood that this approach will result in GH treatment of some children with "idiopathic isolated" GHD or IGF deficiency and that such cases require careful monitoring of both pituitary status and responsiveness to GH treatment. The latter can be assessed relative to recently developed predictive models,[710] and the diagnosis of GHD should be reconsidered in the child with idiopathic isolated GHD, a normal MRI, and a subnormal clinical response to GH.

BOX 10-6 Key History and Physical Examination Findings That May Indicate GH Deficiency

- In the neonate; hypoglycemia, prolonged jaundice, microphallus, or traumatic delivery
- Cranial irradiation
- Head trauma or central nervous system infection
- Consanguinity or an affected family member
- Craniofacial midline abnormalities
- Severe short stature (< − 3 SD)
- Height (< − 2 SD) and a height velocity below over 1 year (< − 1 SD)
- A decrease in height SD of more than 0.5 over in children > 2 years of age
- A height velocity below − 2 SD over 1 year
- A height velocity more than 1.5 SD below the mean sustained over 2 years
- Signs indicative of an intracranial lesion
- Signs of multiple pituitary hormone deficiency
- Neonatal symptoms and signs of growth hormone deficiency

SD, standard deviation.

Diagnosis of GHI. The combination of decreased serum concentrations of IGF-1, IGF-2, and IGFBP-3 plus increased serum concentrations of GH is highly suggestive of a diagnosis of GHI.[110] The possibility of GHRD is supported by a family history consistent with autosomal-recessive transmission. Savage and Rosenfeld[712] devised a scoring system for evaluating short children for the diagnosis of GHRD based on five parameters: basal serum GH greater than 5 ng/mL, serum IGF-1 less than or equal to 50 ng/mL, height SD scores less than –3, serum GHBP less than 10%, and a rise in serum IGF-1 concentrations after a week of GH stimulation of less than twofold the intra-assay variation (approximately 10%).

Blum and colleagues[713] proposed that these criteria could be strengthened by evaluating GH secretory profiles, rather than isolated basal levels; employing an age-dependent range for evaluation of serum IGF-1 concentrations and using the 0.1 percentile as the cutoff level; employing highly sensitive IGF-1 radioimmunoassays and defining a failed GH response as the inability to increase serum IGF-1 concentrations by at least 15 ng/mL; and employing basal and GH-stimulated IGFBP-3 concentrations. These criteria fit well with the population of GHRD patients studied in Ecuador, but that is a remarkably homogeneous population of patients who have severe GHI.[110,709] The universal applicability of these criteria remains to be evaluated. An important biochemical marker will be the IGF-1 (and possibly IGFBP-3) response to GH stimulation. Although such tests were first employed in the early 1980s, normal ranges and age-defined responses of serum IGF-1 concentrations have not yet been determined.[714]

Decreased serum concentrations of GHBP are obviously highly suggestive of a diagnosis of GHRD, but it is important to point out that cases of GHRD with normal serum concentrations of GHBP have already been identified.[642,715] Such cases may represent mutations in the site responsible for dimerization of the GHR, or potentially for abnormalities of the intracellular portion of the receptor or of the postreceptor signal transduction mechanism. On the other hand, polymorphisms of the GHR gene without resulting reductions in IGF-1 and IGFBP-3 concentrations should not be considered GHRD. At this point, definitive diagnosis requires the classic phenotype, decreased

serum concentrations of IGF-1 and IGFBP-3, and identification of an abnormality of the GHR gene.

Constitutional Delay of Growth and Maturation. The diagnosis of constitutional delay has had different meanings to different clinicians.[716,717] To some, it has consisted of delayed adolescent growth and maturation in the presence of decreased (even if only transiently) GH secretion.[717,718] More commonly, it has been considered a normal variant (characterized by short stature) but with relatively normal growth rates during childhood, delayed puberty, a delayed pubertal growth spurt, and attainment of normal adult height.

Most patients with constitutional delay begin to deviate from the normal growth curve during the early years of life and are typically by age 2 years at or slightly below the 5th percentile for height. Such children would be expected to have normal serum IGF-1 and IGFBP-3 concentrations, a normal result of a GH provocative test (if pretreated with sex steroids), and skeletal ages that were delayed (Box 10-7). By definition, children with pure constitutional delay should have bone ages sufficiently delayed to result in normal predicted adult heights (see Box 10-7). When constitutional delay is found in the context of familial short stature, however, children may experience a delayed adolescent growth spurt and a short final height. Such children should be considered to have elements of constitutional delay and familial short stature and should be classified as ISS and considered for GH therapy.

As stated previously, some have attributed constitutional delay to a transient GHD or to a "lazy" pituitary. It is likely that much of this experience can be attributed to the inadequacies of GH testing, especially to the failure to pretreat patients with a brief course of sex steroids.[690] Low serum concentrations of IGF-1 and IGFBP-3 or a poor GH response to provocative testing (after priming with sex steroids) should mandate an investigation for an underlying pathologic process, such as intracranial tumors.

BOX 10-7 Criteria for Presumptive Diagnosis of Constitutional Delay of Growth and Maturation

- No history of systemic illness
- Normal nutrition
- Normal physical examination, including body proportions
- Normal thyroid function test results
- Normal renal function test results
- Normal complete blood cell count, erythrocyte sedimentation rate, electrolytes
- Normal stimulated GH
- Height between −2.5 and −1.5 SDS
- Height velocity > −1 SDS
- Delayed puberty:
- Males: failure to achieve Tanner G2 stage by age 13.8 years or P2 by 15.6 years
- Females: failure to achieve Tanner B2 stage by age 13.3 years
- Delayed bone age (more than 1 year delayed)
- Normal predicted adult height:
- Males: >165 cm (65 inches)
- Females: >153 cm (60 inches)

Idiopathic Short Stature. ISS can be defined as a condition in which the height of an individual is more than 2 SD below the corresponding mean height for a given age, sex, and population group without evidence of systemic, endocrine, nutritional, or chromosomal abnormalities. Specifically, children with ISS have normal birth weight and are growth hormone sufficient. ISS describes a heterogeneous group of children consisting of many presently unidentified causes of short stature. It is estimated that approximately 60% to 80% of all short children at or below –2 SD fit the definition of ISS.

This definition of ISS includes short children labeled with "constitutional delay of growth and puberty" and "familial short stature." The frequency of referral of these children is dependent on the socioeconomic environment. Furthermore, there is a greater perceived disability of short stature in boys compared to girls irrespective of social class. ISS should be subcategorized, principally based on auxologic criteria. The main distinction is between children with a familial history of short stature whose heights are within the expected range for parental target height and those children who are short for their parents. The corrected target height standard deviation score (SDS) is calculated as 0.72 multiplied by the average of the father's and mother's height SD scores and the lower limit of the target height range as corrected target height –1.6 SD.

It is generally accepted that on average adult height achieved in children with ISS is below the parental target height. ISS should also be classified by the presence or absence of bone age delay, indicating the probability of delayed growth and puberty. Subcategorization may help to predict adult height, which would be expected to be greater in a child with delayed maturation. Individuals with no family history of short stature generally have a lower adult height in comparison to target height. In situations in which a specific genetic diagnosis associated with short stature is expected (such as Noonan syndrome or GH insensitivity), the gene(s) of interest should be examined. Online resources exist, such as Genetest (www.genetests.org), which identify laboratories capable of performing these tests.

Although routine analysis of SHOX should not be undertaken in all patients with ISS, SHOX gene analysis should be considered for any patient with clinical findings compatible with SHOX haploinsufficiency. With currently available data it is difficult to generalize the impact of short stature on psychosocial adaptation. Short stature may be a risk factor for psychosocial problems, such as social immaturity, infantilization, low self-esteem, and being bullied—especially for those referred for evaluation. The large interindividual differences in adaptation to short stature and in the impact of being short may be a function of several risk and protective factors, including parental attitudes and prevailing cultural opinions. Stress experiences may be frequent, but true psychopathology is rare.[719]

TREATMENT OF GROWTH DISORDERS

When growth failure is the result of a chronic underlying disease (such as renal failure, cystic fibrosis, or malabsorption syndromes), therapy must be directed at treatment of the underlying condition. Although growth acceleration may be observed with GH or IGF-1 therapy, complete catch-up requires correction of the primary medical problem. If treatment of the underlying condition involves glucocorticoids, growth failure may be profound and is unlikely to be correctable until the patient is weaned from steroids.

Correction of growth failure associated with chronic hypothyroidism requires appropriate thyroid replacement. As discussed previously, thyroid therapy results in dramatic catch-up growth but also markedly accelerates skeletal maturation—potentially limiting adult height. More gradual thyroid replacement, especially in the beginning of the treatment, the use of gonadotropin inhibitors to delay puberty, or the use of aromatase inhibitors to slow skeletal age maturation may be necessary to maximize final adult height.

Treatment of Constitutional Delay

Constitutional delay is a normal variant with (by definition) potential for a normal (although delayed) pubertal maturation and a normal adult height. Most cases can be successfully managed by careful examination and evaluation to rule out other causes of delayed puberty or abnormal growth, combined with appropriate explanation, conservative follow-up, and psychological counseling. The skeletal age and Bayley-Pinneau table are often helpful in explaining the potential for normal growth. A family history of constitutional delay is also frequently a source of reassurance but may not always be elicited. On occasion, however, the stigmas of delayed maturation and short stature may be psychologically disabling for the adolescent.

Studies have demonstrated that some adolescents with constitutional delay have poor self-images and limited social involvement.[720] In such patients, there is a role for the judicious use of short-term sex steroids. In males, therapy should generally be limited to adolescents who meet the following criteria: a minimal age of 14 years; height below the third percentile; prepubertal or early Tanner G2 stage, with a serum testosterone level less than 100 ng/dL; evidence of poor self-image that does not respond to reassurance alone; and predicted adult height well within the normal range. The patients usually have a skeletal age delay that corrects their height to the midparental (target) height range. Therapy in males consists of depot testosterone (enanthate or cypionate), 50 to 100 mg every 3 to 4 weeks for a total of 4 to 6 injections.[721]

Patients will typically show early secondary sex characteristics by the fourth injection and grow an average of 10 cm in the ensuing year. This brief course of therapy has been shown to not cause overly rapid skeletal maturation, compromise adult height, or suppress pubertal maturation.[722] It is important to emphasize to the patient that he is normal, that therapy is short term and designed to provide him with some pubertal development earlier than he would experience on his own, and that treatment will not increase adult height. In such situations, the combination of short-term androgen therapy plus reassurance and counseling has been helpful in assisting the patient with constitutional delay cope with a difficult adolescence.

Patients must be reevaluated to ensure that they spontaneously enter "true" puberty. One year after testosterone treatment, patients should demonstrate testicular enlargement and a serum testosterone level in the pubertal or adult range. If this is not the case, the diagnoses of pituitary insufficiency or hypogonadotropic hypogonadism should be considered. Although a second course of testosterone may be warranted at this time, it is our experience that most such patients eventually prove to be gonadotropin deficient.

The availability of several new forms of testosterone supplementation, which are approved for adults with hypogonadism, has provided pediatricians with an opportunity to offer patients a choice among different androgen replacement therapies. Although these new therapies have not been published as efficacious in children with constitutional delay, we have personal experience with their successful use—with an equivalent response to that obtained with testosterone injections. Testosterone gel is painless and easy to apply, and it has proven popular since its release.[723] Testosterone patches also allow patients to avoid the need for injections but work best when applied to the scrotum and are often accompanied by complaints of itching.[724] The dosing of these alternative forms of therapy in children is not clearly established. Care must be taken, as these preparations increase the risk for administration of too large a dose of androgen, as most preparations are developed for adult androgen replacement.[725] Further experience will undoubtedly accumulate on their use in pediatrics in the coming years. The combined use of androgen therapy with aromatase inhibition is theoretically attractive in patients with constitutional growth delay who have a height prediction below their genetic target height range. Several prospective trials have shown that treatment with aromatase inhibitors delays skeletal maturation and increases predicted adult height. Data on long term follow-up of constitutionally delayed patients treated like this suggest that this may also result in taller adult height.[726] However, use of these agents still is considered experimental until the drug safety profile has been evaluated in more patients with longer follow-up, especially regarding the qualitative effects on skeletal development.

Referrals for constitutional delay are much more common in males than in females, undoubtedly reflecting our cultural values. When constitutional delay is a problem in girls, short-term estrogen therapy can be employed. The use of GH in patients with constitutional delay is discussed later in this chapter.

Treatment of Growth Hormone Deficiency

Patients with proven GHD should be treated with recombinant human GH (rhGH) as soon as possible after

the diagnosis is made. The primary objectives of the therapy for GHD are normalization of height during childhood and attainment of normal adult height. Normally growing patients with craniopharyngioma and GHD should be considered for therapy with GH for metabolic and body composition benefits and for enhancement of pubertal growth.

GH exhibits a high degree of species specificity in its actions. Unlike most other hormones, the only GH biologically active in humans is primate GH. For many years, the only practical source of primate GH for treatment of GHD was human cadaver pituitary glands—first employed in the late 1950s. Over the next 25 years, more than 27,000 children with GHD worldwide were treated. In 1985, distribution of pituitary-derived human growth hormone (hGH) in the United States and most of Europe was halted because of concern about a causal relationship with Creutzfeldt-Jakob disease—a rare and fatal spongiform encephalopathy that had been previously reported to be capable of iatrogenic transmission through human tissue.[727,728]

In North America and Europe, this disorder has an incidence of approximately 1 case per million. Noniatrogenic cases are exceedingly rare before the age of 50 years. To date, more than 20 young adults of about 8000 patients in the United States who had received human cadaver pituitary products have died of Creutzfeldt-Jakob disease. In France, there have been more than 60 cases of Creutzfeldt-Jakob disease among 1700 human cadaver GH recipients—and in England 32 cases among 1900 recipients of human cadaver GH have been reported.

Fortuitously, at the time that the risks of pituitary-derived hGH were discovered, recombinant DNA–derived hGH had already begun extensive testing for safety and efficacy.[312,729,730] The original form of rhGH included an N-terminal methionine, added for use as a start signal for transcription (met-hGH). This preparation was found to mimic pituitary-derived hGH in its anabolic and metabolic actions. Subsequent rhGH preparations were produced without the additional methionine. Over the next decade, rhGH universally replaced pituitary-derived hGH as the treatment of choice for children with GHD.

Dosing of GH

Despite continued variability and lack of consensus, considerable progress and improvement have been made in the standardization of GH dosage and administration. It is well established that GH administration should be initiated as early as possible in the GH-deficient child to optimize final height outcome.[731] Daily administration of GH is clearly more effective than giving the same total dose three times weekly.[732] GH injections are best administered in the evening, better mimicking natural physiology and achieving higher GH peaks.[733] The injected hGH should be administered subcutaneously.

The dosage of GH should be expressed in milligrams (or micrograms) per kilogram per day, although consideration should be given to dosing in micrograms per square meter of body surface per day in patients with obesity. GH is routinely used in the range of 25 to 50 μg/kg/day. A dose-response relationship in terms of height velocity in the first 2 years has been clearly demonstrated within this range (Figure 10-30).[734-736] On this regimen, the typical GH-deficient child accelerates growth from a pretreatment rate of 3 to 4 cm/year to 10 to 12 cm/year in year 1 of therapy and 7 to 9 cm/year in years 2 and 3. This progressive waning of GH efficacy has been observed universally and is still not fully understood. It can, however, be overcome at least in part by increasing the dosage of hGH. In the United States, at a dosage of 50 mcg/kg/day, the approximate current cost of hGH therapy for a 20-kg child is $15,000/year. The practice of individualizing treatment according to the specific needs of each GH-deficient child is gaining acceptance.

Consensus does not exist as to how to formulate individualized treatment plans. Emerging evidence suggests that mathematical prediction models of growth response may be useful for determination of the optimal individual dose.[710,737,738] Although these models will need further improvement in their predictive power (as well as further validation), their potential utility is considerable. GH doses can be calculated to achieve specific therapeutic aims (i.e., catch-up to target height within 2, 3, or 4 years). These models can also enable a comparison between observed and predicted growth, hastening identification of causes of suboptimal growth. Further application of these models to specific diseases and conditions in which GH responsiveness varies will allow disease-specific optimization.

Despite the availability of GH therapy, long-term studies have indicated that many patients fail to achieve normal adult heights—and only a few attain their genetic target heights. Although the development of rhGH has solved the problem of supply experienced in the pituitary GH era, delays in diagnosis and initiation of therapy have still compromised adult height. Data from the National Cooperative Growth Study evaluation of adult heights in 121 patients with childhood GHD indicate a mean adult

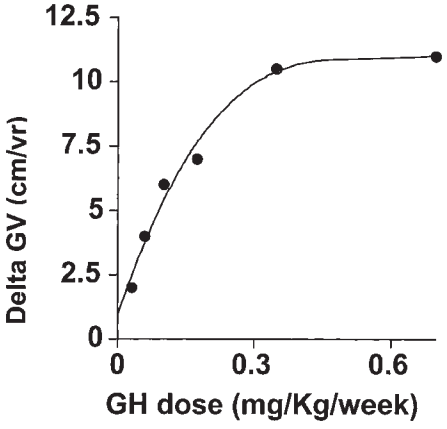


FIGURE 10-30 ■ Meta-analysis of the dose-response relationship between growth hormone (GH) and delta growth velocity (GV) in the first year of treatment of naive GH-deficient children. The lower three doses are from Frasier and colleagues,[501] and the higher three doses are from Cohen and coworkers.[502]

height in male and female patients of < 0.7 SD.[739] By multiple-regression analysis, factors found to correlate with enhanced adult height were baseline height, younger age at onset of treatment, longer treatment duration, and a greater growth velocity during the first year of treatment.

In an effort to increase final height of GHD patients, Mauras and associates[740] have evaluated the use of high-dose GH during puberty—with the rationale that GH secretion normally doubles during the pubertal growth spurt (as indicated by the dramatic rise in serum IGF-1 concentrations during puberty) and that the pubertal growth spurt normally accounts for approximately 17% of adult male height and 12% of adult female height. Earlier studies by Stanhope and associates[741] indicated that little difference in height gain could be observed when adolescent patients were treated with 30 versus 15 IU/m^2/wk of GH (approximately 0.04 versus 0.02 mg/kg/day).

Mauras and associates[740] evaluated higher doses of GH during puberty (100 versus 50 mcg/kg/day) and found that the higher dosage resulted in a 4.6-cm increase in near-final height (defined as height at a bone age of more than 16 years in males and more than 14 years in females). The mean height SD score at near-final height was higher for the 100-mcg/kg/day group. The higher GH dosage did not result in more rapid acceleration of skeletal maturation.

An alternative approach to maximizing height gain during adolescence is to combine GH treatment with suppression of the hypothalamic-pituitary-gonadal axis by GnRH analogues (or, but only in boys, the use of aromatase inhibitors). This combined therapy may lead to significant improvement of final height or predicted adult height of patients, as shown in some studies (but long-term results are still lacking). In addition, the effect of suppression of puberty on bone accretion during the critical pubertal phase (as well as on psychosocial function) has not yet been evaluated adequately. Similar concerns may exist for the combination of GH therapy with aromatase inhibitors, which are designed to prevent the effects of estrogen on epiphyseal fusion.

Novel Modalities for Treatment of GHD

Several novel treatment modalities have emerged (Box 10-8). These include oral secretagogues, GHRH, extended-action depot rhGH preparations, and liquid rhGH formulations. A number of GH-releasing peptides and nonpeptidyl GH secretagogues have been formulated since their discovery in the 1980s.[742] Although these oral agents would be an attractive potential treatment option for GHD secondary to hypothalamic GRF deficiency, their evaluation remains incomplete at this time.[743,744] GHRH has been demonstrated to be safe and more effective than placebo at increasing height velocity in some GHD children. However, further long-term studies are needed.[745,746]

BOX 10-8 New and Emerging Modalities for Treatment of GH Deficiency

- Liquid formulations
- Pen-type delivery devices
- Long-acting GH formulations
- Sustained-release GH formulations
- Dermal patch delivery of GH
- Oral ghrelin-mimetics

The development of sustained-release long-acting depot GH formulations may eventually provide a desirable alternative to daily subcutaneous rhGH injections for some patients. Currently, such formulations remain under study in ongoing clinical trials—and although efficacious they appear less effective than daily rhGH therapy. Further studies will be necessary, however, to ascertain whether such preparations can provide the same level of growth acceleration as is seen with daily GH—and whether side effects will not be increased. Liquid rhGH formulations, which eliminate the need for reconstitution, and new delivery devices such as pen systems have been introduced and appear to improve patient compliance.

Additional novel delivery GH strategies remain under active development, including orally inhaled and intranasally administered rhGH formulations. In time and with further experience, novel treatment approaches may emerge as useful alternatives to conventional rhGH therapy. The combination of GH along with IGF-I therapies may also prove to be a useful treatment of growth disorders.

Management of Multiple Pituitary Hormone Deficiency

In the patient with an initial diagnosis of isolated GHD, particularly those with an ectopic posterior pituitary or other developmental abnormalities, the clinician should be alert to the risk of the development of MPHD. This involves regular repeat biochemical testing and consideration of repeat pituitary imaging. If GHD is part of a multiple pituitary insufficiency, it is necessary to address each endocrine deficiency. TSH deficiency is often "unmasked" during the initial phase of hGH therapy, and thyroid tests should be performed both before the onset of therapy and during the first 3 months of hGH treatment.[747] Even if normal initially, thyroid function should be tested subsequently on at least an annual basis.

The pituitary-adrenal axis is customarily evaluated during the insulin stimulation test for GHD. If ACTH secretion is impaired, patients should be placed on the lowest safe maintenance dose of glucocorticoids—certainly no more than 10 mg/m^2/day of hydrocortisone. Higher doses may impair the growth response to hGH therapy but may be necessary during times of stress. An alternative approach is to avoid maintenance glucocorticoids and treat with steroids only during periods of physiologic stress.

Gonadotropin deficiency may be evident in infancy in the child with microphallus. This can usually be treated with three to four monthly injections of 25 to 50 mg of testosterone enanthate.[748] Management of puberty can be more complicated because the physical and psychological benefits of normalizing sexual maturation must be

balanced against the risk of epiphyseal fusion. When hGH therapy is initiated in childhood and the child's growth is normalized before adolescence, it is appropriate to begin sex steroid replacement at a normal age (e.g., 11 to 12 in females and 12 to 13 in males).

In males, this can be done by beginning with monthly injections of 100 mg of testosterone enanthate—gradually increasing to 200 mg/month, and eventually moving to the appropriate adult replacement regimen. In girls, therapy involves use of conjugated estrogens or ethinyl estradiol, and eventual cycling with estrogen and progesterone (as described elsewhere in this book). On the other hand, in patients in whom normal or precocious puberty may limit the statural response to hGH, it may be appropriate to delay puberty by the use of the GnRH analogue.

Monitoring GH Therapy

Careful monitoring of pediatric patients with GHD is critical. Important aspects of this process are outlined in Box 10-9. The routine follow-up of GHD children should be performed by a pediatric endocrinologist on a 3- to 6-month basis in partnership with the patient's primary care physician. Assessment of the growth response is perhaps the single most important parameter of monitoring. This consists of accurate determination of height velocity and interval height increase (best expressed in terms of the change in height Z score). Establishment of a target height (typically the midparental target height) against which the child's progress can be assessed is important. Careful attention must also be directed toward screening for possible adverse effects and toward assessing compliance.

Monitoring Serum IGF-1 Levels

The consensus is that annual monitoring of serum IGF-1 and IGFBP-3 levels should be an aspect of routine care of the GHD child receiving rhGH therapy.[749] Titration of rhGH dose to maintain these growth factors within age-dependent normal limits is physiologically sound and is standard practice in the treatment of adults with GHD. It is well recognized that IGF-1 and IGFBP-3 levels are low in children with GHD and increase with rhGH injections.[750]

BOX 10-9 Elements of Monitoring GH Therapy

- Close follow-up with a pediatric endocrinologist every 3 to 6 months
- Determination of growth response (change in height Z score)
- Monitoring serum IGF-1 and IGFBP-3 levels
- Screening for potential adverse effects
- Evaluation of compliance
- Consideration of dose adjustment based on IGF values, growth response, and comparison to growth prediction models

IGF, insulin-like growth factor; IGFBP-3, insulin-like growth factor-binding protein-3.

The relationship between the rise in IGF-1 levels and the growth response during therapy has been demonstrated in a study that used a GH-dose titration to achieve target IGF-I levels.[751] In that study, it was demonstrated that at least over the first 2 years of treatment there is a substantial correlation between the level of the IGF-I achieved and the height gain—and that this correlation was valid for both GHD and ISS patients. Growth factor monitoring certainly has important utility in assessment of compliance issues as well as in the assurance of safety. Several epidemiologic studies have linked serum IGF-1 levels and lower IGFBP-3 levels to increased risk of prostate, breast, and colorectal cancer in otherwise healthy subjects.[222,749,752] Although a causal relationship between serum IGF-1 levels and cancer is not proven, monitoring lifetime exposure to IGF-1, and ensuring that IGF-1 and IGFBP-3 levels in the GH-deficient patient are within age-defined normal limits certainly seem prudent at this time.

Role of Serial Bone Age Assessment

Although a well-established diagnostic tool in the initial evaluation of a GH-deficient patient, bone age assessment no longer has a role in the ongoing management of childhood GHD. Previously, many clinicians have included serial bone age assessment as part of their monitoring of a GH-deficient child's progress on rhGH therapy by comparing the observed to predicted heights and by following the Bailey-Pinneau predicted height calculations.[753,754]

When the wide SDs applied to such measures and the lack of clinical evidence that management is enhanced by their use are considered, they have no sound role in the monitoring of GH therapy.[755] It also appears that although GH therapy accelerates bone maturation, there may be an initial delay before this is apparent radiographically and height predictions may be skewed as a result.[755] At this time, the role of bone age assessment during GH therapy lies only in determining remaining growth potential in the patient with GHD approaching final height and in assessing children with concerns about rapid progression of puberty.

Assessment of Treatment Efficacy and Optimizing Growth Response

Clearly defined individual treatment goals need to be established for each patient with GHD. During the initial 2 years of therapy, catch-up growth at a rate twofold to fourfold above pretreatment growth velocity and a gain of 1 to 2 SDs in height should be expected in the majority of patients.[756] This will be influenced by age at diagnosis and severity of GHD. The dose may need to be increased if catch-up growth is inadequate. After the initial catch-up phase, growth velocity should be maintained at a rate at or above the 50th percentile for age.

Mathematical prediction models may be used not only to predict growth response to a specific dose but also to guide the pediatric endocrinologist in modifying therapy when the observed growth falls short of predicted growth.[738] Prediction models have considerable promise

but need to be improved and studied further in a prospective manner before any recommendations regarding their use can be formulated. In instances in which an inadequate response is encountered, it is also important to consider all possible causes—including poor compliance, technical difficulties, underlying hypothyroidism, incorrect diagnosis, poor nutrition, neutralizing antibodies, and intercurrent illness.

It is critical to maximize height with GH therapy before the onset of puberty. If this is not achieved, modulation of the GH dose during puberty may be considered. As stated previously, the growth response to hGH typically attenuates after the first year—but it should continue to be equal to or greater than the normal height velocity for age throughout treatment. In situations in which the clinical response to hGH is suboptimal, the following possibilities must be considered: poor compliance, improper preparation of hGH for administration or incorrect injection techniques, subclinical hypothyroidism, chronic disease, glucocorticoid therapy, history of irradiation of the spine, epiphyseal fusion, anti-GH antibodies,[757] and incorrect diagnosis of GHD as explanation for growth retardation. Some recipients of rhGH have developed detectable anti-GH antibodies, but growth failure resulting from such antibodies has been exceedingly rare.

Maximal growth response to hGH can be obtained by early diagnosis and initiation of therapy and by careful attention to compliance and psychological support. Thus, although some studies have indicated that as many as 50% of males and 85% of females with idiopathic GHD do not achieve adult heights above the third percentile,[739,758] it is our belief that normalization of height (i.e., achieving target height) should be achievable in most cases. Despite the efficacy of hGH in accelerating growth in children with GHD and the ability of such therapy to normalize adult height if treatment is begun sufficiently early, several studies have indicated that the long-term prognosis for such patients is guarded.[759] The educational, vocational, and social outlook for adults with GHD dating from childhood is frequently suboptimal. Whether this suboptimal psychosocial outlook reflects subtle intellectual deficits or the consequences of lower expectations of patients, families, or teachers remains to be determined. In any case, patients with GHD clearly require careful and thorough follow-up throughout childhood and adolescence—and possibly adulthood.

Studies have focused on the clinical consequences of GHD in adults and on the potential benefits of hGH therapy in such patients.[760,761] Signs and symptoms of adult GHD have included reduced lean body mass and musculature, increased body fat, reduced bone mineral density, reduced exercise performance, and increased plasma cholesterol. Adults with GHD have been found to have a significantly increased risk of death from cardiovascular causes, a finding potentially linked to the increase in adiposity and in serum cholesterol.[762]

Adults with GHD have been found to have "impaired psychological well-being and quality of life" characterized by depression, anxiety, reduced energy and vitality, and social isolation. Several placebo-controlled studies have demonstrated that hGH therapy for adults with GHD results in marked alterations in body composition, fat distribution, bone density, and sense of well-being.[763,764] Whether these effects of hGH therapy will be sustained (and if so, what the optimal hGH regimen will be) remains to be determined.

Because of the potential metabolic benefits of treatment of adult GHD, it is necessary to discuss the need for lifelong GH therapy with patients and families at the time of diagnosis. Given the studies that have demonstrated that many (perhaps the majority) of the children diagnosed with childhood GHD demonstrate normal GH levels on repeat provocative testing, it is recommended that on completion of skeletal growth GH therapy be halted for a period of 1 to 3 months and the patient then retested. Toogood and colleagues[765] have reported that the likelihood of GHD persisting in adult life increases with the number of pituitary hormone deficiencies. Approximately 90% of patients with two or three additional pituitary hormone deficiencies had provocative GH levels less than 5 ng/mL. Similarly, patients with documented structural abnormalities of the hypothalamus-pituitary (such as pituitary hypoplasia, pituitary stalk agenesis, posterior pituitary ectopia, or septo-optic dysplasia) have a very high likelihood of retesting as GHD.

A conservative approach would suggest that all children diagnosed as having GHD should be retested by insulin-provocative tests on completion of skeletal growth and before a commitment is made for long-term adult treatment. An argument can be made, however, that patients with multiple pituitary hormone deficiencies, documented structural abnormalities, or documented hypothalamic-pituitary molecular defects do not necessarily require retesting—or at most should have IGF-1 and IGFBP-3 concentrations determined. On the other hand, the child who has carried a diagnosis of idiopathic isolated GHD should always be retested.

Transition to Adult Management

A suggested algorithm to guide the transition to adult management is displayed in Figure 10-31. After attainment of final height, the pediatric endocrinologist should retest the GH-IGF axis using the adult GHD diagnostic criteria as defined by the Growth Hormone Research Society (GRS) Consensus Workshop on Adult GHD.

Standard GH stimulation tests can be performed after an interval of 1 to 3 months of GH therapy.[766] In places where an insulin tolerance test is mandatory for the patient to qualify for further GH therapy, this test should be performed. At the time of retesting, other pituitary hormones and serum IGF-1 and IGFBP-3 levels should be measured.[704] As recommended by the GRS, the opportunity should be taken to assess body composition, bone mineral density, fasting lipids, insulin, and quality of life before and after discontinuation of GH therapy. Patients with severe long-standing MPHD, those with genetic defects, and those with severe organic GHD can probably be excluded from GH retesting.[627]

When the diagnosis of adult GHD is established, continuation of GH therapy is strongly recommended. Caution should be exercised when considering the decision

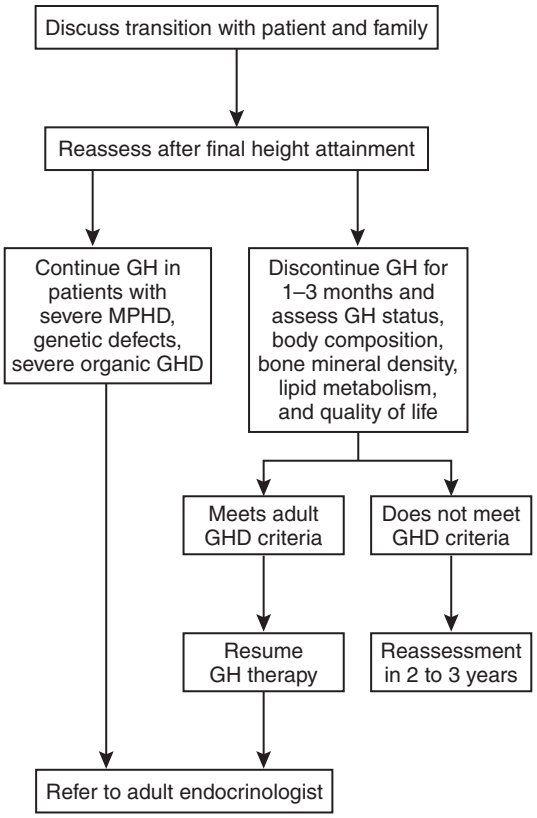


FIGURE 10-31 ■ Algorithm for transition to adult treatment of growth hormone (GH) deficiency. MPHD, multiple pituitary hormone deficiency.

of continuing GH therapy in conditions where there is a known risk of diabetes or malignancy. Although large patient registries have not seen an increase in the incidence of malignancy in pediatric GH recipients, patients with certain high-risk states (such as Bloom syndrome) may be at an increased risk as a result of long-term GH therapy and such patients should be monitored carefully. The transition to adult GH replacement should be arranged as a close collaboration between the pediatric and adult endocrinologists, who should discuss the reinitiation of treatment with the patient.

Growth Hormone Treatment of Other Forms of Short Stature

The development of rhGH has provided the capability for a theoretically unlimited supply of hGH. Although treatment of GHD is the one unequivocal indication for "replacement" therapy, the potential use of hGH for treatment of other forms of short stature has been actively explored. Theoretically, any child with open epiphyses should be capable of accelerating growth and achieving heights greater than indicated by genetic potential—as indicated by experience with cases of pituitary gigantism. Whether such therapy can be done safely and whether it justifies the cost and potential risks of hGH are more complicated issues. In addition, questions have been raised about the appropriateness of "cosmetic" hormonal therapy. Table 10-10 summarizes the different medical indications that the FDA has approved for treatment with rhGH as of September 2012.

TABLE 10-10 Key Indications for the Use of GH Therapy in the United States (USFDA-Approved Indications)

Children	Adults
Growth hormone deficiency	Growth hormone deficiency
Chronic kidney disease	HIV/AIDS wasting syndrome
Turner syndrome	Short bowel syndrome
SGA and lack of catch-up growth	
Prader-Willi syndrome	
Idiopathic short stature	
SHOX gene haploinsufficiency	
Noonan syndrome	

SGA, smallness for gestational age; USFDA, United States Food and Drug Administration.

Chronic Renal Failure

Several studies have now convincingly demonstrated the ability of hGH to accelerate growth over at least several years of therapy.[767,768] These findings have also been confirmed by several double-blind placebo-controlled investigations. Using an hGH dosage of 0.05 mg/kg/day, Fine and coworkers[769] showed a mean first-year growth rate of 10.7 cm in GH recipients and 6.5 cm in the placebo group. In the second year, GH-treated patients had a mean growth rate of 7.8 cm/year versus 5.5 cm/year in placebo recipients. No deleterious effects on renal function were observed. Additional evaluation of the effect of GH treatment on adult height in chronic renal failure patients has been compromised by small sample sizes and restriction to only prepubertal patients treated. However, the impact of GH therapy on final adult height in these patients has been evaluated through the Pfizer International Growth Database (KIGS), which showed long-term catch-up growth and increased near-adult height.[770] Mean height SD scores at near-adult height improved by 1.2 and 1.6 in boys and girls, respectively. The overall treatment response was diminished in patients who were concurrently on dialysis or had severely delayed puberty.

Turner Syndrome

Before the availability of rhGH, a number of uncontrolled studies had produced conflicting data concerning the efficacy of GH therapy in Turner syndrome.[771,772] In 1983, a randomized controlled study of rhGH was initiated.[230,347] First-year results indicated a growth rate of 3.8 cm/year in the control group, 6.6 cm/year in hGH recipients, 7.9 cm/year in oxandrolone recipients, and 9.8 cm/year in subjects receiving hGH plus oxandrolone. At the end of 6 years, heights for 30 subjects who had completed therapy were compared with their projected

adult heights[773] based on the growth curves of Lyon and colleagues.[11]

Mean height achieved after 2 to 6 years of treatment was 151.9 cm, compared to a mean projected adult height of only 143.8 cm. Near-adult heights in these subjects showed that girls receiving GH alone had a final height 8.4 cm greater than their projected adult heights. Girls receiving GH plus oxandrolone had a 10.3-cm increase.[348] Sixteen of 17 girls receiving GH alone achieved adult heights above the 50th percentile for Turner syndrome, and 10 of 17 attained heights above the 90th percentile. All 45 girls receiving combination treatment attained heights above the 50th percentile for Turner syndrome, and 23 of 45 had heights above the 90th percentile.

In a subsequent study, GH was employed in combination with estrogen replacement at either 12 or 15 years of age.[774] Earlier initiation of estrogen therapy resulted in accelerated epiphyseal fusion and a compromise in final height attained, a not surprising observation given the critical role of estrogen in skeletal maturation. Girls who began GH therapy before 11 years of age and estrogen at age 15 had the greatest increase in adult height. These data are in line with the results from the only randomized, controlled trial to adult height. After an average of 5.7 years of therapy plus induction of puberty at near-physiologic age (13 years), the mean height gain due to GH was +7.2 cm.[775]

Even more dramatic results have been observed in Dutch studies, in which the GH dosage was progressively increased to 0.09 mg/kg/day.[349] At the highest GH dosage, increases in adult height over projected heights averaged 16 cm. Estrogen therapy was withheld until subjects had received at least 4 years of GH treatment and reached a minimum age of 12 years. With this regimen, the majority of girls with Turner syndrome attained adult heights within the normal range. A follow-up study further established that GH therapy in Turner syndrome girls allows for height normalization in most patients: 83% reached a normal adult height (final height SD score > -2), and 63% ended up growing into their target height range.[776]

In light of the detailed historical data that exist on natural growth in Turner syndrome, the results to date provide convincing data that hGH can accelerate growth and increase adult height. Furthermore, early initiation of GH treatment should allow for normalization of growth in childhood—as well as the potential to begin estrogen replacement at a physiologically appropriate age. This subject is discussed in detail in Chapter 16.

SHOX Deficiency

SHOX deficiency turns out to be a relatively common cause of short stature. In SHOX deficiency, short stature is mainly due to (mesomelic) shortening of the extremities. Heterozygote mutations, mainly deletions (approximately 80%), have been detected in 2% to 15% of subjects who initially were diagnosed as having idiopathic short stature. Fifty percent to 90% of patients carrying the clinical diagnosis of Leri-Weill dyschondrosteosis have SHOX deficiency, and almost 100% of girls with Turner syndrome have SHOX haploinsufficiency.[777] Several studies have shown that the improvement in statural growth with GH therapy in individuals with SHOX mutations is similar to the effect seen in Turner syndrome patients. In a 2-year prospective, open-label, parallel, multicenter study, GH-treated SHOX-deficient patients grew 3.5 cm and 1.9 cm more that an untreated control group during, respectively, the first and second years of therapy.[778] Final height was evaluated in a group of children with SHOX deficiency in a retrospective study taken after the children had received at least 2 years of GH treatment, and the results revealed an overall height gain of 7 cm, reminiscent of the height gain described in Turner syndrome patients.[779] The FDA has now approved growth hormone therapy for short stature resulting from SHOX deficiency; the recommended weekly dosage is 0.35 mg/kg/week.

Noonan Syndrome

Most of the data on the beneficial effect of GH on statural growth in Noonan syndrome are derived from observational studies using small numbers of patients, without randomization and without the use of controls. In addition, the effect of GH therapy on adult height has only been reported in a couple of studies. In one study, 18 children with Noonan syndrome were treated for a mean duration of 7.5 years until they reached final height. Their mean height SD score increased from −2.9 before GH treatment to −1.2 at the end of GH treatment, which represents a 10.3 cm height increment compared to their predicted adult height.[780] In a large cohort of Noonan syndrome patients from the international postmarketing KIGS database, more than 50% of 402 patients reached adult heights greater than −2 SD scores, whereas other studies yielded overall height gains in the range of 5 to 10 cm only.[781,782] A variety of factors likely influence the observed GH treatment responses. These include the timing of the onset of GH therapy and the duration of prepubertal GH exposure, the height achieved at puberty, and the reduced GH responsiveness seen in Noonan syndrome patients due to a PTPN11 mutation (about 50% of all patients). The FDA has approved GH treatment for short stature associated with Noonan syndrome at doses up to 0.066 mg/kg/day.

Down Syndrome

The encouraging results of hGH trials in Turner and Noonan syndromes have led to studies of hGH in other chromosomal disorders, such as Down syndrome. Several preliminary studies have confirmed the ability of hGH to accelerate growth in such patients, although ethical issues have been raised concerning the appropriateness of such therapy.[783,784] The mean height of children with Down syndrome increased significantly from −1.8 to −0.8 SD score during 3 years of GH therapy in a study from Sweden, whereas the mean height fell in a control group from −1.7 to −2.2 SD score. The study showed no effect on mental or gross-motor development, but some improvement in fine-motor development was reported in the GH-treated subjects.[785] Because no convincing study data exist to indicate that hGH improves neurologic or intellectual function in Down

syndrome patients, therapy with GH is not recommended in these children unless they also have GH deficiency.

Intrauterine Growth Retardation or Small for Gestational Age

A number of studies, employing pituitary-derived or recombinant DNA–derived hGH, have been performed in children with short stature resulting from IUGR/SGA.[786,787] These studies are hampered by the inherent heterogeneity of this group of patients, whose poor growth may reflect maternal factors, chromosomal disorders, dysmorphic syndromes, toxins, and so on. The interpretation of the results from these studies are frequently complicated by an accompanying diagnosis of GHD, often lacking stringency in diagnostic criteria.

Coutant and colleagues,[788] for example, compared children treated with GH for a diagnosis of IUGR associated with "idiopathic" GHD and children with IUGR not treated with GH and found no significant differences in adult height. Furthermore, almost 80% of the alleged GH-deficient IUGR children proved to have normal GH levels when reevaluated after cessation of growth. Two studies have examined the effects of two doses of continuous GH treatment administered over a 6-year period.[362,789,790] GH at doses of 0.033 and 0.067 mg/kg/day resulted in 2- and 2.7-SD increments in height, respectively.

In a prospective, randomized, double-blind, dose–response study, GH treatment for 6 years (with either 0.033 or 0.067 mg/kg/day) caused the majority of SGA patients to reach a height within the normal range.[362] The higher-dose group also had a greater height SD score increase than the lower-dose group, respectively, +2.5 and +2 SD score. After a mean treatment period of 7.8 years, 85% of patients had reached adult heights above −2 SD score and 98% reached adult heights within target height range (Figure 10-32).[791]

A meta-analysis of data from four different randomized controlled trials has been done (n = 391). Adult height of patients from the GH treated groups was significantly greater compared to the control groups: the mean difference was +0.9 SD score, yielding a 5.7 cm greater height ($p < 0.0001$).[792] Taken together, most studies have demonstrated taller final height, leading to approval in most countries of GH therapy for SGA children who lacked catch-up growth. The doses commonly used in SGA children are between 50 and 70 mcg/kg/day, and treatment is typically started at age 3 years.[789]

Prader-Willi Syndrome

One form of IUGR/short stature in which GH therapy has been studied fairly extensively is Prader-Willi syndrome. Early studies in a limited number of subjects demonstrated promising short-term results.[377] Lindgren and colleagues[793] demonstrated that patients with Prader-Willi syndrome had lower serum IGF-1 concentrations than did normal obese controls. Prader-Willi subjects treated with GH at a dosage of 0.033 mg/kg/day showed a 1-year increase in height velocity, whereas untreated

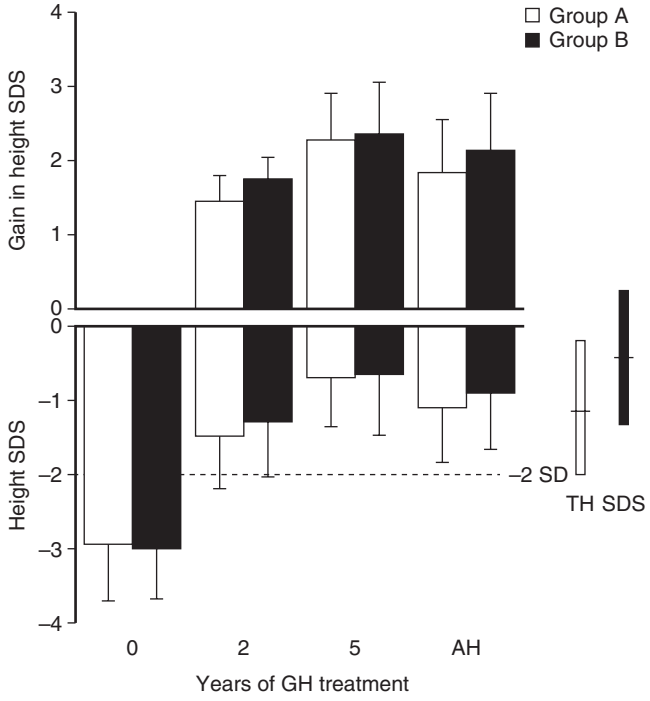


FIGURE 10-32 ■ Bottom panel: Height SD score (SD) during GH treatment and at adult height (AH) in relation to the target height (TH). Top panel: Gain in height SD score from the start until 2 years and until 5 years of GH treatment, and until AH. Group A: 0.033 mg/kg/day GH; group B: 0.067 mg/kg/day GH. (From Van Pareren, Y., Mulder, P., Houdijk, M., et al. (2003). Adult height after long-term, continuous growth hormone (GH) treatment in short children born small for gestational age: results of a randomized, double-blind, dose-response GH trial. *J Clin Endocrinol Metab, 88*, 3584–3590.)

Prader-Willi subjects experienced a decrease in height velocity.

GH treatment also resulted in a reduction of relative fat mass and an increase in fat-free mass. Carrel and coworkers[181] employed a similar dosage of GH in Prader-Willi patients with reduced clonidine-stimulated GH levels and low serum IGF-1 concentrations and demonstrated an increase in height velocity, whereas untreated controls showed no significant change in height velocity.

The FDA has recognized Prader-Willi syndrome to be an accepted diagnosis for GH therapy, even in the absence of demonstrable GHD. The GH treatment is effective in improving growth during childhood, final adult height, and body composition as well.

Randomized controlled studies using a GH dose of 1 mg/m^2/day showed a significant increase in height and height velocity during the first year of treatment, accompanied by reduction in total body fat and an increase in lean body mass, as demonstrated by dual-energy x-ray absorptiometry (DEXA), as well as an increase in muscle strength and agility, sustained in the second year of therapy. After the initial 2 years of GH therapy,[794] 2 additional years demonstrated continued beneficial effects on body composition with GH doses of 1 and 1.5 mg/m^2/day. Bone mineral density also improved.[795] Few studies, however, have reported data on adult height. In the KIGS database analysis, 33 patients were followed to

near-adult or adult height; in nearly two thirds of the patients, height fell above −2 SD score, and median adult height was −1 SD score after a mean of 8.4 years of GH therapy.[796]

The benefits of administering GH treatment as early as 2 years of age are well known, but there is also some evidence of additional benefit when starting GH between 6 and 12 months of age. This further benefit affects the Prader-Willi syndrome child's head growth, muscle function, motor development, and may even positively impact cognition.[797]

Since the 1990s, additional case reports have pointed to a possible GH-associated mortality in patients with Prader-Willi syndrome who have massive obesity. This may be related to a complication from a respiratory tract infection, worsening sleep apnea with or without hypoventillation, adenoid or tonsil hypertrophy, or aspiration associated with obesity.[549] A review that included 64 children (42 boys and 22 girls, 28 on GH treatment) suggested there may be a high-risk period for death during the first 9 months after GH was commenced. For this reason it has been advised that GH should be started at a relatively low dose (e.g., 0.25 to 0.30 mg/m^2/day or 0.009 to 0.012 mg/kg/day) with gradual increases during the first weeks and months toward a standard GH replacement dosage around 1 mg/m^2/day or 0.035 mg/kg·day. Clinicians should monitor clinical effects closely, particularly screening for the presence or worsening of sleep apnea.[798]

Osteochondrodysplasias

Therapy with hGH has been studied in several studies of skeletal dysplasias. The largest initial experience was in achondroplasia and published by Seino and coworkers,[799] who reported on hGH administration to 40 children. During the first year of treatment, the height velocity increased from 3.8 to 6.6 cm/year. In year 2, the height velocity decreased to approximately 5 cm/year. A modest improvement was seen in the ratio of lower limb length to height. Although hGH was well tolerated, it is of note that one patient with atlantoaxial dislocation during hGH therapy has been reported. In another 5-year trial, GH treatment was given to 35 children with achondroplasia to investigate height and body proportion responses (GH therapy was omitted for one of the study years). Mean height velocities improved during the first 2 years of GH therapy, but growth velocity decreased during the third year below baseline. Height SD score increased significantly (by +1.3 to +1.6, based on the GH dose used) during the 5 years of the study. Sitting height SD score also improved during the study. Body proportion did not show any significant change.[800] The effects of 3 years of GH treatment in patients with hypochondroplasia are better than in achondroplasia: respective increase in height SD score of +1.4 versus +0.3.[801] Further studies are needed to determine if continued treatment of hypochondroplasia patients improves adult height or has additional benefit on bone mineralization and quality of life. There has also been limited experience with GH treatment of other skeletal disorders, such as hereditary multiple exostoses, osteogenesis imperfecta, spondyloepiphyseal dysplasia congenita, Schmid type metaphyseal dysplasia, and Ellis-van Creveld syndrome.

Idiopathic Short Stature

Several countries, including the United States, have approved GH therapy of ISS. It is important to recognize that this is a heterogeneous group of patients, as emphasized by Kelnar and coworkers.[802] Even though they are grouped under the title "normal short children" or as having "idiopathic short stature," this group includes children who have passed provocative GH testing but are nevertheless IGF deficient—reflecting the inadequacies of GH testing.

There is abundant evidence that determination of provocative GH levels does not adequately discriminate between true GHD (IGF deficiency) and ISS. In addition, the group labeled "normal short children" will inevitably contain children with unidentified syndromes and possibly with unidentified chronic illnesses or endocrine disorders. These issues have made it difficult to properly evaluate existing clinical trials. Furthermore, published clinical trials have not contained long-term control groups and have indicated considerable variability in growth response.[803-805]

The majority of normal short children treated with hGH have shown growth acceleration that is generally sustained over the first several years of therapy (although attenuation of the response is seen, just as in all other instances of hGH treatment). Longer-term data are inadequate, however, to address the impact of therapy on adult height. Hintz and coworkers[806] treated 80 children with ISS (0.3 mg/kg/wk) for 2 to 10 years and compared results with those in untreated short retrospective control subjects. In children with ISS, GH therapy increased the mean height SDS scores from −2.7 at the start of treatment to −1.4 at final height. The mean increase in final height over the pretreatment predicted adult height was 5 cm for boys and 5.9 cm for girls. Concern has been raised that hGH therapy in normal short children may result in an earlier onset of puberty—and as a result, earlier fusion of the epiphyses—and thereby may offset the positive response observed during the early years of hGH treatment.[807]

Other important questions have been raised about the financial, ethical, and psychosocial impact of hGH therapy on normal short children.[808] Given the current cost of hGH, the financial implications of treating normal short children (whether it is the bottom 5, 3, 1, or 0.1%) is considerable and potentially diverts a limited pool of health care resources away from other needs. The point is well taken that 5% of the population will always be below the 5th percentile whether we treat them with hGH or not. Concern has been raised that focusing on a short child's stature potentially disables an otherwise normal child, handicapping that child psychologically or socially. No convincing data have been presented that hGH treatment of normal short children improves psychological, social, or educational function. Finally, the known and unknown treatment risks of hGH therapy must be considered when treatment of otherwise normal children is an issue.

On the other hand, given the current limitations in our ability to clinically or biochemically discriminate definitively between GHD and normal short stature, our inadequate understanding of neurosecretory defects of GH secretion, our inadequate definition of "partial" GHD, and the need to move to a more global concept of "IGF deficiency," it seems unfair to prevent hGH therapy of short children who do not meet a definition of GHD (i.e., provocative testing) we recognize as inadequate. Many of these children behave clinically and biochemically in a manner identical to those with classic GHD.

The mean improvement in adult height in children with ISS attributable to GH therapy (average duration of 4 to 7 years) is 3.5 to 7.5 cm compared to initial predicted adult height. Responses are highly variable and are dose dependent. Concern has been raised that in ISS, higher GH doses (> 50 ug/kg/day) may advance bone age (BA) and the onset of puberty, but this has not been seen in other studies.

Multiple factors affect the growth response to GH in ISS, most of which are unknown. The first-year response is influenced negatively by age at start (and positively by GH dose), weight at start, and difference from target height—and these factors account for approximately 40% of the variance. Adult height outcome is influenced negatively by age at start and positively by midparental height, height at start, bone age delay, and the first-year response to growth hormone.[807] The utility of baseline and treatment-related biochemical data including IGF-I has not been validated in long-term studies, but 2-year studies suggest that the rise in IGF-I correlates with short-term height gain.

Children treated with GH should be monitored for height, weight, pubertal development, and adverse effects at 3- to 6-month intervals. Periodic monitoring for scoliosis, tonsillar hypertrophy, papilledema, and slipped femoral capital epiphyses (SCFE) should be performed as part of the regular physical exam during follow-up visits. We recommend that after 1 year the response to therapy be assessed by calculating height velocity SDS as well as the change in height SDS. Bone age may be obtained periodically to reassess height prediction and for consideration of manipulation of the tempo of puberty. IGF-1 levels may be helpful in guiding GH dose adjustment, but the significance of abnormally elevated IGF-1 levels remains unknown. Thus far, no cases of elevated blood glucose in GH treated patients with ISS have been reported—but there is controversy regarding the need for routine monitoring of glucose metabolism.

Dosage is usually selected and adjusted by weight. If the growth response is considered inadequate, the dose may be increased. There are no definitive data concerning the long-term safety of doses exceeding 50 ug/kg/day in ISS. The upper limit of GH dosage used in ISS and other indications is approximately 70 ug/kg/day,[789,806] but the possibility of using such doses varies in terms of national health economics. In the future, growth prediction models may improve GH dosing strategies. IGF-1 levels may be helpful in assessing compliance and GH sensitivity. Levels that are consistently elevated (> 2.5 SDS) should prompt consideration of GH dose reduction. Studies on IGF-based dose adjustments in ISS have demonstrated increased short-term growth when higher IGF targets were selected, but this strategy has not been validated in long-term studies in terms of safety or final height effects.

If height prediction is below –2 SDS at the time of pubertal onset in either sex, the addition of GnRH analogues may be considered. Alternatively, in males aromatase inhibitors may also be an option. However, long-term efficacy and safety data are not available for either of these interventions. In addition, the impact of delayed puberty on somatic and psychological development is not known. We do not recommend aromatase inhibitors in girls.

There are two schools of thought about the duration of treatment. One is that treatment should stop when near-adult height is achieved, which is Ht velocity < 2 cm/year or bone age > 16 years (in boys) and > 14 years (in girls). Alternatively, therapy can be discontinued when height is in the "normal" adult range (above –2 SD) or has reached another cutoff for the reference adult population (for example, in Australia the 10th percentile; elsewhere, the 50th percentile). Stopping therapy is influenced by patient/family satisfaction with the result of therapy, ongoing cost-benefit analysis, and when the child wants to stop for other reasons.

Miscellaneous Causes of Growth Failure

In addition to the clinical conditions described previously, hGH has been employed in treatment of short stature associated with a variety of other conditions characterized by postnatal growth failure (e.g., neurofibromatosis or inflammatory bowel disease). In general, such trials have been uncontrolled and have not included sufficient numbers of subjects for adequate evaluation of efficacy. A review of a large database, however, indicates that many of these conditions might in fact be responsive to GH therapy.[809]

Although this database is uncontrolled, 4-year results of GH treatment of Russell-Silver syndrome dwarfism, neural tube defects, neurofibromatosis, and familial hypophosphatemic rickets were indistinguishable from those observed with GH treatment of "idiopathic GHD" using comparable dosages of GH. Furthermore, a variety of other conditions (such as Down syndrome, cystic fibrosis, fetal alcohol syndrome, Crohn's disease, sickle cell anemia, hypochondroplasia, and thalassemia) responded in a manner comparable to that observed with Turner syndrome.

Although management of such patients is often complex and important ethical issues may characterize the use of GH, one must conclude that, at least on auxologic grounds, it becomes difficult to discriminate among GHD (as currently diagnosed), Turner syndrome, and many other medical conditions characterized by growth failure. If, as proposed by Allen and Fost,[810] responsiveness to GH rather than GHD should be the most important criterion for GH treatment, these findings indicate that GH therapy for many of these "other" conditions warrants an open-minded appropriately controlled evaluation. From a practical perspective however, it has

become more difficult for the clinician to consider a GH trial in patients with the aforementioned conditions. Reasons for this include not only the paucity of long-term data from controlled studies but also the cost burden to society associated with the high expense of GH therapy itself. Finally, the lack of strong quality-of-life data warrants that consideration of a GH trial in these "other" patients is done via research protocols. Only with well-designed studies enrolling sufficient patient numbers and continuing until near-adult height has been attained will we determine if GH therapy is both efficacious and safe in these "other" conditions.

Normal Aging and Other Catabolic States

A lengthy discussion of the potential use of hGH in normal aging is beyond the scope of this chapter.[184] The rationale for therapy is based on the concept of the "somatopause," referring to the fact that GH secretion normally declines progressively after 30 years of age—reflected in decreasing serum concentrations of IGF-1. Aging can be viewed as a catabolic state, with the potential that hGH therapy will reverse or retard the loss of muscle mass and strength and the decrease in bone density characteristic of the older population.

It is also conceivable, however, that aging individuals may be more sensitive to the metabolic changes produced by hGH, resulting in fluid accumulation, carpal tunnel syndrome, and glucose intolerance. Clinical studies are currently in progress. hGH therapy is also being investigated in a variety of catabolic states,[185] such as burns, tumor cachexia, major abdominal surgery, AIDS, sepsis, and hyperalimentation. Although the frontiers of new indications of GH therapy are being explored in well-controlled trials, we believe that, outside of appropriate clinical studies, clinicians should not routinely treat aging, catabolic states, and other unapproved indications for GH therapy.

Side Effects of Growth Hormone

Pituitary-derived hGH, which for a quarter of a century had an enviable safety record, proved to be the agent for transmission of the fatal spongiform encephalopathy Creutzfeldt-Jakob disease.[727,805] Although this risk does not exist with recombinant DNA-derived hGH, the experience with pituitary hGH serves as a grim reminder of the potential toxicity that can reside in "normal" products and "physiologic replacement." Nevertheless, 20-plus years of extensive experience with rhGH has been encouraging. Concerns have been raised, however, about a number of potential complications that clearly required continued follow-up and assessment. This evaluation has been greatly facilitated by the extensive databases established by hGH manufacturers.

Development of Leukemia

In 1988, the development of leukemia as a complication of hGH therapy was reported in 5 cases from Japan.[392] Since then, more than 30 cases of leukemia in hGH-treated patients have been reported—a disproportionate number coming from Japan,[393] but with cases also reported in the United States.[394] One of the difficulties in assessing the role of hGH treatment in these cases was that many children with GHD have clinical conditions that may predispose them to the development of leukemia, such as histories of previous malignancies, histories of irradiation, and underlying syndromes known to predispose to the development of leukemia (Bloom syndrome, Down syndrome, Fanconi anemia).

Patients have included recipients of pituitary-derived and recombinant DNA–derived hGH, and leukemia has occurred both during treatment and after termination of therapy. Cases of leukemia have been also reported in GH-deficient individuals without any history of hGH therapy, suggesting that the GHD state by itself might be a predisposing factor. In a comprehensive set of large cohorts of patients from the United States, Europe, and (most recently) Japan, the concern of increased leukemia risk in patients with GHD without predisposing factors has been dispelled.

In a large series describing the entire Japanese experience, the same authors who first reported the increased incidence of leukemia in GHD reanalyzed their data—together with a much larger number of patients treated since the original report—and showed that the risk for leukemia is the same for GH recipients and controls.[811] Consequently, most authorities now agree that hGH treatment is not a causative agent in the development of leukemia in individuals without a predisposing condition. The notion that hGH therapy is linked to development of leukemia should be dispelled and the authors recommend that this issue be discussed with all potential recipients of hGH.

Recurrence of CNS Tumors and Occurrence of Second Malignancies

Because many recipients of hGH have acquired GHD from CNS tumors or their treatment, the possibility of tumor recurrence is of obvious importance. Extensive analysis of the data on 1300 American children treated for a CNS malignancy before receiving hGH has not indicated any increased risk. A similar conclusion has emerged from analysis of a European database. However, one study suggests that GH recipients who had a brain tumor have a slightly higher risk of a second malignancy.[812]

Pseudotumor Cerebri

Pseudotumor cerebri (idiopathic intracranial hypertension) has been reported in at least 26 hGH-treated patients, approximately half of whom had classic GHD.[813] The mechanism of hGH action is unclear, but it may reflect changes in fluid dynamics within the CNS. Pseudotumor cerebri has also been described after thyroid hormone replacement and may represent restoration of a normal physiologic state. In any case, physicians need to be alert to complaints of headache, nausea, dizziness, ataxia, and visual changes; the presence of papilledema warrants temporary discontinuation of GH treatment.

Slipped Capital Femoral Epiphysis

The potential association of slipped capital femoral epiphysis (SCFE) and GHD was first suggested by studies in rats.[814] It is clear that SCFE can be associated with hypothyroidism and GHD.[815,816] The potential role of hGH therapy has been more difficult to determine. This is in part because the incidence of SCFE in the normal population varies by age, sex, race, and geographic locale—being variously reported as between 2 and 142 cases per 100,000. Accordingly, although SCFE cannot at this time be definitively attributed to hGH therapy per se, complaints of hip and knee pain or displaying a limp should receive appropriate evaluation.

Miscellaneous Side Effects

A number of other physical concerns have been raised as possible consequences of hGH treatment, but available data are inadequate to ascribe a causal role to hGH treatment. The potential side effects include prepubertal gynecomastia, increased growth of nevi, behavior changes, scoliosis and kyphosis, worsening of neurofibromatosis, hypertrophy of tonsils and adenoids, and sleep apnea. This list is obviously only partial. It is best for the clinician to remember that GH and the peptide growth factors it stimulates are potent mitogens with diverse metabolic and anabolic actions. All patients receiving hGH treatment, even as replacement therapy, must be carefully monitored for side effects.

For the most part, the side effects of GH are minimal and rare. When they occur, careful history and physical examination are adequate to identify their presence. Management of these side effects may include transient reduction of dosage or temporary discontinuation of GH.[817] The association of GH treatment with insulin resistance is well documented,[818] and one report suggested that there is an increased incidence of development of diabetes in pediatric recipients of GH.[819] Most authorities agree, however, that rather than a side effect of GH this relationship probably represents a common susceptibility of patients to develop growth disorders and diabetes owing to a common genetic trait.[820]

In the absence of other risk factors, there is no evidence that the risk of leukemia, brain tumor recurrence, SCFE, and diabetes is increased in recipients of long-term GH treatment. In any case, any patient receiving GH who has a second major medical condition (such as a tumor survivor receiving GH) should be followed in conjunction with a specialist such as an oncologist or a neurosurgeon when appropriate. Although GH has been shown to increase the mortality of critically ill patients in the intensive care unit,[152] there is no evidence that GH replacement needs to be discontinued during intercurrent illness in children with GHD.

The Question of Long-Term Cancer Risk

Several epidemiologic studies have been published suggesting an association between high serum IGF-1 levels and the incidence of malignancies.[821,822] In addition, the risk of cancer calculated in those studies was increased for patients with low IGFBP-3 levels. Although additional studies are being conducted to verify or disprove the association between serum IGF-1 and cancer risk, the role of GH in this potential phenomenon should be carefully examined.

In a study analyzing the risk of colon cancer, it was shown that IGF-1 was not statistically associated with cancer risk. However, the combination of high IGF-1 and low IGFBP-3 was shown to be related to an increased risk (Table 10-11).[823] Notably, GH positively influences both parameters in parallel. This casts doubt on its role as a driving force in the IGF-cancer equation.

Additional epidemiologic data of note involve the risk of malignancy associated with acromegaly. A number of studies have been published that claimed to identify an association between acromegaly and colon cancer risk,[824-826] whereas others did not find significant associations.[827,828] The interpretation of these studies is hampered by their small size, uncontrolled retrospective nature, and multiple possible sources of bias. The largest study to date, reviewing more than a thousand patients, indicated that overall cancer incidence is not increased in acromegaly.[829] The overall incidence of colon cancer was also not shown to be increased in a study, although mortality from colon cancer was higher in this population—suggesting perhaps an effect of GH or IGF-1 on established tumors.[830]

One prospective analysis of colon cancer and colonic polyps in acromegalics using controlled colonoscopies did not observe an association between these two diseases when using autopsy series or prospective colonoscopy screening series for the control population.[831] It is notable that acromegaly is associated with a dramatic increase in the incidence of benign hyperplasia of several organs, including colonic polyps.[832] These findings raise the possibility that the GH-IGF axis may lead to symptomatic

TABLE 10-11 **Risk of Future Cancer Relative to Serum IGF-I and IGFBP-3 Tertiles** [837]

	IGF-I Lowest Tertile	IGF-I Middle Tertile	IGF-I Upper Tertile
IGFBP-3 upper tertile	—	—	—
IGFBP-3 middle tertile	—	—	—
IGFBP-3 lowest tertile	—	—	Fourfold increased risk

IGF, insulin-like growth factor; IGFBF-3, insulin-like growth factor-binding protein-3.

benign proliferative disease, which could be associated with symptoms (such as rectal bleeding) that would then lead to a potential detection (or ascertainment) bias.

Children receiving GH have not been shown to have an increased risk of de novo tumors. An earlier concern that leukemia risk was increased has been dispelled by the same authors who first published on this topic, showing in a large cohort that the risk for leukemia is the same for GH recipients and controls.[833] Several studies now indicate absolutely no increase in the risk of tumor recurrence in pediatric GH recipients.[834]

In a number of studies, no increased incidence of cancer was found in GH recipients among adults treated for GHD.[835,836] Clearly, these reports represent imperfect uncontrolled studies. However, the experience gained through them demonstrates that even though the IGF-1 levels are normalized, GH therapy is not associated with tumor recurrence or with de novo tumors in the absence of other risk factors. The use of IGF-1 and IGFBP-3 in the monitoring of GH recipients, adult and pediatric, has been recommended and endorsed by international bodies such as the GH Research Society.[817]

Until the issue of cancer risk in GH therapy is fully resolved, the most prudent approach appears to be regular monitoring of IGF-1 and IGFBP-3 and modulation of the GH dose to ensure that the theoretical risk profile induced by GH therapy is favorable. This can be done by avoiding the unlikely situation in which a GH-treated patient will have an IGF-1 level at the upper end and IGFBP-3 levels at the lower end. In the 21st century, many patients with GHD will receive lifetime GH replacement. In that setting, it is especially important that we monitor serum IGF-1 and IGFBP-3 on a regular basis.

Overall, many controversies still surround the issue of the GH-IGF axis and cancer risk, specifically concerning the safety of GH therapy. In general, the currently approved indications for GH therapy in children and adults do not warrant concern regarding future cancer risk. Although additional research coupled with stringent pharmacologic vigilance is clearly warranted, the state of the clinical field mandates that patients' physicians be aware of the vast body of evidence regarding the safety of GH in this regard.[837]

Treatment of Primary Severe IGFD: Use of IGF-I

The production of IGF peptides by recombinant DNA technology has permitted clinical trials of IGF therapy. IGF-I administration to normal adult male volunteers as a single intravenous injection of 100 μg/kg caused hypoglycemia within 15 minutes. On a molar basis, IGF-I has approximately 6% of the hypoglycemic potency of insulin. In contrast, intravenous infusions of IGF-I to normal men at a rate of 20 μg/kg/hr resulted in serum IGF-I levels within the normal range and did not produce hypoglycemia—but did suppress GH levels, increase creatinine clearance, and decrease plasma urea nitrogen.[100,838-846]

The most obvious clinical use of IGF-I therapy is in patients with GHI. A number of short-term growth-related studies with IGF-I treatment at varied doses have been reported since the mid-1990s. Walker and associates[842] reported an increase in growth rate from 6.5 to 11.4 cm/year in a patient with GHRD treated with twice-daily subcutaneous injections of 120 μg/kg. Wilton and coworkers[843] have reported preliminary data in 30 children, aged 3 to 23 years, with GHI (due to GHRD or GHD-IA with anti-GH antibodies). The dosage of IGF-I varied from 40 to 120 μg/kg twice daily. With the exception of the two oldest patients, the growth rates of all subjects increased by at least 2 cm/year. These results have since then been updated and indicate a continued good response by most subjects (n = 32).[844] Observed side effects included hypoglycemia, headache, convulsions, possible urolithiasis, and papilledema—the last indicating the possibility of pseudotumor cerebri, as has been reported with GH therapy. Data from 17 patients in the European collaborative trial, treated for at least 4 years, showed an increase in mean height SD score from –6.5 to –4.9—with two adolescents reaching the third percentile.[846] This emphasizes the importance of early diagnosis and initiation of therapy. Side effects reported were again hypoglycemia, headache, convulsions, and papilledema. The latter, which suggests the possibility of pseudotumor cerebri, resolved spontaneously while the patient was receiving IGF-I. Laron and coworkers[841] reported significant growth acceleration—to rates of 8.8 to 13.6 cm/year in five children treated for 3 to 10 months with a single daily injection of 150 μg/kg. The use of IGF-I in milder forms of primary IGFD has also been the subject of investigation. Vaccarello and coworkers[101] treated six adults with GHRD for 7 days with subcutaneous IGF-I at a dosage of 40 μg/kg every 12 hours. Normal serum IGF-I concentrations were achieved 2 to 6 hours postinjection, followed by a rapid decline—reflecting low serum concentrations of IGFBP-3. Symptomatic hypoglycemia was not observed, but mean 24-hour GH levels were significantly suppressed and urinary calcium level was increased. A randomized double-blind placebo-controlled trial of IGF-I therapy in GHRD was performed in Ecuador, probably the only place where the population is sufficiently large and homogeneous to permit such investigation.[845] Subjects receiving IGF-I showed a significant increase in growth rate (from 2.9 to 8.6 cm/year), which was sustained over the 1-year course of therapy. The placebo group grew 4.4 cm/year during the placebo phase, and their height velocity increased to 8.4 cm/year during the IGF-I phase. Incidents of hypoglycemia were equal in the two experimental groups. One recipient of IGF-I developed papilledema, which resolved spontaneously while the patient was being treated.

In the longest treatment trial from North America, Chernausek and colleagues[840] showed data similar to that found in the European study—with an initial burst of growth followed by slowing to just above baseline by the sixth year of therapy. Height SD score improved from –5.6 to –4.2 by the end of the sixth year (Figure 10-33).

Although these early studies were promising, little was known about the long-term effects of IGF-I or about the optimal dose or frequency of administration. With all clinical studies combined, the total number of children treated is still only several hundred—and relatively few

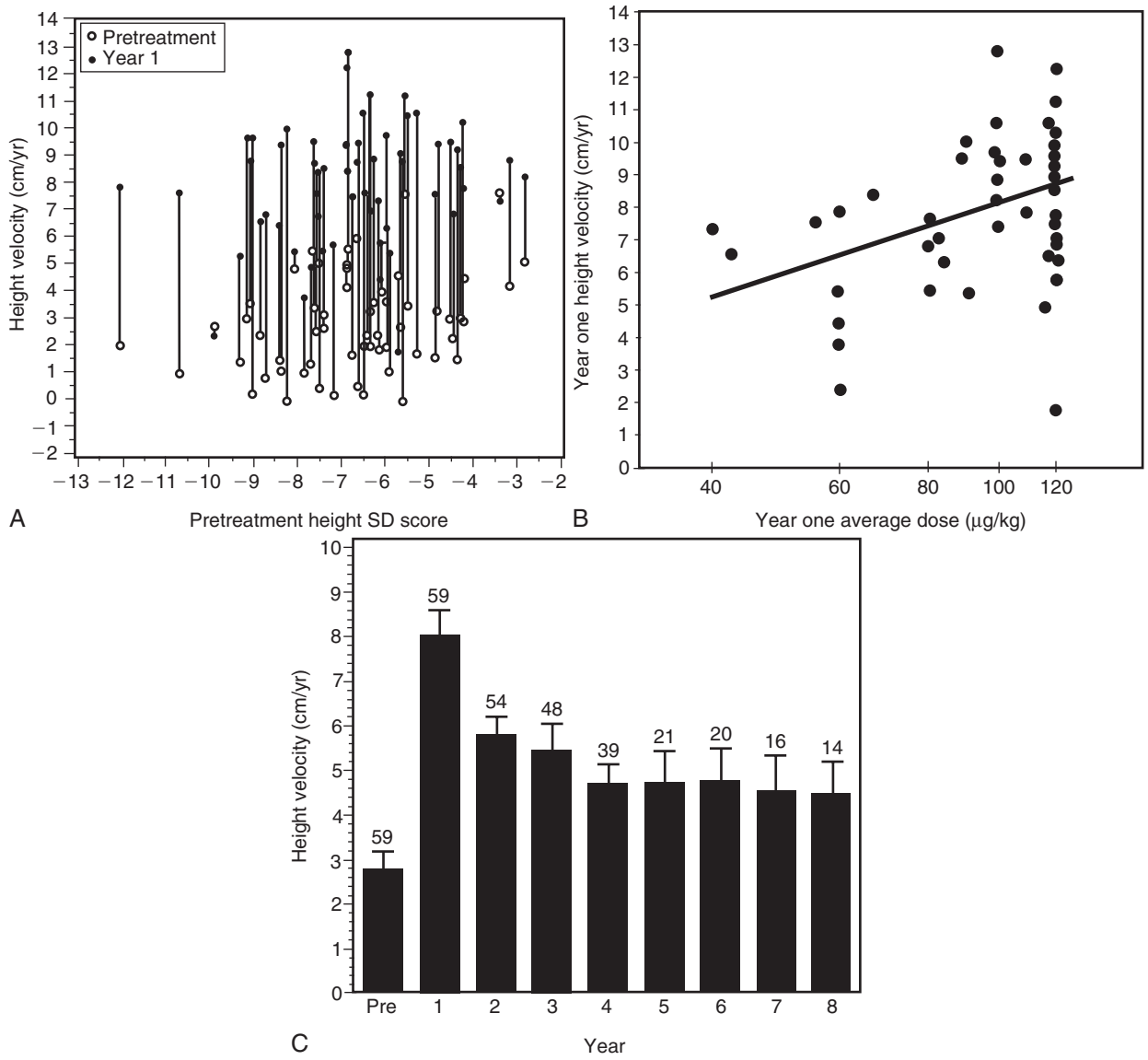


FIGURE 10-33 ■ Linear growth in response to rhIGF-I treatment. **A**, Height velocity (centimeters per year) before (open circle) and during first year of therapy (closed circles) for each child is displayed relative to pretreatment height. **B,** The dose dependency of first-year growth rate is shown. Each point represents a single subject. The equation for the regression line shown is *Height velocity (centimeters per year) = –6.2 + 7.2 log10 rhIGF-I dose (microgram per kilogram, BID)*. **C**, Average growth rates before and during rhIGF-I for first and subsequent years are shown. Error bar shows upper limit of 95% confidence interval. Number of subjects at each year is indicated. (From Chernausek, S. D., Backeljauw, P. F., Frane, J., et al. for the GH Insensitivity Syndrome Collaborative Group (2007). Long-term treatment with recombinant insulin-like growth factor [IGF]-I in children with severe IGF-I deficiency due to growth hormone insensitivity. *J Clin Endocrinol Metab, 92*, 902–910.)

have been treated for more than 5 years. The long-term trial from the United States now has reported on final and near-final adult height from 16 of the patients (9 males, 7 females).[847] Recombinant human IGF-I was administered for a mean of 10 years at a dose of 120 μg/kg subcutaneously twice daily. Mean baseline height SD score improved from −7.1 to −5.2 at last analysis. As a result of IGF-I therapy, the mean estimated gain in height was 13.2 cm (range −0.4 to 23.4 cm). No new safety signals were identified in these patients, which made up a subset of those previously reported.[840] Additional patients from this cohort have completed treatment since then, and data are currently under review for publication. It is likely that this will be the only trial, in the near future, that can provide long-term information on the efficacy and safety of IGF-I therapy in patients who are IGF deficient. Taken together, the IGF-I treatment studies show that, although clinically significant, the growth response is neither as successful nor as long-lived as that of GHD children treated with exogenous GH. The failure of serum levels of IGFBP-3 to increase with IGF-I administration and the suppression of endogenous GH secretion underscores the complexity of the treatment. The possibility that local production of IGF-I at the growth plate may be critical to optimal growth also requires consideration. In addition, suboptimal treatment

adherence with prolonged twice-daily administration may also have contributed to the modest treatment response. Nevertheless, these data indicate that the IGF peptides (long considered to function as autocrine or paracrine growth factors) can act as classic endocrine hormones.

IGF-I has received FDA approval in 2005 for the treatment of severe primary IGF deficiency, defined as serum IGF-I < −3 SD score, height < −3 SD score, and normal GH concentrations. The same indication was also approved in Europe in 2007 (although there the low IGF-I is defined as < 2.5th percentile). The drug shows promise in treatment of severe primary IGFD. However, from analysis of the different cohorts previously treated with IGF-I we learn that the spectrum of GH insensitivity, or IGF deficiency for that matter, is likely much wider that what the small group of patients with GHRD due to homozygous single gene defects would indicate. In the U.S. and European studies, as well as in the Ecuadorian cohort, the height range of the patients was quite variable (from −3 to −4 SD score to −8 to −10 SD score). The best treatment approach to such a broad range of patients still needs to be determined. Additional studies will show which patients will benefit the most from IGF-I therapy. One such trial was a 1-year, randomized, open-label trial of 136 short, prepubertal subjects with low IGF-I (height and IGF-I SD scores < −2, stimulated GH ≥ 7 ng/mL). IGF-I was administered subcutaneously twice daily, using a weight-based approach (80 or 120 μg/kg; n = 111), or subjects were observed without therapy (n = 25). First-year height velocity for subjects completing the trial was increased for both IGF-I treated groups versus the untreated control group (7 ± 1.0, 7.9 ± 1.4, and 5.2 ± 1 cm/year, respectively; all $P < 0.0001$).[848] These results indicate that IGF-I therapy in these short subjects with relatively low IGF-I and normal GH achieved growth responses similar to—but not superior to—those that have been reported in patients with idiopathic short stature on standard GH therapy. Based on this information, a logical management approach for patients with milder forms of IGF deficiency would be to combine GH therapy with IGF-I therapy. The theoretical basis for this combo-treatment approach is the potential enhancement of the direct growth-promoting effects of GH through maintaining normal IGFBPs and ALS concentrations. Preliminary information from such a trial have been presented at endocrinology meetings and seem to indicate a superior growth response with the GH plus IGF-I combination over GH alone in patients with short stature (height < −2 SD score) and low to low-normal IGF-I.[849] As of late 2012, the data from this study have not been subjected to peer review for publication, however. For this reason, it is premature to consider such combination therapy as either efficacious or safe for patients with mild IGF-I deficiency or idiopathic short stature plus low IGF-I.

As promising as all these studies are, IGF-I therapy is still under active investigation and the exact clinical scenarios for which this therapy is most appropriate is still evolving. The failure of serum levels of IGFBP-3 to rise with IGF-I administration underscores the relevance of the IGFBPs to IGF pharmacokinetics.[101,845] Nevertheless, these results also indicate that the IGF peptides (long considered to function primarily as autocrine or paracrine growth factors) are capable of acting as classic endocrine hormones.

EXCESS GROWTH AND TALL STATURE

Tall Stature and Overgrowth Syndromes

On a statistical basis, as many children have heights below −2 SD as have heights above +2 SD, but referral for evaluation of tall stature is much less common than for short stature. This referral pattern speaks eloquently to the psychosocial pressure involved in referral patterns of children with "growth disorders." Nevertheless, it is critical to be able to identify situations in which tall stature or an accelerated growth rate provides a clue to an underlying disorder. Box 10-10 provides a listing of causes of statural overgrowth in infancy and during childhood.

BOX 10-10 Differential Diagnosis of Statural Overgrowth

Fetal Overgrowth

- Maternal diabetes mellitus
- Genetic hyperinsulinism-(KATP channel defects, activating Glud1and GCK, others)
- Cerebral gigantism (Sotos syndrome)
- Weaver syndrome
- Beckwith-Wiedemann syndrome
- Other IGF-2 excess syndromes

Postnatal Overgrowth Leading to Childhood Tall Stature

- Familial (constitutional) tall stature
- Cerebral gigantism
- Beckwith-Wiedemann syndrome
- Exogenous obesity
- Excess GH secretion (pituitary gigantism)
- McCune-Albright syndrome or multiple endocrine neoplasia associated with excess GH secretion
- Precocious puberty
- Marfan syndrome
- Klinefelter syndrome (XXY)
- Weaver syndrome
- Fragile X syndrome
- Homocystinuria
- XYY
- Hyperthyroidism

Postnatal Overgrowth Leading to Adult Tall Stature

- Familial (constitutional) tall stature
- Androgen or estrogen deficiency/estrogen resistance (in males)
- Testicular feminization
- Excess GH secretion
- Marfan syndrome
- Klinefelter syndrome (XXY)
- XYY

GH, growth hormone; IGF, insulin-like growth factor.

Overgrowth in the Fetus

Maternal diabetes constitutes the most common cause of overgrowth in infants who are large for gestational age. Even in the absence of clinical symptoms or a family history, the birth of an excessively large infant should lead to evaluation for maternal (or gestational) diabetes or for a genetic cause of hyperinsulinism most commonly involving inactivating mutations in the KATP genes ABCC8 and KCNJ11 (see Chapter 6). A group of disorders associated with excessive somatic growth and growth of specific organs has been described and collectively referred to as overgrowth syndromes.[850] These overgrowth disorders appear, at least in part, to be caused by excess availability of the IGF-2 encoded by the gene Igf2. The best described of these syndromes is the Beckwith-Wiedemann syndrome, which is an overgrowth malformation syndrome that occurs with an incidence of ~ 1:13,700 births. Children conceived through assisted reproductive techniques (ART) are several-fold more likely to develop Beckwith-Wiedemann syndrome. It is unclear if maternal loss of methylation, seen in children post-ART, is due to the procedure of in vitro fertilization or could be associated to the preexisting decreased fertility or some other genetic/environmental factor inherent to the parents using ART to conceive.

Beckwith-Wiedemann syndrome is manifested as a fetal overgrowth syndrome in which hypertrophy/hemihyperplasia dominates the clinical picture. Typically, macroglossia, omphalocele, hepatosplenomegaly, nephromegaly, and hypoglycemia secondary to pancreatic beta cell hyperplasia in an infant who is large for gestational age constitute the clinical picture at birth. Infants often have the pathognomonic creases and pits of the ear lobes. An additional complication is that these children are predisposed to a specific subset of childhood neoplasms, including Wilms tumor, hepatoblastoma, rhabdomyosarcoma, neuroblastoma, and adrenocortical carcinoma.

Beckwith-Wiedemann syndrome is an imprinting disorder. About 80% to 85% of newly diagnosed cases are sporadic; this means that about 15% 20% of cases are familial. Various lines of investigation have localized several "imprinted" genes involved in Beckwith-Wiedemann syndrome and associated childhood tumors to chromosome 11p15.5.[851] Beckwith-Wiedemann syndrome is due to epigenetic or genomic changes that potentially affect gene clusters in two distinctly imprinted regions on chromosome 11p15.5. The first of these, an area called domain 1, localizes insulin-like growth factor 2 (*IGF2*) and *H19* as two monoallelic expressed genes. In children with Beckwith-Wiedemann syndrome, overexpression of *IGF2* may occur by one of several mechanisms: gene duplication, loss of heterozygosity, and relaxation or loss of maternal imprinting of the Igf2 gene. In each situation, Igf2 gene up-regulation during early embryo and fetal development is thought to play a crucial role in the pathogenesis of Beckwith-Wiedemann syndrome. The *H19* gene, on the other hand, normally encodes a transcript believed to play a role in tumor suppression and has also been implicated in growth restriction. Molecular changes of the domain 1 area are the cause of about 5% of cases of Beckwith-Wiedemann syndrome. The other implicated region, called domain 2, harbors the growth repressor gene *CDKN1C* and the *KCNQ1OT1* gene—also both normally expressed in a monoallelic fashion.[852] Several molecular mechanisms can result in reduced expression of the *CDKN1C* gene. This is a maternally expressed gene that encodes a cyclin-dependent kinase inhibitor that normally negatively regulates cell proliferation. Mutations in *CDKN1C* have been found in about 10% of patients with Beckwith-Wiedemann syndrome. The precise mechanism by which *KCNQ1OT1* leads to Beckwith-Wiedemann syndrome is still not clear yet. Paternal uniparental disomy that incorporates both imprinted gene clusters on chromosome 11p15.5 has now been reported in a total of about 20% of new patients with Beckwith-Wiedemann syndrome.[853] The clinical characteristics of these patients (all with uniparental disomy) include, as noted before, hemihyperplasia of several regions of the body; at the same time, these patients present with varying degrees of somatic mosaicism. For this reason, the molecular defect causing this disorder is most likely due to postzygotic somatic recombination.[854] Finally, other investigations into new cases of Beckwith-Wiedemann syndrome have described mutations outside the chromosome 11p15.5 region.[855] Mutations in GPC3 located at Xq26.2, a glypican gene that codes for an IGF-2 neutralizing membrane receptor, cause the related Simpson-Golabi-Behmel overgrowth syndrome.[856] Key features include large birth weight, tall stature as adults, macrocephaly, coarse facies, vertebral anomalies, polydactyly and syndactyly, and thickened dark skin. There is some evidence for a nonfunctional GPC3 to increase IGF2 signaling. However, certain other defects in cellular signaling, potentially involving Wnt or fibroblast growth factors, also may be responsible for the clinical features of Simpson-Golabi-Behmel syndrome.

Children with cerebral gigantism (also known as Sotos syndrome) are typically above the 90th percentile for both length and weight at birth, and macrocrania may also be noted at that time.[857] Mutations and (micro) deletions of the NSD-1 gene (located at chromosome 5q35.2–35.3) and coding for a histone methyltransferase implicated in transcriptional regulation were first found to be the basis for this disorder. Fluorescence in situ hybridization (FISH) analysis, MLPA, or multiplex quantitative PCR allow the detection of total/partial NSD-1 deletions—and direct sequencing allows detection of NSD-1 mutations. The large majority of NSD-1 abnormalities arise as de novo mutations, and there are few familial cases. The specific cause is haploinsufficiency of the nuclear receptor binding SET domain protein-1, which explains about 60% to 90% of cases. Genome wide SNP array analysis has indicated that other genetic regions (e.g., pathogenic copy number variants on chromosomes 10, 14, 15, and the X chromosome) could also be involved in some of the patients diagnosed with Sotos syndrome.

Although most cases are sporadic, several reports of autosomal-dominant inheritance have been described.[857] Growth is rapid, and by 1 year of age affected infants are over the 97th percentile in length. Accelerated

growth continues for the first 4 to 5 years and then returns to a normal rate. Puberty usually occurs at the normal time but may occur slightly early. Adult height is usually in the upper normal range. The hands and feet are large, with thickened subcutaneous tissue. The head is large and dolichocephalic, the jaw is prominent, there is hypertelorism, and the eyes have an antimongoloid slant. Clumsiness and awkward gait are characteristic, and affected children have great difficulty in sports, in learning to ride a bicycle, and in other tasks requiring coordination.

Some degree of mental retardation affects most patients. In some children, perceptual deficiencies may predominate. Osseous maturation is compatible with the patient's height. Results of tests of GH and IGF-1 levels and of other endocrine studies are usually normal. There are no distinctive chemical or radiologic markers for Sotos syndrome. Abnormal electroencephalograms are common. Imaging studies frequently reveal a dilated ventricular system. Most cases are sporadic. Familial cases are usually consistent with autosomal-dominant inheritance and occasionally with autosomal-recessive inheritance. Affected patients may be at increased risk for neoplasia; particularly hepatic carcinoma and Wilms, ovarian, and parotid tumors have been reported.

Weaver syndrome is a clinical entity of rapid growth and skeletal maturation, combined with craniofacial dysmorphism, a hoarse and low-pitched cry, hypertonia, and camptodactyly. The accelerated growth has a prenatal onset, and weight is more increased that height. Clinical presentation resembles Sotos syndrome in some way, but there are specific differences in facial appearance and the risk of malignancy (elevated risk in Sotos syndrome, not in Weaver syndrome). The genetic basis of Weaver syndrome has not been identified, but mutations in the EZH2 gene or NSD-1 gene have been implicated.[858] Most cases of Weaver syndrome are sporadic, with only a few familial cases reported.

Tall Stature and Postnatal Statural Overgrowth

The normal distribution of height predicts that 2.5% of the population will be taller than 2 SD above the mean. The social acceptability and even desirability of tallness ("heightism") makes tall stature an uncommon complaint, however. In North America, it is extremely unusual for males to seek help regarding excessive height—although in Europe it has been somewhat more common in previous decades. Even in females, tall stature has become more socially acceptable— although an occasional tall girl may still approach the physician with a desire to curb her growth rate.

Differential Diagnosis of Tall Stature

Box 10-10 lists the causes of tall stature in childhood and adolescence. Of these, the normal variant familial or constitutional tall stature is by far the most common cause. Almost invariably, a family history of tallness can be elicited—and no pathologic process is present. The child is often tall throughout childhood and enjoys excellent health. The parent of the constitutionally tall adolescent may reflect unhappily on his or her own adolescence as a tall teenager. There are typically no abnormalities in the physical examination, and the laboratory studies (if obtained) are always negative. Bone maturation tends to be commensurate with chronologic age,

As was described for the child with growth failure, crossing height percentiles between infancy and the onset of puberty always warrants further evaluation. Although such growth patterns frequently are of no concern to parents, an alert pediatrician will recognize that overly rapid statural growth can indicate a serious underlying problem. Furthermore, as with short stature, children with tall stature must be evaluated in the context of their familial growth and pubertal patterns.

Klinefelter syndrome (XXY syndrome) is a relatively common (1:500 to 1:1000 live male births) abnormality associated with tall stature, mild mental retardation, gynecomastia, and decreased upper-to-lower body segment ratio. The testes are invariably small, although androgen production by Leydig cells is often at the low-normal range. Spermatogenesis and Sertoli cell function are defective, resulting in infertility. XYY syndrome is associated with tall stature and possible behavioral and mental problems.

Marfan syndrome is an autosomal-dominant connective tissue disorder consisting of tall stature, increased arm span, and decreased upper-to-lower body segment ratio. The cause of Marfan syndrome is a mutation in the *FBN1* gene located on chromosome 15. FBN1 encodes a glycoprotein called fibrillin-1, which is a crucial in the development of the extracellular matrix and elastin. Additional physical abnormalities include arachnodactyly, ocular abnormalities (including usually an upward/superotemporal lens subluxation), and cardiac anomalies. Homocystinuria is an autosomal-recessive inborn error of amino acid metabolism causing mental retardation when untreated. Homocystinuria is due to a deficiency in cystathionine beta synthase. It has many features resembling Marfan syndrome, particularly ocular manifestations (lens subluxation is usually downward/inferonasal). Hyperthyroidism in adolescence is associated with rapid growth but normal adult height. It is almost always caused by Graves' disease and is much more common in females. Other clinical entities, mostly rare ones, have been associated with increased growth—at least for part of postnatal life.[858] These include a contiguous deletion syndrome known as 22q13 deletion syndrome or Phelan-McDermid syndrome, Bannayan-Riley-Ruvalcaba syndrome (an autosomal dominant disorder caused by haploinsufficiency of the phosphatase and tensin homologue (PTEN) gene at 10q23.31), and Proteus syndrome (asymmetric and disproportionate overgrowth of body parts only now known to be due to an activating mutation in AKT1).[859] In neurofibromatosis type 1, many patients are described to have suboptimal growth in childhood, but some patients have tall stature. Perlman syndrome (macrosomia, nephromegaly, hypotonia, and often death in early infancy), and Nevo syndrome (a subtype of Ehlers-Danlos syndrome with increased length early in life and caused by mutations in the PLOD1 gene on chromosome 1, important for hydroxylation of lysyl residues in collagen proteins) are extremely rare.

Much more common is exogenous obesity, a frequently occurring condition in childhood and adolescence

associated with rapid linear growth and early maturation. This association is so characteristic that the child with a combination of obesity and short stature should always be evaluated for an underlying pathologic process such as hypothyroidism, GHD, Cushing syndrome, and various syndromes (such as Prader-Willi). In exogenous obesity, bone age is usually modestly accelerated—and thus puberty and epiphyseal fusion occur early, resulting in normal adult height. As stated, cerebral gigantism and Beckwith-Wiedemann syndrome are associated with rapid pre- and perinatal growth, but rapid postnatal growth usually ends by early to mid-childhood. Nevertheless, these conditions should be considered when tall stature in childhood is accompanied by the characteristic phenotypic features, a history of unexplained fetal overgrowth, or the presence of the childhood tumors mentioned previously.

Precocious and Delayed Puberty

Precocious puberty, whether mediated centrally (increased gonadotropin secretion) or peripherally (increased secretion of androgens, estrogens, or both), results in accelerated linear growth in childhood—mimicking the pubertal growth spurt. Because skeletal maturation is also accelerated, adult height, when left untreated, can be compromised. The diagnostic evaluation and management of precocious puberty are discussed in Chapters 15 and 17.

Although delayed puberty may be associated with short stature in childhood, as with constitutional delay, failure to eventually enter puberty and complete sexual maturation may result in sustained growth during adult life—with ultimate tall stature. The reports of tall stature in men with open epiphyses, resulting from mutations of the estrogen receptor or of the aromatase gene, underscores the fundamental role of estrogen in promoting epiphyseal fusion and termination of normal skeletal growth.[18,332]

Diagnosing Familial Tall Stature

The purpose of the diagnostic evaluation of tall stature is to distinguish the commonly occurring normal variant constitutional (or familial) variety from the rare pathologic conditions. Often, when the history is suggestive of familial tall stature and the physical examination is entirely normal, laboratory tests are not indicated. It is valuable to obtain a bone age radiograph to be able to predict adult height, which serves as a basis for discussions with the family and for management decisions. If, however, the history is suggestive for any of the previously mentioned disorders or the physical examination reveals abnormalities, additional laboratory tests should be obtained to evaluate for brain disorders, GH excess, chromosomal abnormalities, or other rare causes of tallness. For some of these disorders, genetic screening is available.

IGF-1 and IGFBP-3 are excellent screening tests for GH excess and can be verified with a glucose suppression test. Laboratory evidence of GH excess mandates MRI evaluation of the pituitary and the consideration of the McCune-Albright syndrome with should be made (see below). Thyroid function tests are useful to diagnose or rule out hyperthyroidism when Graves' disease is suspected.

Management of Constitutional and Syndromic Tall Stature

Reassurance of the family and the patients is the key to the management of normal variant tall stature. The use of the bone age evaluation and a careful assessment of pubertal status to predict adult height may provide some comfort for them, as will general supportive discussions on the social acceptability of this condition. Although treatment is available for girls and boys with excessive growth, its use should be restricted to patients with predicted adult height greater than 3 SD above the mean (198 cm in males or 180 cm in females) and evidence of significant psychosocial impairment.

For the child with extreme familial tall stature (or another condition, such as Marfan syndrome) whose parents feel strongly about treatment, a trial of sex steroids is possible. This therapy is designed to accelerate puberty and epiphyseal fusion and is therefore of little benefit when given in late puberty. Therapy is initiated ideally prepubertally or in early puberty. Oral estrogens in various doses have successfully reduced the predicted height of females by 5 to 6 cm on the average (and maximum 10 cm).[860]

This is a direct result of the known effects of sex steroids on promoting epiphyseal fusion, and therapy must therefore begin before the bone age has reached 12 years. Oral ethinyl estradiol at a dose of 0.15 to 0.5 mg/day until cessation of growth occurs has been used successfully in girls. If necessary, a progestational agent can be added after 1 year of unopposed estrogen. In boys, treatment should begin before the bone age reaches 13.5 to 14 years. One trial compared the effect of two treatment approaches using different androgen dosages; testosterone enanthate given at a dose of 250 mg every 2 weeks was as effective in reducing adult height as a dose of 500 mg every 2 weeks[861]; the lower dose is recommended.

Whereas no long-term complications of sex steroid therapy have been clearly documented, short-term side effects are common. These include lipid abnormalities, thromboembolism, cholelithiasis, hypertension, nausea, menstrual irregularities, and acne fulminans. The lack of extensive experience with this form of therapy and the risks involved should be carefully weighed and discussed with the family before embarking on therapy.

The mechanism of estrogen action affects both GH and IGF production. Perhaps more important, however, is the action of estrogen on the epiphyses. Studies have demonstrated that it is estrogen that mediates epiphyseal fusion in both females and males. In prepubertal girls, adult height is reportedly decreased by as much as 5 to 6 cm relative to pretreatment predictions. When therapy is initiated after the onset of puberty, the decrement in adult height is not likely to be as large. Therapy in boys with tall stature is even more problematic. For the reasons discussed, estrogen is likely to be most efficacious in accelerating epiphyseal fusion but is obviously undesirable in males. Androgens will also accelerate skeletal maturation, presumably through aromatization to estrogen, but at the price of rapid virilization.

Although psychoemotional arguments constitute the main reason for treating tall (predominantly) girls, prospective well-structured psychological evaluation of potential treatment candidates is often not pursued. In addition, more than 50 years of experience with growth-attenuating intervention has not yielded objective information indicating that being (too) tall when an adolescent or adult results in unequivocal lifelong psychosocial damage. The practice of treating girls with tall stature has certainly become less prevalent. This may in part be related to data from sociologic studies that confirm a tall woman can experience beneficial effects of her stature. These studies indicate positive influences of height on women's character perception.[862] Taller women are now more often perceived by their peers—and by men as well—as more intelligent, assertive, and ambitious compared to their shorter counterparts.

Excess GH Secretion and Pituitary Gigantism

In young persons with open epiphyses, overproduction of GH results in gigantism. In persons with closed epiphyses, the result is acromegaly. Often, some acromegalic features are seen with gigantism—even in children and adolescents. After closure of the epiphyses, the acromegalic features become more prominent. Famous examples of gigantism include Robert Wadlow (the Alton giant), who stood 271.8 cm at the time of his death in his mid-20s, and the well-known Andre Rousimoff (Andre the Giant), who was 190.5 cm at age 12 and reached a height of 223.5 cm by adulthood.[523] Pituitary gigantism is rare, and its cause is most often a pituitary adenoma—but gigantism has been observed in a 2.5-year-old boy with a hypothalamic tumor that presumably secreted GHRH. Other tumors, particularly in the pancreas, have produced acromegaly by secretion of large amounts of GHRH with resultant hyperplasia of the somatotrophs.[863] Activating mutations of Gsα as seen in the McCune-Albright syndrome can be associated with gigantism-acromegaly as discussed later.

The cardinal clinical feature of gigantism is longitudinal growth acceleration secondary to the GH excess. The usual manifestations consist of coarse facial features and enlarging hands and feet. In young children, rapid growth of the head may precede linear growth. Some patients have behavioral and visual problems. In most of the recorded cases, the abnormal growth became evident at puberty—but the condition has been established as early as the newborn period in one child and at 21 months of age in another. Giants may grow to a height of 243.8 cm or more. Acromegalic features consist chiefly of enlargement of the distal parts of the body, but manifestations of abnormal growth involve all portions.

The circumference of the skull increases, the nose becomes broad, and the tongue is often enlarged—with coarsening of the facial features. The mandible grows excessively, and the teeth become separated. Visual field defects and neurologic abnormalities are common. Signs of increased intracranial pressure appear later. The fingers and toes grow chiefly in thickness. There may be dorsal kyphosis. Fatigue and lassitude are early symptoms. GH levels are elevated and may occasionally exceed 100 ng/mL. There is typically no suppression of GH levels by the hyperglycemia of a glucose tolerance test. IGF-1 and IGFBP-3 levels are consistently elevated in gigantism, whereas other growth factors are not. Gigantism is extremely rare, with only several hundred reported cases. The presentation of gigantism is usually dramatic, unlike the insidious onset of acromegaly in adults.

The tumor mass itself may cause headaches, visual changes from optic nerve compression, and hypopituitarism. About half of the patients also have marked hyperprolactinemia as a result of plurihormonal adenomas that secrete GH and prolactin. This is because mammosomatotrophs are the most common type of GH-secreting cells involved in childhood gigantism. GH-secreting tumors of the pituitary are typically eosinophilic or chromophobe adenomas. Adenomas may compromise other anterior pituitary function through growth or cystic degeneration. Secretion of gonadotropins, thyrotropin, or corticotropin may be impaired. Delayed sexual maturation or hypogonadism may occur. When GH hypersecretion is accompanied by gonadotropin deficiency, accelerated linear growth may persist for decades. In some cases, the tumor spreads outside the sella—invading the sphenoid bone, optic nerves, and brain. GH-secreting tumors in pediatric patients are more likely to be locally invasive or aggressive than are those in adult patients.[522]

The cause of these tumors is uncertain, although studies of acromegalics have suggested that many cases result from mutations that generate constitutively activated G proteins with reduced GTPase activity. The resultant rise in intracellular cyclic AMP in the pituitary leads to increased GH secretion. McCune-Albright syndrome, which can also be caused by mutations resulting in constitutively activated G proteins, may also include the presence of somatotrophic tumors and excess GH secretion. In fact, approximately 20% of patients with gigantism are those with McCune-Albright syndrome (commonly consisting of a triad of precocious puberty, café-au-lait spots, and fibrous dysplasia). GH-secreting tumors have also been reported in multiple endocrine adenomatosis and in association with neurofibromatosis, tuberous sclerosis, and Carney complex.[864,865]

Activating mutations of the stimulatory Gsa protein have been found in the pituitary lesions in patients with McCune-Albright syndrome and are believed to be responsible for the other glandular adenomas observed in this condition as well. Somatic point mutations of the Gsa protein have also been identified in somatotrophs of up to 40% of sporadic GH-secreting pituitary adenomas.

Mutations in several additional genes have been identified. The aryl hydrocarbon receptor interacting protein (AIP) gene is implicated in about 15% of familial isolated pituitary adenomas (FIPA), a small number of cases of sporadic acromegaly, and an unknown number of sporadic prolactinomas. Defects in cyclin-dependent kinase inhibitor (CDKI) genes have also been demonstrated in a few families with features similar to MEN type 1. A report on a large cohort of children and adolescents with pituitary adenomas found that germline AIP and MEN1 gene mutations (found in 5 of 11 [45.5%] cases with GH- or PRL-secreting tumors) are common in pediatric patients with GH-secreting pituitary adenomas.[866] A positive family history is often not present.

Diagnosis of GH Excess

The "gold standard" for making the diagnosis of GH excess is a failure to suppress serum GH levels to less than 5 ng/dL after a 1.75-g/kg oral glucose challenge (maximum, 75 g). This test measures the ability of IGF-1 to suppress GH secretion because the glucose load results in insulin secretion, leading to suppression of IGFBP-1, which results in an acute increase in free IGF-1 levels. The increased free IGF-1 suppresses GH secretion within 30 to 90 minutes.[867] This test can be abnormal in diabetic patients. Note that a single measurement of GH is inadequate because GH is secreted in a pulsatile manner. Therefore, the use of a random GH measurement can lead to both false-positive and false-negative results.

Measurement of serum IGF-1 concentration is a sensitive screening test for GH excess. An excellent linear dose-response correlation between serum IGF-1 levels and 24-hour mean GH secretion has been demonstrated. An elevated IGF-1 level in a patient with appropriate clinical suspicion is almost always indicative of GH excess. Potential confusion may arise when evaluating normal adolescents because significantly higher IGF-1 levels occur during puberty than in adulthood.

For accurate control comparison, the IGF-1 level must be age and gender matched. Serum IGFBP-3 levels may also be useful in the diagnosis of GH excess. In patients with confirmed somatotroph adenomas, increased IGFBP-3 levels have been reported to be a sensitive marker of GH elevations and may be elevated despite normal IGF-1 levels. If laboratory findings suggest GH excess, the presence of a pituitary adenoma should be confirmed using MRI. In rare cases, a pituitary mass may not be identified. This may be an occult pituitary microadenoma or an ectopic tumor. Computed tomography is acceptable when MRI is unavailable.

Treatment of GH Oversecretion

The goals of therapy are to remove or shrink the pituitary mass, to restore GH and secretory patterns to normal, to restore IGF-1 and IGFBP-3 levels to normal, to retain the normal pituitary secretion of other hormones, and to prevent recurrence of disease. For well-circumscribed pituitary adenomas, trans-sphenoidal surgery is the treatment of choice and may be curative.[868] If possible, the tumor should be removed completely. The likelihood of surgical cure depends greatly on the surgeon's expertise as well as on the size and extension of the mass. Intraoperative GH measurements can improve the results of tumor resection. Trans-sphenoidal surgery to resect the tumors has been shown to be as safe in children as in adults. At times, a transcranial approach may be necessary. The primary goal of treatment is to normalize GH levels. GH levels ($<$ 1 ng/mL within 2 hours after a glucose load) and serum IGF-1 levels (age-adjusted normal range) are the best tests to define a biochemical cure.

If GH secretion is not normalized by surgery, the options include pituitary radiation and medical therapy. In general, radiation therapy is recommended if GH hypersecretion is not normalized by surgery. Further growth of the tumor is prevented by radiation in more than 99% of the patients. The main disadvantage is the delayed efficacy in decreasing GH levels. GH is reduced approximately 50% from the initial concentration by 2 years and 75% by 5 years, and approaches 90% by 15 years. Hypopituitarism is a predictable outcome, occurring in 40% to 50% of patients 10 years after irradiation.

It is now clear that surgery fails to cure a significant number of patients, and therefore medical therapy has taken on a more important role in the management of patients with GH excess. Indeed, the greatest progress in recent years in the treatment of GH excess has been within the realm of medical therapy. Treatment has been improved by the availability of effective and well-tolerated long-acting somatostatin analogs and dopamine agonists, as well as by novel GH antagonists.

The somatostatin analogs have been found to be highly effective in the treatment of patients with GH excess. Octreotide suppresses GH to less than 2.5 ng/mL in 65% of patients with acromegaly, and it normalizes IGF-1 levels in 70%. Studies of patients for more than 14 years have shown that the effects of octreotide are well sustained over time. Tumor shrinkage also occurs with octreotide, but it is generally modest. Consistent GH suppression has been obtained with a continuous subcutaneous pump infusion of octreotide in a pubertal boy with pituitary gigantism.[869] New long-acting formulations, including octreotide and lanreotide, have been shown to produce consistent GH and IGF-1 suppression in acromegalic patients with once-monthly intramuscular depot injections. They both appear equivalent for control of these biochemical markers and symptoms. Octreotide as well as lanreotide inhibit GH secretion better than native somatostatin because they possess greater potency and demonstrate a longer plasma half-life. The sustained-release preparations have not been formally tested in children. Octreotide injection in the pediatric population has been used at doses of 1 to 40 mg/kg/day.

Dopamine agonists, such as bromocriptine, bind to pituitary dopamine type 2 (D2) receptors and suppress GH secretion—although the precise mechanism of action remains unclear. Prolactin levels are often adequately suppressed. However, GH levels and IGF-1 levels are rarely normalized with this treatment modality. Less than 20% of patients achieve GH levels less than 5 ng/mL, and less than 10% achieve normalization of IGF-1 levels. Tumor shrinkage occurs in a minority of patients. It is generally used as adjuvant medical treatment for GH excess. Its effectiveness may be additive to that of octreotide.[870] The dose of bromocriptine needed ranges between 10 and 60 mg orally divided four times a day. Only a minority of patients benefit from doses greater than 20 mg/day. It has been found to be safe when used in children for an extended period of time, but side effects may include nausea, vomiting, abdominal pain, arrhythmias, nasal stuffiness, orthostatic hypotension, sleep disturbances, and fatigue. Another dopamine agonist, cabergoline, may inhibit GH secretion more efficiently than bromocriptine and has therefore become the preferred drug in this category. In a meta-analysis of 15 studies of cabergoline

treatment in acromegaly (n = 227), cabergoline achieved normal IGF-1 levels in 34% of patients, whereas when combined with a somatostatin analogue, 52% normalized IGF-1 concentrations.[871]

An addition to the armamentarium for the treatment of GH excess is the novel GHR antagonist, pegvisomant. This is a mutated GH molecule that has a polymer chain attached at several sites to prolong its half-life. Despite its similar binding affinity compared to wild-type GH, pegvisomant blocks endogenous GH from binding to its GHR, and it does not activate the post-GHR intracellular signaling cascade, thereby avoiding the undesirable effect of GH excess—exactly what needs to be blocked. Pegvisomant has now been approved for use in adults with acromegaly. Pegvisomant is administered as a daily subcutaneous injection. The initial daily dose is 10 mg. The serum IGF-1 concentration should be measured every 4 to 6 weeks and the dose can be adjusted, in 5-mg increments, to a maximum of 30 mg/day. The ultimate goal is to keep the serum IGF-1 within the normal range. It has been shown to effectively suppress GH and IGF-1 levels in patients with acromegaly from pituitary tumors as well as ectopic GHRH hypersecretion.[872] Normalization of IGF-1 levels occurs in up to 90% of patients treated daily with this drug for 3 months or longer.[873] More long-term studies need to be performed, as it remains uncertain, based on initial data from postmarketing surveillance studies, if long-term GH suppression continues as was reported in the initial studies. Although experience with this therapy in pediatrics is limited, the well-tolerated long-term efficacy of pegvisomant was reported in an 8-year-old girl who had protracted GH excess after surgical removal of a pituitary macroadenoma and failure to suppress GH with long-acting somatostatin analogs.[874]

CONCLUSIONS

The advancement in our understanding of the molecular mechanisms of growth has resulted in a dramatic enhancement of our ability to diagnose and treat growth disorders. Further rapid changes to the available genetic diagnostic tools are expected. These will continue to alter the manner by which pediatric endocrinologists shall evaluate and manage patients with growth disorders in the near future. Undoubtedly, in the coming years, new and improved methods of identifying specific abnormalities leading to short stature (particularly in the form of genetic tests) will refine even more the diagnostic process. Although hormonal testing is still the mainstay of endocrine assessment in the evaluation of growth disorders, we can now start to envision the age of the "gene-chip" approach. In addition, the therapies available to treat growth disorders will continue to undergo constant improvements and in the future will surely allow more efficacious, safer, and easier-to-tolerate management of growth abnormalities. These changes, both in our diagnostic capabilities and in the area of pharmacogenomics, will allow for a more individualized approach to the patient with a growth disorder.

REFERENCES

1. Cameron, N. (1986). The methods of auxoogical anthropometry. In T. J. Falkner (Ed.), *Human growth: a comprehensive treatise: volume II, postnatal growth, neurobiology* (2nd ed.) (pp. 35–90). New York: Plenum Press.
2. Tanner, J. M. (1986). Normal growth and techniques of growth assessment. *Clin Endocrinol Metab*, *15*, 411.
3. Underwood, L. E., & Van Wyk, J. J. (1992). Normal and aberrant growth. In R. H. Williams, D. W. Foster, & J. D. Wilson (Eds.), *Williams textbook of endocrinology*, (8th ed.) (pp. 1079–1138). Philadelphia: WB Saunders.
4. Whitehouse, R. H., Tanner, J. M., & Healy, M. J. (1974). Diurnal variation in stature and sitting height in 12-14-year-old boys. *Ann Hum Biol*, *1*, 103–106.
5. Marshall, W. A. (1971). Evaluation of growth rate in height over periods of less than one year. *Arch Dis Child*, *46*, 414–420.
6. Hamill, P. V., Drizd, T. A., Johnson, C. L., et al., (1979). Physical growth: National Center for Health Statistics percentiles. *Am J Clin Nutr*, *32*, 607–629.
7. Statistics, N.C.f.H., NCHS Growth Charts (1976). Monthly Vital Statistics Report, H.R. Administration, Editor. Suppl (HRA) (pp. 76–1120). Rockville, MD.
8. Statistics, N.C.f.H., NCHS Growth Curves for Children 0-18 years: United States, Vital and Health Statistics, H.R. Administration, Editor. 1977, Washington, DC: U.S Government Printing Office.
9. Statistics, N.C.f.H., CDC Growth Charts, H.R. Administration, Editor. 2000, Washington, DC: U.S Government Printing Office.
10. Tanner, J. M., & Davies, P. S. (1985). Clinical longitudinal standards for height and height velocity for North American children. *J Pediatr*, *107*, 317–329.
11. Lyon, A. J., Preece, M. A., & Grant, D. B. (1985). Growth curve for girls with Turner syndrome. *Arch Dis Child*, *60*, 932–935.
12. Horton, W. A., Rotter, J. I., Rimoin, D. L., et al. (1978). Standard growth curves for achondroplasia. *J Pediatr*, *93*, 435–438.
13. Myrelid, A., Gustafsson, J., Ollars, B., & Annerén, G. (2002). Growth charts for Down's syndrome from birth to 18 years of age. *Arch Dis Child*, *87*, 97–103.
14. Shalet, S. M., Gibson, B., Swindell, R., & Pearson, D. (1987). Effect of spinal irradiation on growth. *Arch Dis Child*, *62*, 461–464.
15. Bayer, L. M., (1959). *Growth diagnosis*. Chicago: University of Chicago Press.
16. Eveleth, P. B., (1990). *Worldwide variation in human growth*, (2nd ed.). Cambridge: Cambridge University Press.
17. Smith, E. P., Boyd, J., Frank, G. R., et al. (1994). Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. *N Engl J Med*, *331*, 1056–1061.
18. Morishima, A., Grumbach, M. M., Simpson, E. R., et al. (1995). Aromatase deficiency in male and female siblings caused by a novel mutation and the physiological role of estrogens. *J Clin Endocrinol Metab*, *80*, 3689–3698.
19. Greulich, W. W. (1959). *Radiographic atlas of skeletal development of the hand and wrist*, (2nd ed.). Stanford, CA: Stanford University Press.
20. Tanner, J. M., et al. (1975). *Assessment of skeletal maturity and prediction of adult height: TW2 method*. New York: Academic Press.
21. Roche, A., Davila, G. H., & Eyman, S. L. (1971). A comparison between greulich-pyle and tanner-whitehouse assessments of skeletal maturity. *Radiology*, *98*, 273.
22. Bayley, N., & Pinneau, S. R. (1952). Tables for predicting adult height from skeletal age: revised for use with the Greulich-Pyle hand standards. *J Pediatr*, *40*, 423–441.
23. Tanner, J. M., Whitehouse, R. H., Marshall, W. A., & Carter, B. S. (1975). Prediction of adult height from height, bone age, and occurrence of menarche, at ages 4 to 16 with allowance for midparent height. *Arch Dis Child*, *50*, 14–26.
24. Roche, A. F., Wainer, H., & Thissen, D. (1975). The RWT method for the prediction of adult stature. *Pediatrics*, *56*, 1027–1033.
25. Asa, S., & Kovacs, K. (2000). Functional morphology of the human fetal pituitary. *Pathol Annu*, *19*, 275.
26. Ikeda, H., Suzuki, J., Sasano, N., & Niizuma, H. (1988). The development and morphogenesis of the human pituitary gland. *Anat Embryol* (Berl), *178*, 327–336.
27. Goodyer, C. G., (1989). Ontogeny of pituitary hormone secretion. In J. R. Ducharme, R. Collu, & H. J. Guyda (Eds.), *Pediatric endocrinology* (pp. 125–169). New York: Raven Press.

28. Stanfield, J. P. (1960). The blood supply of the human pituitary gland. *J Anat*, *94*, 257–273.
29. Gorczyca, W., & Hardy, J. (1987). Arterial supply of the human anterior pituitary gland. *Neurosurgery*, *20*, 369–378.
30. Treier, M., & Rosenfeld, M. G. (1996). The hypothalamic-pituitary axis: co-development of two organs. *Curr Opin Cell Biol*, *8*, 833–843.
31. Parks, J. S., Adess, M. E., Brown, M. R. (1997). Genes regulating hypothalamic and pituitary development. *Acta Paediatr* (Suppl.), *423*, 28–32.
32. Burrows, H. L., Douglas, K. R., Seasholtz, A. F., & Camper, S. A. (1999). Genealogy of the anterior pituitary gland: tracing a family tree. *Trends Endocrinol Metab*, *10*, 343–352.
33. Cohen, L. E. (2000). Genetic regulation of the embryology of the pituitary gland and somatotrophs. *Endocrine*, *12*, 99–106.
34. Lopez-Bermejo A, Buckway, C. K., & Rosenfeld, R. G. (2000). Genetic defects of the growth hormone-insulin-like growth factor axis. *Trends Endocrinol Metab*, *11*, 39–49.
35. Couly, G. F., & Le Douarin, N. M. (1985). Mapping of the early neural primordium in quail-chick chimeras. I. Developmental relationships between placodes, facial ectoderm, and prosencephalon. *Dev Biol*, *110*, 422–439.
36. Couly, G. F., & Le Douarin, N. M. (1987). Mapping of the early neural primordium in quail-chick chimeras. II. The prosencephalic neural plate and neural folds: implications for the genesis of cephalic human congenital abnormalities. *Dev Biol*, *120*, 198–214.
37. Kelberman, D., Rizzoti, K., Lovell-Badge, R., et al. (2009). Genetic regulation of pituitary gland development in human and mouse. *Endocr Rev*, *30*, 790–829.
38. Davis, S. W., Castinetti, F., Carvalho, L. R., et al. (2010). Molecular mechanisms of pituitary organogenesis: in search of novel regulatory genes. *Mol Cell Endocrinol*, *323*, 4–19.
39. Ericson, J., Norlin, S., Jessell, T. M., & Edlund, T. (1998). Integrated FGF and BMP signaling controls the progression of progenitor cell differentiation and the emergence of pattern in the embryonic anterior pituitary. *Development*, *125*, 1005–1015.
40. Davis, S. W., Mortensen, A. H., & Camper, S. A. (2011). Birthdating studies reshape models for pituitary gland cell specification. *Dev Biol*, *352*, 215–227.
41. Gaston-Massuet, C., Andoniadou, C. L., Signore, M., et al. (2011). Increased Wingless (Wnt) signaling in pituitary progenitor/stem cells gives rise to pituitary tumors in mice and humans. *Proc Natl Acad Sci USA*, *108*, 11482–11487.
42. Andoniadou, C. L., Gaston-Massuet, C., Reddy, R., et al. (20112). Identification of novel pathways involved in the pathogenesis of human adamantinomatous craniopharyngioma. *Acta Neuropathol*, *124*, 259–271.
43. Prince, K. L., Walvoord, E. C., & Rhodes, S. J. (2011). The role of homeodomain transcription factors in heritable pituitary disease. *Nat Rev Endocrinol*, *7*, 727–737.
44. Thorner, M. O., Vance, M. L., Horvath, E., et al. (1992). The anterior pituitary. In J. D. Wilson (Ed.), *Williams textbook of endocrinology*, (8th ed.) (p. 221). Philadelphia: WB Saunders.
45. Scheithauer, B. W., Sano, T., Kovacs, K. T., et al. (1990). The pituitary gland in pregnancy: a clinicopathologic and immunohistochemical study of 69 cases. *Mayo Clin Proc*, *65*, 461–474.
46. Boyd, J. D. (1956). Observations on the human pharyngeal hypophysis. *J Endocrinol*, *14*, 66–77.
47. Underwood, L. E., Radcliffe, W. B., & Guinto, F. C. (1976). New standards for the assessment of sella turcica volume in children. *Radiology*, *119*, 651–654.
48. Hoyt, W. F., Kaplan, S. L., Grumbach, M. M., & Glaser, J. S. (1970). Septo-optic dysplasia and pituitary dwarfism. *Lancet*, *1*, 893–894.
49. Fauquier, T., Rizzoti, K., Dattani, M., et al. (2008). SOX2-expressing progenitor cells generate all of the major cell types in the adult mouse pituitary gland. *Proc Natl Acad Sci USA*, *105*, 2907–2912.
50. Castinetti, F., Davis, S. W., Brue, T., & Camper S. A. (2011). Pituitary stem cell update and potential implications for treating hypopituitarism. *Endocr Rev*, *32*, 453–471.
51. Gleiberman, A. S., Michurina, T., Encinas, J. M., et al. (2008). Genetic approaches identify adult pituitary stem cells. *Proc Natl Acad Sci USA*, *105*, 6332–6337.
52. Suga, H., Kadoshima, T., Minaguchi, M., et al. (2011). Self-formation of functional adenohypophysis in three-dimensional culture. *Nature*, *480*, 57–62.
53. Bonnefont, X., Lacampagne, A., Sanchez-Hormigo, A., et al. (2005). Revealing the large-scale network organization of growth hormone-secreting cells. *Proc Natl Acad Sci USA*, *102*, 16880–16885.
54. Budry, L., Lafont, C., El Yandouzi, T., et al. (2011). Related pituitary cell lineages develop into interdigitated 3D cell networks. *Proc Natl Acad Sci USA*, *108*, 12515–12520.
55. Hodson, D. J., Schaeffer, M., Romanò, N., et al. (2012). Existence of long-lasting experience-dependent plasticity in endocrine cell networks. *Nat Commun*, *3*, 605.
56. Lewis, U. J., Singh, R. N., Tutwiler, G. F., et al. (1980). Human growth hormone: a complex of proteins. *Recent Prog Horm Res*, *36*, 477–508.
57. Gaumann, B., (1988). Heterogeneity of growth hormone. In B. B. Bercu, (Ed.), *Basic and clinical aspects of growth hormone* (pp. 13–31). New York: Plenum Press.
58. de Vos, A. M., Ultsch, M., & Kossiakoff, A. A. (1992). Human growth hormone and extracellular domain of its receptor: crystal structure of the complex. *Science*, *255*, 306–312.
59. Mullis, P. E., Deladoey, J., & Dannies, P. S. (2002). Molecular and cellular basis of isolated dominant-negative growth hormone deficiency, IGHD type II: insights on the secretory pathway of peptide hormones. *Horm Res*, *58*, 53–66.
60. Frankenne, F., Closset, J., Gomez, F., et al. (1988). The physiology of growth hormones (GHs) in pregnant women and partial characterization of the placental GH variant. *J Clin Endocrinol Metab*, *66*, 1171–1180.
61. Cooke, N. E., Ray, J., Watson, M. A., et al. (1988). Human growth hormone gene and the highly homologous growth hormone variant gene display different splicing patterns. *J Clin Invest*, *82*, 270–275.
62. Procter, A. M., Phillips, J. A., 3rd, & Cooper, D. N. (1998). The molecular genetics of growth hormone deficiency. *Hum Genet*, *103*, 255–272.
63. Bennani-Baïti, I. M., Asa, S. L., Song, D., et al. (1998). DNase I-hypersensitive sites I and II of the human growth hormone locus control region are a major developmental activator of somatotrope gene expression. *Proc Natl Acad Sci USA*, *95*, 10655–10660.
64. Miller, W. L., et al. (1983). Structure and evaluation of the growth hormone gene family. *Endocr Rev*, *4*, 97.
65. DeNoto, F. M., Moore, D. D., & Goodman, H. M. (1981). Human growth hormone DNA sequence and mRNA structure: possible alternative splicing. *Nucleic Acids Res*, *9*, 3719–3730.
66. Barinaga, M., Yamonoto, G., Rivier, C., et al. (1983). Transcriptional regulation of growth hormone gene expression by growth hormone-releasing factor. *Nature*, *306*, 84–85.
67. Esch, F. S., Böhlen, P., Ling, N. C., et al. (1983). Primary structure of three human pancreas peptides with growth hormone releasing activity. *J Biol Chem*, *258*, 1806–1812.
68. Holl, R. W., Thorner, M. O., & Leong, D. A. (1988). Intracellular calcium concentration and growth hormone secretion in individual somatotropes: effects of growth hormone-releasing factor and somatostatin. *Endocrinology*, *122*, 2927–2932.
69. Mayo, K. E. (1992). Molecular cloning and expression of a pituitary-specific receptor for growth hormone-releasing hormone. *Mol Endocrinol*, *6*, 1734–1744.
70. Hartman, M. L., Fario, A. C. C., Vance, M. L., et al. (1991). Temporal sequence of in vivo growth hormone secretory events in man. *Am J Physiol*, *260*, E101.
71. Frohman, L. A. (1980). Neurotransmitters as regulators of endocrine function. In J. C. Hughes, & D. T. Krieger (Eds.), *Neuroendocrinology*. Sunderland, MA: Sinauer Associates.
72. Melander, T., Hokfelt, T., & Rokaeus, A. (1986). Distribution of galaninlike immunoreactivity in the rat central nervous system. *J Comp Neurol*, *248*, 475–517.
73. Deller, J. J., Jr., Plunket, D. C., & Forsham, P. H. (1966). Growth hormone studies in growth retardation. Therapeutic response to administration of androgen. *Calif Med*, *104*, 359–362.
74. Zeitler, P., Argente, J., Chowen-Breed, J. A., et al. (1990). Growth hormone-releasing hormone messenger ribonucleic acid in the hypothalamus of the adult male rat is increased by testosterone. *Endocrinology*, *127*, 1362–1368.

75. Ho, K. Y., Evans, W. S., Blizzard, R. M., et al. (1987). Effects of sex and age on the 24-hour profile of growth hormone secretion in man: importance of endogenous estradiol concentrations. *J Clin Endocrinol Metab*, *64*, 51–58.
76. Katz, H. P., Youlton, R., Kaplan, S. L., & Grumbach, M. M. (1969). Growth and growth hormone. 3. Growth hormone release in children with primary hypothyroidism and thyrotoxicosis. *J Clin Endocrinol Metab*, *29*, 346–351.
77. Fratz, A. G., & Rabkin, M. T. (1964). Human growth hormone: clinical measurement, response to hypoglycemia and suppression by corticosteroids. *N Engl J Med*, *271*, 1375.
78. Thompson, R. G., Rodriguez, A., Kowarski, A., & Blizzard, R. M. (1972). Growth hormone: metabolic clearance rates, integrated concentrations, and production rates in normal adults and the effect of prednisone. *J Clin Invest*, *51*, 3193–3199.
79. Martha, P. M., Jr., Rogol, A. D., Veldhuis, J. D., et al. (1989). Alterations in the pulsatile properties of circulating growth hormone concentrations during puberty in boys. *J Clin Endocrinol Metab*, *69*, 563–570.
80. Bowers, C. Y., Momany, F. A., Reynolds, G. A., & Hong, A. (1984). On the in vitro and in vivo activity of a new synthetic hexapeptide that acts on the pituitary to specifically release growth hormone. *Endocrinology*, *114*, 1537–1545.
81. Malozowski, S., Hao, E. H., Ren, S. G., et al. (1991). Growth hormone (GH) responses to the hexapeptide GH-releasing peptide and GH-releasing hormone (GHRH) in the cynomolgus macaque: evidence for non-GHRH-mediated responses. *J Clin Endocrinol Metab*, *73*, 314–317.
82. Smith, R. G., Palyha, O. C., Feighner, S. D., et al. (1999). Growth hormone releasing substances: types and their receptors. *Horm Res*, *51*(Suppl. 3), 1–8.
83. Kojima, M., Hosoda, H., Date, Y., et al. (1999). Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature*, *402*, 656–660.
84. Tschop, M., Smiley, D. L., & Heiman, M. L. (2000). Ghrelin induces adiposity in rodents. Nature 407, 908–913.
85. Hosoda, H., Kojima, M., Matsuo, H., & Kangawa, K. (2000). Purification and characterization of rat des-Gln14-Ghrelin, a second endogenous ligand for the growth hormone secretagogue receptor. *J Biol Chem*, *275*, 21995–2000.
86. Pantel, J., Legendre, M., Cabrol, S., et al. (2006). Loss of constitutive activity of the growth hormone secretagogue receptor in familial short stature. *J Clin Invest*, *116*, 760–768.
87. Sun Y, Butte NF, Garcia JM, Smith RG. Characterization of adult ghrelin and ghrelin receptor knockout mice under positive and negative energy balance. *Endocrinology*. 2008;149(2):843–850.
88. Zhang, J. V., Ren, P. G., Avsian-Kretchmer, O., et al. (2005). Obestatin, a peptide encoded by the ghrelin gene, opposes ghrelin's effects on food intake. *Science*, *310*, 996–999.
89. Yamamoto, D., Ikeshita, N., Daito, R., et al. (2007). Neither intravenous nor intracerebroventricular administration of obestatin affects the secretion of GH, PRL, TSH and ACTH in rats. *Regul Pept*, *138*, 141–144.
90. Nelson, C., Albert, V. R., Elsholtz, H. P., et al. (1988). Activation of cell-specific expression of rat growth hormone and prolactin genes by a common transcription factor. *Science*, *239*, 1400–1405.
91. Quentien, M. H., Barlier, A., Franc, J. L., et al. (2006). Pituitary transcription factors: from congenital deficiencies to gene therapy. *J Neuroendocrinol*, *18*, 633–642.
92. Li, S., Crenshaw, E. B., 3rd, Rawson, E. J., et al. (1990). Dwarf locus mutants lacking three pituitary cell types result from mutations in the POU-domain gene pit-1. *Nature*, *347*, 528–533.
93. Berelowitz, M., Szabo, M., Frohman, L. A., et al. (1981). Somatomedin-C mediates growth hormone negative feedback by effects on both the hypothalamus and the pituitary. *Science*, *212*, 1279–1281.
94. Yamashita, S., & Melmed, S. (1986). Insulin-like growth factor I action on rat anterior pituitary cells: suppression of growth hormone secretion and messenger ribonucleic acid levels. *Endocrinology*, *118*, 176–182.
95. Abe, H., Molitch, M. E., Van Wyk, J. J., & Underwood, L. E. (1983). Human growth hormone and somatomedin C suppress the spontaneous release of growth hormone in unanesthetized rats. *Endocrinology*, *113*, 1319–1324.
96. Rosenfeld, R. G., Ceda, G., Cutler, C. W., et al. (1985). Insulin and insulin-like growth factor (somatomedin) receptors on cloned rat pituitary tumor cells. *Endocrinology*, *117*, 2008–1216.
97. Rosenfeld, R. G., Ceda, G., Wilson, D. M., et al. (1984). Characterization of high affinity receptors for insulin-like growth factors I and II on rat anterior pituitary cells. *Endocrinology*, *114*, 1571–1575.
98. Ceda, G. P., Hoffman, A. R., Silverberg, G. D., et al. (1985). Regulation of growth hormone release from cultured human pituitary adenomas by somatomedins and insulin. *J Clin Endocrinol Metab*, *60*, 1204–1209.
99. Ceda, G. P., Davis, R. G., Rosenfeld, R. G., & Hoffman, A. R. (1987). The growth hormone (GH)-releasing hormone (GHRH)-GH-somatomedin axis: evidence for rapid inhibition of GHRH-elicited GH release by insulin-like growth factors I and II. *Endocrinology*, *120*, 1658–1662.
100. Guler, H. P., Zapf, J., & Froesch, E. R. (1987). Short-term metabolic effects of recombinant human insulin-like growth factor I in healthy adults. *N Engl J Med*, *317*, 137–140.
101. Vaccarello, M. A., Diamond, F. B., Jr., & Guevara-Aguirre, J., et al. (1993). Hormonal and metabolic effects and pharmacokinetics of recombinant insulin-like growth factor-I in growth hormone receptor deficiency/Laron syndrome. *J Clin Endocrinol Metab*, *77*, 273–280.
102. Gluckman, P. D., Grumbach, M. M., & Kaplan, S. L. (1981). The neuroendocrine regulation and function of growth hormone and prolactin in the mammalian fetus. *Endocr Rev*, *2*, 363–395.
103. Dudl, R. J., Ensinck, J. W., Palmer, H. E., & Williams, R. H. (1973). Effect of age on growth hormone secretion in man. *J Clin Endocrinol Metab*, *37*, 11–16.
104. Rudman, D., Kutner, M. H., Rogers, C. M., et al. (1981). Impaired growth hormone secretion in the adult population: relation to age and adiposity. *J Clin Invest*, *67*, 1361–1369.
105. MacGillivray, M. H., Frohman, L. A., & Doe, J. (1970). Metabolic clearance and production rates of human growth hormone in subjects with normal and abnormal growth. *J Clin Endocrinol Metab*, *30*, 632–638.
106. Sassin, J. F., Parker, D. C., Mace, J. W., et al. (1969). Human growth hormone release: relation to slow-wave sleep and sleep-walking cycles. *Science*, *165*, 513–515.
107. Van Cauter, E., Kerkhofs, M., Van Onderbergen, A., et al. (1989). Modulation of spontaneous and GHRH-stimulated growth hormone secretion by sleep. In: 71st Annual Meeting of the Endocrine Society abstract book. *The Endocrine Society*, 1989.
108. Veldhuis, J. D., Carlson, M. L., & Johnson, M. L. (1987). The pituitary gland secretes in bursts: appraising the nature of glandular secretory impulses by simultaneous multiple-parameter deconvolution of plasma hormone concentrations. *Proc Natl Acad Sci USA*, *84*, 7686–7690.
109. Schalch, D. S. (1967). The influence of physical stress and exercise on growth hormone and insulin secretion in man. *J Lab Clin Med*, *69*, 256–2569.
110. Rosenfeld, R. G., Rosenbloom, A. L., & Guevara-Aguirre, J. (1994). Growth hormone (GH) insensitivity due to primary GH receptor deficiency. *Endocr Rev*, *15*, 369–390.
111. Veldhuis, J. D., Iranmanesh, A., Ho, K. K., et al. (1991). Dual defects in pulsatile growth hormone secretion and clearance subserve the hyposomatotropism of obesity in man. *J Clin Endocrinol Metab*, *72*, 51–59.
112. Leung, D. W., Spencer, S. A., Cachianes, G., et al. (1987). Growth hormone receptor and serum binding protein: purification, cloning and expression. *Nature*, *330*, 537–543.
113. Trivedi, B., & Daughaday, W. H. (1988). Release of growth hormone binding protein from IM-9 lymphocytes by endopeptidase is dependent on sulfhydryl group inactivation. *Endocrinology*, *123*, 2201–2206.
114. Smith, W. C., Linzer, D. I., & Talamantes, F. (1988). Detection of two growth hormone receptor mRNAs and primary translation products in the mouse. *Proc Natl Acad Sci USA*, *85*, 9576–9579.
115. Smith, W. C., Kuniyoshi, J., & Talamantes, F. (1989). Mouse serum growth hormone (GH) binding protein has GH receptor extracellular and substituted transmembrane domains. *Mol Endocrinol*, *3*, 984–990.
116. Sadeghi, H., Wang, B. S., Lumanglas, A. L., et al. (1990). Identification of the origin of the growth hormone-binding protein in rat serum. *Mol Endocrinol*, *4*, 1799–1805.

117. Kelly, P. A., Djiane, J., Postel-Vinay, M. C., & Edery, M. (1991). The prolactin/growth hormone receptor family. *Endocr Rev*, *12*, 235–251.
118. Bougneres, P., & Goffin, V. (2007). The growth hormone receptor in growth. *Endocrinol Metab Clin North Am*, *36*, 1–16.
119. Jensen, R. B., Vielwerth, S., Larsen, T., et al. (2007). The presence of the d3-growth hormone receptor polymorphism is negatively associated with fetal growth but positively associated with postnatal growth in healthy subjects. *J Clin Endocrinol Metab*, *92*, 2758–2763.
120. Räz, B., Janner, M., Petkovic, V., et al. (2008). Influence of growth hormone (GH) receptor deletion of exon 3 and full-length isoforms on GH response and final height in patients with severe GH deficiency. *J Clin Endocrinol Metab*, *93*, 974–980.
121. Wilkinson, I. R., Ferrandis, E., Artymiuk, P. J., et al. (2007). A ligand-receptor fusion of growth hormone forms a dimer and is a potent long-acting agonist. *Nat Med*, *13*, 1108–1113.
122. Brown, R. J., Adams, J. J., Pelekanos, R. A., et al. (2005). Model for growth hormone receptor activation based on subunit rotation within a receptor dimer. *Nat Struct Mol Biol*, *12*, 814–821.
123. Rowlinson, S. W., Yoshizato, H., Barclay, J. L., et al. (2008). An agonist-induced conformational change in the growth hormone receptor determines the choice of signalling pathway. *Nat Cell Biol*, *10*, 740–747.
124. Sotiropoulos, A., Perrot-Applanat, M., Dinerstein, H., et al. (1994). Distinct cytoplasmic regions of the growth hormone receptor are required for activation of JAK2, mitogen-activated protein kinase, and transcription. *Endocrinology*, *135*, 1292–1298.
125. Frank, S. J., Gilliland, G., Kraft, A. S., & Arnold, C. S. (1994). Interaction of the growth hormone receptor cytoplasmic domain with the JAK2 tyrosine kinase. *Endocrinology*, *135*, 2228–2239.
126. Soldaini, E., John, S., Moro, S., et al. (2000). DNA binding site selection of dimeric and tetrameric Stat5 proteins reveals a large repertoire of divergent tetrameric Stat5a binding sites. *Mol Cell Biol*, *20*, 389–401.
127. Argetsinger, L. S., Campbell, G. S., Yang, X., et al. (1993). Identification of JAK2 as a growth hormone receptor-associated tyrosine kinase. *Cell*, *74*, 237–244.
128. Levy, D. E., & Darnell, J. E., Jr. (2002). Stats: transcriptional control and biological impact. *Nat Rev Mol Cell Biol*, *3*, 651–662.
129. Teglund, S., McKay, C., Schuetz, E., et al. (1998). Stat5a and Stat5b proteins have essential and nonessential, or redundant, roles in cytokine responses. *Cell*, *93*, 841–850.
130. Liu, X., Robinson, G. W., Wagner, K. U., et al. (1997). Stat5a is mandatory for adult mammary gland development and lactogenesis. *Genes Dev*, *11*, 179–186.
131. Choi, H. K., & Waxman, D. J. (1999). Growth hormone, but not prolactin, maintains, low-level activation of STAT5a and STAT5b in female rat liver. *Endocrinology*, *140*, 5126–5135.
132. Gallego, M. I., Binart, N., Robinson, G. W., et al. (2001). Prolactin, growth hormone, and epidermal growth factor activate Stat5 in different compartments of mammary tissue and exert different and overlapping developmental effects. *Dev Biol*, *229*, 163–175.
133. Holloway, M. G., Cui, Y., Laz, E. V., et al. (2007). Loss of sexually dimorphic liver gene expression upon hepatocyte-specific deletion of Stat5a-Stat5b locus. *Endocrinology*, *148*, 1977–1986.
134. Clodfelter, K. H., Miles, G. D., Wauthier, V., et al. (2007). Role of STAT5a in regulation of sex-specific gene expression in female but not male mouse liver revealed by microarray analysis. *Physiol Genomics*, *31*, 63–74.
135. Udy, G. B., Towers, R. P., Snell, R. G., et al. (1997). Requirement of STAT5b for sexual dimorphism of body growth rates and liver gene expression. *Proc Natl Acad Sci USA*, *94*, 7239–7244.
136. Zhou, Y., Xu, B. C., Maheshwari, H. G., et al. (1997). A mammalian model for Laron syndrome produced by targeted disruption of the mouse growth hormone receptor/binding protein gene (the Laron mouse). *Proc Natl Acad Sci USA*, *94*, 13215–13220.
137. Coschigano, K. T., Clemmons, D., Bellush, L. L., & Kopchick, J. J. (2000). Assessment of growth parameters and life span of GHR/BP gene-disrupted mice. *Endocrinology*, *141*, 2608–2613.
138. Yao, Z., Cui, Y., Watford, W. T., et al. (2006). Stat5a/b are essential for normal lymphoid development and differentiation. *Proc Natl Acad Sci USA*, *103*, 1000–1005.
139. Kelly, J., Spolski, R., Imada, K., et al. (2003). A role for Stat5 in CD8+ T cell homeostasis. *J Immunol*, *170*, 210–217.
140. Iavnilovitch, E., Groner, B., & Barash, I. (2002). Overexpression and forced activation of stat5 in mammary gland of transgenic mice promotes cellular proliferation, enhances differentiation, and delays postlactational apoptosis. *Mol Cancer Res*, *1*, 32–47.
141. Gebert, C. A., Park, S. H., & Waxman, D. J. (1999). Down-regulation of liver JAK2-STAT5b signaling by the female plasma pattern of continuous growth hormone stimulation. *Mol Endocrinol*, *13*, 213–227.
142. Gebert, C. A., Park, S. H., & Waxman, D. J. (1999). Termination of growth hormone pulse-induced STAT5b signaling. *Mol Endocrinol*, *13*, 38–56.
143. Ahluwalia, A., Clodfelter, K. H., & Waxman, D. J. (2004). Sexual dimorphism of rat liver gene expression: regulatory role of growth hormone revealed by deoxyribonucleic acid microarray analysis. *Mol Endocrinol*, *18*, 747–760.
144. Waxman, D. J., & O'Connor, C. (2006). Growth hormone regulation of sex-dependent liver gene expression. *Mol Endocrinol*, *20*, 2613–2629.
145. Davey, H. W., Park, S. H., Grattan, D. R., et al. (1999). STAT5b-deficient mice are growth hormone pulse-resistant. Role of STAT5b in sex-specific liver p450 expression. *J Biol Chem*, *274*, 35331–35336.
146. Clodfelter, K. H., Holloway, M. G., Hodor, P., et al. (2006). Sex-dependent liver gene expression is extensive and largely dependent upon signal transducer and activator of transcription 5b (STAT5b): STAT5b-dependent activation of male genes and repression of female genes revealed by microarray analysis. Mol Endocrinol, 20, 1333–1351.
147. Eleswarapu, S., Gu, Z., & Jiang, H. (2008). Growth hormone regulation of insulin-like growth factor-I gene expression may be mediated by multiple distal signal transducer and activator of transcription 5 binding sites. *Endocrinology*, *149*, 2230–2240.
148. Chia, D. J., Ono, M., Woelfle, J., et al. (2006). Characterization of distinct Stat5b binding sites that mediate growth hormone-stimulated IGF-I gene transcription. *J Biol Chem*, *281*, 3190–3197.
149. Greenhalgh, C. J., Rico-Bautista, E., Lorentzon, M., et al. (2005). SOCS2 negatively regulates growth hormone action in vitro and in vivo. *J Clin Invest*, *115*, 397–406.
150. Colson, A., Le Cam, A., Maiter, D., et al. (2000). Potentiation of growth hormone-induced liver suppressors of cytokine signaling messenger ribonucleic acid by cytokines. *Endocrinology*, *141*, 3687–3695.
151. Mao, Y., Ling, P. R., Fitzgibbons, T. P., et al. (1999). Endotoxin-induced inhibition of growth hormone receptor signaling in rat liver in vivo. *Endocrinology*, *140*, 5505–5515.
152. Takala, J., Ruokonen, E., Webster, N. R., et al. (1999). Increased mortality associated with growth hormone treatment in critically ill adults. *N Engl J Med*, *341*, 785–792.
153. Gu, F., Dubé, N., Kim, J. W., et al. (2003). Protein tyrosine phosphatase 1B attenuates growth hormone-mediated JAK2-STAT signaling. *Mol Cell Biol*, *23*, 3753–3762.
154. Ram, P. A., & Waxman, D. J. (1997). Interaction of growth hormone-activated STATs with SH2-containing phosphotyrosine phosphatase SHP-1 and nuclear JAK2 tyrosine kinase. *J Biol Chem*, *272*, 17694–17702.
155. Binder, G., Neuer, K., Ranke, M. B., & Wittekindt, N. E. (2005). PTPN11 mutations are associated with mild growth hormone resistance in individuals with Noonan syndrome. *J Clin Endocrinol Metab*, *90*, 5377–5381.
156. Argetsinger, L. S., & Carter-Su, C. (1997). Mechanism of signaling by growth hormone receptor. *Physiol Rev*, *76*, 1089–1107.
157. Schindler, C., & Darnell, J. E., Jr. (1995). Transcriptional responses to polypeptide ligands: the JAK-STAT pathway. *Annu Rev Biochem*, *64*, 621–651.
158. Baumann, G., Stolar, M. W., Amburn, K., et al. (1986). A specific growth hormone-binding protein in human plasma: initial characterization. *J Clin Endocrinol Metab*, *62*, 134–141.
159. Herington, A. C., Ymer, S., & Stevenson, J. (1986). Identification and characterization of specific binding proteins for growth hormone in normal human sera. *J Clin Invest*, *77*, 1817–1823.
160. Herington, A. C., Ymer, S. I., & Stevenson, J. L. (1986). Affinity purification and structural characterization of a specific binding

protein for human growth hormone in human serum. *Biochem Biophys Res Commun*, *139*, 150–155.
161. Zhang, Y., Jiang, J., Black, R. A., et al. (2000). Tumor necrosis factor-alpha converting enzyme (TACE) is a growth hormone binding protein (GHBP) sheddase: the metalloprotease TACE/ADAM-17 is critical for (PMA-induced) GH receptor proteolysis and GHBP generation. *Endocrinology*, *141*, 4342–4348.
162. Carmignac, D. F., Gabrielsson, B. G., & Robinson, I. C. (1993). Growth hormone binding protein in the rat: effects of gonadal steroids. *Endocrinology*, *133*, 2445–2452.
163. Gevers, E. F., Wit, J. M., & Robinson, I. C. (1996). Growth, growth hormone (GH)-binding protein, and GH receptors are differentially regulated by peak and trough components of the GH secretory pattern in the rat. *Endocrinology*, *137*, 1013–1018.
164. Baumann, G. (2002). Growth hormone binding protein: the soluble growth hormone receptor. *Minerva Endocrinol*, *27*, 265–276.
165. Amit, T., Youdim, M. B., & Hochberg, Z. (2000). Clinical review 112: does serum growth hormone (GH) binding protein reflect human GH receptor function? *J Clin Endocrinol Metab*, *85*, 927–932.
166. Carlsson, L. M., Rowland, A. M., Clark, R. G., et al. (1991). Ligand-mediated immunofunctional assay for quantitation of growth hormone-binding protein in human blood. *J Clin Endocrinol Metab*, *73*, 1216–1223.
167. Daughaday, W. H., & Trivedi, B. (1987). Absence of serum growth hormone binding protein in patients with growth hormone receptor deficiency (Laron dwarfism). *Proc Natl Acad Sci USA*, *84*, 4636–4640.
168. Baumann, G., Shaw, M. A., & Winter, R. J. (1987). Absence of the plasma growth hormone-binding protein in Laron-type dwarfism. *J Clin Endocrinol Metab*, *65*, 814–816.
169. Buchanan, C. R., Maheshwari, H. G., Norman, M. R., et al. (1991). Laron-type dwarfism with apparently normal high affinity serum growth hormone-binding protein. *Clin Endocrinol* (Oxf), *35*, 179–185.
170. Hansen, J. A., Lindberg, K., Hilton, D. J., et al. (1999). Mechanism of inhibition of growth hormone receptor signaling by suppressor of cytokine signaling proteins. *Mol Endocrinol*, *13*, 1832–1843.
171. Metcalf, D., Greenhalgh, C. J., Viney, E., et al. (2000). Gigantism in mice lacking suppressor of cytokine signalling-2. *Nature*, *405*, 1069–1073.
172. Ram, P. A., & Waxman, D. J. (1999). SOCS/CIS protein inhibition of growth hormone-stimulated STAT5 signaling by multiple mechanisms. *J Biol Chem*, *274*, 35553–35561.
173. Salmon, W. D., Jr., & Daughaday, W. H. (1957). A hormonally controlled serum factor which stimulates sulfate incorporation by cartilage in vitro. *J Lab Clin Med*, *49*, 825–836.
174. Daughaday, W. H., & Rotwein, P. (1989). Insulin-like growth factors I and II. Peptide, messenger ribonucleic acid and gene structures, serum, and tissue concentrations. *Endocr Rev*, *10*, 68–91.
175. Sherwin, R. S., Schulman, G. A., Hendler, R., et al. (1983). Effect of growth hormone on oral glucose tolerance and circulating metabolic fuels in man. *Diabetologia*, *24*, 155–161.
176. Rosenfeld, R. G., Wilson, D. M., Dollar, L. A., et al. (1982). Both human pituitary growth hormone and recombinant DNA-derived human growth hormone cause insulin resistance at a postreceptor site. *J Clin Endocrinol Metab*, *54*, 1033–1038.
177. Green, H., Morikawa, M., & Nixon, T. (1985). A dual effector theory of growth-hormone action. *Differentiation*, *29*, 195–198.
178. Gerich, J. E., Lorenzi, M., Bier, D. M., et al. (1976). Effects of physiologic levels of glucagon and growth hormone on human carbohydrate and lipid metabolism: studies involving administration of exogenous hormone during suppression of endogenous hormone secretion with somatostatin. *J Clin Invest*, *57*, 875–884.
179. Kostyo, J. L., Hotchkiss, J., & Knobil, E. (1959). Stimulation of amino acid transport in isolated diaphragm by growth hormone added in vitro. *Science*, *130*, 1653–1654.
180. Hjalmarson A, Isaksson, O., & Ahren, K. (1969). Effects of growth hormone and insulin on amino acid transport in perfused rat heart. *Am J Physiol*, *217*, 1795–1802.
181. Carrel, A. L., & Allen, D. B. (2000). Effects of growth hormone on body composition and bone metabolism. *Endocrine*, *12*, 163–172.
182. Lupu, F., Terwilliger, J. D., Lee, K., et al. (2001). Roles of growth hormone and insulin-like growth factor 1 in mouse postnatal growth. *Dev Biol*, *229*, 141–162.
183. Kaplan, S. A., & Cohen, P. (2007). The somatomedin hypothesis 2007: 50 years later. *J Clin Endocrinol Metab*, *92*, 4529–4535.
184. Rudman, D., Feller, A. G., Nagraj, H. S., et al. (1990). Effects of human growth hormone in men over 60 years old. *N Engl J Med*, *323*, 1–6.
185. Horber, F. F., & Haymond, M. W. (1990). Human growth hormone prevents the protein catabolic side effects of prednisone in humans. *J Clin Invest*, *86*, 265–272.
186. Bürgi, H., Müller, W. A., Humbel, R. E., et al. (1966). Non-suppressible insulin-like activity of human serum. I. Physicochemical properties, extraction and partial purification. *Biochim Biophys Acta*, *121*, 349–359.
187. Froesch, E. R., Zapf, J., Meuli, C., et al. (1975). Biological properties of NSILA-S. *Adv Metab Disord*, *8*, 211–235.
188. Dulak, N. C., & Temin, H. M. (1973). A partially purified polypeptide fraction from rat liver cell conditioned medium with multiplication-stimulating activity for embryo fibroblasts. *J Cell Physiol*, *81*, 153–160.
189. Daughaday, W. H., Hall, K., Raben, M. S., et al. (1972). Somatomedin: proposed designation for sulphation factor. *Nature*, *235*, 107.
190. Hall, K., Takano, K., Fryklund, L., & Sievertsson, H. (1975). Somatomedins. *Adv Metab Disord*, *8*, 19–46.
191. Van Wyk, J. J., Underwood, L. E., Hintz, R. L., et al. (1974). The somatomedins: a family of insulinlike hormones under growth hormone control. *Recent Prog Horm Res*, *30*, 259–318.
192. Rinderknecht, E., & Humbel, R. E. (1978). The amino acid sequence of human insulin-like growth factor I and its structural homology with proinsulin. *J Biol Chem*, *253*, 2769–2776.
193. Rinderknecht, E., & Humbel, R. E. (1978). Primary structure of human insulin-like growth factor II. *FEBS Lett*, 89, 283–286.
194. Sussenbach, J. S. (1989). The gene structure of the insulin-like growth factor family. *Prog Growth Factor Res*, *1*, 33–48.
195. Lund, P. K., Moats-Staats, B. M., Hynes, M. A., et al. (1986). Somatomedin-C/insulin-like growth factor-I and insulin-like growth factor-II mRNAs in rat fetal and adult tissues. *J Biol Chem*, *261*, 14539–14544.
196. Brown, A. L., Graham, D. E., Nissley, S. P., et al. (1986). Developmental regulation of insulin-like growth factor II mRNA in different rat tissues. *J Biol Chem*, *261*, 13144–13150.
197. Brissenden, J. E., Ullrich, A., & Francke, U. (1984). Human chromosomal mapping of genes for insulin-like growth factors I and II and epidermal growth factor. *Nature*, *310*, 781–784.
198. Tricoli, J. V., Rall, L. B., Scott, J., et al. (1984). Localization of insulin-like growth factor genes to human chromosomes 11 and 12. *Nature*, *310*, 784–786.
199. Bell, G. I., Gerhard, D. S., Fong, N. M., et al. (1985). Isolation of the human insulin-like growth factor genes: insulin-like growth factor II and insulin genes are contiguous. *Proc Natl Acad Sci USA*, *82*, 6450–6454.
200. Hall, K. (1970). Quantitative determination of the sulphation factor activity in human serum. *Acta Endocrinol* (Copenh), *63*, 338–350.
201. Phillips, L. S., Herington, A. C., & Daughaday, W. H. (1974). Somatomedin stimulation of sulfate incorporation in porcine costal cartilage discs. *Endocrinology*, *94*, 856–863.
202. Garland, J. T., Lottes, M. E., Kozak, S., & Daughaday, W. H. (1972). Stimulation of DNA synthesis in isolated chondrocytes by sulfation factor. *Endocrinology*, *90*, 1086–1090.
203. Garland, J. T., & Buchanan, F. (1976). Stimulation of RNA and protein synthesis in isolated chondrocytes by human serum. *J Clin Endocrinol Metab*, *43*, 842–846.
204. Meuli, C. (1975). Effects of insulin and of NSILA-S on the perfused rat heart: glucose uptake, lactate production and efflux of 3-0-methyl glucose. *Eur J Clin Invest*, *5*, 93–99.
205. Hall, K., Takano, K., & Fryklund, L. (1974). Radioreceptor assay for somatomedin A. *J Clin Endocrinol Metab*, *39*, 973–976.
206. van Wyk, J. J., Underwood, L. E., Baseman, J. B., et al. (1975). Explorations of the insulinlike and growth-promoting properties of somatomedin by membrane receptor assays. *Adv Metab Disord*, *8*, 127–150.
207. Zapf, J., Kaufmann, U., Eigenmann, E. J., & Froesch, E. R. (1977). Determination of nonsuppressible insulin-like activity in

human serum by a sensitive protein-binding assay. *Clin Chem*, *23*, 677–682.
208. Schalch, D. S., Heinrich, U. E., Koch, J. G., et al. (1978). Non-suppressible insulin-like activity (NSILA). I. Development of a new sensitive competitive protein-binding assay for determination of serum levels. *J Clin Endocrinol Metab*, *46*, 664–671.
209. Furlanetto, R. W., Underwood, L. E., Van Wyk, J. J., & D'Ercole, A. J. (1977). Estimation of somatomedin-C levels in normals and patients with pituitary disease by radioimmunoassay. *J Clin Invest*, *60*, 648–657.
210. Zapf, J., Walter, H., & Froesch, E. R. (1981). Radioimmunological determination of insulinlike growth factors I and II in normal subjects and in patients with growth disorders and extrapancreatic tumor hypoglycemia. *J Clin Invest*, *68*, 1321–1330.
211. Bala, R. M., & Bhaumick, B. (1979). Radioimmunoassay of a basic somatomedin: comparison of various assay techniques and somatomedin levels in various sera. *J Clin Endocrinol Metab*, *49*, 770–777.
212. Rosenfeld, R. G., Wilson, D. M., Lee, P. D., & Hintz, R. L. (1986). Insulin-like growth factors I and II in evaluation of growth retardation. *J Pediatr*, *109*, 428–433.
213. Clemmons, D. R. (2007). Value of insulin-like growth factor system markers in the assessment of growth hormone status. *Endocrinol Metab Clin North Am*, *36*, 109–129.
214. Daughaday, W. H., Kapadia, M., & Mariz, I. (1987). Serum somatomedin binding proteins: physiologic significance and interference in radioligand assay. *J Lab Clin Med*, *109*, 355–363.
215. Powell, D. R., Rosenfeld, R. G., Baker, B. K., et al. (1986). Serum somatomedin levels in adults with chronic renal failure: the importance of measuring insulin-like growth factor I (IGF-I) and IGF-II in acid-chromatographed uremic serum. *J Clin Endocrinol Metab*, *63*, 1186–1192.
216. Horner, J. M., Liu, F., & Hintz, R. L. (1978). Comparison of [125I]somatomedin A and[125I]somatomedin C radioreceptor assays for somatomedin peptide content in whole and acid-chromatographed plasma. *J Clin Endocrinol Metab*, *47*, 1287–1295.
217. Daughaday, W. H., Mariz, I. K., & Blethen, S. L. (1980). Inhibition of access of bound somatomedin to membrane receptor and immunobinding sites: a comparison of radioreceptor and radioimmunoassay of somatomedin in native and acid-ethanol-extracted serum. *J Clin Endocrinol Metab*, *51*, 781–778.
218. Blum, W. F., Ranke, M. B., & Bierich, J. R. (1988). A specific radioimmunoassay for insulin-like growth factor II: the interference of IGF binding proteins can be blocked by excess IGF-I. *Acta Endocrinol* (Copenh), *118*, 374–380.
219. Bang, P., Eriksson, U., Sara, V., et al. (1991). Comparison of acid ethanol extraction and acid gel filtration prior to IGF-I and IGF-II radioimmunoassays: improvement of determinations in acid ethanol extracts by the use of truncated IGF-I as radioligand. *Acta Endocrinol* (Copenh), *124*, 620–629.
220. Bystrom, C., Sheng, S., Zhang, K., et al. (2012). Clinical utility of insulin-like growth factor 1 and 2; determination by high resolution mass spectrometry. *PLoS One*, 7, e43457.
221. Khosravi, M. J., Diamandi, A., Mistry, J., Lee, P. D., et al. (1996). Noncompetitive ELISA for human serum insulin-like growth factor-I. *Clin Chem*, *42*(8 Pt. 1), 1147–1154.
222. Cohen, P. (1998). Serum insulin-like growth factor-I levels and prostate cancer risk—interpreting the evidence. *J Natl Cancer Inst*, *90*, 876–879.
223. Bennett, A., Wilson, D. M., Liu, F., et al. (1983). Levels of insulin-like growth factors I and II in human cord blood. *J Clin Endocrinol Metab*, *57*, 609–612.
224. Gluckman, P. D., Johnson-Barrett, J. J., Butler, J. H., et al. (1983). Studies of insulin-like growth factor -I and -II by specific radioligand assays in umbilical cord blood. Clin Endocrinol (Oxf) 19, 405–413.
225. Lassarre, C., Hardouin, S., Daffos, F., et al. (1991). Serum insulin-like growth factors and their binding proteins in the human fetus: relationships with growth in normal subjects and in subjects with intrauterine growth retardation. *Pediatr Res*, *29*, 219–225.
226. Hall, K., Hansson, U., Lundin, G., et al. (1986). Serum levels of somatomedins and somatomedin-binding protein in pregnant women with type I or gestational diabetes and their infants. *J Clin Endocrinol Metab*, *63*, 1300–1306.
227. Luna, A. M., Wilson, D. M., Wibbelsman, C. J., et al. (1983). Somatomedins in adolescence: a cross-sectional study of the effect of puberty on plasma insulin-like growth factor I and II levels. *J Clin Endocrinol Metab*, *57*, 268–271.
228. Cara, J. F., Rosenfield, R. L., & Furlanetto, R. W. (1987). A longitudinal study of the relationship of plasma somatomedin-C concentration to the pubertal growth spurt. *Am J Dis Child*, *141*, 562–564.
229. Cuttler, L., Van Vliet, G., Conte, F. A., et al. (1985). Somatomedin-C levels in children and adolescents with gonadal dysgenesis: differences from age-matched normal females and effect of chronic estrogen replacement therapy. *J Clin Endocrinol Metab*, *60*, 1087–1092.
230. Rosenfeld, R. G., Hintz, R. L., Johanson, A. J., et al. (1986). Methionyl human growth hormone and oxandrolone in Turner syndrome: preliminary results of a prospective randomized trial. *J Pediatr*, *109*, 936–943.
231. Copeland, K. C. (1988). Effects of acute high dose and chronic low dose estrogen on plasma somatomedin-C and growth in patients with Turner's syndrome. *J Clin Endocrinol Meta*, *66*, 1278–1282.
232. Ranke, M. B., Schweizer, R., Elmlinger, M. W., et al. (2000). Significance of basal IGF-I, IGFBP-3 and IGFBP-2 measurements in the diagnostics of short stature in children. *Horm Res*, *54*, 60–68.
233. Johanson, A. J., & Blizzard, R. M. (1981). Low somatomedin-C levels in older men rise in response to growth hormone administration. *Johns Hopkins Med J*, *149*, 115–117.
234. Donovan, S. M., Oh, Y., Pham, H., & Rosenfeld, R. G. (1989). Ontogeny of serum insulin-like growth factor binding proteins in the rat. *Endocrinology*, *125*, 2621–2627.
235. Glasscock, G. F., Gelber, S. E., Lamson, G., et al. (1990). Pituitary control of growth in the neonatal rat: effects of neonatal hypophysectomy on somatic and organ growth, serum insulin-like growth factors (IGF)-I and -II levels, and expression of IGF binding proteins. *Endocrinology*, *127*, 1792–1803.
236. Nunez, S. B., Municchi, G., Barnes, K. M., & Rose, S. R. (1996). Insulin-like growth factor I (IGF-I) and IGF-binding protein-3 concentrations compared to stimulated and night growth hormone in the evaluation of short children—a clinical research center study. *J Clin Endocrinol Metab*, *81*, 1927–1932.
237. Hintz, R. L., Clemmons, D. R., Underwood, L. E., & Van Wyk, J. J. (1972). Competitive binding of somatomedin to the insulin receptors of adipocytes, chondrocytes, and liver membranes. *Proc Natl Acad Sci USA*, *69*, 2351–2353.
238. Megyesi, K., Kahn, C. R., Roth, J., et al. (1974). Insulin and non-suppressible insulin-like activity (NSILA-s): evidence for separate plasma membrane receptor sites. *Biochem Biophys Res Commun*, *57*, 307–315.
239. Massague, J., & Czech, M. P. (1982). The subunit structures of two distinct receptors for insulin-like growth factors I and II and their relationship to the insulin receptor. *J Biol Chem*, *257*, 5038–5045.
240. Kasuga, M., Van Obberghen, E., Nissley, S. P., & Rechler, M. M. (1981). Demonstration of two subtypes of insulin-like growth factor receptors by affinity cross-linking. *J Biol Chem*, *256*, 5305–5308.
241. Chernausek, S. D., Jacobs, S., & Van Wyk, J. J. (1981). Structural similarities between human receptors for somatomedin C and insulin: analysis by affinity labeling. *Biochemistry*, *20*, 7345–7350.
242. Oh, Y., Müller, H. L., Neely, E. K., et al. (1993). New concepts in insulin-like growth factor receptor physiology. *Growth Regul*, *3*, 113–123.
243. Werner, H., & Le Roith, D. (1997). The insulin-like growth factor-I receptor signaling pathways are important for tumorigenesis and inhibition of apoptosis. *Crit Rev Oncog*, *8*, 71–92.
244. LeRoith, D., & Yakar, S. (2007). Mechanisms of disease: metabolic effects of growth hormone and insulin-like growth factor 1. *Nat Clin Pract Endocrinol Metab*, *3*, 302–310.
245. Denley, A., Carroll, J. M., Brierley, G. V., et al. (2007). Differential activation of insulin receptor substrates 1 and 2 by insulin-like growth factor-activated insulin receptors. *Mol Cell Biol*, *27*, 3569–3577.
246. Kadowaki, T., Tamemoto, H., Tobe, K., et al. (1996). Insulin resistance and growth retardation in mice lacking insulin receptor

substrate-1 and identification of insulin receptor substrate-2. *Diabet Med*, *13*(9 Suppl. 6), S103–S108.
247. Tsuruzoe, K., Emkey, R., Kriauciunas, K. M., et al. (2001). Insulin receptor substrate 3 (IRS-3) and IRS-4 impair IRS-1- and IRS-2-mediated signaling. *Mol Cell Biol*, *21*, 26–38.
248. Wu, J. D., Haugk, K., Coleman, I., et al. (2006). Combined in vivo effect of A12, a type 1 insulin-like growth factor receptor antibody, and docetaxel against prostate cancer tumors. *Clin Cancer Res*, *12*(20 Pt. 1), 6153–6160.
249. Yang, J., Anzo, M., & Cohen, P. (2005). Control of aging and longevity by IGF-I signaling. Exp Gerontol 40, 867–872.
250. Suh, Y., Atzmon, G., Cho, M. O., et al. (2008). Functionally significant insulin-like growth factor I receptor mutations in centenarians. *Proc Natl Acad Sci USA*, *105*, 3438–3442.
251. Morgan, D. O., Edman, J. C., Standring, D. N., et al. (1987). Insulin-like growth factor II receptor as a multifunctional binding protein. *Nature*, *329*, 301–307.
252. MacDonald, R. G., Pfeffer, S. R., Coussens, L., et al. (1988). A single receptor binds both insulin-like growth factor II and mannose-6-phosphate. *Science*, *239*, 1134–1137.
253. Kornfeld, S. (1987). Trafficking of lysosomal enzymes. *FASEB J*, *1*, 462–468.
254. Rosenfeld, R. G., Conover, C. A., Hodges, D., et al. (1987). Heterogeneity of insulin-like growth factor-I affinity for the insulin-like growth factor-II receptor: comparison of natural, synthetic and recombinant DNA-derived insulin-like growth factor-I. *Biochem Biophys Res Commun*, *143*, 199–205.
255. Conover, C. A., Misra, P., Hintz, R. L., & Rosenfeld, R. G. (1986). Effect of an anti-insulin-like growth factor I receptor antibody on insulin-like growth factor II stimulation of DNA synthesis in human fibroblasts. *Biochem Biophys Res Commun*, *139*, 501–508.
256. Furlanetto, R. W., DiCarlo, J. N., & Wisehart, C. (1987). The type II insulin-like growth factor receptor does not mediate deoxyribonucleic acid synthesis in human fibroblasts. *J Clin Endocrinol Metab*, *64*, 1142–1149.
257. Mottola, C., Czech, M. P. (1984). The type II insulin-like growth factor receptor does not mediate increased DNA synthesis in H-35 hepatoma cells. *J Biol Chem*, *259*, 12705–12713.
258. Kiess, W., Haskell, J. F., Lee, L., et al. (1987). An antibody that blocks insulin-like growth factor (IGF) binding to the type II IGF receptor is neither an agonist nor an inhibitor of IGF-stimulated biologic responses in L6 myoblasts. *J Biol Chem*, *262*, 12745–12751.
259. Adashi, E. Y., Resnick, C. E., & Rosenfeld, R. G. (1990). Insulin-like growth factor-I (IGF-I) hormonal action in cultured rat granulosa cells: mediation via type I but not type II IGF receptors. *Endocrinology*, *126*, 216–222.
260. Beukers, M. W., Oh, Y., Zhang, H., et al. (1991). [Leu27] insulin-like growth factor II is highly selective for the type-II IGF receptor in binding, cross-linking and thymidine incorporation experiments. *Endocrinology*, *128*, 1201–1203.
261. Canfield, W. M., & Kornfeld, S. (1989). The chicken liver cation-independent mannose 6-phosphate receptor lacks the high affinity binding site for insulin-like growth factor II. *J Biol Chem*, *264*, 7100–7103.
262. Clairmont, K. B., & Czech, M. P. (1989). Chicken and Xenopus mannose 6-phosphate receptors fail to bind insulin-like growth factor II. J Biol Chem 264, 16390–16392.
263. Rogers, S. A., & Hammerman, M. R. (1988). Insulin-like growth factor II stimulates production of inositol triphosphate in proximal tubular basolateral membranes from canine kidney. *Proc Natl Acad Sci USA*, *85*, 4037–4041.
264. Tally, M., Li, C. H., & Hall, K. (1987). IGF-2 stimulated growth mediated by the somatomedin type 2 receptor. *Biochem Biophys Res Commun*, *148*, 811–816.
265. Minniti, C. P., Kohn, E. C., Grubb, J. H., et al. (1992). The insulin-like growth factor II (IGF-II)/mannose 6-phosphate receptor mediates IGF-II-induced motility in human rhabdomyosarcoma cells. *J Biol Chem*, *267*, 9000–9004.
266. Nishimoto, I., Murayama, Y., Katada, T., et al. (1989). Possible direct linkage of insulin-like growth factor-II receptor with guanine nucleotide-binding proteins. *J Biol Chem*, *264*, 14029–14038.
267. Braulke, T. (1999). Type-2 IGF receptor: a multi-ligand binding protein. *Horm Metab Res*, *31*, 242–246.
268. Kang, J. X., Bell, J., Beard, R. L., & Chandraratna, R. A. (1999). Mannose 6-phosphate/insulin-like growth factor II receptor mediates the growth-inhibitory effects of retinoids. *Cell Growth Differ*, *10*, 591–600.
269. Melnick, M., Chen, H., Buckley, S., et al. (1998). Insulin-like growth factor II receptor, transforming growth factor-beta, and Cdk4 expression and the developmental epigenetics of mouse palate morphogenesis and dysmorphogenesis. *Dev Dyn*, *211*, 11–25.
270. Misra, P., Hintz, T. L., & Rosenfeld, R. G. (1986). Structural and immunological characterization of insulin-like growth factor II binding to IM-9 cells. *J Clin Endocrinol Metab*, *63*, 1400–1405.
271. Jonas, H. A., & Cox, A. J. (1990). Insulin-like growth factor binding to the atypical insulin receptors of a human lymphoid-derived cell line (IM-9). *Biochem J*, *266*, 737–742.
272. Feltz, S. M., Swanson, M. L., Wemmie, J. A., & Pessin, J. E. (1988). Functional properties of an isolated alpha beta heterodimeric human placenta insulin-like growth factor 1 receptor complex. *Biochemistry*, *27*, 3234–3242.
273. Treadway, J. L., Morrison, B. D., Goldfine, I. D., & Pessin, J. E. (1989). Assembly of insulin/insulin-like growth factor-1 hybrid receptors in vitro. *J Biol Chem*, *264*, 21450–21453.
274. Soos, M. A., & Siddle, K. (1989). Immunological relationships between receptors for insulin and insulin-like growth factor I. Evidence for structural heterogeneity of insulin-like growth factor I receptors involving hybrids with insulin receptors. *Biochem J*, *263*, 553–563.
275. Rosenfeld, R. G., Lamson, G., Pham, H., et al. (1990). Insulin-like growth factor binding proteins. *Recent Prog Horm Res*, *46*, 99–159.
276. Lamson, G., Giudice, L. C., & Rosenfeld, R. G. (1991). Insulin-like growth factor binding proteins: structural and molecular relationships. *Growth Factors*, *5*, 19–28.
277. Rechler, M. M. (1993). Insulin-like growth factor binding proteins. *Vitam Horm*, *47*, 1–114.
278. Hintz, R. L., & Liu, F. (1977). Demonstration of specific plasma protein binding sites for somatomedin. *J Clin Endocrinol Metab*, *45*, 988–995.
279. Hossenlopp, P., Seurin, D., Segovia-Quinson, B., et al. (1986). Analysis of serum insulin-like growth factor binding proteins using western blotting: use of the method for titration of the binding proteins and competitive binding studies. *Anal Biochem*, *154*, 138–143.
280. Shimasaki, S., Shimonaka, M., Zhang, H. P., & Ling, N. (1991). Identification of five different insulin-like growth factor binding proteins (IGFBPs) from adult rat serum and molecular cloning of a novel IGFBP-5 in rat and human. *J Biol Chem*, *266*, 10646–10653.
281. Brewer, M. T., Stetler, G. L., Squires, C. H., et al. (1988). Cloning, characterization, and expression of a human insulin-like growth factor binding protein. *Biochem Biophys Res Commun*, *152*, 1289–1297.
282. Oh, Y., Müller, H. L., Pham, H., et al. (1992). Non-receptor mediated, post-transcriptional regulation of insulin-like growth factor binding protein (IGFBP)-3 in Hs578T human breast cancer cells. *Endocrinology*, *131*, 3123–3125.
283. Oh, Y., Müller, H. L., Lamson, G., & Rosenfeld, R. G. (1993). Insulin-like growth factor (IGF)-independent action of IGF-binding protein-3 in Hs578T human breast cancer cells. Cell surface binding and growth inhibition. *J Biol Chem*, *268*, 14964–14971.
284. Oh, Y., Müller, H. L., Pham, H., & Rosenfeld, R. G. (1993). Demonstration of receptors for insulin-like growth factor binding protein-3 on Hs578T human breast cancer cells. *J Biol Chem*, *268*, 26045–26048.
285. Ritvos, O., Ranta, T., Jalkanen, J., et al. (1988). Insulin-like growth factor (IGF) binding protein from human decidua inhibits the binding and biological action of IGF-I in cultured choriocarcinoma cells. *Endocrinology*, *122*, 2150–2157.
286. Ross, M., Francis, G. L., Szabo, L., et al. (1989). Insulin-like growth factor (IGF)-binding proteins inhibit the biological activities of IGF-1 and IGF-2 but not des-(1-3)-IGF-1. *Biochem J*, *258*, 267–272.
287. Clemmons, D. R., Cascieri, M. A., Camacho-Hubner, C., et al. (1990). Discrete alterations of the insulin-like growth factor I molecule which alter its affinity for insulin-like growth factor-binding proteins result in changes in bioactivity. *J Biol Chem*, *265*, 12210–12216.

288. Okajima, T., Nakamura, K., Zhang, H., et al. (1992). Sensitive colorimetric bioassays for insulin-like growth factor (IGF) stimulation of cell proliferation and glucose consumption: use in studies of IGF analogs. *Endocrinology*, *130*, 2201–2212.
289. Cohen, P., Lamson, G., Okajima, T., & Rosenfeld, R. G. (1993). Transfection of the human insulin-like growth factor binding protein-3 gene into Balb/c fibroblasts inhibits cellular growth. *Mol Endocrinol*, *7*, 380–386.
290. Elgin, R. G., Busby, W. H., Jr., & Clemmons, D. R. (1987). An insulin-like growth factor (IGF) binding protein enhances the biologic response to IGF-I. *Proc Natl Acad Sci USA*, *84*, 3254–3258.
291. Hwa, V., Oh, Y., Rosenfeld, R. G. (1999). The insulin-like growth factor-binding protein (IGFBP) superfamily. *Endocr Rev*, *20*, 761–787.
292. Oh, Y., Müller, H. L., Ng, L., & Rosenfeld, R. G. (1995). Transforming growth factor-beta-induced cell growth inhibition in human breast cancer cells is mediated through insulin-like growth factor-binding protein-3 action. *J Biol Chem*, *270*, 13589–13592.
293. Conover, C. A., Bale, L. K., Durham, S. K., & Powell, D. R. (2000). Insulin-like growth factor (IGF) binding protein-3 potentiation of IGF action is mediated through the phosphatidylinositol-3-kinase pathway and is associated with alteration in protein kinase B/AKT sensitivity. *Endocrinology*, *141*, 3098–3103.
294. Rajah, R., Valentinis, B., & Cohen, P. (1997). Insulin-like growth factor (IGF)-binding protein-3 induces apoptosis and mediates the effects of transforming growth factor-beta1 on programmed cell death through a p53- and IGF-independent mechanism. *J Biol Chem*, *272*, 12181–12188.
295. Liu, B., Lee, H. Y., Weinzimer, S. A., et al. (2000). Direct functional interactions between insulin-like growth factor-binding protein-3 and retinoid X receptor-alpha regulate transcriptional signaling and apoptosis. *J Biol Chem*, *275*, 33607–613.
296. Clark, R. G., Mortensen, D., Reifsynder, D., et al. (1993). Recombinant human insulin-like growth factor binding protein-3 (rhIGFBP-3): effects on the glycemic and growth promoting activities of rhIGF-1 in the rat. *Growth Regul*, *3*, 50–52.
297. Giudice, L. C., Farrell, E. M., Pham, H., et al. (1990). Insulin-like growth factor binding proteins in maternal serum throughout gestation and in the puerperium: effects of a pregnancy-associated serum protease activity. *J Clin Endocrinol Metab*, *71*, 806–816.
298. Hossenlopp, P., Segovia, B., Lassarre, C., et al. (1990). Evidence of enzymatic degradation of insulin-like growth factor-binding proteins in the 150K complex during pregnancy. *J Clin Endocrinol Metab*, *71*, 797–805.
299. Müller, H. L., Oh, Y., Gargosky, S. E., et al. (1993). Concentrations of insulin-like growth factor (IGF)-binding protein-3 (IGFBP-3), IGF, and IGFBP-3 protease activity in cerebrospinal fluid of children with leukemia, central nervous system tumor, or meningitis. *J Clin Endocrinol Metab*, *77*, 1113–1119.
300. Lee, D. Y., Park, S. K., Yorgin, P. D., et al. (1994). Alteration in insulin-like growth factor-binding proteins (IGFBPs) and IGFBP-3 protease activity in serum and urine from acute and chronic renal failure. *J Clin Endocrinol Metab*, *79*, 1376–1382.
301. Gargosky, S. E., Pham, H. M., Wilson, K. F., et al., (1992). Measurement and characterization of insulin-like growth factor binding protein-3 in human biological fluids: discrepancies between radioimmunoassay and ligand blotting. *Endocrinology*, *131*, 3051–3060.
302. Drop, S. L., Valiquette, G., Guyda, H. J., et al. (1979). Partial purification and characterization of a binding protein for insulin-like activity (ILAs) in human amniotic fluid: a possible inhibitor of insulin-like activity. *Acta Endocrinol* (Copenh), *90*, 505–518.
303. Rosenfeld, R. G., Pham, H., Conover, C. A., et al. (1989). Structural and immunological comparison of insulin-like growth factor binding proteins of cerebrospinal and amniotic fluids. *J Clin Endocrinol Metab*, *68*, 638–646.
304. Rosenfeld, R. G., Pham, H., Oh, Y., et al. (1990). Identification of insulin-like growth factor binding protein-2 (IGF-BP-2) and a low molecular weight IGF-BP in human seminal plasma. *J Clin Endocrinol Metab*, *70*, 551–553.
305. Martin, J. L., & Baxter, R. C. (1986). Insulin-like growth factor-binding protein from human plasma. Purification and characterization. *J Biol Chem*, *261*, 8754–8760.
306. Baxter, R. C. (1988). Characterization of the acid-labile subunit of the growth hormone-dependent insulin-like growth factor binding protein complex. *J Clin Endocrinol Metab*, *67*, 265–272.
307. Povoa, G., Roovete, A., & Hall, K. (1984). Cross-reaction of serum somatomedin-binding protein in a radioimmunoassay developed for somatomedin-binding protein isolated from human amniotic fluid. *Acta Endocrinol* (Copenh), *107*, 563–570.
308. Baxter, R., & Cowell, C. T. (1987). Diurnal variation of growth hormone independent binding protein for insulin-like growth factors in humans. *Clin Endocrinol Metab*, *65*, 432.
309. Cohen, P., Peehl, D. M., Stamey, T. A., et al. (1993). Elevated levels of insulin-like growth factor-binding protein-2 in the serum of prostate cancer patients. *J Clin Endocrinol Metab*, *76*, 1031–1035.
310. Baxter, R. C., & Martin, J. L. (1986). Radioimmunoassay of growth hormone-dependent insulinlike growth factor binding protein in human plasma. *J Clin Invest*, *78*, 1504–1512.
311. Blum, W. F., Ranke, M. B., Kietzmann, K., et al. (1990). A specific radioimmunoassay for the growth hormone (GH)-dependent somatomedin-binding protein: its use for diagnosis of GH deficiency. *J Clin Endocrinol Metab*, *70*, 1292–1298.
312. Rosenfeld, R. G., Aggarwal, B. B., Hintz, R. L., & Dollar, L. A. (1982). Recombinant DNA-derived methionyl human growth hormone is similar in membrane binding properties to human pituitary growth hormone. *Biochem Biophys Res Commun*, *106*, 202–209.
313. Rosenfeld, R. G. (1995). Broadening the growth hormone insensitivity syndrome. *N Engl J Med*, *333*, 1145–1146.
314. Rosenfeld, R. G., Albertsson-Wikland, K., Cassorla, F., et al. (1995). Diagnostic controversy: the diagnosis of childhood growth hormone deficiency revisited. *J Clin Endocrinol Metab*, *80*, 1532–1540.
315. Rosenfeld, R. G., & Gargosky, S. E. (1996). Assays for insulin-like growth factors and their binding proteins: practicalities and pitfalls. *J Pediatr*, *128*(5 Pt. 2), S52–S57.
316. Vu, T. H., Jirtle, R. L., & Hoffman, A. R. (2006). Cross-species clues of an epigenetic imprinting regulatory code for the IGF2R gene. *Cytogenet Genome Res*, *113*, 202–208.
317. Finklestein, S. P., (2001). Growth factors. In L. P. Miller, R. L. Hayes, & J. K. Newcomb (Eds.), *Head trauma: basical, preclinical and clinical directions* (pp. 165–187). New York: Wiley.
318. Itoh, N., & Ornitz, D. M. (2011). Fibroblast growth factors: from molecular evolution to roles in development, metabolism and disease. *J Biochem*, *149*, 121–130.
319. Inagaki, T., Choi, M., Moschetta, A., & Mohammadi, M. (2005). Fibroblast growth factor 15 functions as an enterohepatic signal to regulate bile acid homeostasis. *Cell Metab*, *2*, 217–225.
320. Plotnikov, A. N., Schlessinger, J., Hubbard, S. R., et al. (1999). Structural basis for FGF receptor dimerization and activation. *Cell*, *98*, 641–650.
321. Ladher, R. K., Anakwe, K. U., Gurney, A. L., et al. (2000). Identification of synergistic signals initiating inner ear development. *Science*, *290*, 1965–1967.
322. Endo, I., Fukumoto, S., Ozono, K., et al. (2008). Clinical usefulness of measurement of fibroblast growth factor 23 (FGF23) in hypophosphatemic patients: proposal of diagnostic criteria using FGF23 measurement. *Bone*, *42*, 1235–1239.
323. Rousseau, F., Bonaventure, J., Legeai-Mallet, L., et al. (1996). Mutations of the fibroblast growth factor receptor-3 gene in achondroplasia. *Horm Res*, *45*, 108–110.
324. Winterpacht, A., Hilbert, K., Stelzer, C., et al. (2000). A novel mutation in FGFR-3 disrupts a putative N-glycosylation site and results in hypochondroplasia. P *hysiol Genomics*, *2*, 9–12.
325. Cohen, M. M., Jr. (1997).Transforming growth factor beta s and fibroblast growth factors and their receptors: role in sutural biology and craniosynostosis. *J Bone Miner Res*, *12*, 322–331.
326. Merrill, A. E., Sarukhanov, A., Krejci, P., et al. (2012). Bent bone dysplasia-FGFR2 type, a distinct skeletal disorder, has deficient canonical FGF signaling. *Am J Hum Genet*, *90*, 550–557.
327. Casci, T., & Freeman, M. (1999). Control of EGF receptor signalling: lessons from fruitflies. *Cancer Metastasis Rev*, *18*, 181–201.
328. Herbst, R. S. (2004). Review of epidermal growth factor receptor biology. *Int J Radiat Oncol Biol Phys*, *59*(2 Suppl.), 21–26.

329. Sansone P, & Bromberg, J. (2012). Targeting the interleukin-6/Jak/stat pathway in human malignancies. *J Clin Oncol*, *30*, 1005–10014.
330. George, P. M., Badiger, R., Alazawi, W., et al. (2012). Pharmacology and therapeutic potential of interferons. *Pharmacol Ther*, *135*, 44–53.
331. Sherr, C. J. (2000). The Pezcoller lecture: cancer cell cycles revisited. *Cancer Res*, *60*, 3689–3695.
332. Jones, M. E., Boon, W. C., McInnes, K., et al. (2007). Recognizing rare disorders: aromatase deficiency. *Nat Clin Pract Endocrinol Metab*, *3*, 414–421.
333. Demura, M., Martin, R. M., Shozu, M., et al. (2007). Regional rearrangements in chromosome 15q21 cause formation of cryptic promoters for the CYP19 (aromatase) gene. *Hum Mol Genet*, *16*, 2529–2541.
334. Horton, W. A., Hall, J. G., & Hecht, J. T. (2007). Achondroplasia. *Lancet*, *370*, 162–172.
335. Spranger, J. (1992). International classification of osteochondrodysplasias. The International Working Group on Constitutional Diseases of Bone. *Eur J Pediatr*, *151*, 407–415.
336. Shiang, R., Thompson, L. M., Zhu, Y. Z., et al. (1994). Mutations in the transmembrane domain of FGFR3 cause the most common genetic form of dwarfism, achondroplasia. *Cell*, *78*, 335–342.
337. Alatzoglou, K. S., Hindmarsh, P. C., Brain, C., et al. (2009). Acanthosis nigricans and insulin sensitivity in patients with achondroplasia and hypochondroplasia due to FGFR3 mutations. *J Clin Endocrinol Metab*, *94*, 3959–3963.
338. Cronk, C., Crocker, A. C., Pueschel, S. M., et al. (1988). Growth charts for children with Down syndrome: 1 month to 18 years of age. *Pediatrics*, *81*, 102–110.
339. Rosenfeld, R. G., & Grumbach, M. M. (1990). *Turner syndrome*. New York: Marcel Dekker.
340. Ranke, M. B., & Rosenfeld, R. G., (1991). *Turner syndrome: growth promoting therapies*. Amsterdam: Excerpta Medica.
341. Hibi, I., & Takano, K., (1993). *Basic and clinical approach to Turner syndrome*. Amsterdam: Excerpta Medica.
342. Ranke, M. B., Pflüger, H., Rosendahl, W., et al. (1983). Turner syndrome: spontaneous growth in 150 cases and review of the literature. *Eur J Pediatr*, *141*, 81–88.
343. Davenport, M. L., Punyasavatsut, N., Gunther, D., et al. (1999). Turner syndrome: a pattern of early growth failure. *Acta Paediatr Suppl*, *88*, 118–121.
344. Davenport, M. L., Punyasavatsut, N., Gunther, D., et al. (2000). Longitudinal analysis of growth over the first 3 years of life in Turner's syndrome. *J Pediatr*, *137*, 460–464.
345. Blaschke, R. J., & Rappold, G. A. (2000). SHOX: growth, Leri-Weill and Turner syndromes. *Trends Endocrinol Metab*, *11*, 227–230.
346. Ross, J. L., Long, L. M., Loriaux, D. L., & Cutler, G. B., Jr. (1985). Growth hormone secretory dynamics in Turner syndrome. *J Pediatr*, *106*, 202–206.
347. Rosenfeld, R. G., Hintz, R. L., Johanson, A. J., et al. (1988). Three-year results of a randomized prospective trial of methionyl human growth hormone and oxandrolone in Turner syndrome. *J Pediatr*, *113*, 393–400.
348. Quigley, C. A. (2007). Growth hormone treatment of non-growth hormone-deficient growth disorders. *Endocrinol Metab Clin North Am*, *36*, 131–186.
349. Sas, T. C., de Muinck Keizer-Schrama, S. M., Stijnen, T., et al. (1999). Normalization of height in girls with Turner syndrome after long-term growth hormone treatment: results of a randomized dose-response trial. *J Clin Endocrinol Metab*, *84*, 4607–4612.
350. Hale, D. E., Cody, J. D., Baillargeon, J., et al. (2000). The spectrum of growth abnormalities in children with 18q deletions. *J Clin Endocrinol Metab*, *85*, 4450–4454.
351. Saenger, P., Czernichow, P., Hughes, I., & Reiter, E. O. (2007). Small for gestational age: short stature and beyond. *Endocr Rev*, *28*, 219–251.
352. Chernausek, S. D. (2006). Mendelian genetic causes of the short child born small for gestational age. *J Endocrinol Invest*, *29*(1 Suppl.), 16–20.
353. Gluckman, P. D., & Harding, J. (1992). The regulation of fetal growth. In J. Argente, & M. Hernandez (Ed.), *Human growth: basic and clinical aspects*, New York: Elsevier, 253–276.
354. Cooke, P. S., Nicoll, C. S. (1983). Hormonal control of fetal growth. Physiologist 26, 317–323.
355. Kim, J. D., Näntö-Salonen, K., Szczepankiewicz, J. R., et al. (1993). Evidence \for pituitary regulation of somatic growth, insulin-like growth factors-I and -II, and their binding proteins in the fetal rat. *Pediatr Res*, *33*, 144–151.
356. Liu, J. P., Baker, J., Perkins, A. S., et al. (1993). Mice carrying null mutations of the genes encoding insulin-like growth factor I (Igf-1) and type 1 IGF receptor (Igf1r). *Cell*, *75*, 59–72.
357. Woods, K. A., Camacho-Hübner, C., Savage, M. O., & Clark, A. J. (1996). Intrauterine growth retardation and postnatal growth failure associated with deletion of the insulin-like growth factor I gene. *N Engl J Med*, *335*, 1363–1367.
358. Rosenfeld, R., Thorsson, A. V., & Hintz, R. L. (1979). Increased somatomedin receptor sites in newborn circulating mononuclear cells. *J Clin Endocrinol Metab*, *48*, 456–461.
359. D'Ercole, A. J. (1987). Somatomedins/insulin-like growth factors and fetal growth. *J Dev Physiol*, *9*, 481–495.
360. Han, V. K., D'Ercole, A. J., & Lund, P. K. (1987). Cellular localization of somatomedin (insulin-like growth factor) messenger RNA in the human fetus. *Science*, *236*, 193–197.
361. Giudice, L. C., de Zegher, F., Gargosky, S. E., et al. (1995). Insulin-like growth factors and their binding proteins in the term and preterm human fetus and neonate with normal and extremes of intrauterine growth. *J Clin Endocrinol Metab*, *80*, 1548–1555.
362. Sas, T., de Waal, W., Mulder, P., et al. (1999). Growth hormone treatment in children with short stature born small for gestational age: 5-year results of a randomized, double-blind, dose-response trial. *J Clin Endocrinol Metab*, *84*, 3064–3070.
363. Simmons, R. A. (2007). Developmental origins of diabetes: the role of epigenetic mechanisms. *Curr Opin Endocrinol Diabetes Obes*, *14*, 13–16.
364. Barker, D. J. P. (1995). *Fetal origins of coronary heart disease*. London: BMJ Publishing Group.
365. Russell, A. (1954). A syndrome of intra-uterine dwarfism recognizable at birth with cranio-facial dysostosis, disproportionately short arms, and other anomalies (5 examples). *Proc R Soc Med*, *47*, 1040–1044.
366. Gicquel, C., Rossignol, S., Cabrol, S., et al. (2005). Epimutation of the telomeric imprinting center region on chromosome 11p15 in Silver-Russell syndrome. *Nat Genet*, *37*, 1003–1007.
367. Netchine, I., Rossignol, S., Dufourg, M. N., et al. (2007). 11p15 imprinting center region 1 loss of methylation is a common and specific cause of typical Russell-Silver syndrome: clinical scoring system and epigenetic-phenotypic correlations. *J Clin Endocrinol Metab*, *92*, 3148–3154.
368. Bruce, S., Hannula-Jouppi, K., Peltonen, J., et al. (2009). Clinically distinct epigenetic subgroups in Silver-Russell syndrome: the degree of H19 hypomethylation associates with phenotype severity and genital and skeletal anomalies. *J Clin Endocrinol Metab*, *94*, 579–587.
369. Binder, G., Seidel, A. K., Martin, D. D., et al. (2008). The endocrine phenotype in Silver-Russell syndrome is defined by the underlying epigenetic alteration. *J Clin Endocrinol Metab*, *93*, 1402–1407.
370. Arboleda, V. A., Lee, H., Parnaik, R., et al. (2012). Mutations in the PCNA-binding domain of CDKN1C cause IMAGe syndrome. *Nat Genet*, *44*, 788–792.
371. Mann, T. P., & Russell, A. (1959). Study of a microcephalic midget of extreme type. *Proc R Soc Med*, *52*, 1024–1027.
372. Harper, R. G., Orti, E., & Baker, R. K. (1967). Bird-beaded dwarfs (Seckel's syndrome): a familial pattern of developmental, dental, skeletal, genital, and central nervous system anomalies. *J Pediatr*, *70*, 799–804.
373. Allanson, J. E. (2007). Noonan syndrome. *Am J Med Genet C Semin Med Genet*, *145C*, 274–279.
374. Rosenbloom, A. L., & DeBusk, F. L. (1971). Progeria of Hutchinson-Gilford: a caricature of aging. *Am Heart J*, *82*, 287–289.
375. Macdonald, W. B., Fitch, K. D., & Lewis, I. C. (1960). Cockayne's syndrome: an heredo-familial disorder of growth and development. *Pediatrics*, *25*, 997–1007.
376. Bray, G. A., Dahms, W. T., Swerdloff, R. S., et al. (1983). The Prader-Willi syndrome: a study of 40 patients and a review of the literature. *Medicine* (Baltimore), *62*, 59–80.

377. Lee, P. D. K., Wilson, D. M., Hintz, R. L., & Rosenfeld, R. G. (1987). Growth hormone treatment of short stature in Prader-Willi syndrome. *J Pediatr Endocrinol*, *2*, 31.
378. Angulo, M., Castro-Magana, M., & Uly, J. (1991). Pituitary evaluation and growth hormone treatment in Prader-Willi syndrome. *J Pediatr Endocrinol*, *3*, 167.
379. de Lind van Wijngaarden, R. F., Otten, B. J., Festen, D. A., et al. (2008). High prevalence of central adrenal insufficiency in patients with Prader-Willi syndrome. *J Clin Endocrinol Metab*, *93*, 1649–1654.
380. Edwards, L. E., Alton, I. R., Barrada, M. I., & Hakanson, E. Y. (1979). Pregnancy in the underweight woman: course, outcome, and growth patterns of the infant. *Am J Obstet Gynecol*, *135*, 297–302.
381. Ouellette, E. M., Rosett, H. L., Rosman, N. P., & Weiner, L. (1977). Adverse effects on offspring of maternal alcohol abuse during pregnancy. *N Engl J Med*, *297*, 528–530.
382. Jones, K. L., Smith, D. W., Streissguth, A. P., & Myrianthopoulos, N. C. (1974). Outcome in offspring of chronic alcoholic women. *Lancet*, *1*, 1076–1978.
383. Abel, E. L. (1982). Consumption of alcohol during pregnancy: a review of effects on growth and development of offspring. *Hum Biol*, *54*, 421–453.
384. Chasnoff, I. J., Griffith, D. R., MacGregor, S., et al. (1989). Temporal patterns of cocaine use in pregnancy. Perinatal outcome. *JAMA*, *261*, 1741–1744.
385. Zuckerman, B., Frank, D. A., Hingson, R., et al. (1989). Effects of maternal marijuana and cocaine use on fetal growth. *N Engl J Med*, *320*, 762–768.
386. Abel, E. L. (1980). Smoking during pregnancy: a review of effects on growth and development of offspring. *Hum Biol*, *52*, 593–625.
387. Pringle, P. J., Geary, M. P., Rodeck, C. H., et al. (2005). The influence of cigarette smoking on antenatal growth, birth size, and the insulin-like growth factor axis. *J Clin Endocrinol Metab*, *90*, 2556–2562.
388. Chaussain, J. L., Colle, M., & Ducret, J. P. (1994). Adult height in children with prepubertal short stature secondary to intrauterine growth retardation. *Acta Paediatr* (Suppl.), *399*, 72–73.
389. Barker, D. J., Gluckman, P. D., Godfrey, K. M., et al. (1993). Fetal nutrition and cardiovascular disease in adult life. *Lancet*, *341*, 938–941.
390. Tanner, J. M., Goldstein, H., & Whitehouse, R. H. (1970). Standards for children's height at ages 2-9 years allowing for heights of parents. *Arch Dis Child*, *45*, 755–762.
391. Graham, G. G., Adrianzen, B., Rabold, J., & Mellits, E. D. (1982). Later growth of malnourished infants and children. Comparison with 'healthy' siblings and parents. *Am J Dis Child*, *136*, 348–352.
392. Pimstone, B., Barbezat, G., Hansen, J. D., & Murray, P. (1967). Growth hormone and protein-calorie malnutrition. Impaired suppression during induced hyperglycaemia. *Lancet*, *2*, 1333–1334.
393. Beas, F., Contreras, I., Maccioni, A., & Arenas S. (1971). Growth hormone in infant malnutrition: the arginine test in marasmus and kwashiorkor. *Br J Nutr*, *26*, 169–175.
394. Grant, D. B., Hambley, J., Becker, D., Pimstone, B. L. (1973). Reduced sulphation factor in undernourished children. *Arch Dis Child*, *48*, 596–600.
395. Soliman, A. T., Hassan, A. E., Aref, M. K., et al. (1986). Serum insulin-like growth factors I and II concentrations and growth hormone and insulin responses to arginine infusion in children with protein-energy malnutrition before and after nutritional rehabilitation. *Pediatr Res*, *20*, 1122–1130.
396. Donovan, S. M., Atilano, L. C., Hintz, R. L., et al. (1991). Differential regulation of the insulin-like growth factors (IGF-I and -II) and IGF binding proteins during malnutrition in the neonatal rat. *Endocrinology*, *129*, 149–157.
397. Pugliese, M. T., Lifshitz, F., Grad G, et al. (1983). Fear of obesity: a cause of short stature and delayed puberty. *N Engl J Med*, *309*, 513–518.
398. Preece, M. A., Law, C. M., & Davies, P. S. (1986). The growth of children with chronic paediatric disease. *Clin Endocrinol Metab*, *15*, 453–477.
399. Groll, A., Candy, D. C., Preece, M. A., et al. (1980). Short stature as the primary manifestation of coeliac disease. *Lancet*, *2*, 1097–1099.
400. Mock, D. M. (1986). Growth retardation in chronic inflammatory bowel disease. *Gastroenterology*, *91*, 1019–1021.
401. Rosenthal, S. R., Snyder, J. D., Hendricks, K. M., & Walker, W. A. (1983). Growth failure and inflammatory bowel disease: approach to treatment of a complicated adolescent problem. *Pediatrics*, *72*, 481–490.
402. Lecornu, M., David, L., & Francois, R. (1978). Low serum somatomedin activity in celiac disease: a misleading aspect in growth failure from asymptomatic celiac disease. *Helv Paediatr Acta*, *33*, 509–516.
403. Greco, L., Troncone, R., De Vizia, B., et al. (1987). Discriminant analysis for the diagnosis of childhood celiac disease. *J Pediatr Gastroenterol Nutr*, *6*, 538–542.
404. Sblattero, D., Berti, I., Trevisiol, C., et al. (2000). Human recombinant tissue transglutaminase ELISA: an innovative diagnostic assay for celiac disease. *Am J Gastroenterol*, *95*, 1253–1257.
405. Booth, I. W., & Harries, J. T. (1984). Inflammatory bowel disease in childhood. *Gut*, *25*, 188–202.
406. Bayer, L. M., & Robinson, S. J. (1969). Growth history of children with congenital heart defects: size according to sex, age decade, surgical status, and diagnostic category. *Am J Dis Child*, *117*, 564–572.
407. Feldt, R. H., Strickler, G. B., & Weidman, W. H. (1969). Growth of children with congenital heart disease. *Am J Dis Child*, *117*, 573–579.
408. Holliday, M. A., Chantler, C., & Potter, D. E. (1978). Symposium on metabolism and growth in children with kidney disease: introduction. *Kidney Int*, *14*, 299–300.
409. Rizzoni, G., Broyer, M., Guest, G., et al. (1986). Growth retardation in children with chronic renal disease: scope of the problem. *Am J Kidney Dis*, *7*, 256–261.
410. Hokken-Koelega, A. C., van Zaal, M. A., van Bergen, W., et al. (1994). Final height and its predictive factors after renal transplantation in childhood. *Pediatr Res*, *36*, 323–328.
411. Platt, O. S., Rosenstock, W., & Espeland, M. A. (1984). Influence of sickle hemoglobinopathies on growth and development. *N Engl J Med*, *311*, 7–12.
412. Stevens, M. C., Maude, G. H., Cupidore, L., et al. (1986). Prepubertal growth and skeletal maturation in children with sickle cell disease. *Pediatrics*, *78*, 124–132.
413. Phebus, C. K., Gloninger, M. F., & Maciak, B. J. (1984). Growth patterns by age and sex in children with sickle cell disease. *J Pediatr*, *105*, 28–33.
414. Borgna-Pignatti, C., De Stefano, P., Zonta, L., et al. (1985). Growth and sexual maturation in thalassemia major. *J Pediatr*, *106*, 150–155.
415. De Luca, F., Simone, E., Corona, G., et al. (1987). Adult height in thalassaemia major without hormonal treatment. *Eur J Pediatr*, *146*, 494–496.
416. Saenger, P., Schwartz, E., Markenson, A. L., et al. (1980). Depressed serum somatomedin activity in beta-thalassemia. *J Pediatr*, *96*, 214–218.
417. Werther, G. A., Matthews, R. N., Burger, H. G., & Herington, A. C. (1981). Lack of response of nonsuppressible insulin-like activity to short term administration of human growth hormone in thalassemia major. *J Clin Endocrinol Metab*, *53*, 806–809.
418. Zadik, Z., Chalew, S. A., McCarter, R. J., Jr., et al. (1985). The influence of age on the 24-hour integrated concentration of growth hormone in normal individuals. *J Clin Endocrinol Metab*, *60*, 513–516.
419. Menon, R. K., & Sperling, M. A. (1996). Insulin as a growth factor. *Endocrinol Metab Clin North Am*, *25*, 633–647.
420. Zadik, Z., Chalew, S. A., Gilula, Z., & Kowarski, A. A. (1990). Reproducibility of growth hormone testing procedures: a comparison between 24-hour integrated concentration and pharmacological stimulation. *J Clin Endocrinol Metab*, *71*, 1127–1130.
421. Infections as deterrents of growth. (1981). *Nutr Rev*, *39*, 328–330.
422. Rivkees, S. A., Bode, H. H., & Crawford, J. D. (1988). Long-term growth in juvenile acquired hypothyroidism: the failure to achieve normal adult stature. *N Engl J Med*, *318*, 599–602.
423. Van Wyk, J. J., & Grumbach, M. (1960). Syndrome of precocious menstruation and galactorrhea. *J Pediatr*, *57*, 416–435.
424. Magiakou, M. A., Mastorakos, G., Oldfield, E. H., et al. (1994). Cushing's syndrome in children and adolescents. Presentation, diagnosis, and therapy. *N Engl J Med*, *331*, 629–636.

425. Mosier, H. D., Jr., Smith, F. G., Jr., & Schultz, M. A. (1972). Failure of catch-up growth after Cushing's syndrome in childhood. *Am J Dis Child*, *124*, 251–253.
426. Schwindinger, W. F., & Levine, M. A. (1994). Albright hereditary osteodystrophy. *Endocrinologist*, *4*, 17.
427. Chan, J. C. (1982). Renal hypophosphatemic rickets: a review. *Int J Pediatr Nephrol*, *3*, 305–310.
428. Efstratiadis, A. (1998). Genetics of mouse growth. *Int J Dev Biol*, *42*, 955–976.
429. Hintz, R. L., Menking, M., & Sotos, J. F. (1968). Familial holoprosencephaly with endocrine dysgenesis. *J Pediatr*, *72*, 81–87.
430. Lieblich, J. M., Rosen, S. E., Guyda, H., et al. (1978). The syndrome of basal encephalocele and hypothalamic-pituitary dysfunction. *Ann Intern Med*, *89*, 910–916.
431. Roitman, A., & Laron, Z. (1978). Hypothalamo-pituitary hormone deficiency associated with cleft lip and palate. *Arch Dis Child*, *53*, 952–925.
432. Rudman, D., Davis, T., Priest, J. H., et al. (1978). Prevalence of growth hormone deficiency in children with cleft lip or palate. *J Pediatr*, *93*, 378–382.
433. Patel, L., McNally, R. J., Harrison, E., et al. (2006). Geographical distribution of optic nerve hypoplasia and septo-optic dysplasia in Northwest England. *J Pediatr*, *148*, 85–88.
434. Arslanian, S. A., Rothfus, W. E., Foley, T. P., Jr., & Becker, D. J. (1984). Hormonal, metabolic, and neuroradiologic abnormalities associated with septo-optic dysplasia. *Acta Endocrinol* (Copenh), *107*, 282–288.
435. Izenberg, N., Rosenblum, M., & Parks, J. S. (1984). The endocrine spectrum of septo-optic dysplasia. *Clin Pediatr* (Phila), *23*, 632–636.
436. Morishima, A., & Aranoff, G. S. (1986). Syndrome of septo-optic-pituitary dysplasia: the clinical spectrum. *Brain Dev*, *8*, 233–239.
437. Brodsky, M. C., & Glasier, C. M. (1993). Optic nerve hypoplasia. Clinical significance of associated central nervous system abnormalities on magnetic resonance imaging. *Arch Ophthalmol*, *111*, 66–74.
438. Zeki, S. M., Hollman, A. S., & Dutton, G. N. (1992). Neuroradiological features of patients with optic nerve hypoplasia. *J Pediatr Ophthalmol Strabismus*, *29*, 107–112.
439. Birkebaek, N. H., Patel, L., Wright, N. B., et al. (2003). Endocrine status in patients with optic nerve hypoplasia: relationship to midline central nervous system abnormalities and appearance of the hypothalamic-pituitary axis on magnetic resonance imaging. *J Clin Endocrinol Metab*, *88*, 5281–5286.
440. Nanduri, V. R., & Stanhope, R. (1999). Why is the retention of gonadotrophin secretion common in children with panhypopituitarism due to septo-optic dysplasia? *Eur J Endocrinol*, *140*, 48–50.
441. Pagon, R. A., & Stephan, M. J. (1984). Septo-optic dysplasia with digital anomalies. *J Pediatr*, *105*, 966–968.
442. Wales, J. K., & Quarrell, O. W. (1996). Evidence for possible Mendelian inheritance of septo-optic dysplasia. *Acta Paediatr*, *85*, 391–392.
443. Murray, P. G., Paterson, W. F., & Donaldson, M. D. (2005). Maternal age in patients with septo-optic dysplasia. *J Pediatr Endocrinol Metab*, *18*, 471–476.
444. Hermesz, E., Mackem, S., & Mahon, K. A. (1996). Rpx: a novel anterior-restricted homeobox gene progressively activated in the prechordal plate, anterior neural plate and Rathke's pouch of the mouse embryo. *Development*, *122*, 41–52.
445. Dattani, M. T., Martinez-Barbera, J. P., Thomas, P. Q., et al. (1998). Mutations in the homeobox gene HESX1/Hesx1 associated with septo-optic dysplasia in human and mouse. *Nat Genet*, *19*, 125–133.
446. Alatzoglou, K. S., & Dattani, M. T. (2009). Genetic forms of hypopituitarism and their manifestation in the neonatal period. *Early Hum Dev*, *85*, 705–712.
447. Sajedi, E., Gaston-Massuet, C., Signore, M., et al. (2008). Analysis of mouse models carrying the I26T and R160C substitutions in the transcriptional repressor HESX1 as models for septo-optic dysplasia and hypopituitarism. *Dis Model Mech*, *1*, 241–254.
448. McNay, D. E., Turton, J. P., Kelberman, D., et al. (2007). HESX1 mutations are an uncommon cause of septooptic dysplasia and hypopituitarism. *J Clin Endocrinol Metab*, *92*, 691–697.
449. Wilson, D. M., Enzmann, D. R., Hintz, R. L., & Rosenfeld, G. (1984). Computed tomographic findings in septo-optic dysplasia: discordance between clinical and radiological findings. *Neuroradiology*, *26*, 279–283.
450. Mortensen, A. H., MacDonald, J. W., Ghosh, D., & Camper, S. A. (2011). Candidate genes for panhypopituitarism identified by gene expression profiling. *Physiol Genomics*, *43*, 1105–1116.
451. Diaczok, D., DiVall, S., Matsuo, I., et al. (2011). Deletion of Otx2 in GnRH neurons results in a mouse model of hypogonadotropic hypogonadism. *Mol Endocrinol*, *25*, 833–846.
452. Nolen, L. D., Amor, D., Haywood, A., et al. (2006). Deletion at 14q22-23 indicates a contiguous gene syndrome comprising anophthalmia, pituitary hypoplasia, and ear anomalies. *Am J Med Genet A*, *140*, 1711–1718.
453. Tajima, T., Ohtake, A., Hoshino, M., et al. (2009). OTX2 loss of function mutation causes anophthalmia and combined pituitary hormone deficiency with a small anterior and ectopic posterior pituitary. *J Clin Endocrinol Metab*, *94*, 314–319.
454. Dateki, S., Fukami, M., Sato, N., et al. (2008). OTX2 mutation in a patient with anophthalmia, short stature, and partial growth hormone deficiency: functional studies using the IRBP, HESX1, and POU1F1 promoters. *J Clin Endocrinol Metab*, *93*, 3697–3702.
455. Diaczok, D., Romero, C., Zunich, J., et al. (2008). A novel dominant negative mutation of OTX2 associated with combined pituitary hormone deficiency. *J Clin Endocrinol Metab*, *93*, 4351–4359.
456. Roessler, E., Belloni, E., Gaudenz, K., et al. (1996). Mutations in the human Sonic Hedgehog gene cause holoprosencephaly. *Nat Genet*, *14*, 357–360.
457. Roessler, E., Du, Y. Z., Mullor, J. L., et al. (2003). Loss-of-function mutations in the human GLI2 gene are associated with pituitary anomalies and holoprosencephaly-like features. *Proc Natl Acad Sci USA*, *100*, 13424–13429.
458. Fran√ßa, M. M., Jorge, A. A., Carvalho, L. R., et al. (2010). Novel heterozygous nonsense GLI2 mutations in patients with hypopituitarism and ectopic posterior pituitary lobe without holoprosencephaly. *J Clin Endocrinol Metab*, *95*, E384–E391.
459. Kelberman, D., Rizzoti, K., Avilion, A., et al. (2006). Mutations within Sox2/SOX2 are associated with abnormalities in the hypothalamo-pituitary-gonadal axis in mice and humans. *J Clin Invest*, *116*, 2442–2455.
460. Kelberman, D., de Castro, S. C., Huang, S., et al. (2008). SOX2 plays a critical role in the pituitary, forebrain, and eye during human embryonic development. *J Clin Endocrinol Metab*, *93*, 1865–1873.
461. Alatzoglou, K. S., Andoniadou, C. L., Kelberman, D., et al. (2011). SOX2 haploinsufficiency is associated with slow progressing hypothalamo-pituitary tumours. *Hum Mutat*, *32*, 1376–1380.
462. Laumonnier, F., Ronce, N., Hamel, B. C., et al. (2002). Transcription factor SOX3 is involved in X-linked mental retardation with growth hormone deficiency. *Am J Hum Genet*, *71*, 1450–1455.
463. Woods, K. S., Cundall, M., Turton, J., et al. (2005). Over- and underdosage of SOX3 is associated with infundibular hypoplasia and hypopituitarism. *Am J Hum Genet*, *76*, 833–849.
464. Alatzoglou, K. S., Kelberman, D., Cowell, C. T., et al. (2011). Increased transactivation associated with SOX3 polyalanine tract deletion in a patient with hypopituitarism. *J Clin Endocrinol Metab*, *96*, E685–E690.
465. França, M. M., Jorge, A. A., Alatzoglou, K. S., et al. (2011). Absence of GH-releasing hormone (GHRH) mutations in selected patients with isolated GH deficiency. *J Clin Endocrinol Metab*, *96*, E1457–E1460.
466. Wajnrajch, M. P., Gertner, J. M., Harbison, M. D., et al. (1996). Nonsense mutation in the human growth hormone-releasing hormone receptor causes growth failure analogous to the little (lit) mouse. *Nat Genet*, *12*, 88–90.
467. Baumann, G., & Maheshwari, H. (1997). The Dwarfs of Sindh: severe growth hormone (GH) deficiency caused by a mutation in the GH-releasing hormone receptor gene. *Acta Paediatr Suppl*, *423*, 33–38.
468. Netchine, I., Talon, P., Dastot, F., et al. (1998). Extensive phenotypic analysis of a family with growth hormone (GH) deficiency caused by a mutation in the GH-releasing hormone receptor gene. *J Clin Endocrinol Metab*, *83*, 432–436.
469. Salvatori, R., Hayashida, C. Y., Aguiar-Oliveira, M. H., et al. (1999). Familial dwarfism due to a novel mutation of the growth

hormone-releasing hormone receptor gene. *J Clin Endocrinol Metab*, *84*, 917–923.
470. Alba M, & Salvatori, R. (2005). Naturally-occurring missense mutations in the human growth hormone-releasing hormone receptor alter ligand binding. *J Endocrinol*, *186*, 515–521.
471. Salvatori, R., Fan, X., Phillips, J. A., 3rd, et al. (2001). Three new mutations in the gene for the growth hormone (gh)-releasing hormone receptor in familial isolated gh deficiency type ib. *J Clin Endocrinol Metab*, *86*, 273–279.
472. Alatzoglou, K. S., Turton, J. P., Kelberman, D., et al. (2009). Expanding the spectrum of mutations in GH1 and GHRHR: genetic screening in a large cohort of patients with congenital isolated growth hormone deficiency. *J Clin Endocrinol Metab*, *94*, 3191–3199.
473. Acerini, C. L., & Tasker, R. C. (2007). Traumatic brain injury induced hypothalamic-pituitary dysfunction: a paediatric perspective. *Pituitary*, *10*, 373–380.
474. Oertel, M., Boscardin, W. J., Obrist, W. D., et al. (2005). Posttraumatic vasospasm: the epidemiology, severity, and time course of an underestimated phenomenon: a prospective study performed in 299 patients. *J Neurosurg*, *103*, 812–824.
475. Urban, R. J. (2006). Hypopituitarism after acute brain injury. *Growth Horm IGF Res*, *16*(Suppl. A), S25–S29.
476. Agha, A., & Thompson, C. J. (2006). Anterior pituitary dysfunction following traumatic brain injury (TBI). *Clin Endocrinol* (Oxf), *64*, 481–488.
477. Benvenga, S., Campenní, A., Ruggeri, R. M., & Trimarchi, F. (2000). Clinical review 113: hypopituitarism secondary to head trauma. *J Clin Endocrinol Metab*, *85*, 1353–1361.
478. Kelly, D. F., Gonzalo, I. T., Cohan, P., et al. (2000). Hypopituitarism following traumatic brain injury and aneurysmal subarachnoid hemorrhage: a preliminary report. *J Neurosurg*, *93*, 743–752.
479. Tanriverdi, F., Senyurek, H., Unluhizarci, K., et al. (2006). High risk of hypopituitarism after traumatic brain injury: a prospective investigation of anterior pituitary function in the acute phase and 12 months after trauma. *J Clin Endocrinol Metab*, *91*, 2105–2111.
480. Klose, M., Juul, A., Struck, J., et al. (2007). Acute and long-term pituitary insufficiency in traumatic brain injury: a prospective single-centre study. *Clin Endocrinol* (Oxf), *67*, 598–606.
481. Dimopoulou, I., Tsagarakis, S., Theodorakopoulou, M., et al. (2004). Endocrine abnormalities in critical care patients with moderate-to-severe head trauma: incidence, pattern and predisposing factors. *Intensive Care Med*, *30*, 1051–1057.
482. Lieberman, S. A., Oberoi, A. L., Gilkison, C. R., et al. (2001). Prevalence of neuroendocrine dysfunction in patients recovering from traumatic brain injury. *J Clin Endocrinol Metab*, *86*, 2752–2756.
483. Agha, A., Sherlock, M., Phillips, J., et al. (2005). The natural history of post-traumatic neurohypophysial dysfunction. *Eur J Endocrinol*, *152*, 371–377.
484. Leal-Cerro, A., Flores, J. M., Rincon, M., et al. (2005). Prevalence of hypopituitarism and growth hormone deficiency in adults long-term after severe traumatic brain injury. *Clin Endocrinol* (Oxf), *62*, 525–532.
485. Aimaretti, G., Ambrosio, M. R., Di Somma, C., et al. (2005). Residual pituitary function after brain injury-induced hypopituitarism: a prospective 12-month study. *J Clin Endocrinol Metab*, *90*, 6085–6092.
486. Einaudi, S., Matarazzo, P., Peretta, P., et al. (2006). Hypothalamo-hypophysial dysfunction after traumatic brain injury in children and adolescents: a preliminary retrospective and prospective study. *J Pediatr Endocrinol Metab*, *19*, 691–703.
487. Niederland, T., Makovi, H., Gál, V., et al. (2007). Abnormalities of pituitary function after traumatic brain injury in children. *J Neurotrauma*, *24*, 119–127.
488. Keenan, H. T., Hooper, S. R., Wetherington, C. E., et al. (2007). Neurodevelopmental consequences of early traumatic brain injury in 3-year-old children. *Pediatrics*, *119*, e616–e623.
489. Acerini, C. L., Tasker, R. C., Bellone, S., et al. (2006). Hypopituitarism in childhood and adolescence following traumatic brain injury: the case for prospective endocrine investigation. *Eur J Endocrinol*, *155*, 663–669.
490. Einaudi, S., & Bondone, C. (2007). The effects of head trauma on hypothalamic-pituitary function in children and adolescents. *Curr Opin Pediatr*, *19*, 465–470.
491. Ghigo, E., Masel, B., Aimaretti, G., et al. (2005). Consensus guidelines on screening for hypopituitarism following traumatic brain injury. *Brain Inj*, *19*, 711–724.
492. Miller, W. L., Kaplan, S. L., & Grumbach, M. M. (1980). Child abuse as a cause of post-traumatic hypopituitarism. *N Engl J Med*, *302*, 724–728.
493. Craft, W. H., Underwoood, L. E., & Van Wyk, J. J. (1980). High incidence of perinatal insult in children with idiopathic hypopituitarism. *J Pediatr*, *96*(3 Pt. 1), 397–402.
494. Mayfield, R. K., Levine, J. H., Gordon, L., et al. (1980). Lymphadenoid hypophysitis presenting as a pituitary tumor. *Am J Med*, *69*, 619.
495. Bartsocas, C. S., Pantelakis, S. N. (1973). Human growth hormone therapy in hypopituitarism due to tuberculous meningitis. *Acta Paediatr Scand*, *62*, 304–306.
496. Schaefer, S., Boegershausen, N., Meyer, S., et al. (2008). Hypothalamic-pituitary insufficiency following infectious diseases of the central nervous system. *Eur J Endocrinol*, *158*, 3–9.
497. Stuart, C. A., Neelon, F. A., & Lebovitz, H. E. (1978). Hypothalamic insufficiency: the cause of hypopituitarism in sarcoidosis. *Ann Intern Med*, *88*, 589–594.
498. Iannuzzi, M. C., Rybicki, B. A., & Teirstein, A. S. (2007). Sarcoidosis. *N Engl J Med*, *357*, 2153–2165.
499. Bihan, H., Christozova, V., Dumas, J. L., et al. (2007). Sarcoidosis: clinical, hormonal, and magnetic resonance imaging (MRI) manifestations of hypothalamic-pituitary disease in 9 patients and review of the literature. *Medicine* (Baltimore), *86*, 259–268.
500. Takano, K. (2004). Sarcoidosis of the hypothalamus and pituitary. *Intern Med*, *43*, 894–895.
501. Murialdo G, & Tamagno, G. (2002). Endocrine aspects of neurosarcoidosis. *J Endocrinol Invest*, *25*, 650–662.
502. Molina, A., Mañá, J., Villabona, C., et al. (2002). Hypothalamic-pituitary sarcoidosis with hypopituitarism: long-term remission with methylprednisolone pulse therapy. *Pituitary*, *5*, 33–36.
503. Caturegli, P., Newschaffer, C., Olivi, A., et al. (2005). Autoimmune hypophysitis. *Endocr Rev*, *26*, 599–614.
504. Mikami-Terao, Y., Akiyama, M., Yanagisawa, T., et al. (2006). Lymphocytic hypophysitis with central diabetes insipidus and subsequent hypopituitarism masking a suprasellar germinoma in a 13-year-old girl. *Childs Nerv Syst*, *22*, 1338–1343.
505. Fehn, M., Bettendorf, M., Lüdecke, D. K., et al. (1999). Lymphocytic hypophysitis masking a suprasellar germinoma in a 12-year-old girl—a case report. *Pituitary*, *1*, 303–307.
506. Bettendorf, M., Fehn, M., Grulich-Henn, J., et al. (1999). Lymphocytic hypophysitis with central diabetes insipidus and consequent panhypopituitarism preceding a multifocal, intracranial germinoma in a prepubertal girl. *Eur J Pediatr*, *158*, 288–292.
507. Cemeroglu, A. P., Blaivas, M., Muraszko, K. M., et al. (1997). Lymphocytic hypophysitis presenting with diabetes insipidus in a 14-year-old girl: case report and review of the literature. *Eur J Pediatr*, *156*, 684–688.
508. Younes, J. S., & Secord, E. A. (2002). Panhypopituitarism in a child with common variable immunodeficiency. *Ann Allergy Asthma Immunol*, *89*, 322–325.
509. de Graaf, J. H., & Egeler, R. M. (1997). New insights into the pathogenesis of Langerhans cell histiocytosis. *Curr Opin Pediatr*, *9*, 46–50.
510. Bhatia, S., Nesbit, M. E., Jr., Egeler, R. M., et al. (1997). Epidemiologic study of Langerhans cell histiocytosis in children. *J Pediatr*, *130*, 774–784.
511. Howarth, D. M., Gilchrist, G. S., Mullan, B. P., et al. (1999). Langerhans cell histiocytosis: diagnosis, natural history, management, and outcome. *Cancer*, *85*, 2278–2290.
512. Bernstrand, C., Sandstedt, B., Ahström, L., & Henter, J. I. (2005). Long-term follow-up of Langerhans cell histiocytosis: 39 years' experience at a single centre. *Acta Paediatr*, *94*, 1073–1084.
513. Amato, M. C., Elias, L. L., Elias, J., et al. (2006). Endocrine disorders in pediatric: onset Langerhans Cell Histiocytosis. *Horm Metab Res*, *38*, 746–751.
514. Nanduri, V. R., Bareille, P., Pritchard, J., & Stanhope, R. (2000). Growth and endocrine disorders in multisystem Langerhans' cell histiocytosis. *Clin Endocrinol* (Oxf), *53*, 509–515.
515. Donadieu, J., Rolon, M. A., Thomas, C., et al. (2004). Endocrine involvement in pediatric-onset Langerhans' cell histiocytosis: a population-based study. *J Pediatr*, *144*, 344–350.

516. Maghnie, M., Cosi, G., Genovese, E., et al. (2000). Central diabetes insipidus in children and young adults. *N Engl J Med*, *343*, 998–1007.
517. Donadieu, J., Rolon, M. A., Pion, I., et al. (2004). Incidence of growth hormone deficiency in pediatric-onset Langerhans cell histiocytosis: efficacy and safety of growth hormone treatment. *J Clin Endocrinol Metab*, *89*, 604–609.
518. Maghnie, M., Aricò, M., Villa, A., et al. (1992). MR of the hypothalamic-pituitary axis in Langerhans cell histiocytosis. *AJNR Am J Neuroradiol*, *13*, 1365–1371.
519. Kaltsas, G. A., Powles, T. B., Evanson, J., et al. (2000). Hypothalamo-pituitary abnormalities in adult patients with Langerhans cell histiocytosis: clinical, endocrinological, and radiological features and response to treatment. *J Clin Endocrinol Metab*, *85*, 1370–1376.
520. Grois, N., Prayer, D., Prosch, H., et al. (2004). Course and clinical impact of magnetic resonance imaging findings in diabetes insipidus associated with Langerhans cell histiocytosis. *Pediatr Blood Cancer*, *43*, 59–65.
521. Costin, G. (1979). Endocrine disorders associated with tumors of the pituitary and hypothalamus. *Pediatr Clin North Am*, *26*, 15–31.
522. Lafferty, A. R., & Chrousos, G. P. (1999). Pituitary tumors in children and adolescents. *J Clin Endocrinol Metab*, *84*, 4317–4323.
523. Kunwar, S., & Wilson, C. B. (1999). Pediatric pituitary adenomas. J Clin Endocrinol Metab, 84, 4385–4389.
524. Ezzat, S., & Asa, S. L. (2006). Mechanisms of disease: the pathogenesis of pituitary tumors. *Nat Clin Pract Endocrinol Metab*, *2*, 220–230.
525. Walker, D. (2003). Recent advances in optic nerve glioma with a focus on the young patient. *Curr Opin Neurol*, *16*, 657–664.
526. Czyzyk, E., Jóźwiak, S., Roszkowski, M., & Schwartz, R. A. (2003). Optic pathway gliomas in children with and without neurofibromatosis 1. *J Child Neurol*, *18*, 471–478.
527. Jahraus, C. D., & Tarbell, N. J. (2006). Optic pathway gliomas. *Pediatr Blood Cancer*, *46*, 586–596.
528. Adan, L., Trivin, C., Sainte-Rose, C., et al. (2001). GH deficiency caused by cranial irradiation during childhood: factors and markers in young adults. *J Clin Endocrinol Metab*, *86*, 5245–5251.
529. el-Mahdy, W., & Powell, M. (1998). Transsphenoidal management of 28 symptomatic Rathke's cleft cysts, with special reference to visual and hormonal recovery. *Neurosurgery*, *42*, 7–16, discussion 16-17.
530. Billeci, D., Marton, E., Tripodi, M., et al. (2004). Symptomatic Rathke's cleft cysts: a radiological, surgical and pathological review. *Pituitary*, *7*, 131–137.
531. Brauner, R., Rappaport, R., Prevot, C., et al. (1989). A prospective study of the development of growth hormone deficiency in children given cranial irradiation, and its relation to statural growth. *J Clin Endocrinol Metab*, *68*, 346–351.
532. Shalet, S. M. (1986). Irradiation-induced growth failure. *Clin Endocrinol Metab*, *15*, 591–606.
533. Blatt, J., Bercu, B. B., Gillin, J. C., et al. (1984). Reduced pulsatile growth hormone secretion in children after therapy for acute lymphoblastic leukemia. *J Pediatr*, *104*, 182–186.
534. Stubberfield, T. G., Byrne, G. C., & Jones, T. W. (1995). Growth and growth hormone secretion after treatment for acute lymphoblastic leukemia in childhood. 18-Gy versus 24-Gy cranial irradiation. *J Pediatr Hematol Oncol*, *17*, 167–171.
535. Albertsson-Wikland, K., Lannering, B., Márky, I., et al. (1987). A longitudinal study on growth and spontaneous growth hormone (GH) secretion in children with irradiated brain tumors. *Acta Paediatr Scand*, *76*, 966–973.
536. Abreu, A. P., Dauber, A., Macedo, D. B., et al. (2013). Central precocious puberty caused by mutations in the imprinted gene MKRN3. *N Engl J Med*, *368*, 2467–2475.
537. Parkin, J. M. (1974). Incidence of growth hormone deficiency. *Arch Dis Child*, *49*, 904.
538. Vimpani, G. V., Vimpani, A. F., Lidgard, G. P., et al. (1977). Prevalence of severe growth hormone deficiency. *Br Med J*, *2*, 427–430.
539. Lindsay, R., Feldkamp, M., Harris, D., et al. (1994). Utah Growth Study: growth standards and the prevalence of growth hormone deficiency. *J Pediatr*, *125*, 29–35.
540. Rajab, A., Kelberman, D., de Castro, S. C., et al. (2008). Novel mutations in LHX3 are associated with hypopituitarism and sensorineural hearing loss. *Hum Mol Genet*, *17*, 2150–2159.
541. Netchine, I., Sobrier, M. L., Krude, H., et al. (2000). Mutations in LHX3 result in a new syndrome revealed by combined pituitary hormone deficiency. *Nat Genet*, *25*, 182–186.
542. Pfaeffle, R. W., Savage, J. J., Hunter, C. S., et al. (2007). Four novel mutations of the LHX3 gene cause combined pituitary hormone deficiencies with or without limited neck rotation. *J Clin Endocrinol Metab*, *92*, 1909–1919.
543. Bhangoo, A. P., Hunter, C. S., Savage, J. J., et al. (2006). Clinical case seminar: a novel LHX3 mutation presenting as combined pituitary hormonal deficiency. *J Clin Endocrinol Metab*, *91*, 747–753.
544. Sobrier, M. L., Attié-Bitach, T., Netchine, I., et al. (2004). Pathophysiology of syndromic combined pituitary hormone deficiency due to a LHX3 defect in light of LHX3 and LHX4 expression during early human development. *Gene Expr Patterns*, *5*, 279–284.
545. Machinis, K., Pantel, J., Netchine, I., et al. (2001). Syndromic short stature in patients with a germline mutation in the LIM homeobox LHX4. *Am J Hum Genet*, *69*, 961–968.
546. Pfaeffle, R. W., Hunter, C. S., Savage, J. J., et al. (2008). Three novel missense mutations within the LHX4 gene are associated with variable pituitary hormone deficiencies. *J Clin Endocrinol Metab*, *93*, 1062–1071.
547. Castinetti, F., Saveanu, A., Reynaud, R., et al. (2008). A novel dysfunctional LHX4 mutation with high phenotypical variability in patients with hypopituitarism. *J Clin Endocrinol Metab*, *93*, 2790–2799.
548. Sornson, M. W., Wu, W., Dasen, J. S., et al. (1996). Pituitary lineage determination by the Prophet of Pit-1 homeodomain factor defective in Ames dwarfism. *Nature*, *384*, 327–333.
549. Deladoëy, J., Flück, C., Büyükgebiz, A., et al. (1999). "Hot spot" in the PROP1 gene responsible for combined pituitary hormone deficiency. *J Clin Endocrinol Metab*, *84*, 1645–1650.
550. Fofanova, O., Takamura, N., Kinoshita, E., et al. (1998). Compound heterozygous deletion of the PROP-1 gene in children with combined pituitary hormone deficiency. *J Clin Endocrinol Metab*, *83*, 2601–2604.
551. Wu, W., Cogan, J. D., Pfäffle, R. W., et al. (1998). Mutations in PROP1 cause familial combined pituitary hormone deficiency. *Nat Genet*, *18*, 147–149.
552. Brown, M. R., et al. (1998). Prop-1 mutations and hypopituitarism in Poland. *In Proceedings of the 80th Annual Meeting of the Endocrine Society*.
553. Parks, J. S., et al. (1998). Natural history and molecular mechanisms of hypopituitarism with large sella turcica. *In: Proceedings of the 80th Annual Meeting of the Endocrine Society*.
554. Cogan, J. D., Wu, W., Phillips, J. A., 3rd, et al. (1998). The PROP1 2-base pair deletion is a common cause of combined pituitary hormone deficiency. *J Clin Endocrinol Metab*, *83*, 3346–3349.
555. Turton, J. P., Mehta, A., Raza, J., et al. (2005). Mutations within the transcription factor PROP1 are rare in a cohort of patients with sporadic combined pituitary hormone deficiency (CPHD). *Clin Endocrinol* (Oxf), *63*, 10–18.
556. Duquesnoy, P., Roy, A., Dastot, F., et al. (1998). Human Prop-1: cloning, mapping, genomic structure. Mutations in familial combined pituitary hormone deficiency. *FEBS Lett*, *437*, 216–220.
557. Osorio, M. G., Kopp, P., Marui, S., et al. (2000). Combined pituitary hormone deficiency caused by a novel mutation of a highly conserved residue (F88S) in the homeodomain of PROP-1. *J Clin Endocrinol Metab*, *85*, 2779–2785.
558. Kelberman, D., Turton, J. P., Woods, K. S., et al. (2009). Molecular analysis of novel PROP1 mutations associated with combined pituitary hormone deficiency (CPHD). *Clin Endocrinol* (Oxf), *70*, 96–103.
559. Reynaud, R., Barlier, A., Vallette-Kasic, S., et al. (2005). An uncommon phenotype with familial central hypogonadism caused by a novel PROP1 gene mutant truncated in the transactivation domain. *J Clin Endocrinol Metab*, *90*, 4880–4887.
560. Arroyo, A., Pernasetti, F., Vasilyev, V. V., et al. (2002). A unique case of combined pituitary hormone deficiency caused by a PROP1 gene mutation (R120C) associated with normal height and absent puberty. *Clin Endocrinol* (Oxf), *57*, 283–291.
561. Mendonca, B. B., Osorio, M. G., Latronico, A. C., et al. (1999). Longitudinal hormonal and pituitary imaging changes in two females with combined pituitary hormone deficiency due to

deletion of A301,G302 in the PROP1 gene. *J Clin Endocrinol Metab*, *84*, 942–945.
562. Asteria, C., Oliveira, J. H., Abucham, J., & Beck-Peccoz, P. (2000). Central hypocortisolism as part of combined pituitary hormone deficiency due to mutations of PROP-1 gene. Eur J Endocrinol 143, 347–352.
563. Pernasetti, F., Toledo, S. P., Vasilyev, V. V., et al. (2000). Impaired adrenocorticotropin-adrenal axis in combined pituitary hormone deficiency caused by a two-base pair deletion (301-302delAG) in the prophet of Pit-1 gene. *J Clin Endocrinol Metab*, *85*, 390–397.
564. Riepe, F. G., Partsch, C. J., Blankenstein, O., et al. (2001). Longitudinal imaging reveals pituitary enlargement preceding hypoplasia in two brothers with combined pituitary hormone deficiency attributable to PROP1 mutation. *J Clin Endocrinol Metab*, *86*, 4353–4357.
565. Agarwal, G., Bhatia, V., Cook, S., & Thomas, P. Q. (2000). Adrenocorticotropin deficiency in combined pituitary hormone deficiency patients homozygous for a novel PROP1 deletion. *J Clin Endocrinol Metab*, *85*, 4556–4561.
566. Böttner, A., Keller, E., Kratzsch, J., et al. (2004). PROP1 mutations cause progressive deterioration of anterior pituitary function including adrenal insufficiency: a longitudinal analysis. *J Clin Endocrinol Metab 89*, 5256–5265.
567. Voutetakis, A., Argyropoulou, M., Sertedaki, A., et al. (2004). Pituitary magnetic resonance imaging in 15 patients with Prop1 gene mutations: pituitary enlargement may originate from the intermediate lobe. *J Clin Endocrinol Metab*, *89*, 2200–2206.
568. Ward, R. D., Raetzman, L. T., Suh, H., et al. (2005). Role of PROP1 in pituitary gland growth. *Mol Endocrinol*, *19*, 698–710.
569. Parks, J. S., Brown, M. R., Hurley, D. L., et al. (1999). Heritable disorders of pituitary development. *J Clin Endocrinol Metab*, *84*, 4362–4370.
570. Camper, S. A., Saunders, T. L., Katz, R. W., & Reeves, R. H. (1990). The Pit-1 transcription factor gene is a candidate for the murine Snell dwarf mutation. *Genomics*, *8*, 586–590.
571. Pfäffle, R. W., DiMattia, G. E., Parks, J. S., et al. (1992). Mutation of the POU-specific domain of Pit-1 and hypopituitarism without pituitary hypoplasia. *Science*, *257*, 1118–1121.
572. Radovick, S., Nations, M., Du, Y., et al. (1992). A mutation in the POU-homeodomain of Pit-1 responsible for combined pituitary hormone deficiency. *Science*, *257*, 1115–1118.
573. Fofanova, O. V., Takamura, N., Kinoshita, E., et al. (1998). Rarity of PIT1 involvement in children from Russia with combined pituitary hormone deficiency. *Am J Med Genet*, *77*, 360–365.
574. Tatsumi, K., Miyai, K., Notomi, T., et al. (1992). Cretinism with combined hormone deficiency caused by a mutation in the PIT1 gene. *Nat Genet*, *1*, 56–58.
575. Ohta, K., Nobukuni, Y., Mitsubuchi, H., et al. (1992). Mutations in the Pit-1 gene in children with combined pituitary hormone deficiency. *Biochem Biophys Res Commun*, *189*, 851–855.
576. Turton, J. P., Reynaud, R., Mehta, A., et al. (2005). Novel mutations within the POU1F1 gene associated with variable combined pituitary hormone deficiency. *J Clin Endocrinol Metab*, *90*, 4762–4770.
577. Sadeghi-Nejad, A., & Senior, B. (1974). Autosomal dominant transmission of isolated growth hormone deficiency in iris-dental dysplasia (Rieger's syndrome). *J Pediatr*, *85*, 644–648.
578. Semina, E. V., Reiter, R., Leysens, N. J., et al. (1996). Cloning and characterization of a novel bicoid-related homeobox transcription factor gene, RIEG, involved in Rieger syndrome. *Nat Genet*, *14*, 392–399.
579. Phillips, J. A., 3rd, Hjelle, B. L., Seeburg, P. H., & Zachmann, M. (1981). Molecular basis for familial isolated growth hormone deficiency. *Proc Natl Acad Sci USA*, *78*, 6372–6375.
580. Phillips, J. A., 3rd, & Cogan, J. D. (1994). Genetic basis of endocrine disease. 6. Molecular basis of familial human growth hormone deficiency. *J Clin Endocrinol Metab*, *78*, 11–16.
581. Illig, R., Prader, A., Ferrandez, A., et al. (1971). Hereditary prenatal growth hormone deficiency with increased tendency to growth hormone antibody formation: "A-type" of isolated growth hormone deficiency. *Acta Paediatr Scand*, *60*, 607.
582. Cogan, J. D., & Phillips, J. A., 3rd (1998). Growth disorders caused by genetic defects in the growth hormone pathway. *Adv Pediatr*, *45*, 337–361.
583. Wagner, J. K., Eblé, A., Hindmarsh, P. C., & Mullis, P. E. (1998). Prevalence of human GH-1 gene alterations in patients with isolated growth hormone deficiency. *Pediatr Res*, *43*, 105–110.
584. Cogan, J. D., Phillips, J. A., 3rd, Sakati, N., et al. (1993). Heterogeneous growth hormone (GH) gene mutations in familial GH deficiency. *J Clin Endocrinol Metab*, *76*, 1224–1228.
585. McGuinness, L., Magoulas, C., Sesay, A. K., et al. (2003). Autosomal dominant growth hormone deficiency disrupts secretory vesicles in vitro and in vivo in transgenic mice. *Endocrinology*, *144*, 720–731.
586. Ryther, R. C., Flynt, A. S., Harris, B. D., et al. (2004). GH1 splicing is regulated by multiple enhancers whose mutation produces a dominant-negative GH isoform that can be degraded by allele-specific small interfering RNA (siRNA). *Endocrinology*, *145*, 2988–2996.
587. Salemi, S., Yousefi, S., Eblé, A., et al. (2006). Impact of del32-71-GH (exon 3 skipped GH) on intracellular GH distribution, secretion and cell viability: a quantitative confocal microscopy analysis. *Horm Res*, *65*, 132–141.
588. Salemi, S., Yousefi, S., Lochmatter, D., et al. (2007). Isolated autosomal dominant growth hormone deficiency: stimulating mutant GH-1 gene expression drives GH-1 splice-site selection, cell proliferation, and apoptosis. *Endocrinology*, *148*, 45–53.
589. Ryther, R. C., McGuinness, L. M., Phillips, J. A., 3rd, et al. (2003). Disruption of exon definition produces a dominant-negative growth hormone isoform that causes somatotroph death and IGHD II. *Hum Genet*, *113*, 140–148.
590. Moseley, C. T., Mullis, P. E., Prince, M. A., & Phillips, J. A., 3rd (2002). An exon splice enhancer mutation causes autosomal dominant GH deficiency. *J Clin Endocrinol Metab*, *87*, 847–852.
591. Deladoey, J., Stocker, P., & Mullis, P. E. (2001). Autosomal dominant GH deficiency due to an Arg183His GH-1 gene mutation: clinical and molecular evidence of impaired regulated GH secretion. *J Clin Endocrinol Metab*, *86*, 3941–3947.
592. Salemi, S., Yousefi, S., Baltensperger, K., et al. (2005). Variability of isolated autosomal dominant GH deficiency (IGHD II): impact of the P89L GH mutation on clinical follow-up and GH secretion. *Eur J Endocrinol*, *153*, 791–802.
593. Hess, O., Hujeirat, Y., Wajnrajch, M. P., et al. (2007). Variable phenotypes in familial isolated growth hormone deficiency caused by a G6664A mutation in the GH-1 gene. *J Clin Endocrinol Metab*, *92*, 4387–43893.
594. Binder, G., Nagel, B. H., Ranke, M. B., & Mullis, P. E. (2002). Isolated GH deficiency (IGHD) type II: imaging of the pituitary gland by magnetic resonance reveals characteristic differences in comparison with severe IGHD of unknown origin. *Eur J Endocrinol*, *147*, 755–760.
595. Binder, G., Keller, E., Mix, M., et al. (2001). Isolated GH deficiency with dominant inheritance: new mutations, new insights. *J Clin Endocrinol Metab*, *86*, 3877–3881.
596. Mullis, P. E., Robinson, I. C., Salemi, S., et al. (2005). Isolated autosomal dominant growth hormone deficiency: an evolving pituitary deficit? A multicenter follow-up study. *J Clin Endocrinol Metab*, *90*, 2089–2096.
597. Turton, J. P., Buchanan, C. R., Robinson, I. C., et al. (2006). Evolution of gonadotropin deficiency in a patient with type II autosomal dominant GH deficiency. *Eur J Endocrinol*, *155*, 793–799.
598. Petkovic, V., Lochmatter, D., Turton, J., et al. (2007). Exon splice enhancer mutation (GH-E32A) causes autosomal dominant growth hormone deficiency. *J Clin Endocrinol Metab*, *92*, 4427–4435.
599. Fujisawa, I., Kikuchi, K., Nishimura, K., et al. (1987). Transection of the pituitary stalk: development of an ectopic posterior lobe assessed with MR imaging. *Radiology*, *165*, 487–489.
600. Abrahams, J. J., Trefelner, E., & Boulware, S. D. (1991). Idiopathic growth hormone deficiency: MR findings in 35 patients. *AJNR Am J Neuroradiol*, *12*, 155–160.
601. Kuroiwa, T., Okabe, Y., Hasuo, K., et al. (1991). MR imaging of pituitary dwarfism. *AJNR Am J Neuroradiol*, *12*, 161–164.
602. Hamilton, J., Blaser, S., & Daneman, D. (1998). MR imaging in idiopathic growth hormone deficiency. *AJNR Am J Neuroradiol*, *19*, 1609–1615.
603. Kornreich, L., Horev, G., Lazar, L., et al. (1998). MR findings in growth hormone deficiency: correlation with severity of hypopituitarism. *AJNR Am J Neuroradiol*, *19*, 1495–1459.

604. Léger, J., Danner, S., Simon, D., et al. (2005). Do all patients with childhood-onset growth hormone deficiency (GHD) and ectopic neurohypophysis have persistent GHD in adulthood? *J Clin Endocrinol Metab*, *90*, 650–656.
605. Gelwane, G., Garel, C., Chevenne, D., et al. (2007). Subnormal serum insulin-like growth factor-I levels in young adults with childhood-onset nonacquired growth hormone (GH) deficiency who recover normal gh secretion may indicate less severe but persistent pituitary failure. *J Clin Endocrinol Metab*, *92*, 3788–3795.
606. Jenkins, J. S., Gilbert, C. J., & Ang, V. (1976). Hypothalamic-pituitary function in patients with craniopharyngiomas. *J Clin Endocrinol Metab*, *43*, 394–349.
607. Thomsett, M. J., Conte, F. A., Kaplan, S. L., & Grumbach, M. M. (1980). Endocrine and neurologic outcome in childhood craniopharyngioma: review of effect of treatment in 42 patients. *J Pediatr*, *97*, 728–735.
608. Karavitaki, N., Cudlip, S., Adams, C. B., & Wass, J. A. (2006). Craniopharyngiomas. *Endocr Rev*, 27, 371–397.
609. Kaatsch, P., Rickert, C. H., Kühl, J., et al. (2001). Population-based epidemiologic data on brain tumors in German children. *Cancer*, *92*, 3155–3164.
610. May, J. A., Krieger, M. D., Bowen, I., & Geffner, M. E. (2006). Craniopharyngioma in childhood. *Adv Pediatr*, 53, 183–209.
611. Bunin, G. R., Surawicz, T. S., Witman, P. A., et al. (1998). The descriptive epidemiology of craniopharyngioma. *J Neurosurg*, *89*, 547–551.
612. Müller, H. L., Gebhardt, U., Etavard-Gorris, N., et al. (2004). Prognosis and sequela in patients with childhood craniopharyngioma—results of HIT-ENDO and update on KRANIOPHARYNGEOM 2000. *Klin Padiatr*, *216*, 343–348.
613. Karavitaki, N., Brufani, C., Warner, J. T., et al. (2005). Craniopharyngiomas in children and adults: systematic analysis of 121 cases with long-term follow-up. *Clin Endocrinol* (Oxf), *62*, 397–409.
614. Fahlbusch, R., Honegger, J., Paulus, W., et al. (1999). Surgical treatment of craniopharyngiomas: experience with 168 patients. *J Neurosurg*, *90*, 237–250.
615. Sekine, S., Shibata, T., Kokubu, A., et al. (2002). Craniopharyngiomas of adamantinomatous type harbor beta-catenin gene mutations. *Am J Pathol*, *161*, 1997–2001.
616. Oikonomou, E., Barreto, D. C., Soares, B., et al. (2005). Beta-catenin mutations in craniopharyngiomas and pituitary adenomas. *J Neurooncol*, *73*, 205–209.
617. de Vries L, Lazar, L., & Phillip, M. (2003). Craniopharyngioma: presentation and endocrine sequelae in 36 children. *J Pediatr Endocrinol Metab*, *16*, 703–710.
618. Lena, G., Paz Paredes, A., Scavarda, D., & Giusiano, B. (2005). Craniopharyngioma in children: Marseille experience. *Childs Nerv Syst*, *21*, 778–784.
619. Müller, H. L., Albanese, A., Calaminus, G., et al. (2006). Consensus and perspectives on treatment strategies in childhood craniopharyngioma: results of a meeting of the Craniopharyngioma Study Group (SIOP), Genova, 2004. *J Pediatr Endocrinol Metab*, *19*(Suppl. 1), 453–454.
620. Spoudeas, H. A., Saran, F., & Pizer, B. (2006). A multimodality approach to the treatment of craniopharyngiomas avoiding hypothalamic morbidity: a UK perspective. *J Pediatr Endocrinol Metab*, *19*(Suppl. 1), 447–451.
621. Braunstein, G. D., & Kohler, P. O. (1972). Pituitary function in Hand-Schuller-Christian disease: evidence for deficient growth-hormone release in patients with short stature. *N Engl J Med*, *286*, 1225–1229.
622. Powell, G. F., Brasel, J. A., & Blizzard, R. M. (1967). Emotional deprivation and growth retardation simulating idiopathic hypopituitarism. I. Clinical evaluation of the syndrome. *N Engl J Med*, *276*, 1271–1278.
623. Blizzard, R. M., (1985). Psychosocial short stature. In F. Lipshitz (Ed.), *Pediatric endocrinology* (pp. 87–107). New York: Marcel Dekker.
624. Spiliotis, B. E., August, G. P., Hung, W., et al. (1984). Growth hormone neurosecretory dysfunction. A treatable cause of short stature. *JAMA*, *251*, 2223–2230.
625. Bercu, B. B., Shulman, D., Root, A. W., & Spiliotis, B. E. (1986). Growth hormone (GH) provocative testing frequently does not reflect endogenous GH secretion. *J Clin Endocrinol Metab*, *63*, 709–716.
626. Tauber, M., Moulin, P., Pienkowski, C., et al. (1997). Growth hormone (GH) retesting and auxological data in 131 GH-deficient patients after completion of treatment. *J Clin Endocrinol Metab*, *82*, 352–356.
627. Maghnie, M., Strigazzi, C., Tinelli, C., et al. (1999). Growth hormone (GH) deficiency (GHD) of childhood onset: reassessment of GH status and evaluation of the predictive criteria for permanent GHD in young adults. *J Clin Endocrinol Metab*, *84*, 1324–1328.
628. Toogood, A. A., & Shalet, S. M. (1997). Diagnosis of severe growth hormone (GH) deficiency in young adults who received GH replacement therapy during childhood. *Acta Paediatr Suppl*, *423*, 117–120.
629. Valenta, L. J., Sigel, M. B., Lesniak, M. A., et al. (1985). Pituitary dwarfism in a patient with circulating abnormal growth hormone polymers. *N Engl J Med*, *312*, 214–217.
630. Takahashi, Y., Kaji, H., Okimura, Y., et al. (1996). Brief report: short stature caused by a mutant growth hormone. *N Engl J Med*, *334*, 432–436.
631. Takahashi, Y., Shirono, H., Arisaka, O., et al. (1997). Biologically inactive growth hormone caused by an amino acid substitution. *J Clin Invest*, *100*, 1159–1165.
632. Laron, Z., Pertzelan, A., & Mannheimer, S. (1966). Genetic pituitary dwarfism with high serum concentration of growth hormone—a new inborn error of metabolism? *Isr J Med Sci*, *2*, 152–155.
633. Rosenbloom, A. L., Guevara Aguirre, J., Rosenfeld, R. G., & Fielder, P. J. (1990). The little women of Loja—growth hormone-receptor deficiency in an inbred population of southern Ecuador. *N Engl J Med*, *323*, 1367–1374.
634. Golde, D. W., Bersch, N., Kaplan, S. A., et al. (1980). Peripheral unresponsiveness to human growth hormone in Laron dwarfism. *N Engl J Med*, *303*, 1156–1159.
635. Eshet, R., Laron, Z., Pertzelan, A., et al. (1984). Defect of human growth hormone receptors in the liver of two patients with Laron-type dwarfism. *Isr J Med Sci*, *20*, 8–11.
636. Godowski, P. J., Leung, D. W., Meacham, L. R., et al. (1989). Characterization of the human growth hormone receptor gene and demonstration of a partial gene deletion in two patients with Laron-type dwarfism. *Proc Natl Acad Sci USA*, *86*, 8083–8087.
637. Amselem, S., Duquesnoy, P., Attree, O., et al. (1989). Laron dwarfism and mutations of the growth hormone-receptor gene. *N Engl J Med*, *321*, 989–995.
638. Berg, M. A., Guevara-Aguirre, J., Rosenbloom, A. L., et al. (1992). Mutation creating a new splice site in the growth hormone receptor genes of 37 Ecuadorean patients with Laron syndrome. *Hum Mutat*, *1*, 24–32.
639. David, A., Hwa, V., Metherell, L. A., et al. (2011). Evidence for a continuum of genetic, phenotypic, and biochemical abnormalities in children with growth hormone insensitivity. *Endocr Rev*, *32*, 472–497.
640. Amselem, S., et al. (1993). Molecular analysis of two families with Laron syndrome and positive growth hormone binding protein. *Ped Res*, *33*;5.
641. Kou, K., Lajara, R., & Rotwein, P. (1993). Amino acid substitutions in the intracellular part of the growth hormone receptor in a patient with the Laron syndrome. *J Clin Endocrinol Metab*, *76*, 54–59.
642. Woods, K. A., Fraser, N. C., Postel-Vinay, M. C., et al. (1996). A homozygous splice site mutation affecting the intracellular domain of the growth hormone (GH) receptor resulting in Laron syndrome with elevated GH-binding protein. *J Clin Endocrinol Metab*, *81*, 1686–1690.
643. Silbergeld, A., Dastot, F., Klinger, B., et al. (1997). Intronic mutation in the growth hormone (GH) receptor gene from a girl with Laron syndrome and extremely high serum GH binding protein: extended phenotypic study in a very large pedigree. *J Pediatr Endocrinol Metab*, *10*, 265–274.
644. Ayling, R. M., Ross, R., Towner, P., et al. (1997). A dominant-negative mutation of the growth hormone receptor causes familial short stature. *Nat Genet*, *16*, 13–14.
645. Metherell, L. A., Akker, S. A., Munroe, P. B., et al. (2001). Pseudoexon activation as a novel mechanism for disease resulting in atypical growth-hormone insensitivity. *Am J Hum Genet*, *69*, 641–646.

646. David, A., Camacho-Hübner, C., Bhangoo, A., Rose, S. J., et al. (2007). An intronic growth hormone receptor mutation causing activation of a pseudoexon is associated with a broad spectrum of growth hormone insensitivity phenotypes. *J Clin Endocrinol Metab*, *92*, 655–659.
647. Fang, P., Riedl, S., Amselem, S., et al. (2007). Primary growth hormone (GH) insensitivity and insulin-like growth factor deficiency caused by novel compound heterozygous mutations of the GH receptor gene: genetic and functional studies of simple and compound heterozygous states. *J Clin Endocrinol Metab*, *92*, 2223–2231.
648. Aisenberg, J., Auyeung, V., Pedro, H. F., et al. (2010). Atypical GH insensitivity syndrome and severe insulin-like growth factor-I deficiency resulting from compound heterozygous mutations of the GH receptor, including a novel frameshift mutation affecting the intracellular domain. *Horm Res Paediatr*, *74*, 406–411.
649. Derr, M. A., Aisenberg, J., Fang, P., et al. (2011). The growth hormone receptor (GHR) c.899dupC mutation functions as a dominant negative: insights into the pathophysiology of intracellular GHR defects. *J Clin Endocrinol Metab*, *96*, E1896–E1904.
650. Rosenfeld, R. G., Belgorosky, A., Camacho-Hubner, C., et al. (2007). Defects in growth hormone receptor signaling. *Trends Endocrinol Metab*, *18*, 134–141.
651. Nadeau, K., Hwa, V., & Rosenfeld, R. G. (2011). STAT5b deficiency: an unsuspected cause of growth failure, immunodeficiency, and severe pulmonary disease. *J Pediatr*, *158*, 701–708.
652. Hwa, V., Haeusler, G., Pratt, K. L., et al. (2006). Total absence of functional acid labile subunit, resulting in severe insulin-like growth factor deficiency and moderate growth failure. *J Clin Endocrinol Metab*, *91*, 1826–1831.
653. Domené, H. M., Hwa, V., Jasper, H. G., & Rosenfeld, R. G. (2011). Acid-labile subunit (ALS) deficiency. *Best Pract Res Clin Endocrinol Metab*, *25*, 101–113.
654. Fofanova-Gambetti, O. V., Hwa, V., Wit, J. M., et al. (2010). Impact of heterozygosity for acid-labile subunit (IGFALS) gene mutations on stature: results from the international acid-labile subunit consortium. *J Clin Endocrinol Metab*, *95*, 4184–4191.
655. Baker, J., Liu, J. P., Robertson, E. J., & Efstratiadis, A. (1993). Role of insulin-like growth factors in embryonic and postnatal growth. *Cell*, *75*, 73–82.
656. Woods, K. A., Camacho-Hübner, C., Bergman, R. N., et al. (2000). Effects of insulin-like growth factor I (IGF-I) therapy on body composition and insulin resistance in IGF-I gene deletion. *J Clin Endocrinol Metab*, *85*, 1407–1411.
657. Walenkamp, M. J., Karperien, M., Pereira, A. M., et al. (2005). Homozygous and heterozygous expression of a novel insulin-like growth factor-I mutation. *J Clin Endocrinol Metab*, *90*, 2855–2864.
658. Netchine, I., Azzi, S., Houang, M., et al. (2009). Partial primary deficiency of insulin-like growth factor (IGF)-I activity associated with IGF1 mutation demonstrates its critical role in growth and brain development. *J Clin Endocrinol Metab*, *94*, 3913–3921.
659. Bonapace, G., Concolino, D., Formicola, S., & Strisciuglio, P. (2003). A novel mutation in a patient with insulin-like growth factor 1 (IGF1) deficiency. *J Med Genet*, *40*, 913–917.
660. Walenkamp, M. J., & Wit, J. M. (2006). Genetic disorders in the growth hormone - insulin-like growth factor-I axis. *Horm Res*, *66*, 221–230.
661. Klammt, J., Kiess, W., & Pfaffle, R. (2011). IGF1R mutations as cause of SGA. *Best Pract Res Clin Endocrinol Metab*, *25*, 191–206.
662. Fang, P., Schwartz, I. D., Johnson, B. D., et al. (2009). Familial short stature caused by haploinsufficiency of the insulin-like growth factor i receptor due to nonsense-mediated messenger ribonucleic acid decay. *J Clin Endocrinol Metab*, *94*, 1740–1747.
663. Wallborn, T., Wüller, S., Klammt, J., et al. (2010). A heterozygous mutation of the insulin-like growth factor-I receptor causes retention of the nascent protein in the endoplasmic reticulum and results in intrauterine and postnatal growth retardation. *J Clin Endocrinol Metab*, *95*, 2316–2324.
664. Abuzzahab, M. J., Schneider, A., Goddard, A., et al. (2003). IGF-I receptor mutations resulting in intrauterine and postnatal growth retardation. *N Engl J Med*, *349*, 2211–2222.
665. Fang, P., Cho, Y. H., Derr, M. A., et al. (2012). Severe short stature caused by novel compound heterozygous mutations of the insulin-like growth factor 1 receptor (IGF1R). *J Clin Endocrinol Metab*, *97*, E243–E247.
666. Copeland, K. C., Franks, R. C., & Ramamurthy, R. (1981). Neonatal hyperbilirubinemia and hypoglycemia in congenital hypopituitarism. *Clin Pediatr* (Phila), *20*, 523–526.
667. Lovinger, R. D., Kaplan, S. L., & Grumbach, M. M. (1975). Congenital hypopituitarism associated with neonatal hypoglycemia and microphallus: four cases secondary to hypothalamic hormone deficiencies. *J Pediatr*, *87*(6 Pt 2), 1171–1181.
668. Frasier, S. D. (1974). A preview of growth hormone stimulation tests in children. *Pediatrics*, *53*, 929–937.
669. Underwood, L. E., Azumi, K., Voina, S. J., & Van Wyk, J. J. (1971). Growth hormone levels during sleep in normal and growth hormone deficient children. *Pediatrics*, *48*, 946–954.
670. Buckler, J. M. (1973). Plasma growth hormone response to exercise as diagnostic aid. *Arch Dis Child*, *48*, 565–567.
671. Lacey, K. A., Hewison, A., & Parkin, J. M. (1973). Exercise as a screening test for growth hormone deficiency in children. *Arch Dis Child*, *48*, 508–512.
672. Collu, R., & Letarte, J. (1975). Stimulation of growth hormone secretion by levodopa-propanolol in children and adolescents. *Pediatrics*, *56*, 262.
673. Lanes, R., & Hurtado, E. (1982). Oral clonidine-an effective growth hormone-releasing agent in prepubertal subjects. *J Pediatr*, *100*, 710–714.
674. Mitchell, M. L., Byrne, M. J., Sanchez, Y., & Sawin, C. T. (1970). Detecon of growth-hormone deficiency. *N Engl J Med*, *282*, 539–541.
675. Merimee, T. J., Rabinowtitz, D., & Fineberg, S. E. (1969). Arginine-initiated release of human growth hormone. Factors modifying the response in normal man. *N Engl J Med*, *280*, 1434–1438.
676. Kaplan, S. L., Abrams, C. A., Bell, J. J., et al. (1968). Growth and growth hormone. I. Changes in serum level of growth hormone following hypoglycemia in 134 children with growth retardation. *Pediatr Res*, *2*, 43–63.
677. Root, A. W., Rosenfield, R. L., Bongiovanni, A. M., & Eberlein, W. R. (1967). The plasma growth hormone response to insulin-induced hypoglycemia in children with retardation of growth. *Pediatrics*, *39*, 844–852.
678. Penny, R., Blizzard, R. M., & Davis, W. T. (1969). Sequential arginine and insulin tolerance tests on the same day. *J Clin Endocrinol Metab*, *29*, 1499–1501.
679. Fass, B., Lippe, B. M., & Kaplan, S. A. (1979). Relative usefulness of three growth hormone stimulation screening tests. *Am J Dis Child*, *133*, 931–933.
680. Weldon, V. V., Gupta, S. K., Klingensmith, G., et al. (1975). Evaluation of growth hormone release in children using arginine and L-dopa in combination. *J Pediatr*, *87*, 540–544.
681. Reiter, E. O., & Martha, P. M., Jr. (1990). Pharmacological testing of growth hormone secretion. *Horm Res*, *33*, 121–126; discussion 126-127.
682. Raiti, S., Davis, W. T., & Blizzard, R. M. (1967). A comparison of the effects of insulin hypoglycaemia and arginine infusion on release of human growth hormone. *Lancet*, *2*, 1182–1183.
683. Guyda, H. J. (2000). Growth hormone testing and the short child. *Pediatr Res*, *48*, 579–580.
684. Physicians' desk reference (48th ed.) (1994). Montvale, NJ: Medical Economics Data Production.
685. Martha, P. M., Jr., Gorman, K. M., Blizzard, R. M., et al. (1992). Endogenous growth hormone secretion and clearance rates in normal boys, as determined by deconvolution analysis: relationship to age, pubertal status, and body mass. *J Clin Endocrinol Metab*, *74*, 336–344.
686. Lippe, B., Wong, S. L., & Kaplan, S. A. (1971). Simultaneous assessment of growth hormone and ACTH reserve in children pretreated with diethylstilbestrol. *J Clin Endocrinol Metab*, *33*, 949–956.
687. Chernausek, S. D., (1987). Laboratory diagnosis of growth disorders. In R. L. Hintz (Ed.), *Growth abnormalities: contemporary issues in endocrinology and metabolism* (p. 231). New York: Churchill Livingstone.
688. Gourmelen, M., Pham-Huu-Trung, M. T., & Girard, F. (1979). Transient partial hGH deficiency in prepubertal children with delay of growth. *Pediatr Res*, *13*(4 Pt. 1), 221–224.
689. Cacciari, E., Tassoni, P., Parisi, G., et al. (1992). Pitfalls in diagnosing impaired growth hormone (GH) secretion: retesting after

replacement therapy of 63 children defined as GH deficient. *J Clin Endocrinol Metab*, *74*, 1284–1289.
690. Marin, G., Domené, H. M., Barnes, K. M., et al. (1994). The effects of estrogen priming and puberty on the growth hormone response to standardized treadmill exercise and arginine-insulin in normal girls and boys. *J Clin Endocrinol Metab*, *79*, 537–541.
691. Reiter, E. O., Morris, A. H., MacGillivray, M. H., & Weber, D. (1988). Variable estimates of serum growth hormone concentrations by different radioassay systems. *J Clin Endocrinol Metab*, *66*, 68–71.
692. Celniker, A. C., Chen, A. B., Wert, R. M., Jr., & Sherman, B. M. (1989). Variability in the quantitation of circulating growth hormone using commercial immunoassays. *J Clin Endocrinol Metab*, *68*, 469–476.
693. Mauras, N., Walton, P., Nicar, M., et al. (2000). Growth hormone stimulation testing in both short and normal statured children: use of an immunofunctional assay. *Pediatr Res*, *48*, 614–618.
694. Shah, A., Stanhope, R., & Matthew, D. (1992). Hazards of pharmacological tests of growth hormone secretion in childhood. *BMJ*, *304*, 173–174.
695. Eddy, R. L., Gilliland, P. F., Ibarra, J. D., Jr., et al. (1974). Human growth hormone release. Comparison of provocative test procedures. *Am J Med*, *56*, 179–185.
696. Zadik, Z., Chalew, S. A., Raiti, S., & Kowarski, A. A. (1985). Do short children secrete insufficient growth hormone? *Pediatrics*, *76*, 355–360.
697. Tassoni, P., Cacciari, E., Cau, M., et al. (1990). Variability of growth hormone response to pharmacological and sleep tests performed twice in short children. *J Clin Endocrinol Metab*, *71*, 230–234.
698. Donaldson, D. L., Hollowell, J. G., Pan, F. P., et al. (1989). Growth hormone secretory profiles: variation on consecutive nights. *J Pediatr*, *115*, 51–56.
699. Rose, S. R., Ross, J. L., Uriarte, M., et al. (1988). The advantage of measuring stimulated as compared with spontaneous growth hormone levels in the diagnosis of growth hormone deficiency. *N Engl J Med*, *319*, 201–207.
700. Lanes, R. (1989). Diagnostic limitations of spontaneous growth hormone measurements in normally growing prepubertal children. *Am J Dis Child*, *143*, 1284–1286.
701. Hourd, P., & Edwards, R. (1994). Current methods for the measurement of growth hormone in urine. *Clin Endocrinol* (Oxf), *40*, 155–170.
702. Albini, C. H., Quattrin, T., Vandlen, R. L., & MacGillivray, M. H. (1988). Quantitation of urinary growth hormone in children with normal and abnormal growth. *Pediatr Res*, *23*, 89–92.
703. Granada, M. L., Sanmartí, A., Lucas, A., et al. (1992). Clinical usefulness of urinary growth hormone measurements in normal and short children according to different expressions of urinary growth hormone data. *Pediatr Res*, *32*, 73–76.
704. Juul, A., Kastrup, K. W., Pedersen, S. A., & Skakkebaek, N. E. (1997). Growth hormone (GH) provocative retesting of 108 young adults with childhood-onset GH deficiency and the diagnostic value of insulin-like growth factor I (IGF-I) and IGF-binding protein-3. *J Clin Endocrinol Metab*, *82*, 1195–1201.
705. Hasegawa, Y., Hasegawa, T., Aso, T., et al. (1994). Clinical utility of insulin-like growth factor binding protein-3 in the evaluation and treatment of short children with suspected growth hormone deficiency. *Eur J Endocrinol*, *131*, 27–32.
706. Hasegawa, Y., Hasegawa, T., Aso, T., et al. (1992). Usefulness and limitation of measurement of insulin-like growth factor binding protein-3 (IGFBP-3) for diagnosis of growth hormone deficiency. *Endocrinol Jpn*, *39*, 585–591.
707. Smith, W. J., Nam, T. J., Underwood, L. E., et al. (1993). Use of insulin-like growth factor-binding protein-2 (IGFBP-2), IGFBP-3, and IGF-I for assessing growth hormone status in short children. *J Clin Endocrinol Metab*, 77, 1294–1299.
708. Savage, M. O., Blum, W. F., Ranke, M. B., et al. (1993). Clinical features and endocrine status in patients with growth hormone insensitivity (Laron syndrome). *J Clin Endocrinol Metab*, 77, 1465–1471.
709. Guevara-Aguirre, J., Rosenbloom, A. L., Fielder, P. J., et al. (1993). Growth hormone receptor deficiency in Ecuador: clinical and biochemical phenotype in two populations. *J Clin Endocrinol Metab*, *76*, 417–423.
710. Ranke, M. B., Lindberg, A., Chatelain, P., et al. (1999). Derivation and validation of a mathematical model for predicting the response to exogenous recombinant human growth hormone (GH) in prepubertal children with idiopathic GH deficiency. KIGS International Board. Kabi Pharmacia International Growth Study. *J Clin Endocrinol Metab*, *84*, 1174–1183.
711. Bhala, A., Harris, M. C., Zirin, S., et al. (1998). Insulin-like growth factor axis parameters in sick hospitalized neonates. *J Pediatr Endocrinol Metab*, *11*, 451–459.
712. Savage, M. O., & Rosenfeld, R. G. (1999). Growth hormone insensitivity: a proposed revised classification. *Acta Paediatr Suppl*, *428*, 147.
713. Blum, W. F., Ranke, M. B., Savage, M. O., & Hall, K. (1992). Insulin-like growth factors and their binding proteins in patients with growth hormone receptor deficiency: suggestions for new diagnostic criteria. The Kabi Pharmacia Study Group on Insulin-like Growth Factor I Treatment in Growth Hormone Insensitivity Syndromes. *Acta Paediatr Suppl*, *383*, 125–126.
714. Rosenfeld, R. G., Kemp, S. F., & Hintz, R. L. (1981). Constancy of somatomedin response to growth hormone treatment of hypopituitary dwarfism, and lack of correlation with growth rate. *J Clin Endocrinol Metab*, *53*, 611–617.
715. Duquesnoy, P., Sobrier, M. L., Duriez, B., et al. (1994). A single amino acid substitution in the exoplasmic domain of the human growth hormone (GH) receptor confers familial GH resistance (Laron syndrome) with positive GH-binding activity by abolishing receptor homodimerization. *EMBO J*, *13*, 1386–1395.
716. Bierich, J. R. (1982). Constitutional delay of growth and adolescent development. *Eur J Pediatr*, *139*.
717. Clayton, P. E., Shalet, S. M., & Price, D. A. (1966). Endocrine manipulation of constitutional delay in growth and puberty. *J Endocrinol*, *116*, 321–323.
718. Eastman, C. J., Lazarus, L., Stuart, M. C., & Casey, J. H. (1971). The effect of puberty on growth hormone secretion in boys with short stature and delayed adolescence. *Aust N Z J Med*, *1*, 154–159.
719. Lee, M. M. (2006). Clinical practice. Idiopathic short stature. *N Engl J Med*, *354*, 2576–2582.
720. Rosenfeld, R. G., Northcraft, G. B., & Hintz, R. L. (1982). A prospective, randomized study of testosterone treatment of constitutional delay of growth and development in male adolescents. *Pediatrics*, *69*, 681–687.
721. Richman, R. A., & Kirsch, L. R. (1988). Testosterone treatment in adolescent boys with constitutional delay in growth and development. *N Engl J Med*, *319*, 1563–1567.
722. Wilson, D. M., Kei, J., Hintz, R. L., & Rosenfeld, R. G. (1988). Effects of testosterone therapy for pubertal delay. *Am J Dis Child*, *142*, 96–99.
723. Wang, C., Swerdloff, R. S., Iranmanesh, A., et al. (2000). Transdermal testosterone gel improves sexual function, mood, muscle strength, and body composition parameters in hypogonadal men. *J Clin Endocrinol Metab*, *85*, 2839–2853.
724. Ahmed, S. R., Boucher, A. E., Manni, A., et al. (1988). Transdermal testosterone therapy in the treatment of male hypogonadism. *J Clin Endocrinol Metab*, *66*, 546–551.
725. Rogol, A. D. (2005). New facets of androgen replacement therapy during childhood and adolescence. *Expert Opin Pharmacother*, *6*, 1319–1336.
726. Dunkel, L. (2009). Update on the role of aromatase inhibitors in growth disorders. *Horm Res*, *71*(Suppl. 1), 57–63.
727. Gibbs, C. J., Jr., Asher, D. M., Brown, P. W., et al. (1993). Creutzfeldt-Jakob disease infectivity of growth hormone derived from human pituitary glands. *N Engl J Med*, *328*, 358–359.
728. Buchanan, C. R., Preece, M. A., & Milner, R. D. (1991). Mortality, neoplasia, and Creutzfeldt-Jakob disease in patients treated with human pituitary growth hormone in the United Kingdom. *BMJ*, *302*, 824–828.
729. Hintz, R. L., Rosenfeld, R. G., Wilson, D. M., et al. (1982). Biosynthetic methionyl human growth hormones is biologically active in adult man. *Lancet*, *1*, 1276–1279.
730. Kaplan, S. L., Underwood, L. E., August, G. P., et al. (1986). Clinical studies with recombinant-DNA-derived methionyl human growth hormone in growth hormone deficient children. *Lancet*, *1*, 697–700.

731. Grimberg, A., & Cohen, P. (1997). Optimizing growth hormone therapy in children. *Horm Res*, *48*(Suppl. 5), 11–15.
732. Albertsson-Wikland, K., Westphal, O., & Westgren, U. (1986). Daily subcutaneous administration of human growth hormone in growth hormone deficient children. *Acta Paediatr Scand*, *75*, 89–97.
733. Jørgensen, J. O., Møller, N., Lauritzen, T., et al. (1990). Evening versus morning injections of growth hormone (GH) in GH-deficient patients: effects on 24-hour patterns of circulating hormones and metabolites. *J Clin Endocrinol Metab*, *70*, 207–214.
734. Saggese, G., Ranke, M. B., Saenger, P., et al. (1998). Diagnosis and treatment of growth hormone deficiency in children and adolescents: towards a consensus. Ten years after the Availability of Recombinant Human Growth Hormone Workshop held in Pisa, Italy, March 27–28, 1998. *Horm Res*, *50*, 320–340.
735. Frasier, S. D., Costin, G., Lippe, B. M., et al. (1981). A dose-response curve for human growth hormone. *J Clin Endocrinol Metab*, *53*, 1213–1217.
736. Cohen, P., et al (1997). Dose response effects of growth hormone on auxological and biochemical parameters in GH deficient children. *Endocrinol Metab*, *4S*, 59.
737. Ranke, M. B., Schweizer, R., Wollmann, H. A., & Schwarze, P. (1999). Dosing of growth hormone in growth hormone deficiency. *Horm Res*, *51*(Suppl. 3), 70–74.
738. Südfeld, H., Kiese, K., Heinecke, A., & Brämswig, J. H. (2000). Prediction of growth response in prepubertal children treated with growth hormone for idiopathic growth hormone deficiency. *Acta Paediatr*, *89*, 34–37.
739. Blethen, S. L., Baptista, J., Kuntze, J., et al. (1997). Adult height in growth hormone (GH)-deficient children treated with biosynthetic GH. The Genentech Growth Study Group. *J Clin Endocrinol Metab*, *82*, 418–420.
740. Mauras, N., Attie, K. M., Reiter, E. O., et al. (2000). High dose recombinant human growth hormone (GH) treatment of GH-deficient patients in puberty increases near-final height: a randomized, multicenter trial. Genentech, Inc., Cooperative Study Group. *J Clin Endocrinol Metab*, *85*, 3653–3660.
741. Stanhope, R., Uruena, M., Hindmarsh, P., et al. (1991). Management of growth hormone deficiency through puberty. *Acta Paediatr Scand Suppl*, *372*, 47–52; discussion 53.
742. Bowers, C. Y. (1996). Xenobiotic growth hormone secretagogues: growth hormone releasing peptides. In B. B. Bercu, & R. F. Walker (Eds.), *Growth hormone secretagogues* (pp. 9–28). New York: Springer.
743. Svensson, J. A., & Bengtsson, B. (1999). Clinical and experimental effects of growth hormone secretagogues on various organ systems. *Horm Res*, *51* (Suppl. 3), 16–20.
744. Ghigo, E., Arvat, E., & Camanni, F. (1998). Orally active growth hormone secretagogues: state of the art and clinical perspectives. *Ann Med*, *30*, 159–168.
745. Thorner, M., Rochiccioli, P., Colle, M., et al. (1996). Once daily subcutaneous growth hormone-releasing hormone therapy accelerates growth in growth hormone-deficient children during the first year of therapy. Geref International Study Group. *J Clin Endocrinol Metab*, *81*, 1189–1196.
746. Duck, S. C., & Rapaport, R. (1999). Long-term treatment with GHRH[1-44] amide in prepubertal children with classical growth hormone deficiency. *J Pediatr Endocrinol Metab*, *12*, 531–536.
747. Lippe, B. M., Van Herle, A. J., LaFranchi, S. H., et al. (1975). Reversible hypothyroidism in growth hormone-deficient children treated with human growth hormone. *J Clin Endocrinol Metab*, *40*, 612–618.
748. Guthrie, R. D., Smith, D. W., & Graham, C. B. (1973). Testosterone treatment for micropenis during early childhood. *J Pediatr*, *83*, 247–252.
749. Juul, A. (1999). Determination of insulin-like growth factor-I in the monitoring of growth hormone treatment with respect to efficacy of treatment and side effects: should potential risks of cardiovascular disease and cancer be considered? *Horm Res*, *51*(Suppl. 3), 141–148.
750. Cowell, C. T., & Baxter, R. C. (1993). The response of the insulin-like growth factor binding protein 3 complex to growth hormone. *Clin Pediatr Endocrinol*, *2*, 45.
751. Cohen, P., Rogol, A. D., Howard, C. P., et al. (2007). Effects of IGF-based dosing on the efficacy of GH therapy in children: results of a randomized concentration-controlled study. *J Clin Endocrinol Metab*, *92*, 2480–2486.
752. Shim, M., & Cohen, P. (1999). IGFs and human cancer: implications regarding the risk of growth hormone therapy. *Horm Res*, *51*(Suppl. 3), 42–51.
753. Ranke, M. B. (1995). Growth hormone therapy in children: when to stop? *Horm Res*, *43*, 122–125.
754. Kaufman, R., & Sy, J. P. (1999). Regular monitoring of bone age is useful in children treated with growth hormone. *Pediatrics*, *104*(4 Pt. 2), 1039–1042.
755. Wilson, D. M. (1999). Regular monitoring of bone age is not useful in children treated with growth hormone. *Pediatrics*, *104* (4 Pt. 2), 1036–1039.
756. Guidelines for the use of growth hormone in children with short stature. A report by the Drug and Therapeutics Committee of the Lawson Wilkins Pediatric Endocrine Society. (1995). *J Pediatr*, *127*, 857–867.
757. Retegui, L. A., Masson, P. L., & Paladini, A. C. (1985). Specificities of antibodies to human growth hormone (hGH) in patients treated with hGH: longitudinal study and comparison with the specificities of animal antisera. *J Clin Endocrinol Metab*, *60*, 184–190.
758. Burns, E. C., Tanner, J. M., Preece, M. A., & Cameron, N. (1981). Final height and pubertal development in 55 children with idiopathic growth hormone deficiency, treated for between 2 and 15 years with human growth hormone. *Eur J Pediatr*, *137*, 155–164.
759. Dean, H. J., McTaggart, T. L., Fish, D. G., & Friesen, H. G. (1985). The educational, vocational, and marital status of growth hormone-deficient adults treated with growth hormone during childhood. *Am J Dis Child*, *139*, 1105–1110.
760. Salomon, F., Cuneo, R. C., Hesp, R., & Sönksen, P. H. (1989). The effects of treatment with recombinant human growth hormone on body composition and metabolism in adults with growth hormone deficiency. *N Engl J Med*, *321*, 1797–1803.
761. Bengtsson, B. A., Edén, S., Lönn, L., et al. (1993). Treatment of adults with growth hormone (GH) deficiency with recombinant human GH. *J Clin Endocrinol Metab*, *76*, 309–317.
762. Rosen, T., & Bengtsson, B. A. (1990). Premature mortality due to cardiovascular disease in hypopituitarism. *Lancet*, *336*, 285–288.
763. Sönksen, P. H., Cuneo, R. C., Salomon, F., et al. (1991). Growth hormone therapy in adults with growth hormone deficiency. *Acta Paediatr Scand Suppl*, *379*, 139–146; discussion 147.
764. Mardh, G., Lundin, K., Borg, B., et al. (1994). Growth hormone replacement therapy in adult hypopituitary patients with growth hormone deficiency: combined data from 12 European placebo-controlled clinical trials. *Endocrinol Metab*, *1(A)*.
765. Toogood, A. A., Beardwell, C. G., & Shalet, S. M. (1994). The severity of growth hormone deficiency in adults with pituitary disease is related to the degree of hypopituitarism. *Clin Endocrinol* (Oxf), *41*, 511–516.
766. Consensus guidelines for the diagnosis and treatment of adults with growth hormone deficiency: summary statement of the Growth Hormone Research Society Workshop on Adult Growth Hormone Deficiency. (1998). *J Clin Endocrinol Metab*, *83*, 379–381.
767. Tönshoff, B., Mehis, O., Heinrich, U., et al. (1990). Growth-stimulating effects of recombinant human growth hormone in children with end-stage renal disease. *J Pediatr*, *116*, 561–566.
768. Hokken-Koelega, A. C., Stijnen, T., de Muinck Keizer-Schrama, S. M., et al. (1991). Placebo-controlled, double-blind, cross-over trial of growth hormone treatment in prepubertal children with chronic renal failure. *Lancet*, *338*, 585–590.
769. Fine, R. N., Kohaut, E. C., Brown, D., & Perlman, A. J. (1994). Growth after recombinant human growth hormone treatment in children with chronic renal failure: report of a multicenter randomized double-blind placebo-controlled study. Genentech Cooperative Study Group. *J Pediatr*, *124*, 374–382.
770. Nissel, R., Lindberg, A., Mehls, O., et al. (2008). Factors predicting the near-final height in growth hormone-treated children and adolescents with chronic kidney disease. *J Clin Endocrinol Metab*, *93*, 1359–1365.
771. Rudman, D., Goldsmith, M., Kutner, M., & Blackston, D. (1980). Effect of growth hormone and oxandrolone singly and together on growth rate in girls with X chromosome abnormalities. *J Pediatr*, *96*, 132–135.
772. Forbes, A. P., Jacobsen, J. G., Carroll, E. L., & Pechet, M. M. (1962). Studies of growth arrest in gonadal dysgenesis: response to exogenous human growth hormone. *Metabolism*, *11*, 56–75.

773. Rosenfeld, R. G., Frane, J., Attie, K. M., et al. (1992). Six-year results of a randomized, prospective trial of human growth hormone and oxandrolone in Turner syndrome. *J Pediatr*, *121*, 49–55.
774. Chernausek, S. D., Attie, K. M., Cara, J. F., et al. (2000). Growth hormone therapy of Turner syndrome: the impact of age of estrogen replacement on final height. Genentech, Inc., Collaborative Study Group. *J Clin Endocrinol Metab*, *85*, 2439–2445.
775. Stephure, D. K., & Canadian Growth Hormone Advisory (2005). Impact of growth hormone supplementation on adult height in turner syndrome: results of the Canadian randomized controlled trial. *J Clin Endocrinol Metab*, *90*, 3360–3366.
776. van Pareren, Y. K., de Muinck Keizer-Schrama, S. M., Stijnen, T., et al. (2003). Final height in girls with turner syndrome after long-term growth hormone treatment in three dosages and low dose estrogens. *J Clin Endocrinol Metab*, *88*, 1119–1125.
777. Binder, G. (2011). Short stature due to SHOX deficiency: genotype, phenotype, and therapy. *Horm Res Paediatr*, *75*, 81–89.
778. Blum, W. F., Crowe, B. J., Quigley, C. A., et al. (2007). Growth hormone is effective in treatment of short stature associated with short stature homeobox-containing gene deficiency: two-year results of a randomized, controlled, multicenter trial. *J Clin Endocrinol Metab*, *92*, 219–228.
779. Blum, W. F., Cao, D., Hesse, V., et al. (2009). Height gains in response to growth hormone treatment to final height are similar in patients with SHOX deficiency and Turner syndrome. *Horm Res*, *71*, 167–172.
780. Osio, D., Dahlgren, J., Wikland, K. A., & Westphal, O. (2005). Improved final height with long-term growth hormone treatment in Noonan syndrome. *Acta Paediatr*, *94*, 1232–1237.
781. Noordam, C., Peer, P. G., Francois, I., et al. (2008). Long-term GH treatment improves adult height in children with Noonan syndrome with and without mutations in protein tyrosine phosphatase, non-receptor-type 11. *Eur J Endocrinol*, *159*, 203-208.
782. Romano, A. A., Dana, K., Bakker, B., et al. (2009). Growth response, near-adult height, and patterns of growth and puberty in patients with noonan syndrome treated with growth hormone. *J Clin Endocrinol Metab*, *94*, 2338–2344.
783. Annerén, G., Sara, V. R., Hall, K., & Tuvemo, T. (1986). Growth and somatomedin responses to growth hormone in Down's syndrome. *Arch Dis Child*, *61*, 48–52.
784. Torrado, C., Bastian, W., Wisniewski, K. E., & Castells, S. (1991). Treatment of children with Down syndrome and growth retardation with recombinant human growth hormone. *J Pediatr*, *119*, 478–483.
785. Annerén, G., Tuvemo, T., & Gustafsson, J. (2000). Growth hormone therapy in young children with Down syndrome and a clinical comparison of Down and Prader-Willi syndromes. *Growth Horm IGF Res*, *10*, 87–91.
786. Chatelain, P. G. (1993). Auxology and response to growth hormone treatment of patients with intrauterine growth retardation or Silver-Russell syndrome: analysis of data from the Kabi Pharmacia International Growth Study. International Board of the Kabi Pharmacia International Growth Study. *Acta Paediatr Suppl*, *82*(Suppl. 391), 79–81.
787. de Zegher, F., Maes, M., Heinrichs, C., et al. (1994). High-dose growth hormone therapy for short children born small for gestational age. Belgian Study Group for Paediatric Endocrinology. *Acta Paediatr Suppl*, *399*, 77; discussion 78.
788. Coutant, R., Carel, J. C., Letrait, M., et al. (1998). Short stature associated with intrauterine growth retardation: final height of untreated and growth hormone-treated children. *J Clin Endocrinol Metab*, *83*, 1070–1074.
789. Clayton, P. E., Cianfarani, S., Czernichow, P., et al. (2007). Management of the child born small for gestational age through to adulthood: a consensus statement of the International Societies of Pediatric Endocrinology and the Growth Hormone Research Society. *J Clin Endocrinol Metab*, *92*, 804–810.
790. de Zegher, F., Albertsson-Wikland, K., Wollmann, H. A., et al. (2000). Growth hormone treatment of short children born small for gestational age: growth responses with continuous and discontinuous regimens over 6 years. *J Clin Endocrinol Metab*, *85*, 2816–2821.
791. Van Pareren, Y., Mulder, P., Houdijk, M., et al. (2003). Adult height after long-term, continuous growth hormone (GH) treatment in short children born small for gestational age: results of a randomized, double-blind, dose-response GH trial. *J Clin Endocrinol Metab*, *88*, 3584–3590.
792. Maiorana, A., & Cianfarani, S. (2009). Impact of growth hormone therapy on adult height of children born small for gestational age. *Pediatrics*, *124*, e519–e531.
793. Lindgren, A. C., Hagenäs, L., Müller, J., et al. (1998). Growth hormone treatment of children with Prader-Willi syndrome affects linear growth and body composition favourably. *Acta Paediatr*, *87*, 28–31.
794. Carrel, A. L., Myers, S. E., Whitman, B. Y., & Allen, D. B. (2002). Benefits of long-term GH therapy in Prader-Willi syndrome: a 4-year study. *J Clin Endocrinol Metab*, *87*, 1581–1585.
795. Allen, D. B., & Carrel, A. L. (2004). Growth hormone therapy for Prader-Willi syndrome: a critical appraisal. *J Pediatr Endocrinol Metab*, *17*(Suppl. 4), 1297–306.
796. Tauber, M. (2007). Effects of growth hormone treatment in children presenting with Prader-Willi syndrome: the KIGS experience. In M. B. Ranke, D. A. Price, & E. O. Reiter (Eds.), *Growth hormone therapy in pediatrics: 20 years of KIGS* (pp. 377–387). Basel: Karger.
797. Festen, D. A., Wevers, M., Lindgren, A. C., et al. (2008). Mental and motor development before and during growth hormone treatment in infants and toddlers with Prader-Willi syndrome. *Clin Endocrinol* (Oxf), *68*, 919–925.
798. Goldstone, A. P., Holland, A. J., Hauffa, B. P., et al. (2008). Recommendations for the diagnosis and management of Prader-Willi syndrome. *J Clin Endocrinol Metab*, *93*, 4183–4197.
799. Seino, Y., Yamate T, Kanzaki, S., et al. (1994). Achondroplasia: effect of growth hormone in 40 patients. *Clin Pediatr Endocrinol*, *3*(4).
800. Hertel, N. T., Eklöf, O., Ivarsson, S., et al. (2005). Growth hormone treatment in 35 prepubertal children with achondroplasia: a five-year dose-response trial. *Acta Paediatr*, *94*, 1402–1410.
801. Tanaka, N., Katsumata, N., Horikawa, R., & Tanaka, T. (2003). The comparison of the effects of short-term growth hormone treatment in patients with achondroplasia and with hypochondroplasia. *Endocr J*, *50*, 69–75.
802. Kelnar, C. J., Albertsson-Wikland, K., Hintz, R. L., et al. (1999). Should we treat children with idiopathic short stature? *Horm Res*, *52*, 150–157.
803. Van Vliet, G., Styne, D. M., Kaplan, S. L., & Grumbach, M. M. (1983). Growth hormone treatment for short stature. *N Engl J Med*, *309*, 1016–1022.
804. Gertner, J. M., Genel, M., Gianfredi, S. P., et al. (1984). Prospective clinical trial of human growth hormone in short children without growth hormone deficiency. *J Pediatr*, *104*, 172–176.
805. Hopwood, N. J., Hintz, R. L., Gertner, J. M., et al. (1993). Growth response of children with non-growth-hormone deficiency and marked short stature during three years of growth hormone therapy. *J Pediatr*, *123*, 215–222.
806. Hintz, R. L., Attie, K. M., Baptista, J., & Roche, A. (1999). Effect of growth hormone treatment on adult height of children with idiopathic short stature. Genentech Collaborative Group. *N Engl J Med*, *340*, 502–507.
807. Ranke, M. B., Lindberg, A., Price, D. A., et al. (2007). Age at growth hormone therapy start and first-year responsiveness to growth hormone are major determinants of height outcome in idiopathic short stature. *Horm Res*, *68*, 53–62.
808. Allen, D. B., Brook, C. G., Bridges, N. A., et al. (1994). Therapeutic controversies: growth hormone (GH) treatment of non-GH deficient subjects. *J Clin Endocrinol Metab*, *79*, 1239–1248.
809. Rosenfeld, R. G., & Buckway, C. K. (2000). Should we treat genetic syndromes? *J Pediatr Endocrinol Metab*, *13*(Suppl 2), 971-81.
810. Allen, D. B., & Fost, N. C. (1990). Growth hormone therapy for short stature: panacea or Pandora's box? *J Pediatr*, *117*(1 Pt. 1), 16–21.
811. Herber, S. M., & Dunsmore, I. R. (1988). Does control affect growth in diabetes mellitus? *Acta Paediatr Scand*, *77*, 303–305.
812. Ergun-Longmire, B., Mertens, A. C., Mitby, P., et al. (2006). Growth hormone treatment and risk of second neoplasms in the childhood cancer survivor. *J Clin Endocrinol Metab*, *91*, 3494–3498.
813. Malozowski, S., Tanner, L. A., Wysowski, D., & Fleming, G. A. (1993). Growth hormone, insulin-like growth factor I, and benign intracranial hypertension. *N Engl J Med*, *329*, 665–666.

814. Harris, W. R., (1950). The endocrine basis for slipping of the upper femoral epiphysis, an experimental study. *J Bone Joint Surg*, 5B–32B.
815. Kelsey, J. L. (1973). Epidemiology of slipped capital femoral epiphysis: a review of the literature. *Pediatrics*, *51*, 1042–1050.
816. Rappaport, E. B., & Fife, D. (1985). Slipped capital femoral epiphysis in growth hormone-deficient patients. *Am J Dis Child*, *139*, 396–399.
817. Growth Hormone Research Society (2000). Consensus guidelines for the diagnosis and treatment of growth hormone (GH) deficiency in childhood and adolescence: summary statement of the GH Research Society. GH Research Society. *J Clin Endocrinol Metab*, *85*, 3990–3993.
818. Alford, F. P., Hew, F. L., Christopher, M. C., & Rantzau, C. (1999). Insulin sensitivity in growth hormone (GH)-deficient adults and effect of GH replacement therapy. *J Endocrinol Invest*, *22*(Suppl. 5), 28–32.
819. Cutfield, W. S., Wilton, P., Bennmarker, H., et al. (2000). Incidence of diabetes mellitus and impaired glucose tolerance in children and adolescents receiving growth-hormone treatment. *Lancet*, *355*, 610–613.
820. Wetterau, L., & Cohen, P. (2000). New paradigms for growth hormone therapy in children. *Horm Res*, *53*(Suppl. 3), 31–36.
821. Chan, J. M., Stampfer, M. J., Giovannucci, E., et al. (1998). Plasma insulin-like growth factor-I and prostate cancer risk: a prospective study. Science, 279, 563–566.
822. Hankinson, S. E., Willett, W. C., Colditz, G. A., et al. (1998). Circulating concentrations of insulin-like growth factor-I and risk of breast cancer. *Lancet*, *351*, 1393–1396.
823. Ma, J., Pollak, M. N., Giovannucci, E., Chan, J. M., et al. (1999). Prospective study of colorectal cancer risk in men and plasma levels of insulin-like growth factor (IGF)-I and IGF-binding protein-3. *J Natl Cancer Inst*, *91*, 620–625.
824. Ron, E., Gridley, G., Hrubec, Z., et al. (1991). Acromegaly and gastrointestinal cancer. *Cancer*, *68*, 1673–1677.
825. Popovic, V., Damjanovic, S., Micic, D., et al. (1998). Increased incidence of neoplasia in patients with pituitary adenomas. The Pituitary Study Group. *Clin Endocrinol* (Oxf), *49*, 441–445.
826. Bengtsson, B. A., Edén, S., Ernest, I., et al. (1988). Epidemiology and long-term survival in acromegaly. A study of 166 cases diagnosed between 1955 and 1984. *Acta Med Scand*, *223*, 327–335.
827. Delhougne, B., Deneux, C., Abs, R., et al. (1995). The prevalence of colonic polyps in acromegaly: a colonoscopic and pathological study in 103 patients. *J Clin Endocrinol Metab*, *80*, 3223–3226.
828. Ladas, S. D., Thalassinos, N. C., Ioannides, G., & Raptis, S. A. (1994). Does acromegaly really predispose to an increased prevalence of gastrointestinal tumours? *Clin Endocrinol* (Oxf), *41*, 597–601.
829. Orme, S. M., McNally, R. J., Cartwright, R. A., & Belchetz, P. E. (1998). Mortality and cancer incidence in acromegaly: a retrospective cohort study. United Kingdom Acromegaly Study Group. *J Clin Endocrinol Metab*, *83*, 2730–2734.
830. Sönksen, P., Jacobs, H., Orme, S., & Belchetz, P. (1997). Acromegaly and colonic cancer. *Clin Endocrinol* (Oxf), *47*, 647–648.
831. Renehan, A. G., O'dwyer, S. T., & Shalet, S. M. (2000). Colorectal neoplasia in acromegaly: the reported increased prevalence is overestimated. *Gut*, *46*, 440–441.
832. Colao, A., Balzano, A., Ferone, D., et al. (1997). Increased prevalence of colonic polyps and altered lymphocyte subset pattern in the colonic lamina propria in acromegaly. *Clin Endocrinol* (Oxf), *47*, 23–28.
833. Nishi, Y., Tanaka, T., Takano, K., et al. (1999). Recent status in the occurrence of leukemia in growth hormone-treated patients in Japan. GH Treatment Study Committee of the Foundation for Growth Science, Japan. *J Clin Endocrinol Metab*, *84*, 1961–1965.
834. Swerdlow, A. J., Reddingius, R. E., Higgins, C. D., et al. (2000). Growth hormone treatment of children with brain tumors and risk of tumor recurrence. *J Clin Endocrinol Metab*, *85*, 4444–4449.
835. Abs, R., Bengtsson, B. A., Hernberg-Stâhl, E., et al. (1999). GH replacement in 1034 growth hormone deficient hypopituitary adults: demographic and clinical characteristics, dosing and safety. *Clin Endocrinol* (Oxf), *50*, 703–713.
836. Tuffli, G. A., Johanson, A., Rundle, A. C., & Allen, D. B. (1995). Lack of increased risk for extracranial, nonleukemic neoplasms in recipients of recombinant deoxyribonucleic acid growth hormone. *J Clin Endocrinol Metab*, *80*, 1416–1422.
837. Cohen P, Clemmons, D. R., & Rosenfeld, R. G. (2000). Does the GH-IGF axis play a role in cancer pathogenesis? *Growth Horm IGF Res*, *10*, 297–305.
838. Guler, H. P., Schmid, C., Zapf, J., & Froesch, E. R. (1989). Effects of recombinant insulin-like growth factor I on insulin secretion and renal function in normal human subjects. *Proc Natl Acad Sci USA*, *86*, 2868–2872.
839. Laron, Z., Klinger, B., Erster, B., & Anin, S. (1988). Effect of acute administration of insulin-like growth factor I in patients with Laron-type dwarfism. *Lancet*, *2*, 1170–1172.
840. Chernausek, S. D., Backeljauw, P. F., Frane, J., et al. (2007). Long-term treatment with recombinant insulin-like growth factor (IGF)-I in children with severe IGF-I deficiency due to growth hormone insensitivity. *J Clin Endocrinol Metab*, *92*, 902–910.
841. Laron, Z., Anin, S., Klipper-Aurbach, Y., & Klinger, B. (1992). Effects of insulin-like growth factor on linear growth, head circumference, and body fat in patients with Laron-type dwarfism. *Lancet*, *339*, 1258–1261.
842. Walker, J. L., Van Wyk, J. J., & Underwood, L. E. (1992). Stimulation of statural growth by recombinant insulin-like growth factor I in a child with growth hormone insensitivity syndrome (Laron type). *J Pediatr*, *121*, 641–646.
843. Wilton, P. (1992). Treatment with recombinant human insulin-like growth factor I of children with growth hormone receptor deficiency (Laron syndrome). Kabi Pharmacia Study Group on Insulin-like Growth Factor I Treatment in Growth Hormone Insensitivity Syndromes. *Acta Paediatr Suppl*, *383*, 137–142.
844. Savage, M. O., Wilton P., & Ranke, M. B. (1993). Therapeutic response to recombinant IGF-I in thirty-two patients with growth hormone insensitivity. *Pediatr Res*, *33*, 5.
845. Guevara-Aguirre, J., Vasconez, O., Martinez, V., et al. (1995). A randomized, double blind, placebo-controlled trial on safety and efficacy of recombinant human insulin-like growth factor-I in children with growth hormone receptor deficiency. *J Clin Endocrinol Metab*, *80*, 1393–1398.
846. Ranke, M. B., Savage, M. O., Chatelain, P. G., et al. (19999). Long-term treatment of growth hormone insensitivity syndrome with IGF-I. Results of the European Multicentre Study. The Working Group on Growth Hormone Insensitivity Syndromes. *Horm Res*, *51*, 128–134.
847. Backeljauw, P. F., Untze, J., Frane, J. W., et al (2009). Final and near-final adult height in patients with severe primary insulin-like growth factor-I deficiency (IGFD) after long-term treatment with recombinant human insulin-like growth factor-I (rhIGF-I). *Horm Res*, *72*(Suppl. 3), 46, 2009.
848. Midyett, L. K., Rogol, A. D., Van Meter, Q. L., et al. (2010). Recombinant insulin-like growth factor (IGF)-I treatment in short children with low IGF-I levels: first-year results from a randomized clinical trial. *J Clin Endocrinol Metab*, *95*, 611–619.
849. Midyett, L. K., Rogol, A. D., et al. (2009). Preliminary results of a phase II, randomized, open-label, active-treatment controlled trial of rh GH & rhIGF-I combination therapy in prepubertal children with nonsevere primary IGF-I deficiency. 8th Joint LWPES/ESPE Congress, New York. Abstract FC10-006. *Horm Res*, *Vol. 72*(Suppl. 3), 47.
850. Morison, I. M., Becroft, D. M., Taniguchi, T., et al. (1996). Somatic overgrowth associated with overexpression of insulin-like growth factor II. *Nat Med*, *2*, 311–316.
851. Weksberg, R., & Shuman C. (2000). Beckwith-Wiedemann syndrome and hemihypertrophy. In J. E. Allanson, & S. B. Cassidy (Eds.), *Management of genetic syndromes* (2nd ed.) (pp. 101–116). New York: John Wiley & Sons.
852. Weksberg, R., Shuman, C., & Smith, A. C. (2005). Beckwith-Wiedemann syndrome. *Am J Med Genet C Semin Med Genet*, *137C*, 12–23.
853. Zollino, M., Orteschi, D., Marangi, G., et al. (2010). A case of Beckwith-Wiedemann syndrome caused by a cryptic 11p15 deletion encompassing the centromeric imprinted domain of the BWS locus. *J Med Genet*, *47*, 429–432.
854. Shuman, C., Smith, A. C., Steele, L., et al. (2006). Constitutional UPD for chromosome 11p15 in individuals with isolated hemihyperplasia is associated with high tumor risk and occurs

following assisted reproductive technologies. *Am J Med Genet A*, *140*, 1497–1503.
855. Choufani, S., Shuman, C., & Weksberg, R. (2010). Beckwith-Wiedemann syndrome. *Am J Med Genet C Semin Med Genet*, *154C*, 343–354.
856. Sakazume, S., Okamoto, N., Yamamoto, T., et al. (2007). GPC3 mutations in seven patients with Simpson-Golabi-Behmel syndrome. *Am J Med Genet A*, *143A*, 1703–1707.
857. Saugier-Veber, P., Bonnet, C., Afenjar, A., et al. (2007). Heterogeneity of NSD1 alterations in 116 patients with Sotos syndrome. *Hum Mutat*, *28*, 1098–1107.
858. Sabin, M. A., Werther, G. A., & Kiess, W. (2011). Genetics of obesity and overgrowth syndromes. *Best Pract Res Clin Endocrinol Metab*, *25*, 207–220.
859. Lindhurst, M. J., Sapp, J. C., Teer, J. K., et al. (2011). A mosaic activating mutation in AKT1 associated with the Proteus syndrome. *N Engl J Med*, *365*, 611–619.
860. Sorgo, W., Scholler, K., Heinze, F., et al. (1984). Critical analysis of height reduction in oestrogen-treated tall girls. *Eur J Pediatr*, *142*, 260–265.
861. Reinehr, T., Gueldensupp, M., Wunsch, R., & Bramswig, J. H. (2011). Treatment of tall stature in boys: comparison of two different treatment regimens. *Horm Res Paediatr*, *76*, 343–347.
862. Chu, S., & Geary, K. (2005). Physical stature influences character perception in women. *Pers Individ Dif*, *38*, 1927–1934.
863. Thapar, K., Kovacs, K., Stefaneanu, L., et al. (1997). Overexpression of the growth-hormone-releasing hormone gene in acromegaly-associated pituitary tumors: an event associated with neoplastic progression and aggressive behavior. *Am J Pathol*, *151*, 769–784.
864. Fuqua, J. S., & Berkovitz, G. D. (1998). Growth hormone excess in a child with neurofibromatosis type 1 and optic pathway tumor: a patient report. *Clin Pediatr* (Phila), *37*, 749–752.
865. Stratakis, C. A., Carney, J. A., Lin, J. P., et al. (1996). Carney complex, a familial multiple neoplasia and lentiginosis syndrome. Analysis of 11 kindreds and linkage to the short arm of chromosome 2. *J Clin Invest*, *97*, 699–705.
866. Stratakis, C. A., Tichomirowa, M. A., Boikos, S., et al. (2010). The role of germline AIP, MEN1, PRKAR1A, CDKN1B and CDKN2C mutations in causing pituitary adenomas in a large cohort of children, adolescents, and patients with genetic syndromes. *Clin Genet*, *78*, 457–463.
867. Holl, R. W., Bucher, P., Sorgo, W., et al. (1999). Suppression of growth hormone by oral glucose in the evaluation of tall stature. *Horm Res*, *51*, 20–24.
868. Abe, T., Tara, L. S., & Ludecke, D. K. (1999). Growth hormone-secreting pituitary adenomas in childhood and adolescence: features and results of transnasal surgery. *Neurosurgery*, *45*, 1–10.
869. Näntö-Salonen, K., Koskinen, P., Sonninen, P., & Toppari, J. (1999). Suppression of GH secretion in pituitary gigantism by continuous subcutaneous octreotide infusion in a pubertal boy. *Acta Paediatr*, *88*, 29–33.
870. Moran, A., & Pescovitz, O. (1994). Long-term treatment of gigantism with combination octreotide and bromocriptine in a child with McCune-Albright syndrome. *Endocr J*, *2*, 111–113.
871. Sandret, L., Maison, P., & Chanson, P. (2011). Place of cabergoline in acromegaly: a meta-analysis. *J Clin Endocrinol Metab*, *96*, 1327–1335.
872. Trainer, P. J., Drake, W. M., Katznelson, L., et al. (2000). Treatment of acromegaly with the growth hormone-receptor antagonist pegvisomant. *N Engl J Med*, *342*, 1171–1177.
873. Herman-Bonert, V. S., Zib, K., Scarlett, J. A., & Melmed, S. (2000). Growth hormone receptor antagonist therapy in acromegalic patients resistant to somatostatin analogs. *J Clin Endocrinol Metab*, *85*, 2958–2961.
874. Bergamaschi, S., Ronchi, C. L., Giavoli, C., et al. (2010). Eight-year follow-up of a child with a GH/prolactin-secreting adenoma: efficacy of pegvisomant therapy. *Horm Res Paediatr*, *73*, 74–79.
875. Kelberman, D., & Dattani, M. T. (2006). The role of transcription factors implicated in anterior pituitary development in the aetiology of congenital hypopituitarism. *Ann Med*, *38*, 560–577.
876. Rimoin, D. L., Cohn, D., Krakow, D., et al. (2007). The skeletal dysplasias: clinical-molecular correlations. *Ann NY Acad Sci*, *1117*, 302–309.
877. Rosenfeld, R. G., & Hwa, V. (2004). New molecular mechanisms of GH resistance. *Eur J Endocrinol*, *151*(Suppl. 1), S11–S15.
878. Kofoed, E. M., Hwa, V., Little, B., et al. (2003). Growth-hormone insensitivity associated with a STAT5b mutation. *N Engl J Med*, *349*, 1139-1147.
879. Hwa, V., Little, B., Adiyaman, P., et al. (2005). Severe growth hormone insensitivity resulting from total absence of signal transducer and activator of transcription 5b. *J Clin Endocrinol Metab*, *90*, 4260–4426.
880. Bernasconi, A., Marino, R., Ribas, A., et al. (2006). Characterization of immunodeficiency in a patient with growth hormone insensitivity secondary to a novel STAT5b gene mutation. *Pediatrics*, *118*, e1584–e1592.
881. Boyanovsky, A., Lozano, A., Testa, G., et al. (2009). Growth hormone insensitivity and immunodeficiency: mutation in the STAT5B gene. *Proceedings of the 8th Joint Meeting of the Lawson Wilkins Pediatric Endocrine Society/European Society for Paediatric Endocrinology, New York* (Abstract P01-067).
882. Vidarsdottir, S., Walenkamp, M. J., Pereira, A. M., et al. (2006). Clinical and biochemical characteristics of a male patient with a novel homozygous STAT5b mutation. *J Clin Endocrinol Metab*, *91*, 3482–3485.
883. Hwa, V., Camacho-Hübner, C., Little, B. M., et al. (2007). Growth hormone insensitivity and severe short stature in siblings: a novel mutation at the exon 13-intron 13 junction of the STAT5b gene. *Horm Res*, *68*, 218–224.
884. Martinez, A., Scaglia, P. A., Rivas, M., et al. (2007). Novel STAT5b gene mutation in a patient presenting GH insensitivity and immunodeficiency. *Horm Res*, *68*(Suppl. 1), 33.
885. Pugliese-Pires, P. N., Tonelli, C. A., Dora, J. M., et al. (2010). A novel STAT5B mutation causing GH insensitivity syndrome associated with hyperprolactinemia and immune dysfunction in two male siblings. *Eur J Endocrinol*, *163*, 349–355.
886. Domené, H. M., Bengolea, S. V., Martinez, A. S., et al. (2004). Deficiency of the circulating insulin-like growth factor system associated with inactivation of the acid-labile subunit gene. *N Engl J Med*, *350*, 570–577.
887. Domené, H. M., Scaglia, P. A., Lteif, A., et al. (2007). Phenotypic effects of null and haploinsufficiency of acid-labile subunit in a family with two novel IGFALS gene mutations. *J Clin Endocrinol Metab*, *92*, 4444–4450.
888. Heath, K. E., Argente, J., Barrios, V., et al. (2008). Primary acid-labile subunit deficiency due to recessive IGFALS mutations results in postnatal growth deficit associated with low circulating insulin growth factor (IGF)-I, IGF binding protein-3 levels, and hyperinsulinemia. *J Clin Endocrinol Metab*, *93*, 1616–1624.
889. van Duyvenvoorde, H. A., van Doorn, J., Koenig, J., et al. (2011). The severe short stature in two siblings with a heterozygous IGF1 mutation is not caused by a dominant negative effect of the putative truncated protein. *Growth Horm IGF Res*, *21*, 44–50.
890. Kawashima, Y., Kanzaki, S., Yang, F., et al. (2005). Mutation at cleavage site of insulin-like growth factor receptor in a short-stature child born with intrauterine growth retardation. *J Clin Endocrinol Metab*, *90*, 4679–4687.
891. Walenkamp, M. J., van der Kamp, H. J., Pereira, A. M., et al. (2006). A variable degree of intrauterine and postnatal growth retardation in a family with a missense mutation in the insulin-like growth factor I receptor. *J Clin Endocrinol Metab*, *91*, 3062–3470.

QUESTIONS

1. You are seeing a 2.8-year-old boy for severe growth failure. His mother reports he has a good appetite, but he often wakes up once at night to take a bottle. His exam reveals frontal bossing, midface hypoplasia, and micrognathia. He also has small hands and feet. His abdomen is slightly protuberant, with a fat distribution that is more pronounced on his trunk than on his extremities. You are very concerned about his growth and obtain laboratory tests that include the following:
   - Random GH = 10 ng/mL
   - IGF-I = 11 ng/mL (normal 31 to 160)
   - IGFBP-3 = 0.7 mg/L (normal 1.4 to 3)
   - ALS, acid-labile subunit = 0.2 mg/L (normal 0.9 to 9.3)
   - GHBP = 59 pmol/L (normal 125 to 762)
   - Prolactin = 13 ng/mL (normal 3 to 18)
   - Free T4 = 2.2 ng/dL (normal 1 to 2.8)
   - TSH = 2.45 μIU/mL (normal 0.5 to 4)

   What is the most likely diagnosis for this patient?
   a. IGF-I gene defect
   b. GH gene defect
   c. STAT5 B defect
   d. ALS defect
   e. GH receptor defect

   Answer: e

2. An 8-year-old boy is referred by his pediatrician because of an advanced skeletal age. The film was obtained because the pediatrician was worried about rapid growth in this patient. The boy's mother states that her son has always been tall compared to his classmates. Midparental height is at the 50th to 75th percentile. The boy has mild developmental delay with some speech impairment. On physical exam, his height and weight are both at +3 SD score. He has a flat nasal bridge with a wide tip, large ears, broad philtrum, and a small chin. His skin is relatively loose. He has deep-set nails. He has clinodactyly and overriding toes. There is limited extension at the elbows and the knees, mild kyphosis, and obvious scoliosis by inspection. He underwent an orchidopexy early in life. Small testes are palpable in the scrotal sac. This boy's constellation of features is most consistent with which of the following diagnoses?
   a. Weaver syndrome
   b. Fragile X syndrome
   c. Beckwith-Wiedemann syndrome
   d. Marfan syndrome
   e. McCune-Albright syndrome

   Answer: a

3. You are preparing a lecture for medical students addressing genetic abnormalities that result in combined pituitary hormone deficiency. Which of the following does *not* lead to this multiple hormone insufficiency?
   a. PROP1 gene mutation
   b. HESX1 gene mutation
   c. STAT5B gene mutation
   d. LHX3 gene mutation
   e. POU1F1 gene mutation

   Answer: c

4. The measurement of serum IGF-I concentrations, as part of the evaluation of short stature, can be affected by all *except* which of the following factors?
   a. Estradiol
   b. Melatonin
   c. Growth hormone
   d. Thyroxine
   e. Testosterone

   Answer: b

5. The parents of a 6-year-old girl want to know how tall their daughter will be as an adult. The mother is 155 cm (5′1″). The father is 178 cm (5′10″). Despite all the nuances involved in the determination of the predicted adult height that you plan to discuss with them, you calculate her midparental height to be closest to which of the following?
   a. 155 cm
   b. 168 cm
   c. 165 cm
   d. 160 cm
   e. 175 cm

   Answer: d

CHAPTER 11

Disorders of the Posterior Pituitary

Joseph A. Majzoub, MD • Louis J. Muglia, MD, PhD • Abhinash Srivatsa, MD

CHAPTER OUTLINE

INTRODUCTION

Maintenance of the tonicity of extracellular fluids within a very narrow range is crucial for proper cell function.[1,2] Extracellular osmolality regulates cell shape as well as intracellular concentrations of ions and other osmolytes. Furthermore, proper extracellular ionic concentrations are necessary for the correct function of ion channels, action potentials, and other modes of intercellular communication. Extracellular fluid tonicity is regulated almost exclusively by the amount of water intake and excretion, whereas extracellular volume is regulated by the level of sodium chloride intake and excretion. In children and adults, normal blood tonicity is maintained over a 10-fold variation in water intake by a coordinated interaction among thirst, vasopressin, and renal systems. Dysfunction in any of these systems can result in abnormal regulation of blood osmolality, which if not properly recognized and treated may cause life-threatening dysfunction in neuronal and other cellular activities.

The posterior pituitary, or the neurohypophysis, secretes the nonapeptide hormones vasopressin and oxytocin. Vasopressin controls water homeostasis, and oxytocin regulates smooth muscle contraction during parturition and lactation. Disorders of vasopressin secretion and action lead to clinically important derangements in water metabolism. This chapter summarizes the physiology of water and volume regulation, presents a symptom-based approach to the differential diagnosis of the diseases of water homeostasis, and provides a review of the pathology and treatment of disorders involving these systems. (See Chapter 13 for a discussion of defects in mineralocorticoid regulation that result in disturbances in volume regulation.)

PHYSIOLOGY OF OSMOTIC AND VOLUME REGULATION

The control of plasma tonicity and intravascular volume involves a complex integration of endocrine, neural, and paracrine pathways. Osmotic sensor and effector pathways control the regulation of vasopressin release and signal transduction, whereas volume homeostasis is determined largely through the action of the renin-angiotensin-aldosterone system, with contributions from both vasopressin and the natriuretic peptide family. An improved understanding of the anatomic structures and molecules involved has developed through detailed molecular biologic and physiologic studies.

Osmotic Sensor and Effector Pathways

Vasopressin and Oxytocin Biochemistry

Vasopressin and oxytocin are evolutionarily related peptides (paralogs), having arisen from gene duplication of a phylogenetically common molecule approximately 450 million years ago.[3,4] Both peptides consist of a 6-amino-acid disulfide ring plus a 3-amino-acid tail, with amidation of the carboxy terminus.[5,6] As early as 1895, a potent biologic principle—consisting of vascular pressor activity, "birth quickening," and milk secretory effects—was recognized in neurohypophyseal extracts.[7] The sequences of the

individual peptides with pressor and antidiuretic capacity (vasopressin) and oxytocic capacity were determined by du Vigneaud and colleagues during the mid-1950s,[6] culminating in the synthesis of each hormone in its biologically active form.[8,9] In most mammals, vasopressin and oxytocin differ in only two amino acids—one substitution within the ring and one within the tail structure (Figure 11-1). Exploration of the structure-function relationship of specific amino acids within both vasopressin and oxytocin has allowed characterization of molecules with substantial clinical use. Most notably, by replacement of l-arginine with d-arginine at position 8 of the vasopressin molecule, and amino-terminal deamidation, an analog with enhanced, prolonged antidiuretic to pressor activity was found (desmopressin [desamino-d-arginine vasopressin; dDAVP], see Figure 11-1).[10] Desmopressin, with an antidiuretic potency more than double that of its parent vasopressin, is now routinely used in clinical practice.

The association of vasopressin and oxytocin with specific proteins, the neurophysins, while stored in the neurohypophysis, was apparent as early as 1900.[11] Subsequent isolation and characterization of the neurophysins revealed two distinct forms, one type exclusively associated with vasopressin and the other exclusively associated with oxytocin.[12,13] Both are single-polypeptide chains of molecular weight 10,000 daltons. Despite extensive biophysical characterization, including crystallography of the oxytocin-neurophysin complex,[14,15] the biologic function of the neurophysins remains unclear. Possible roles for the neurophysins include stabilization against degradation during intracellular storage, more efficient packaging within secretory granules, and enhancement of posttranslation processing by the proenzyme convertases.

The common origin of vasopressin and its neurophysin from a single larger precursor was first proposed by Sachs and colleagues,[16,17] who showed increased incorporation of ^{35}S cysteine, infused into the canine third ventricle, into vasopressin isolated from the hypothalamus compared with vasopressin isolated from the posterior pituitary. Isolation of the larger precursor from the hypothalamus followed by trypsin digestion produced fragments of size similar to that of vasopressin and its neurophysin, with vasopressin immunoreactivity in the 1000-dalton component.[18,19]

Since 1990, molecular genetic analyses have furthered the understanding of the synthesis, the processing, and the evolution of the vasopressin and oxytocin preprohormones. Human, mouse, rat, and bovine vasopressin and oxytocin genes each consist of three exons (Figure 11-2).[20,21] The first exon encodes the 19-amino-acid signal peptide, followed by vasopressin or oxytocin nonapeptides. This is followed by a 3-amino-acid protease cleavage site leading into the first nine amino acids of neurophysin II (for vasopressin) or neurophysin I (for oxytocin). After interruption of the coding region by an intron, exon 2 continues with neurophysin coding sequences. The third exon completes the sequence of the neurophysin and, for vasopressin only, is followed by coding information for an additional 39-amino-acid glycopeptide (copeptin) whose function is unclear. Preprovasopressin contains 16 cysteines, which likely participate in eight disulfide bridges that determine the tertiary structure of the protein (Figure 11-3). One cysteine pair is present in vasopressin peptide, whereas the rest are in neurophysin.

In all mammalian species analyzed thus far, oxytocin and vasopressin genes are adjacent in chromosomal location (chromosome 20 in the human[22]) and linked tail to tail, in opposite transcriptional orientation. In the human, they are separated by 12 kb.[22] This likely explains their origin from the ancient duplication of a common ancestral gene.[23] Whether this adjacent linkage is of regulatory significance is under investigation.

Vasopressin and oxytocin genes are expressed in the hypothalamic paraventricular and supraoptic nuclei.[18,24] The magnocellular components of each of these nuclei are the primary neuronal populations involved in water balance, with vasopressin synthesized in these areas carried by means of axonal transport to the posterior pituitary, its primary site of storage and release into the systemic circulation (Figure 11-4). The bilaterally paired hypothalamic paraventricular and supraoptic nuclei are separated from one another by relatively large distances (approximately 1 cm). Their axons course caudally, converge

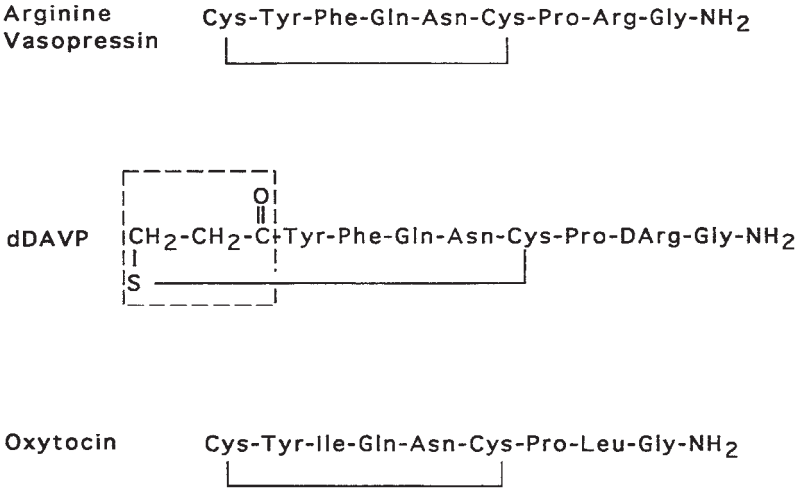


FIGURE 11-1 ■ Structures of vasopressin, dDAVP, and oxytocin. In dDAVP, the de-amidated cysteine is enclosed in the box.

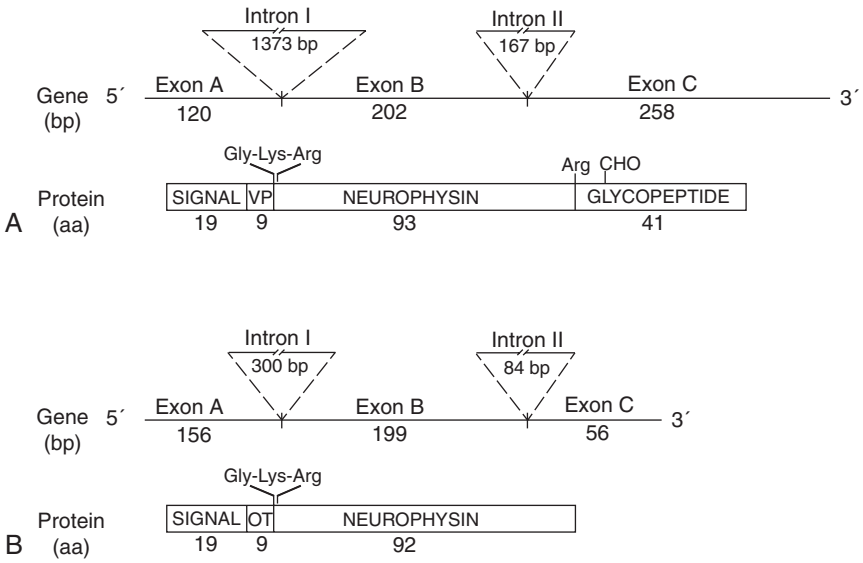


FIGURE 11-2 ■ Structure of the human genes and peptide products of vasopressin (VP) (**A**) and oxytocin (OT) (**B**). Shown are the sizes of exons and intron, in nucleotide base pairs (bp) and peptide products in amino acids (aa). Depicted are the amidation-dibasic cleavage signal (Gly-Lys-Arg) at the carboxy terminus of vasopressin and oxytocin and the monobasic cleavage signal at the end of neurophysin. Signal, signal peptide; VP, vasopressin; OT, oxytocin; CHO, carbohydrate.

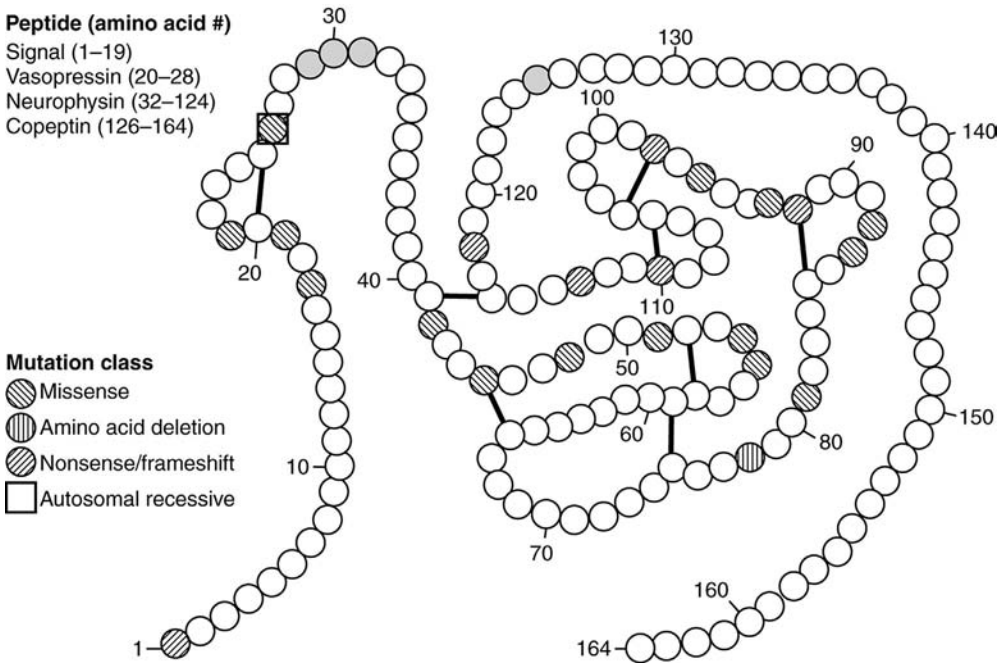


FIGURE 11-3 ■ Structure of preprovasopressin peptide. The 164-amino-qcid preprovasopressin peptide consists of signal peptide, vasopressin, neurophysin, and copeptin. The latter three entities are separated by basic residues (gray), which serve as cleavage sites for proconvertase enzymes. The 16 cysteines are connected by eight putative disulfide bridges. Amino acid mutations are classified as missense, in-frame deletion, or nonsense/frameshift mutations. Most mutations are inherited with an autosomal dominant pattern. The one that has an autosomal recessive pattern is boxed. (Reproduced with permission from *Uyeki TM, Barry FL, Rosenthal SM, et al.* Successful treatment with hydrochlorothiazide and amiloride in an infant with congenital nephrogenic diabetes insipidus. *Pediatr Nephrol 1993;* 7:554-6.)

at the infundibulum, and terminate at different levels within the pituitary stalk and the posterior pituitary gland (see Figure 11-4). Vasopressin is also synthesized in the parvocellular neurons of the paraventricular nucleus, where it has a role in modulating hypothalamic-pituitary-adrenal axis activity. In this site, vasopressin is colocalized in cells that synthesize corticotropin-releasing hormone,[25,26] and both are secreted at the median eminence and carried through the portal-hypophyseal capillary system to the anterior pituitary, where together they act as the major regulators of adrenocorticotropic hormone synthesis and release.[27] Vasopressin is also present in the hypothalamic suprachiasmatic nucleus, the circadian pacemaker of the body, where its function is unknown.

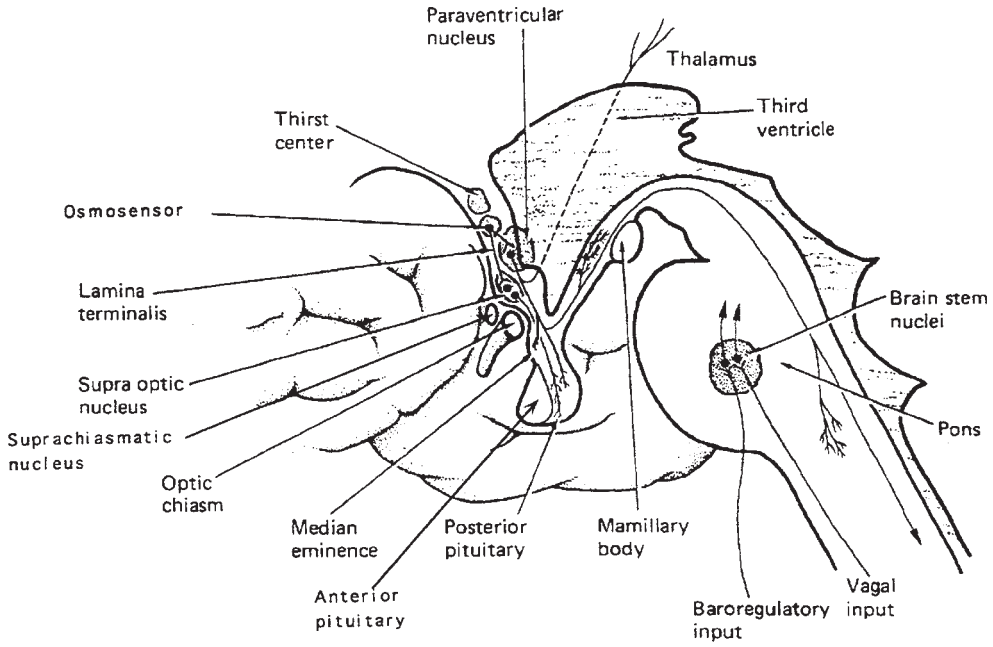


FIGURE 11-4 ■ Vasopressin cells in the hypothalamus. Diagram of vasopressin cell bodies in the supraoptic, paraventricular, and suprachiasmatic hypothalamic nuclei, and axonal termination in the posterior pituitary and median eminence. Because vasopressin axons terminate at different levels in the pituitary stalk and posterior pituitary, the amount of permanent cell loss following neurosurgical insult is determined by the highest level of damage, which will dictate the degree of vasopressin axon transection and retrograde neuronal degeneration. (Modified with permission from reference 507 Baylis, P. H. (1989). Vasopressin and its neurophysin. In L. D. Degroot (Ed.), *Endocrinology*, (2nd ed.) (p. 213). Philadelphia: WB Saunders.)

Regulation of Vasopressin Secretion and Thirst

Osmotic Regulation. The rate of secretion of vasopressin from the paraventricular and supraoptic nuclei is influenced by several physiologic variables, including plasma osmolality and intravascular volume, as well as nausea and a number of pharmacologic agents. The major osmotically active constituents of blood are sodium, chloride, and glucose (with insulin deficiency). Normal blood osmolality ranges between 280 and 290 mOsm/kg H_2O.

The work of Verney[28] first demonstrated the relationship of increased vasopressin release in response to increasing plasma osmolality, as altered by infusion of sodium chloride or sucrose. At that time, it was postulated that there existed intracranial sensors sensitive to changes in plasma osmolality. Multiple researchers have subsequently confirmed that plasma vasopressin concentration increases in response to increasing plasma tonicity, although the exact nature of the osmosensor has not been defined.[29,30] Neurons of the supraoptic nucleus can respond directly to hypertonic stimuli with depolarization and vasopressin secretion,[31] but the majority of evidence indicates that osmosensor- and vasopressin-secreting neurons are anatomically distinct.[32,33] The osmosensor is likely to reside outside the blood-brain barrier, as implicated by differential vasopressin secretory response to similar changes in plasma osmolality depending on whether the change was induced by salt, sucrose, or urea.[28,34] The organ vasculosum of the lamina terminalis (OVLT) and the subfornical organ (SFO), areas of the preoptic hypothalamus outside the blood-brain barrier, are likely sites of osmosensing, because lesions of the OVLT result in impaired vasopressin secretion and hypernatremia.[32,33] Also, the site of action of angiotensin II infused intracerebrally or peripherally to produce vasopressin secretion and antidiuresis resides within the OVLT.[35-37]

The pattern of secretion of vasopressin into blood has been characterized extensively in normal individuals and in those with abnormalities in water homeostasis. Normally, at a serum osmolality of less than 280 mOsm/kg, plasma vasopressin concentration is at or below 1 pg/mL, the lower limit of detection of most radioimmunoassays.[29,30] Above 283 mOsm/kg—the normal threshold for vasopressin release—plasma vasopressin concentration increases in proportion to plasma osmolality, up to a maximum concentration of about 20 pg/mL at a blood osmolality of approximately 320 mOsm/kg (Figure 11-5). The osmosensor can detect as little as a 1% change in blood osmolality. Plasma concentrations in excess of 5 pg/mL are also found with nausea, hypotension, hypovolemia, and insulin-induced hypoglycemia, but further increments in urine concentration do not occur, because the peak antidiuretic effect is achieved at 5 pg/mL. The rate of increase of plasma vasopressin concentration, and thus the sensitivity of the osmosensor, exhibits substantial (as much as 10-fold) interindividual variation as plasma osmolality increases.[38] The set point for vasopressin secretion varies in a single individual in relation to changes in volume status and hormonal environment (e.g., pregnancy[39]) or glucocorticoid status.[40,41] After the seventh week of gestation, osmotic thresholds for both vasopressin release and thirst are reduced by

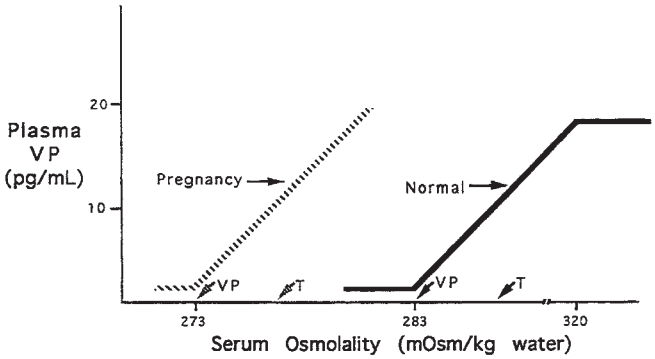


FIGURE 11-5 ■ Osmotic thresholds for vasopressin and thirst. The threshold for vasopressin release is below that for thirst. In nonpregnant persons there is linear increase in vasopressin (VP) release up to a serum osmolality of 320 mOsm/kg, after which no further increase occurs. In pregnancy, there is a decreased threshold for vasopressin release and thirst sensation, with no change in the sensitivity (slope) of the vasopressin-osmolality relationship. Vasopressin secretion in pregnancy presumably also plateaus at some level of hyperosmolality, although this has not been studied. Normal nonpregnant persons indicated by solid line and arrows; pregnant women indicated by dashed line and arrows.

approximately 10 mOsm/kg (see Figure 11-5), such that normal blood osmolality during pregnancy is approximately 273 mOsm/kg (serum sodium 135 mEq/L).[39,42] Similarly, thresholds for vasopressin release and thirst during the luteal phase of the menstrual cycle are approximately 5 mOsm/kg lower than those in the follicular phase.[43,44] Human chorionic gonadotropin during pregnancy[45] and luteinizing hormone during the second half of the menstrual cycle may contribute to these changes in osmotic thresholds.

The sensation of thirst, a more integrated cortical activity, is determined by other anatomically distinct hypothalamic neurons, with afferents involving the ventromedial nucleus.[46] The activation of the thirst mechanism is also probably mediated by angiotensin II.[47] Whether the osmosensor for thirst and vasopressin release are the same is not certain, although this is suggested by lesions in the anteroventral region of the third ventricle that abolish both thirst sensation and vasopressin release.[48] It makes physiologic sense that the threshold for thirst (293 mOsm/kg) is approximately 10 mOsm/kg higher than that for vasopressin release (see Figure 11-5). Otherwise, during the development of hyperosmolality, the initial activation of thirst and water ingestion would result in polyuria without activation of vasopressin release, causing a persistent diuretic state. Immediately after water ingestion, before a change in blood osmolality or volume, vasopressin concentration falls and thirst ceases.[49] The degree of suppression is directly related to the coldness[50] and volume[51] of the ingested fluid. This effect is probably mediated by chemoreceptors present in the oropharynx, which guard against the rapid overdrinking of fluids after intense thirst during the time before the lowering of blood osmolality.

As noted earlier, water balance is regulated in two ways: (1) vasopressin secretion stimulates water reabsorption by the kidney, thereby reducing future water loss, and (2) thirst stimulates water ingestion, thereby restoring previous water loss. Ideally, these two systems work in parallel to efficiently regulate extracellular fluid tonicity (Figure 11-6); however, each system by itself can maintain plasma osmolality in the near-normal range. For example, in the absence of vasopressin secretion but with free access to water, thirst drives water ingestion up to the 5 to 10 L/m^2 of urine output seen with vasopressin deficiency. Conversely, an intact vasopressin secretory system can compensate for some degree of disordered thirst regulation. When both vasopressin secretion and thirst are compromised, however, by either disease or iatrogenic means, there is great risk of the occurrence of life-threatening abnormalities in plasma osmolality.

Nonosmotic Regulation. Separate from osmotic regulation, vasopressin has been shown to be secreted in response to alterations in intravascular volume. Afferent baroreceptor pathways arising from the right and left atria and the aortic arch (carotid sinus) are stimulated by increasing intravascular volume and stretch of vessel walls, and they send signals through the vagus and glossopharyngeal nerves, respectively, to the brainstem nucleus tractus solitarius.[52,53] Noradrenergic fibers from the nucleus tractus solitarius synapse on the hypothalamic paraventricular nucleus and the supraoptic nucleus and, on stimulation, inhibit vasopressin secretion.[54,55] Experimental verification of this pathway has included demonstration of increased vasopressin concentration after interruption of baroreceptor output to the brainstem and decreased plasma vasopressin concentration after mechanical stimulation of baroreceptors, an effect diminished by vagotomy.[56,57]

The pattern of vasopressin secretion in response to volume as opposed to osmotic stimuli is markedly different (Figure 11-7). Although minor changes in plasma

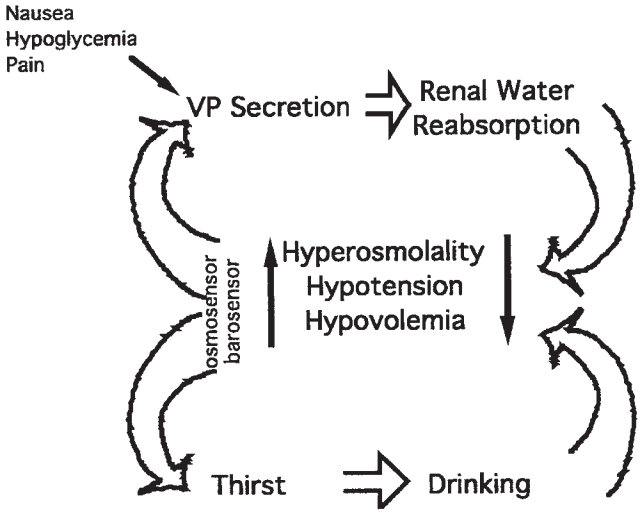


FIGURE 11-6 ■ Regulation of vasopressin secretion and serum osmolality. Hyperosmolality, hypovolemia, or hypotension are sensed by osmosensors, volume sensors, or barosensors, respectively. These stimulate both vasopressin (VP) secretion and thirst. Vasopressin, acting on the kidney, causes increased reabsorption of water (antidiuresis). Thirst causes increased water ingestion. The results of these dual negative feedback loops cause a reduction in hyperosmolality or hypotension/hypovolemia. Additional stimuli for vasopressin secretion include nausea, hypoglycemia, and pain.

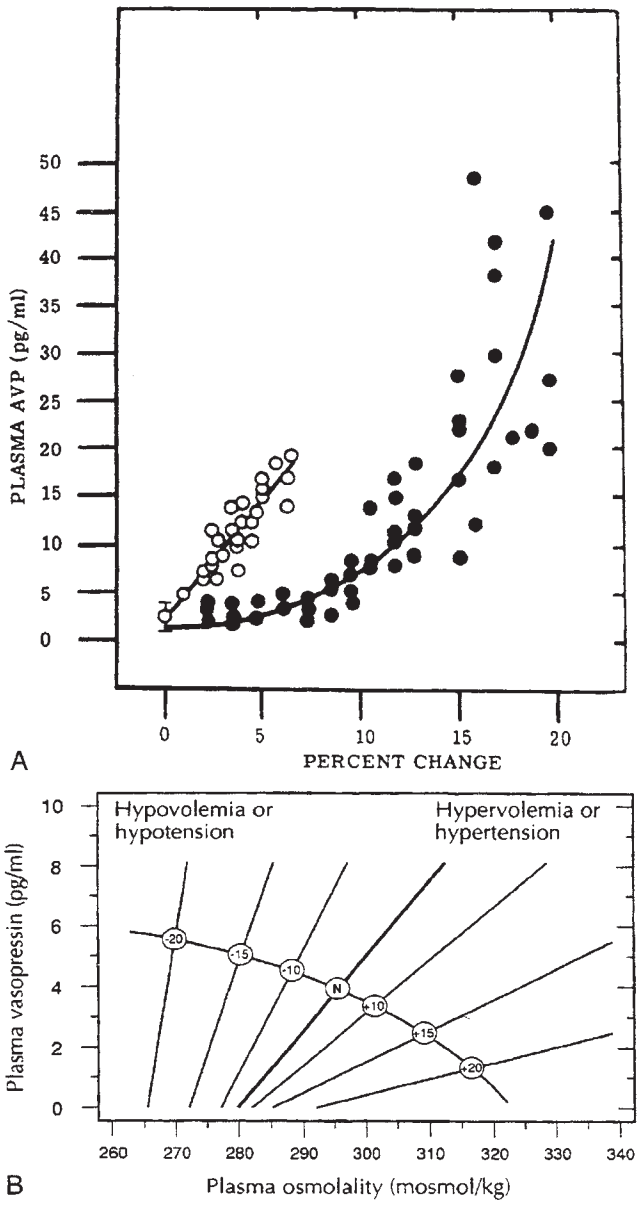


FIGURE 11-7 ■ Relationships between osmotic and nonosmotic stimuli for vasopressin release. **A,** Relationship of plasma vasopressin (AVP) concentration to the percentage increase in blood osmolality (open circles) or decrease in blood volume (closed circles). **B,** Alteration of sensitivity of osmotic stimulation of vasopressin secretion by volume or pressure stimuli. (Reproduced with permission from referene (508) Dunn Brennan, T. J., Nelson, A. E., et al. (1973). The role of blood osmolality and volume regulating vasopressin secretion in the rat. *J Clin Invest, 52,* 3212; reference (509) Robertson, G. L. (1985). *The kidney, physiology and pathophysiology* Regulation of vasopressin secretion. In D. W. Seldin, & G. Giebisch (Eds.), New York: Raven Press.) (p. 869).

function of several tissue types by binding to members of a family of G protein–coupled cell surface receptors, which subsequently transduce ligand binding into alterations of intracellular second messenger pathways.[79] Biochemical and cell biologic studies have defined at least three receptor types, designated V1, V2, and V3 (or V1b). The major sites of V1 receptor expression are on vascular smooth muscle[80] and hepatocytes,[81-84] where receptor activation results in vasoconstriction[85,86] and osmolality above 280 mOsm/kg evoke linear increases in plasma vasopressin, substantial alteration in intravascular volume is required for alteration in vasopressin output.[58-60] No change in vasopressin secretion is seen until blood volume decreases by approximately 8%. With intravascular volume deficits exceeding 8%, vasopressin concentration increases exponentially. Furthermore, osmotic and hemodynamic stimuli can interact in a mutually synergistic fashion, so that the response to either stimulus may be enhanced by the concomitant presence of the other (see Figure 11-7). When blood volume (or blood pressure[61-63]) decreases by approximately 25%, vasopressin concentrations are evident of 20- to 30-fold above normal and vastly exceeding those required for maximal antidiuresis. Surprisingly, the use of vasopressin antagonists has suggested that the high concentration of vasopressin observed with hypotension does not contribute to the maintenance of blood pressure in humans.[64]

Nausea—as evoked by apomorphine,[65] motion sickness,[66] and vasovagal reactions—is a very potent stimulus for vasopressin secretion. This effect is likely mediated by afferents from the area postrema of the brainstem and may result in vasopressin concentrations two to three orders of magnitude above basal levels. Nicotine is also a strong stimulus for vasopressin release.[67] These pathways probably do not involve osmotic or hemodynamic sensor systems, because blockade of the emetic stimulus with dopamine or opioid antagonists does not alter the vasopressin response to hypernatremia or hypovolemia.

Vasopressin secretion is inhibited by glucocorticoids; for this reason, loss of negative regulation of vasopressin secretion occurs in the setting of primary or secondary glucocorticoid insufficiency.[68,69] The effects of cortisol loss of both enhancing hypothalamic vasopressin production and directly impairing free water excretion[70] are important considerations in the evaluation of the patient with hyponatremia, as is subsequently discussed.

Vasopressin Metabolism

Once in the circulation, vasopressin has a half-life of only 5 to 10 minutes, owing to its rapid degradation by a cysteine amino-terminal peptidase called vasopressinase. A synthetic analog of vasopressin, desmopressin, is insensitive to aminoterminal degradation and thus has a much longer half-life of 8 to 24 hours. During pregnancy, the placenta secretes increased amounts of this vasopressinase,[71] resulting in a fourfold increase in the metabolic clearance rate of vasopressin.[72] Normal women compensate with an increase in vasopressin secretion, but women with preexisting deficits in vasopressin secretion or action,[73] or those with increased concentrations of placental vasopressinase associated with liver dysfunction[74] or multiple gestations,[75] may develop diabetes insipidus in the last trimester, which resolves in the immediate postpartum period.[76] As expected, this form of diabetes insipidus responds to treatment with desmopressin but not with vasopressin.[77,78]

Sites of Vasopressin Action

Vasopressin Receptors. Vasopressin released from the posterior pituitary and the median eminence affects the

glycogenolysis,[87] respectively. The latter activity may be augmented by stimulation of glucagon secretion from the pancreas.[87] The V1 receptor on platelets also stimulates platelet aggregation.[88] V1 receptor activation mobilizes intracellular calcium stores through phosphatidylinositol hydrolysis.[86,89] Despite its initial characterization as a powerful pressor agent, the concentration of vasopressin needed to significantly increase blood pressure is several-fold higher than that required for maximal antidiuresis,[90] although substantial vasoconstriction in renal and splanchnic vasculature can occur at physiologic concentrations.[91] The cloning of the V1 receptor[80,81,83] has greatly elucidated the relationship of the vasopressin (and oxytocin[92,93]) receptors and, through sensitive in situ hybridization analysis, has further localized V1 expression to the liver and the vasculature of the renal medulla, as well as to many sites within the brain, including the hippocampus, the amygdala, the hypothalamus, and the brainstem.[82,84] Compared to their normal counterparts, mice genetically modified to be deficient in the V1 receptor (V1a KO) have been found to manifest insulin resistance, increased hepatic glucose production, decreased hepatic glycogen content, decreased aldosterone secretion despite a lower plasma volume, lower basal blood pressure, a greater degree of lipolysis, and impaired nuclear transport of the renal tubular mineralocorticoid receptor.[94] The V3 (or V1b) receptor is present on corticotrophs in the anterior pituitary[95] and acts through the phosphatidylinositol pathway[96] to increase adrenocorticotropic hormone secretion. Its binding profile for vasopressin analogs resembles more closely that of the V1 than the V2 receptor. The structure of this receptor has been determined in humans by cloning of its complementary DNA.[96,97] Its structure is similar to that of the V1 and oxytocin receptors, and it is expressed in the kidney as well as in the pituitary. Mice with deletion of the V1b (V3) receptor gene (V1bKO) have been created and studied.[97-99] As expected, they have defective activation of the pituitary-adrenal axis following some acute and chronic stressors. Male V1bKO mice were also found to have decreased aggression and social motivation. [100]

Modulation of water balance occurs through the action of vasopressin on V2 receptors located primarily in the renal collecting tubule, along with other sites in the kidney, including the thick ascending limb of the loop of Henle and periglomerular tubules.[82,84,101] It is also present on vascular endothelial cells in some systemic vascular beds, where vasopressin stimulates vasodilation,[102] possibly through the activation of nitric oxide synthase.[103] Vasopressin also stimulates von Willebrand factor, factor VIIIa, and tissue plasminogen activator through V2-mediated actions. For this reason, desmopressin is used to improve the prolonged bleeding times characteristic of uremia, type I von Willebrand disease, and hemophilia.[104] The V2 receptor consists of 370 amino acids encoding seven transmembrane domains characteristic of the G protein–coupled receptors.[101,105] These transmembrane domains share approximately 60% sequence identity with the V1 receptor but substantially less with other members of this family (Figure 11-8). Unlike the V1 and

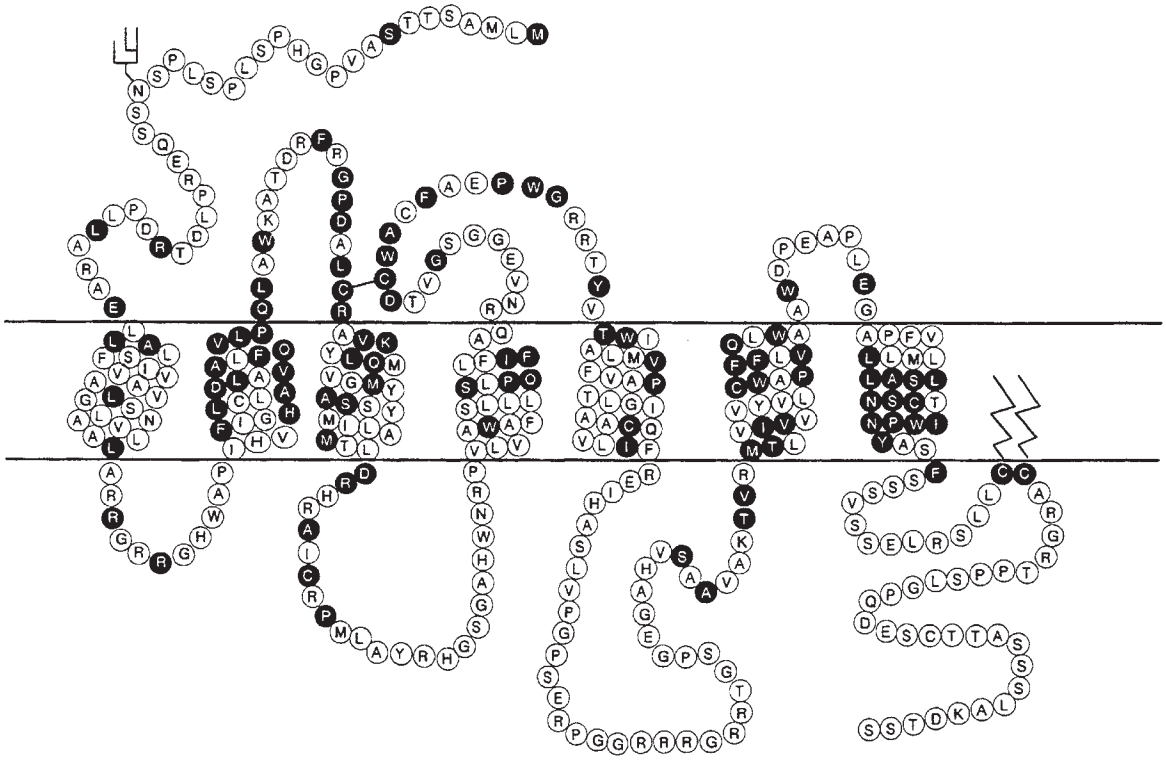
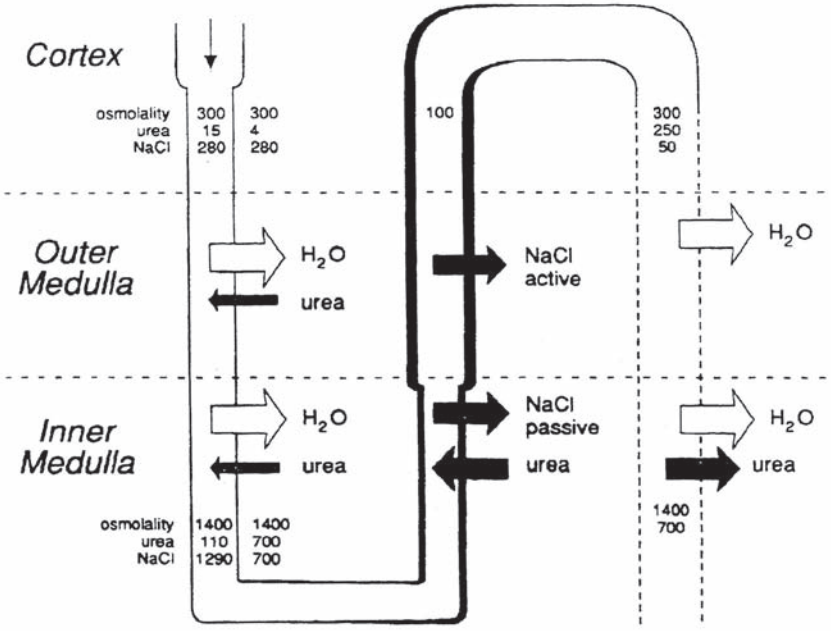


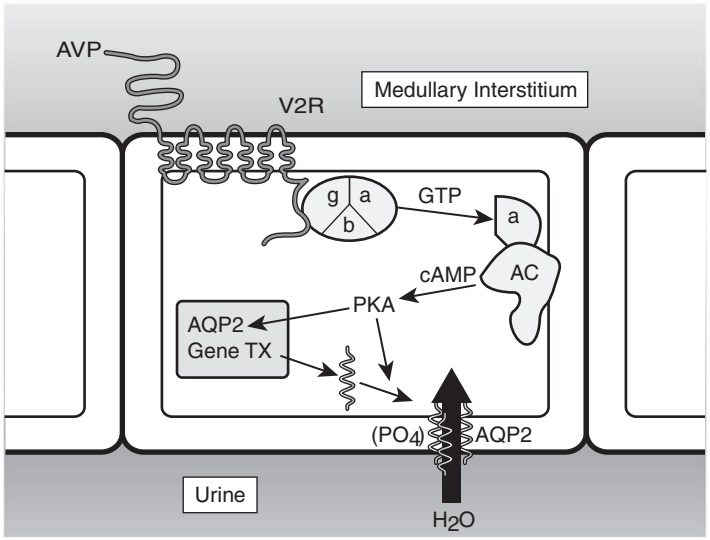
FIGURE 11-8 ■ Structure of the V1 and V2 vasopressin receptors and the oxytocin receptor. Depicted are predicted membrane topology, with the extracellular domain at the top of the figure and amino acids in the one-letter code. Amino acids in open circles encode the V1 receptor, whereas those in black circles are common to all three receptors. (Reproduced with permission from reference (510) Bichet, D. G. (1995). The posterior pituitary. In S. Melmed (Ed.), *The pituitary* (p. 277). Cambridge: Blackwell Science.)

V3 receptors, the V2 receptor acts through adenylate cyclase to increase intracellular cyclic adenosine monophosphate (AMP) concentration. The human V2 receptor gene is located on the long arm of the X chromosome (Xq28),[106,107] at the locus associated with congenital, X-linked vasopressin-resistant diabetes insipidus. Mice in which *V2R* has been deleted have a similar phenotype. [108]

Renal Cascade of Vasopressin Function. Vasopressin-induced increases in intracellular cyclic AMP as mediated by the V2 receptor triggers a complex pathway of events resulting in increased permeability of the collecting duct to water and efficient water transit across an otherwise minimally permeable epithelium (Figure 11-9).[109] Activation of a cyclic AMP-dependent protein kinase imparts



A



B

FIGURE 11-9 ■ Vasopressin action in the kidney. **A,** Solute and water handling in the kidney. **B,** Action of vasopressin in the collecting duct cell. Vasopressin (AVP) binds to the V2 receptor (V2R), causing the binding of GTP to the stimulatory alpha G protein subunit (α). This activates adenylate cyclase (AC), resulting in an increase in cAMP and activation of protein kinase A (PKA). The catalytic subunit of PKA, via phosphorylation of serine 256 of the water channel, aquaporin-2 (AQP2), causes aggregation of AQP2 homotetramers in membrane vesicles and their fusion with the collecting duct luminal membrane, resulting in an increase in water flow from the urine into the renal medullary interstitium. Demeclocycline, lithium, high calcium, and low potassium interfere with these processes, possibly at the level of cAMP generation and AQP2 synthesis or action. (**A** from reference (511) Reeves, W. B., & Andreoli, T. E. (1989). Nephrogenic diabetes insipidus. In C. R. Scriver, A. L. *The metabolic basis of inherited disease* Beaudet, & W. S. Sly (Eds.), (6th ed.) (p. 1985) New York: McGraw-Hill.)

remodeling of cytoskeletal microtubules and microfilaments that culminate in the insertion of aggregates of water channels into the apical membrane.[110] These mechanisms may involve a vesicle-associated membrane protein-2–like protein (VAMP-2), which also regulates synaptic vesicle activity in neuronal terminals[111] and its associated receptor syntaxin-4.[112]

Insertion of the water channels causes an up to 100-fold increase in water permeability of the apical membrane, allowing water movement along its osmotic gradient into the hypertonic inner medullary interstitium from the tubule lumen and excretion of a concentrated urine (see Figure 11-9). The molecular analysis of the water channels has revealed a family of related proteins, designated aquaporins, that differ in their sites of expression and pattern of regulation.[113] Each protein consists of a single polypeptide chain with six membrane-spanning domains (Figure 11-10). Although functional as monomers, they are believed to form homotetramers in the plasma membrane.[109]

Aquaporin-2 is expressed mostly within the kidney,[114] primarily within the collecting duct.[115] It is also expressed in the vas deferens, at least in the rat, although it is not regulated by vasopressin in this location.[116] Studies with immunoelectron microscopy have demonstrated large amounts of aquaporin-2 in the apical plasma membrane and subapical vesicles of the collecting duct, consistent with the "membrane shuttling" model of water channel aggregate insertion into the apical membrane after vasopressin stimulation.[116] Studies analyzing the mechanism by which aquaporin-2 traffics to the apical plasma membrane have demonstrated that vasopressin-induced, protein kinase A–mediated serine phosphorylation at amino acid 256 is required for its exocytosis,[117] a process also requiring a heterotrimeric G protein of the G_i family.[118] In response to water restriction or desmopressin infusion in humans, the content of urinary aquaporin-2 in both soluble and membrane-bound forms has been found to increase.[119] Mice with targeted deletion of the aquaporin-2 gene have been made.[120] As expected, they have nephrogenic diabetes insipidus that is unresponsive to treatment with vasopressin.

In addition to aquaporin-2, different aquaporins appear to be involved in other aspects of renal water handling. In contrast to the apical localization of aquaporin-2, aquaporin-3 and aquaporin-4 are expressed on the basolateral membrane of the collecting duct epithelium. They appear to be involved in the flow of water and urea from the inside of the collecting duct cell into the extracellular renal medullary space. Mice made genetically deficient in aquaporin-4 demonstrate a mild urinary concentrating defect,[121] whereas those with deficiency of aquaporin-3 alone, or together with aquaporin-4, demonstrate more severely impaired urinary concentrating ability.[122] Mice made genetically deficient in aquaporin-1 demonstrate a urinary concentrating defect caused by decreased water permeability in the proximal tubule.[123]

Volume Sensor and Effector Pathways

Renin-Angiotensin-Aldosterone System

In contrast to the vasopressin system, the classic, or peripheral, renin-angiotensin system primarily affects

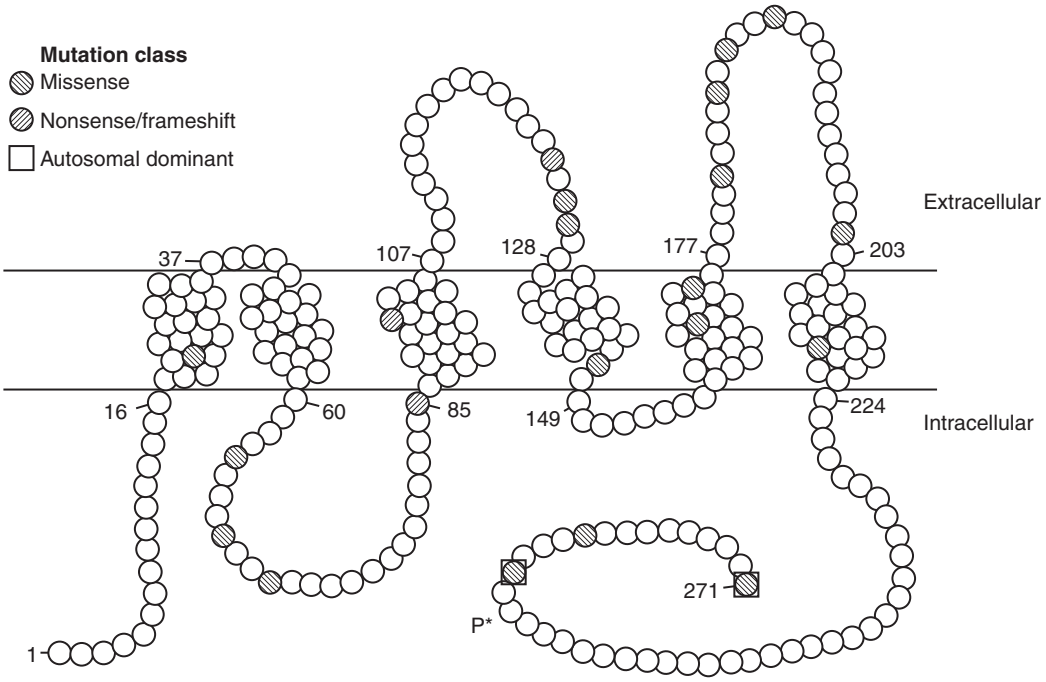


FIGURE 11-10 ■ Structure of the aquaporin-2 protein inserted into the luminal membrane of the distal tubule. The 271-amino-acid protein consists of five transmembrane domains, four intracellular domains, and three extracellular domains. Amino acid mutations are denoted by filled circles. Most mutations are transmitted with an autosomal recessive pattern; the two dominant mutations are bounded by squares. Vasopressin-dependent, protein kinase A–mediated phosphorylation of serine at amino acid 256 (P*) is noted. (Reproduced with permission from *Uyeki TM, Barry FL, Rosenthal SM, et al.* Successful treatment with hydrochlorothiazide and amiloride in an infant with congenital nephrogenic diabetes insipidus. *Pediatr Nephrol 1993;* 7:554-6.)

maintenance of intravascular volume as opposed to plasma tonicity. In addition to the well-established endocrine regulatory system, several local renin-angiotensin systems have emerged, with both autocrine and paracrine effects in their tissue of synthesis, whose regulation is independent of the classic system. Finally, brain and pituitary angiotensin systems involved in blood pressure, autonomic function, and fluid balance have been characterized with extensive interaction with the vasopressin system, and vasopressin has been found to play a role in the normal action of aldosterone on the renal tubular epithelium.[124]

Endocrine Renin-Angiotensin-Aldosterone System

Anatomy and Biochemistry. Renin, which is synthesized by the renal juxtaglomerular apparatus, is a proteolytic enzyme that catalyzes the cleavage of angiotensinogen, synthesized by hepatocytes, into the decapeptide angiotensin I.[125,126] Angiotensin I possesses no intrinsic vasoreactive or mineralocorticoid secretagogue activity but is efficiently cleaved by angiotensin-converting enzyme in the lungs, as well as other peripheral sites, to generate the octapeptide angiotensin II. Angiotensin II is further metabolized to the heptapeptide angiotensin III by removal of one aminoterminal amino acid. Angiotensin II possesses greater vasopressor activity and is present in approximately a fourfold greater amount than angiotensin III. Angiotensins II and III possess equivalent mineralocorticoid secretory activity on the adrenal glomerulosa cells.

Angiotensin II and III act through cell surface receptors (AT_1) on the adrenal glomerulosa cells to activate the phospholipase C/protein kinase C pathway.[127-131] This activation results in increased production of pregnenolone from cholesterol by side-chain cleavage enzyme (20,22-desmolase) and of aldosterone from corticosterone by the glomerulosa-specific corticosterone methyloxidase I and II activities (18-hydroxylation and dehydrogenation, respectively).[132-135] A distinct receptor subtype for angiotensin II, the AT_2 receptor, is not G protein coupled and is of unclear physiologic significance in the periphery.[136-138] Aldosterone, the primary and most potent endogenous mineralocorticoid released by the zona glomerulosa, acts on target tissues expressing the nuclear mineralocorticoid (or type I glucocorticoid) receptor to promote sodium absorption and potassium excretion. For control of intravascular volume, the primary target of action of aldosterone is the distal nephron. Here, aldosterone increases synthesis of apical membrane sodium channels, mitochondrial enzymes involved in adenosine triphosphate production, and components of Na^+,K^+ adenosine triphosphatase to increase sodium reabsorption and potassium excretion.[139]

Regulation of Secretion. Decreased intravascular volume as sensed by the renal juxtaglomerular apparatus results in release of renin.[125,140] Increased plasma renin activity then allows increased conversion of angiotensinogen to angiotensin I, which in turn is converted peripherally to angiotensins II and III. Increased angiotensin II activity causes vasoconstriction and blood pressure elevation, whereas both angiotensins II and III stimulate aldosterone release from the zona glomerulosa and subsequent salt and water retention and potassium excretion by the distal tubule of the kidney. Conversely, expanded intravascular volume causes decreased renin output and less sodium and water resorption in the kidney, serving to decrease intravascular volume and restore homeostasis.

Changes in vascular volume are not the only regulators of the renin-angiotensin-aldosterone system. Serum potassium concentration directly modulates aldosterone release by the adrenal glomerulosa by its effects on plasma membrane potential and activation of voltage-gated calcium channels.[126,141] By membrane depolarization, increased serum potassium leads to increased aldosterone synthesis, which promotes renal potassium excretion, whereas low serum potassium reduces aldosterone synthesis and decreases urinary potassium losses. Pituitary adrenocorticotropin hormone and vasopressin act through their respective receptors on the glomerulosa cells to increase acute aldosterone secretion. These effects are of short duration because long-term chronic infusions do not chronically elevate aldosterone concentrations. Direct inhibitors of aldosterone secretion, and thus promoters of natriuresis, include atrial natriuretic peptide (ANP),[142,143] somatostatin,[144-146] and dopamine.[147,148]

Local Renin-Angiotensin Systems

Anatomy and Biochemistry. In addition to the well-defined endocrine circuit, the components of the renin-angiotensin system have been found in a wide variety of tissues, including brain, pituitary, arterial wall, heart, ovary, kidney, and adrenal where paracrine and autocrine regulatory functions[149-153] have been postulated, undergoing regulation independent of the systemic counterpart. From the standpoint of regulation of water and volume homeostasis, the brain renin-angiotensin system merits further description.[154] It has long been known that peripherally synthesized angiotensin II could increase blood pressure by effects on the brain outside the blood-brain barrier, at sites such as the OVLT, SFO, area postrema, and median eminence as revealed by ligand-binding studies.[35,154-156] Since the early 2000s, it has become clear that the complete system for generation of angiotensin II is present within the brain. Angiotensinogen has been localized to astrocytes by both immunohistochemical peptide localization and in situ hybridization analysis of messenger RNA.[157] In contrast, renin has been found in high concentration in nerve terminals, with enhanced release on nerve depolarization.[158] Angiotensin-converting enzyme has been found within vascular, choroid plexus, and neuronal components of the central nervous system,[159-161] most notably the SFO and many hypothalamic nuclei, sites of endogenous angiotensin II receptor expression, primarily of the AT_1 subtype, as well as sites not expressing the angiotensin II receptor such as the basal ganglia. The primary effector molecule, angiotensin II, has been localized specifically to neurons and subcellularly to synaptic vesicles.[162] Two of the most significant sites include the circumventricular organs and the paraventricular nucleus of the hypothalamus. Within the paraventricular nucleus, angiotensin II immunoreactivity colocalizes with magnocellular vasopressin, whereas its receptors are within the parvocellular region of the paraventricular nucleus.[163]

Regulation of Secretion. The forebrain angiotensin II pathway, of which the paraventricular nucleus is one component, and circumventricular organ angiotensin II pathway are important control centers for maintenance of osmotic and volume homeostasis.[164] Increased concentration of peripheral angiotensin II, as would be expected in intravascular volume depletion, stimulates drinking behavior.[35] This action of peripheral angiotensin II can be abolished by destruction of the OVLT or SFO, regions whose destruction has long been recognized as causing adipsia.[36] Further effects of central angiotensin II action include augmentation of sodium appetite and stimulation of vasopressin release, all serving, as with peripheral angiotensin II, to restore intravascular volume and maintain blood pressure.[35] The signal of hypovolemia is transduced through the vagal nerve from volume sensors to the brainstem and the region of the nucleus tractus solitarius. Efferents from these brainstem centers project to the median preoptic nucleus and paraventricular nucleus, as does the forebrain angiotensin II pathway, where drinking and pressor effects as well as vasopressin release are elicited.[165-170]

Separate pathways for vasopressin release mediate the response to either peripheral angiotensin II or purely osmotic stimulation of the osmosensors.[171] The release of vasopressin in response to osmotic stimulation is not increased by peripheral angiotensin II, and pure osmotic stimulation does not increase salt appetite. Central angiotensin II, in contrast, may function as a transmitter in the osmosensing circuit, leading to vasopressin release.[172]

The Natriuretic Peptide System

In addition to the classic vasopressin and renin-angiotensin-aldosterone systems, the natriuretic peptide families of ligands and their receptors add further potential for the modulation of salt and water balance. The interaction of the natriuretic peptide system occurs both in the central nervous system, through effects on vasopressin secretion, and peripherally, through its ability to both directly promote natriuresis in the kidney and indirectly inhibit adrenal aldosterone production.

Anatomy and Biochemistry. Atrial natriuretic peptide was initially discovered as a component of cardiac atrial muscle that was able to induce natriuresis, a decrease in blood pressure, and an increase in hematocrit when injected into rats.[173,174]

The biologically active form of ANP consists of a 28-amino-acid peptide that includes a 17-amino-acid ring structure[175] (Figure 11-11). The primary sequence of the peptide has been conserved among mammalian species and, in addition to synthesis in cardiac atrial tissue,[176] has been detected in brain, spinal cord, pituitary, and adrenal gland.[177-180] Within the brain, ANP synthesis occurs at several critical neuroendocrine regulatory sites, including the periventricular, arcuate, anteroventral preoptic, and lateral hypothalamic nuclei.[181,182] ANP is synthesized as a 151-amino-acid preprohormone and is stored as a 126-amino-acid prohormone after removal of the signal peptide sequence.[175,183] Coupled with secretion of pro-ANP is its cleavage between amino acids 98 and 99 to yield the mature 28-amino-acid 99–126 fragment.

Subsequent investigation defined a second peptide from porcine brain with structural homology to ANP.[184] This peptide, designated brain natriuretic peptide (BNP), was later found to be secreted by the heart as well, in this case from both ventricular and atrial tissue.[185-187] Human BNP consists of a 32-amino-acid processed from a larger preprohormone[188] sharing a central ring structure with

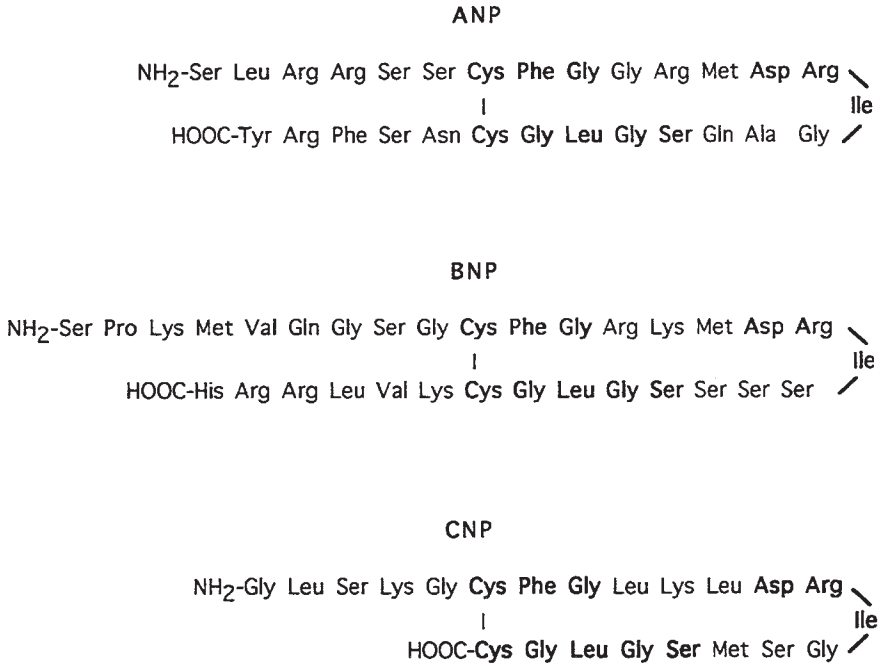


FIGURE 11-11 ■ Amino acid composition of the human natriuretic peptides. Amino acids identical between the three peptides are indicated by bold letters, and the disulfide bond between Cys residues is shown.

ANP (see Figure 11-11), although it is less conserved between species than ANP.

A third member of this family, C-type natriuretic peptide (CNP), was also isolated from porcine brain.[189] In brain, CNP is the most abundant member of the natriuretic peptide family. Within the hypothalamus, specific sites of synthesis largely overlap sites of ANP expression.[182] Little CNP can be detected in plasma, and in marked contrast to ANP and BNP, CNP does not increase in plasma in the setting of cardiac failure.[190,191] Outside the brain, CNP is synthesized in endothelial and vascular smooth muscle. In tissues capable of CNP gene expression, two forms of the peptide are produced, a 53-amino-acid peptide and a less abundant 22-amino-acid molecule[192] (see Figure 11-11).

Three distinct endogenous receptors exist for the natriuretic peptides. The first of these receptors, isolated NPR-A or GC-A, was cloned by virtue of its homology to sea urchin sperm guanylyl cyclase and was later found to have ANP and BNP as its normal ligands.[193-195] A second guanylyl cyclase type receptor (NPR-B) has substantial homology to NPR-A; however, it binds CNP with substantially greater affinity than ANP or BNP.[196] A third receptor, NPR-C,[197] does not possess guanylyl cyclase activity and probably functions to clear all three natriuretic peptides from the circulation.[198] In situ hybridization studies using probes capable of distinguishing the different receptor types have revealed some interspecies discrepancy in distribution. The NPR-A receptor has been localized to kidney, adrenal, pituitary, brain, and heart in the monkey, with NPR-B limited to adrenal, pituitary, and brain.[199] In the rat, broad tissue distribution of both NPR-A and NPR-B has been described.[200] The NPR-C receptor has similarly been found in adrenal, heart, brain, and pituitary.[199]

Regulation of Secretion and Action. Secretion of ANP by cardiac tissue occurs in response to increasing atrial transmural pressure, from both left and right atria.[191,201] Studies employing intravascular volume expansion, exercise, and hypoxia demonstrate increased plasma concentration of ANP after these stimuli in both animal and human paradigms.[190,201-203] Also, increased heart rate, especially increased atrial contractile frequency, results in increased ANP secretion. In the setting of supraventricular tachycardia, high plasma concentration of ANP as well as suppressed concentration of vasopressin (both probably caused by an increase in atrial volume and pressure) contribute to the polyuria associated with this syndrome.[204-206] Ventricular production of ANP has also been demonstrated; it is increased in states of left-sided overload associated with ventricular hypertrophy.[186] ANP synthesized within the central nervous system varies in a volume-dependent fashion in a manner similar to peripheral ANP, suggesting similar function.[177]

The physiologic ramifications of increased ANP production are several. Infusion of ANP in the setting of normovolemia causes natriuresis, diuresis, and a small increase in divalent cation excretion.[190,191,207] ANP, through the NPR-A receptor, primarily inhibits sodium reabsorption within the renal inner medullary collecting duct but also opposes the salt-retaining effects of angiotensin II at the level of the proximal tubule.[207] ANP similarly inhibits the actions of vasopressin and aldosterone in the renal tubules.[208-210] Direct cardiovascular effects of ANP include arterial smooth muscle relaxation, both acutely and with chronic administration. In part, this effect may be mediated through opposition of angiotensin II action.[211]

ANP modulates mineralocorticoid production in a manner that results in the reduction of intravascular volume or pressure. Although direct reduction in plasma renin activity has been described with ANP infusion,[212,213] the most dramatic response to ANP occurs at the level of the adrenal glomerulosa cell. ANP inhibits aldosterone production by inhibiting action of most aldosterone secretagogues, with the most pronounced reduction being angiotensin II activity.[190,201,207] The serum concentration of ANP at which the effects on PRA and aldosterone production occur is within the physiologic range, although the importance of this pathway in normal human physiology remains incompletely defined.

Direct injection of ANP into the central nervous system of animals has suggested an important role for ANP (or CNP) in cardiovascular and salt homeostasis. Vasodepression and bradycardia have both been observed,[214] as has inhibition of vasopressin, adrenocorticotropic hormone, and gonadotropin-releasing hormone secretion.[177,215] Thus, the antagonistic actions of ANP and angiotensin II on intravascular volume and blood pressure remain congruent between central and peripheral systems.

BNP synthesis and secretion from cardiac ventricular tissue are augmented in congestive heart failure and, as for ANP, with hypertension, chronic renal, and chronic liver failure.[187,191] BNP binds the NPR-A receptor, where it is capable of stimulating cyclic guanosine monophosphate production.[193] Infusion of BNP inhibits aldosterone production and results in natriuresis similar to that reported for ANP. With infusion rates generating BNP concentrations 10-fold greater than baseline, reduction in blood pressure has also been found. In addition to modulating sodium homeostasis, ANP and BNP, via NPR-A receptors, stimulate the transition of white to beige fat, and therefore may be involved in thermoregulation and energy balance.[216]

In contrast to ANP and BNP, CNP expression primarily causes activation of the NPR-B receptor.[196] Plasma concentration of CNP does not change significantly with volume overload, and it is believed most CNP action occurs in a paracrine fashion both within the brain and vasculature.[191] CNP synthesized within vascular endothelium acts on receptors in vascular smooth muscle to cause relaxation.[217] CNP infusions in dogs acutely reduce blood pressure and right atrial pressure but do not result in natriuresis, whereas, in humans, moderately supraphysiologic doses cause neither hypotension nor natriuresis.[218] In contrast to ANP, intracerebroventricular infusion of CNP leads to a reduction in blood pressure, suggesting a role for CNP in central control of arterial pressure.[219] CNP inhibits angiotensin II–stimulated vasopressin secretion but stimulates thirst.[220] The overall importance of the CNP central pathways in the modulation of water balance in humans remains to be defined.

APPROACH TO THE PATIENT: DIFFERENTIAL DIAGNOSIS OF DISORDERS OF WATER METABOLISM

Hyponatremia

Hyponatremia (serum sodium, 130 mEq/L) in children is usually associated with severe systemic disorders. It is most often caused by intravascular volume depletion or excessive salt loss and is also encountered with hypotonic fluid overload, especially in infants. Inappropriate vasopressin excess is one of the least common causes of hyponatremia in children, except after vasopressin administration for treatment of diabetes insipidus.

In evaluating the cause of hyponatremia, one should first determine whether the patient is dehydrated and hypovolemic. This is usually evident from the physical examination (decreased weight, skin turgor, central venous pressure) and laboratory data (high blood urea nitrogen, renin, aldosterone, uric acid). With a decrease in the glomerular filtration rate, proximal tubular reabsorption of sodium and water will be high, leading to a urinary sodium value less than 10 mEq/L. Patients with decreased "effective" intravascular volume from congestive heart failure, cirrhosis, nephrotic syndrome, or lung disease will present with similar laboratory data but will also have obvious signs of their underlying disease, which often includes peripheral edema. Patients with primary salt loss will also appear volume depleted. If the salt loss is from the kidney (e.g., diuretic therapy or polycystic kidney disease), the urine sodium level will be elevated, as may urine volume. Salt loss from other regions (e.g., the gut in gastroenteritis or the skin in cystic fibrosis) will cause urine sodium to be low, as in other forms of systemic dehydration. Cerebral salt wasting is encountered with central nervous system insults and results in high serum ANP concentrations, leading to high urine sodium and urine excretion.

The syndrome of inappropriate antidiuresis (SIAD) exists when a primary elevation in vasopressin secretion or inappropriate activation of the vasopressin V2 receptor is the cause of hyponatremia. It is characterized by hyponatremia, an inappropriately concentrated urine (>100 mOsm/kg), normal or slightly elevated plasma volume, and a normal-to-high urine sodium (because of volume-induced suppression of aldosterone and elevation of ANP). Serum uric acid is low in patients with SIAD, whereas it is high in those with hyponatremia caused by systemic dehydration or other causes of decreased intravascular volume.[221] Measurement of plasma vasopressin is often not very useful because it is elevated in nearly all causes of hyponatremia except for primary hypersecretion of ANP[222] or mutations in the vasopressin receptor that lead to inappropriate regulation of its activity. Because cortisol and thyroid deficiency cause hyponatremia by several mechanisms, discussed subsequently, they should be considered in all hyponatremic patients. Drug-induced hyponatremia should be considered in patients on potentially offending medications, as discussed later. In children with SIAD who do not have an obvious cause, a careful search for a tumor (thymoma, glioma, bronchial carcinoid) should be considered.

Polyuria, Polydipsia, and Hypernatremia

In children, it must first be determined whether pathologic polyuria or polydipsia (exceeding 2 L/m2/day) is present. The following questions are asked: Is there a psychosocial reason for either polyuria or polydipsia? Can either be quantitated? Has either polyuria or polydipsia interfered with normal activities? Is nocturia or enuresis present? If so, does the patient also drink after nocturnal awakening? Does the history (including longitudinal growth data) or physical examination suggest other deficient or excessive endocrine secretion or an intracranial neoplasm?

If pathologic polyuria or polydipsia is present, the following should be obtained. In the outpatient setting: serum osmolality; serum concentrations of sodium, potassium, glucose, calcium, and blood urea nitrogen; and urinalysis, including measurement of urine osmolality, specific gravity, and glucose concentration. A serum osmolality greater than 300 mOsm/kg, with urine osmolality less than 300 mOsm/kg, establishes the diagnosis of diabetes insipidus. If serum osmolality is less than 270 mOsm/kg or urine osmolality is greater than 600 mOsm/kg, the diagnosis of diabetes insipidus is unlikely. If, on initial screening, the patient has a serum osmolality less than 300 mOsm/kg but the intake/output record at home suggests significant polyuria and polydipsia that cannot be attributed to primary polydipsia (i.e., the serum osmolality is greater than 270 mOsm/kg), the patient should undergo a water deprivation test to establish a diagnosis of diabetes insipidus and to differentiate central from nephrogenic causes.

After a maximally tolerated overnight fast (based on the outpatient history), the child is admitted to the outpatient testing center in the early morning of a day when an 8- to 10-hour test can be carried out, and the child is deprived of water.[223,224] The physical signs and biochemical parameters shown in the accompanying protocol are measured (Figure 11-12). If at any time during the test, the urine osmolality exceeds 1000 mOsm/kg, or 600 mOsm/kg and is stable over 1 hour, the patient does not have diabetes insipidus. If at any time the serum osmolality exceeds 300 mOsm/kg and the urine osmolality is less than 600 mOsm/kg, the patient has diabetes insipidus. If the serum osmolality is less than 300 mOsm/kg and the urine osmolality is less than 600 mOsm/kg, the test should be continued unless vital signs disclose hypovolemia.

A common error is to stop a test too soon, based on the amount of body weight lost, before either urine osmolality has plateaued above 600 mOsm/kg or a serum osmolality above 300 mOsm/kg has been achieved. Unless the serum osmolality increases above the threshold for vasopressin release, a lack of vasopressin action (as inferred by a nonconcentrated urine) cannot be deemed pathologic. If the diagnosis of diabetes insipidus is made, aqueous vasopressin (Pitressin, 1 U/m2) should be given subcutaneously. If the patient has central diabetes insipidus, urine volume should fall and osmolality should at least double during the next hour, compared with the value before vasopressin therapy. If there is less than a twofold increase in urine osmolality after vasopressin

Water Deprivation Test

ENDOCRINE FUNCTION TEST | Patient Name

DIAGNOSIS: Suspected Diabetes Insipidus

TEST: Water Deprivation

Present Health _____ Good _____ Fair _____ Poor

Diet for previous two days (attach diet history), to avoid tobacco, ethanol: _____

Initial period of fast_____hr

Initial body weight_____ kg Recent Medications: _____

Thirst sensation normal?_____

No	Hour	Interval Minutes	Body weight	Vital signs	Serum Na	Serum OSM	Serum BUN	Plasma VP	Urine Na	Urine OSM	Urine S.G.	Urine vol/hr
		-30	Place IV hep lock									
		0	X	X	X	X	X	X	X	X	X	X
		60	X	X	X	X				X	X	X
		120	X	X	X	X			X	X	X	X
		180	X	X	X	X				X	X	X
		240	X	X	X	X		X	X	X	X	X
		300	X	X	X	X				X	X	X
		360	X	X	X	X			X	X	X	X
		420	X	X	X	X				X	X	X
		480	X	X	X	X	X	X*	X	X	X	X

*If patient has DI, last VP sample at last time point before VP admininstration (see below)

AT ANY TIME DURING TEST:

If serum osm <300 (Na<145), urine osm <600, continue test unless vital signs disclose hypovolemia

If urine osm >1000, or >600 and stable (<30 mosm change for 2 time points), stop test = NORMAL

If serum osm >300 and urine osm<600=DIABETES INSIPIDUS. Give Pitressin, 1U/m2 SQ and measure:

TIME AFTER PITRESSIN ADMINISTRATION:

		0		X						X	X	X
		30		X						X	X	X
		60		X					X	X	X	X

COMMENTS:

FIGURE 11-12 ■ Protocol for evaluation of diabetes insipidus using water deprivation. IV, intravenous; OSM, osmolality; S.G., urinary specific gravity; SQ, subcutaneous.

administration, the patient probably has nephrogenic diabetes insipidus. Desmopressin should not be used for this test, because it has been associated with water intoxication in small children in this setting.[225] Patients with long-standing primary polydipsia may have mild nephrogenic diabetes insipidus because of dilution of their renal medullary interstitium. This should not be confused with primary nephrogenic diabetes insipidus, because patients with primary polydipsia should have a tendency toward hyponatremia, rather than hypernatremia, in the basal state. Patients with a family history of X-linked nephrogenic diabetes insipidus can be evaluated

for the disorder in the prenatal or perinatal period by DNA sequence analysis, thus allowing therapy to be initiated without delay.[226]

The water deprivation test should be sufficient in most patients to establish the diagnosis of diabetes insipidus and to differentiate central from nephrogenic causes. Plasma vasopressin concentrations may be obtained during the procedure (see Figure 11-12), although they are rarely needed for diagnostic purposes in children.[227] They are particularly helpful in differentiating between partial central diabetes insipidus and nephrogenic diabetes insipidus, in that they are low in the former and high in the latter situation.[228] If urine osmolality concentrates normally, but only after serum osmolality is well above 300 mOsm/kg, the patient may have an altered threshold for vasopressin release, also termed a reset osmostat. This may occur after head trauma, neurosurgery, or brain tumors.[229] More recently, an immunoassay for copeptin, the carboxy-terminus of the vasopressin precursor, has been developed, which may replace the measurement of vasopressin in the evaluation of diabetes insipidus.[230] Copeptin is more stable than vasopressin, and blood concentrations of the two peptides are highly correlated.[230]

Magnetic resonance imaging (MRI) is not very helpful in distinguishing central diabetes insipidus from nephrogenic diabetes insipidus.[231] Normally, the posterior pituitary is seen as an area of enhanced brightness in T1-weighted images after administration of gadolinium.[232] The posterior pituitary "bright spot" is diminished or absent in both forms of diabetes insipidus, presumably because of decreased vasopressin synthesis in central, and increased vasopressin release in nephrogenic, disease.[232-234] In primary polydipsia, the bright spot is normal, probably because vasopressin accumulates in the posterior pituitary during chronic water ingestion,[232] whereas it is decreased in SIADH, presumably because of increased vasopressin secretion.[231] Dynamic, fast-frame, MRI analysis has allowed the estimation of blood flow to the posterior pituitary.[235] With this technique, both central and nephrogenic diabetes insipidus are associated with delayed enhancement in the area of the neurohypophysis.[236]

In the inpatient, post-neurosurgical setting, central diabetes insipidus is likely if hyperosmolality (serum osmolality $>$ 300 mOsm/kg) is associated with urine osmolality less than serum osmolality. One must beware of intraoperative fluid expansion with subsequent hypo-osmolar polyuria masquerading as diabetes insipidus.

SPECIFIC DISORDERS OF WATER METABOLISM

Hyponatremia with Normal Regulation of Vasopressin

Hyponatremia with Appropriate Decreased Secretion of Vasopressin

Increased Water Ingestion (Primary Polydipsia). In a hypo-osmolar state with vasopressin secretion normally suppressed, the kidney can excrete urine with an osmolality as low as 50 mOsm/kg. Under these conditions, a daily solute load of 500 mOsm/m^2 could be excreted in 10 L/m^2 of urine per day. Neonates cannot dilute their urine to this degree and are prone to develop water intoxication at levels of water ingestion above 4 L/m^2/day (approximately 60 mL/hr in a newborn). This may happen when concentrated infant formula is diluted with excess water, either by accident or in a misguided attempt to make it last longer.[237] A primary increase in thirst, without apparent cause, leading to hyponatremia has been reported in infants as young 5 weeks of age.[238] In older children, with a normal kidney and the ability to suppress vasopressin secretion, hyponatremia does not occur unless water intake exceeds 10 L/m^2/day, a feat that is almost impossible to accomplish. Long-standing ingestion of large volumes of water will decrease the hypertonicity within the renal medullary interstitium, which will impair water reabsorption and guard against water intoxication.[239] Hyponatremia will occur at lower rates of water ingestion when renal water clearance is impaired, either because of inappropriately elevated vasopressin secretion or for other reasons.

The rare patient in whom the osmotic thresholds for thirst and vasopressin release are reversed illustrates the importance of the normal relationship between these two responses to osmotic stimulation.[240] If thirst is activated below the threshold for vasopressin release, water intake and hypo-osmolality will occur and suppress vasopressin secretion, leading to persistent polydipsia and polyuria. As long as daily fluid intake is less than 10 L/m^2, hyponatremia will not occur. Despite the presence of polyuria and polydipsia, this entity should not be confused with diabetes insipidus because of the absence of hypernatremia. Desmopressin treatment of such a patient may lower serum osmolality below the threshold for thirst, suppressing water ingestion and the consequent polyuria.

Decreased Renal Free Water Clearance. Adrenal insufficiency, either primary or secondary, has long been known to result in compromised free water excretion.[40,70] The mechanisms by which glucocorticoids and mineralocorticoids modulate water diuresis have been the subject of substantial investigation. Some studies have demonstrated increased plasma vasopressin activity in the context of glucocorticoid insufficiency,[241,242] consistent with more recent molecular biologic evidence that glucocorticoids inhibit transcription of the vasopressin gene.[243] Other investigators, however, have failed to detect vasopressin in plasma of patients with adrenal insufficiency and abnormal water clearance.[244] Consistent with vasopressin-independent actions of adrenal steroids on water metabolism, Brattleboro rats with hypothalamic diabetes insipidus manifest impaired excretion of a water load after adrenalectomy.[70] In adrenalectomized Brattleboro rats, glucocorticoid administration restored urine flow rate but did not restore maximal urinary diluting capacity. Conversely, mineralocorticoid administration restored maximal urinary diluting capacity but not flow rate. Thus, both mineralocorticoids and glucocorticoids are required for normal free water clearance. In part, these vasopressin-independent actions of mineralocorticoids and glucocorticoids have been attributed to the increased glomerular filtration rate arising

from reexpansion of extracellular fluid volume (reduced owing to salt wasting) and improved cardiovascular tone, respectively.[41,245,246] By restoring the glomerular filtration rate, more free water is delivered to the distal tubule for excretion. Additionally, volume repletion reduces the nonosmotic stimuli for vasopressin release of volume depletion and hypotension. Nitric oxide has been found to stimulate cyclic guanosine monophosphate–dependent membrane insertion of aquaporin-2 into renal epithelial cells.[247] Because glucocorticoid has been shown to inhibit endothelial nitric oxide synthase,[248] it is possible that under conditions of glucocorticoid deficiency high levels of nitric oxide synthase result in elevated levels of endothelial nitric oxide in the renal vasculature, which in the distal renal tubule stimulate increased, vasopressin-independent, aquaporin-2 activity and decreased free water clearance.

Direct effects of glucocorticoid or mineralocorticoid insufficiency on aquaporin expression and function have not been reported. In addition to impairing maximal renal diluting capacity, adrenal insufficiency compromises maximal urine-concentrating capacity.[249] This effect has been shown to result from reduced tubular response to vasopressin.

Thyroid hormone is also required for normal free water clearance, and its deficiency likewise results in decreased renal water clearance and hyponatremia. Although some studies suggest that vasopressin mediates the hyponatremia of hypothyroidism because ethanol increases free water excretion in hypothyroid patients, this effect has not been found in other reports.[250] Additionally, in severe hypothyroidism, hypovolemia is not present and hyponatremia is accompanied by appropriate suppression of vasopressin.[251] Similar to the consequences of isolated glucocorticoid deficiency described earlier, hypothyroidism impairs free water clearance more than maximal urine diluting capacity.[252] This decrease in free water clearance may result from a diminished glomerular filtration rate and delivery of free water to the diluting segment of distal nephron, as suggested by both animal[253] and human studies.[254]

Given the often subtle clinical findings associated with adrenal and thyroid deficiency, all patients with hyponatremia should be suspected of having these disease states and have appropriate diagnostic tests performed if indicated. Moreover, patients with coexisting adrenal failure and diabetes insipidus may have no symptoms of the latter until glucocorticoid therapy unmasks the need for vasopressin replacement.[255,256] Similarly, resolution of diabetes insipidus in chronically polyuric and polydipsic patients may suggest inadequate glucocorticoid supplementation or noncompliance with glucocorticoid replacement.

Some drugs may cause hyponatremia by inhibiting renal water excretion without stimulating secretion of vasopressin (Table 11-1), an action that could be called nephrogenic SIAD. In addition to augmenting vasopressin release, both

TABLE 11-1 **Drugs Impairing Free Water Clearance**

Class	Drug	Increases AVP Secretion	Increases AVP Effect	AVP-independent Renal Effects	Hyponatremia
Angiotensin-converting enzyme inhibitors	Lisinopril				Yes
Anticonvulsants	Carbamazepine/ oxcarbazepine	Yes	Yes	Possibly	Yes
	Valproic acid				Yes
Antineoplastics	Cis-platinum			Yes	Yes
	Cyclophosphamide	No		Yes	Yes
	Vinblastine	Yes		Yes	Yes
	Vincristine	Yes			Yes
Antiparkinsonian	Amantadine, trihexyphenidyl	Yes			Yes
Antipsychotics	Haloperidol, thioridazine				Yes
Antipyretics	Acetaminophen		Yes		
Hypolipidemics	Clofibrate	Yes	No		
Oral hypoglycemics	Chlorpropamide, tolbutamide	Yes	Yes	No	Yes
Selective serotonin Uptake inhibitors	Fluoxetine, sertraline, others	Likely			Yes
Tricyclic antidepressants	Imipramine, amitriptyline	Yes			Yes

Note: Proven actions of the drugs, if known, and whether the drugs have resulted in hyponatremia in humans, are indicated. Adapted from references 258-260, 265, and 500-506.

carbamazepine[257,258] and chlorpropamide[259,260] increase the cellular response to vasopressin. Acetaminophen also increases the response of the kidney to vasopressin[259]; however, this has not been found to cause hyponatremia. High-dose cyclophosphamide treatment (15 to 20 mg/kg intravenous bolus) is often associated with hyponatremia, particularly when it is followed by a forced water diuresis to prevent hemorrhagic cystitis.[261-263] Plasma vasopressin concentrations are normal, suggesting a direct effect of the drug to increase water resorption.[264] Similarly, vinblastine, independent of augmentation of plasma vasopressin concentration or vasopressin action,[265] and cisplatin[266,267] cause hyponatremia. These drugs may damage the collecting duct tubular cells, which are normally highly impermeable to water, or they may enhance aquaporin-2 water channel activity and thereby increase water reabsorption down its osmotic gradient into the hypertonic renal interstitium.

Treatment. Hyponatremia due to cortisol or thyroid hormone deficiency reverses promptly after the institution of hormone replacement. Because the hyponatremia is often chronic, too rapid an increase in the serum sodium concentration should be avoided if possible to reduce the risk of developing central pontine myelinolysis. When drugs that impair free water excretion must be used, water intake should be limited, as if the patient has SIADH, to 1 $L/m^2/24$ hr, using the regimen discussed.

Hyponatremia with Appropriate Increased Secretion of Vasopressin

Increased vasopressin secretion causing hyponatremia may be either an appropriate response or an inappropriate response to a pathologic state. Inappropriate secretion of vasopressin or V2 receptor activity (SIAD) is the much less common of the two entities.[268,269] Whatever the cause, hyponatremia is a worrisome sign often associated with increased morbidity and mortality.[270]

Causes

Systemic Dehydration. Systemic dehydration (water in excess of salt depletion) initially results in hypernatremia, hyperosmolality, and activation of vasopressin secretion, as discussed earlier. In addition, the associated fall in the renal glomerular filtration rate results in an increase in proximal tubular sodium and water reabsorption, with a concomitant decrease in distal tubular water excretion. This limits the ability to form a dilute urine and, along with the associated stimulation of the renin-angiotensin-aldosterone system and suppression of ANP secretion, results in the excretion of urine that is very low in sodium. As dehydration progresses, hypovolemia and hypotension become major stimuli for vasopressin release, much more potent than hyperosmolality. This effect, by attempting to preserve volume, decreases free water clearance further and may lead to water retention and hyponatremia, especially if water replacement in excess of salt is given. In many cases, hyponatremia caused by intravascular volume depletion is evident from physical and laboratory signs such as decreased skin turgor, low central venous pressure, hemoconcentration, and elevated blood urea nitrogen levels. The diagnosis may be subtle, however. For example, patients with meningitis may present with hyponatremia, for which water restriction has been advocated in the belief that it is due to central SIAD. Several studies have found that volume depletion, rather than SIAD, is often the cause of the hyponatremia[271,272] and that it resolves more readily when supplemental, rather than restricted, fluid and solute are administered.[273] In patients with hyponatremia after head trauma, volume depletion rather than central SIAD is the cause in approximately half of cases.[274] Similarly, many patients with gastroenteritis who present with mild hyponatremia and elevated plasma vasopressin levels[275] have these on the basis of systemic dehydration rather than SIAD, and they benefit from volume expansion rather than fluid restriction.[276] More generally, most hospitalized pediatric patients with hyponatremia benefit from isotonic rather than hypotonic fluid replacement, suggesting that the underlying cause of the electrolyte disturbance is dehydration.[277]

Primary Loss of Sodium Chloride. The kidney can lose salt, as it does in patients with congenital polycystic kidney disease, acute interstitial nephritis, and chronic renal failure. Mineralocorticoid deficiency, pseudohypoaldosteronism (sometimes seen in children with urinary tract obstruction or infection), diuretic use, and gastrointestinal disease (usually gastroenteritis with diarrhea or vomiting) can also result in an excess loss of sodium chloride. Hyponatremia can also result from salt loss in sweat in cystic fibrosis, although obstructive lung disease with an elevation of plasma vasopressin probably plays a more prominent role, as has been discussed. With the onset of salt loss, any tendency toward hyponatremia will initially be countered by suppression of vasopressin and increased water excretion. With continuing salt loss, hypovolemia or hypotension ensues, causing nonosmotic stimulation of vasopressin. This, plus increased thirst, which leads to ingestion of hypotonic fluids with low solute content, results in hyponatremia. Weight loss is usually evident, as is the source of sodium wasting. If it is the kidney, it is accompanied by a rate of urine output and a urine sodium content greater than those associated with most other causes of hyponatremia except a primary increase in ANP secretion.

Decreased Effective Plasma Volume. Congestive heart failure, cirrhosis, nephrotic syndrome, positive-pressure mechanical ventilation,[278] severe burns,[279] lung disease (bronchopulmonary dysplasia[280-282] [in neonates]), cystic fibrosis with obstruction,[283,284] and severe asthma[285,286] are all characterized by a decrease in "effective" intravascular volume.[250,287] This occurs because of impaired cardiac output, an inability to keep fluid within the vascular space, or impaired blood flow into the heart, respectively. As with systemic dehydration, in an attempt to preserve intravascular volume, water and salt excretions by the kidney are reduced; and decreased barosensor stimulation results in a compensatory, appropriate increase in vasopressin secretion, leading to an antidiuretic state and hyponatremia.[288] Because of the associated stimulation of the renin-angiotensin-aldosterone system, these patients also have an increase in the total-body content of sodium chloride and may have peripheral edema, which distinguishes them from those with systemic dehydration.

In patients with impaired cardiac output and elevated atrial volume (e.g., congestive heart failure or lung disease), ANP concentrations are elevated, which contributes to hyponatremia by promoting natriuresis.

Treatment. Patients with systemic dehydration and hypovolemia should be rehydrated with salt-containing fluids such as normal saline or lactated Ringer solution. Because of activation of the renin-angiotensin-aldosterone system, the administered sodium will be avidly conserved and a water diuresis will quickly ensue as volume is restored and vasopressin concentrations fall.[289] Under these conditions, caution must be taken to prevent too rapid a correction of hyponatremia, which may itself result in brain damage.

Hyponatremia caused by a decrease in effective plasma volume from cardiac, hepatic, renal, or pulmonary dysfunction is more difficult to reverse. The most effective therapy is the least easily achieved: treatment of the underlying systemic disorder. Patients weaned from positive-pressure ventilation undergo a prompt water diuresis and resolution of hyponatremia as cardiac output is restored and vasopressin concentrations fall. The only other effective route is to limit water intake to that required for the renal excretion of the obligate daily solute load of approximately 500 mOsm/m^2 and to replenish insensible losses. In a partial antidiuretic state with a urine osmolality of 750 mOsm/kg H_2O and insensible losses of 500 mL/m^2, oral intake would have to be limited to approximately 1200 mL/m^2/day. Because of concomitant hyperaldosteronism, the dietary restriction of sodium chloride needed to control peripheral edema in patients with heart failure may reduce the daily solute load and further limit the amount of water that can be ingested without exacerbating hyponatremia. Hyponatremia in these settings is often slow to develop, rarely causes symptoms, and usually does not need treatment. If the serum sodium falls below 125 mEq/L, water restriction to 1 L/m^2/day is usually effective in preventing a further decline. Because water retention in these disorders is a compensatory response to decreased intravascular volume, an attempt to reverse it with drugs such as demeclocycline or specific V2 receptor antagonists (which induce nephrogenic diabetes insipidus as discussed subsequently) could worsen hypovolemia, with potentially dire consequences.[290]

In general, patients with hyponatremia caused by salt loss require ongoing supplementation with sodium chloride and fluids. Initially, intravenous replacement of urine volume with fluid containing sodium chloride (150 to 450 mEq/L depending on the degree of salt loss) may be necessary; oral salt supplementation may be required subsequently.[222] This treatment contrasts to that of SIAD, in which water restriction without sodium supplementation is the mainstay.

Precautions in the Emergency Treatment of Hyponatremia. Most children with hyponatremia develop the disorder gradually, are asymptomatic, and should be treated with water restriction alone. The development of acute hyponatremia, or a serum sodium concentration below 120 mEq/L, may be associated with lethargy, psychosis, coma, or generalized seizures, especially in younger children. Acute hyponatremia causes cell swelling owing to the entry of water into cells (Figure 11-13), which can lead to neuronal dysfunction from alterations in the ionic environment or to cerebral herniation because of the

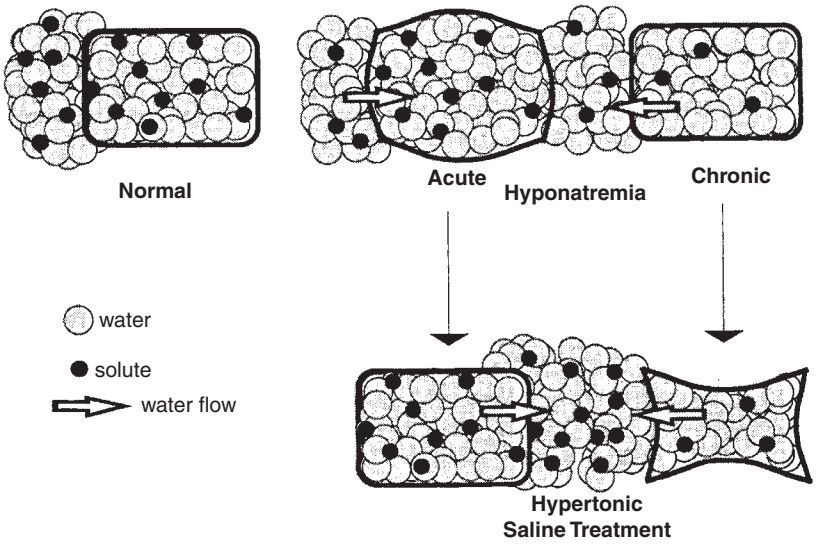


FIGURE 11-13 ■ Changes in organic osmolytes with hyponatremia and after its correction. Under normal conditions, osmotic balance exists between extracellular and intracellular compartments. With acute hyponatremia, water enters cells, causing cell swelling. After approximately 24 hours of continued hyponatremia, intracellular organic osmolytes decrease, restoring cell volume toward normal. Hypertonic saline treatment of acute hyponatremia results in restoration of normal cell volume, whereas the same treatment of chronic hyponatremia results in cell shrinkage. Large circle, water; closed smaller circle, solute; arrow, direction of water flow.

encasement of the brain in the cranium. If present for more than 24 hours, cell swelling triggers a compensatory decrease in intracellular organic osmolytes, resulting in the partial restoration of normal cell volume in chronic hyponatremia.[291]

The proper emergency treatment of cerebral dysfunction depends on whether the hyponatremia is acute or chronic.[1,292] In all cases, water restriction should be instituted. If hyponatremia is acute, and therefore probably not associated with a decrease in intracellular organic osmolyte concentration, rapid correction with hypertonic 3% sodium chloride administered intravenously may be indicated. As a general guide, this solution, given in the amount of 12 mL/kg, will result in an increase in serum sodium concentration of approximately 10 mEq/L. If hyponatremia is chronic, hypertonic saline treatment must be undertaken with caution, because it may result in both cell shrinkage (see Figure 11-13) and the associated syndrome of central pontine myelinolysis.[293] This syndrome, affecting the central portion of the basal pons as well as other brain regions, is characterized by axonal demyelination, with sparing of neurons. It becomes evident within 24 to 48 hours after too rapid correction of hyponatremia, has a characteristic appearance by computed tomography and MRI, and often causes irreversible brain damage.[293-295] If hypertonic saline treatment is undertaken, the serum sodium concentration should be raised only high enough to cause an improvement in mental status, and in no case faster than 0.5 mEq/L/hr or 12 mEq/L/day.[292-295] In the case of systemic dehydration, the increase in serum sodium level may occur rapidly using this regimen. The associated hyperaldosteronism will cause avid retention of the administered sodium, leading to rapid restoration of volume and suppression of vasopressin secretion and resulting in a brisk water diuresis and an increase in the serum sodium concentration.[289]

Acute treatment of hyponatremia is more difficult in patients with decreased effective plasma volume. This is both because the underlying disorder makes it difficult to maintain the administered fluid within the intravascular space and because an associated increase in ANP promotes natriuresis and loss of the administered salt. Furthermore, patients with cardiac disease who are administered hypertonic saline may require concomitant treatment with a diuretic such as furosemide to prevent worsening of heart failure, which will also increase natriuresis.

Hyponatremia with Abnormal Regulation of Vasopressin

Hyponatremia with Inappropriate Increased Secretion of Vasopressin or Increased Vasopressin V2 Receptor Activity (Syndrome of Inappropriate Antidiuresis [SIAD])

Causes of SIAD. SIAD is uncommon in children.[268,269,296] It can occur with encephalitis, brain tumor,[297] head trauma,[274,298] or psychiatric disease[299]; in the postictal period after generalized seizures[300]; and after prolonged nausea,[301,302] pneumonia,[303,304] or AIDS.[305] Many drugs have been associated with impaired free water clearance as indicated in Table 11-1. Impaired free water clearance can result from alteration in vasopressin release, increased vasopressin effect at the same plasma vasopressin concentration, or vasopressin-independent changes in distal collecting tubule water permeability. Common drugs that have been shown to increase antidiuretic hormone (ADH) secretion and result in hyponatremia include carbamazepine,[258] chlorpropamide,[306] vinblastine,[265] vincristine,[307] and tricyclic antidepressants.[308,309] Newer sulfonylurea agents, including glyburide, are not associated with SIAD.[310] Other rarer causes of SIAD in children are listed in Table 11-2. Although it has been believed to be the cause of hyponatremia associated with viral meningitis, volume depletion is more commonly the etiology.[271,273] In contrast, the majority of children with tuberculous meningitis have hyponatremia and SIADH, which predict more severe disease and poor outcome.[311-313] SIAD is the cause of the hyponatremic second phase of the triple-phase response seen after hypothalamic-pituitary surgery. Hyponatremia with elevated vasopressin secretion is found in up to 35% of patients 1 week after transsphenoidal pituitary surgery.[314,315] The mechanism is most likely retrograde neuronal degeneration with cell death and vasopressin release. Secondary adrenal insufficiency causing stimulation of vasopressin release[69] may also play a role, because hyponatremia most commonly follows the removal of adrenocorticotropin hormone-secreting corticotroph adenomas.[315] In the majority of children with SIAD, the cause is the excessive administration of vasopressin to treat either central diabetes insipidus,[225,316] or, less commonly, bleeding disorders[317] (as has been discussed previously), or, most uncommonly, following dDAVP therapy for enuresis.

TABLE 11-2 **Causes of Syndrome of Inappropriate Secretion of Antidiuretic Hormone (Vasopressin)**

Central Nervous System	Cancer	Infections	Pulmonary
Head trauma	Small cell of lung	Herpes zoster	Viral pneumonia
Subarachnoid hemorrhage	Duodenum	Respiratory syncytial virus	Bacterial pneumonia
Brain abscess	Pancreas	Tuberculosis	Abscess
Guillain-Barré syndrome	Thymoma	Aspergillosis	
Hydrocephalus	Bladder	Botulism	
Meningitis	Ureter		
	Lymphoma		
	Ewing sarcoma		

Two unrelated infants with mutations in the vasopressin V2 receptor that presented with severe hyponatremia in the first months of life heralded a new genetic cause of hyponatremia.[318] These two infants had missense mutations at codon 137 that converted arginine to cysteine or leucine and lead to constitutive activation of the V2 receptor with appropriately suppressed arginine vasopressin plasma concentration. This genetic disorder has been termed "nephrogenic syndrome of inappropriate antidiuresis (NSIAD)." It remains unclear what portion of isolated early onset chronic SIAD results from activating mutations of the V2 receptor, though the incidence is likely to be very low. Interestingly, this same codon is also the site of a loss-of-function mutation (R137H) that leads to X-linked nephrogenic diabetes insipidus.[319] A total of 16 patients with NSIAD have been reported.[320]

Treatment of SIAD. Chronic SIAD is best treated by chronic oral fluid restriction. Under full vasopressin antidiuretic effect (urine osmolality of 1,000 mOsm/L), a normal daily obligate renal solute load of 500 mOsm/m^2 would be excreted in 500 mL/m^2 H_2O. This, plus a daily nonrenal water loss of 500 mL/m^2, would require that oral fluid intake be limited to 1000 mL/m^2/day to avoid hyponatremia, as has been discussed more fully. In young children, this degree of fluid restriction may not provide adequate calories for growth. In this situation, the creation of nephrogenic diabetes insipidus using demeclocycline therapy may be indicated to allow sufficient fluid intake for normal growth.[315]

Demeclocycline is superior to lithium for this purpose.[316] Lithium and demeclocycline, however, are associated with significant toxicities—which may limit their use in pediatric patients. Oral urea has been effectively used to treat adult patients with chronic SIAD by virtue of its ability to induce an effective osmotic diuresis. This therapy was also demonstrated to be safe and effective in four children with chronic SIAD, including two with mutations in the vasopressin V2 receptor.[317] Specific nonpeptide V2 receptor antagonists (vaptans) have also been developed for use in subacute or chronic SIAD due to inappropriate increased vasopressin secretion.[318-320] The aquaretic effects of the vaptans, after either parental or oral administration, have a rapid onset of action, exert peak effects within a few hours, and subside within 24 hours.[321,322] In a large series of adult patients with euvolemic or hypervolemic hyponatremia resulting from cirrhosis, heart failure, or SIAD, these vasopressin receptor antagonists were effective in sustained elevation of serum sodium concentration.[320] Another study in adults, limited to subjects with SIAD arising from inappropriate vasopressin secretion, demonstrated the efficacy of long-term treatment with an orally active vaptan in conjunction with fluid restriction to 1.5 L per day.[323] Substantial variability in the degree of serum sodium elevation was observed, however. This variability resulted from both interindividual differences in drug efficacy/disposition and the failure to adequately restrict water consumption. The primary adverse effect from these agents is inflammation at infusion sites, though rises in serum sodium above rates recommended to prevent myelinolysis have also been found.[321] Limited experience with vaptans has been reported in children, though they have been used to promote hydration during chemotherapy with malignancy-associated SIAD.[324] These agents have not been effective in treating activating mutations of the V2 receptor,[325] although low-dose urea has been useful.[320] Because the predictability of hypertonic saline administration for acute, severe forms of SIAD is greater than that of vaptans, hypertonic saline infusion for symptomatic hyponatremia due to inappropriate vasopressin secretion remains the recommended intervention.[321] Of note, some of these V2 receptor antagonists facilitate the proper transport of loss-of-function V2 receptor mutants to the cell surface.[321]

Acute treatment of hyponatremia due to SIAD is only indicated if cerebral dysfunction is present. In that case, treatment is dictated by the duration of hyponatremia and the extent of cerebral dysfunction. Because patients with SIAD have volume expansion, salt administration is not very effective in raising the serum sodium concentration because it is rapidly excreted in the urine due to suppressed aldosterone and elevated atrial natriuretic peptide concentrations.

Hyponatremia with Inappropriate Decreased Secretion of Vasopressin, Due to Increased Secretion of Atrial Natriuretic Peptide

Although atrial natriuretic peptide does not usually play a primary role in the pathogenesis of disorders of water metabolism, it may have an important secondary role.[280,326-328] Patients with SIAD have elevated atrial natriuretic peptide concentrations, probably due to hypervolemia, which may contribute to the elevated natriuresis of SIAD and which decrease as water intake is restricted.[326] Likewise, the suppressed atrial natriuretic peptide concentrations found in central diabetes insipidus, probably due to the associated hypovolemia, rise after dDAVP therapy.[326] However, hyponatremia in some patients, primarily those with central nervous system disorders including brain tumor, head trauma, hydrocephalus, neurosurgery, cerebral vascular accidents, and brain death, may be due to the primary hypersecretion of atrial natriuretic peptide.[222,329-331] This syndrome, called cerebral salt wasting, is defined by hyponatremia accompanied by elevated urinary sodium excretion (often more than 150 mEq/L), excessive urine output, hypovolemia, suppressed vasopressin, and elevated atrial natriuretic peptide concentrations (> 20 pmol/L). Thus, it is distinguished from SIAD, in which normal or decreased urine output, euvolemia, only modestly elevated urine sodium concentration, and elevated vasopressin concentration occur. Direct measurement of intravascular volume status with a central venous line is often helpful. The distinction is important because the therapies of the two disorders are markedly different. There is controversy regarding the prevalence of cerebral salt wasting.[332] In patients in an intensive care setting with the initial diagnosis of cerebral salt wasting, none of them upon further investigation were hypovolemic, one of the cardinal criteria of the syndrome.[333]

Treatment of Cerebral Salt Wasting

Treatment of patients with cerebral salt wasting consists of restoring intravascular volume with sodium chloride and water, as with the treatment of other causes of systemic dehydration. The underlying cause of the disorder, which is usually acute brain injury, should also be treated if possible.

Other Causes of True and Factitious Hyponatremia

True hyponatremia occurs with hyperglycemia, which causes the influx of water into the intravascular space. Serum sodium will decrease by 1.6 mEq/L for every 100-mg/dL increment in blood glucose above 100 mg/dL. Glucose is not ordinarily an osmotically active agent and does not stimulate vasopressin release, probably because it is able to equilibrate freely across plasma membranes. However, in the presence of insulin deficiency and hyperglycemia, glucose acts as an osmotic agent, presumably because its normal intracellular access to osmosensor sites is prevented.[334] Under these circumstances, an osmotic gradient exists, and this stimulates vasopressin release. In diabetic ketoacidosis, this, together with the hypovolemia caused by the osmotic diuresis secondary to glycosuria, results in marked stimulation of vasopressin secretion.[335-338] Rapid correction of hyponatremia may follow soon after the institution of fluid and insulin therapy. Whether this contributes to the pathogenesis of cerebral edema occasionally seen following treatment of diabetic ketoacidosis is not known. Elevated concentrations of triglycerides may cause factitious hyponatremia, as can obtaining a blood sample downstream from an intravenous infusion of hypotonic fluid.

Hypernatremia with Inappropriate Decreased Vasopressin Secretion or Action

Central Diabetes Insipidus

Causes of Central Diabetes Insipidus. Central (hypothalamic, neurogenic, or vasopressin-sensitive) diabetes insipidus can be caused by disorders of vasopressin gene structure; accidental or surgical trauma to vasopressin neurons; congenital anatomic hypothalamic or pituitary defects; neoplasms; infiltrative, autoimmune, and infectious diseases affecting vasopressin neurons or fiber tracts; and increased metabolism of vasopressin. The etiology of central diabetes insipidus is not apparent in anywhere between 9% and 55% of children and young adults in different series published in the literature.[339,340] *Long-term surveillance can identify an underlying cause that is not apparent at the time of initial diagnosis sometimes after as long as 21 years.*[341]

Genetic Causes. Familial, autosomal dominant central diabetes insipidus is manifest within the first half of the first decade of life.[342] Vasopressin secretion, initially normal, gradually declines until diabetes insipidus of variable severity ensues. Patients respond well to vasopressin replacement therapy. The disease has a high degree of penetrance, but it may be of variable severity within a family[343] and may spontaneously improve in middle age.[343,344] Vasopressin-containing neurons are absent from the magnocellular paraventricular neurons[345] but present in parvocellular regions.[346] Several different oligonucleotide mutations in the vasopressin structural gene have been found to cause the disease (www.medcon.mcgill.ca/nephros/avp_npii.html). To date, more than 25 mutations have been detected in the coding region of the vasopressin gene (see Figure 11-3). Most mutations are in the neurophysin portion of the vasopressin precursor, except for five in either the signal peptide or vasopressin peptide regions of the gene. This suggests that neurophysin has a valuable function, possibly in the proper intracellular sorting or packaging of vasopressin into secretory granules. There are no disease-causing mutations in the copeptin region of the vasopressin precursor. This suggests either that this region has a low mutation rate or more likely that it does not serve a critical function in vasopressin biology.

A family with a missense mutation within the vasopressin peptide region (Proline→Leucine at amino acid 7) of the gene, causing markedly reduced biologic activity, was reported.[347] The disease in this family is transmitted with an autosomal recessive pattern. This indicates that haploinsufficiency is not the basis for the autosomal dominant nature of the disease in families with the more common mutations in the neurophysin region of the gene. Rather, the abnormal gene product may interfere with the processing and secretion of the product of the normal allele,[348] or it may cause neuronal degeneration and cell death.[349] In support of this theory, mutant vasopressin precursors impair the secretion of the normal protein in cell models,[348] and in a transgenic mouse model of the disease, a progressive loss of hypothalamic vasopressin-containing neurons occurs as the mice develop diabetes insipidus.[350] Heterozygous mice with a mutation (C67X) in the vasopressin gene, known to cause autosomal dominant familial neurohypophyseal diabetes insipidus in humans, developed diabetes insipidus by 2 months of age. They were found to have retention of the precursor of vasopressin within the neurons and the induction of an endoplasmic reticulum chaperone protein (BiP).[351]

Vasopressin deficiency is also found in the DIDMOAD syndrome, consisting of diabetes insipidus, diabetes mellitus, optic atrophy, and deafness.[352,353] The gene for this syndrome complex, also known as Wolfram syndrome, was localized to human chromosome 4p16 by polymorphic linkage analysis[348] and isolated.[349] The Wolfram syndrome gene, *WFS1*, encodes an 890-amino-acid tetrameric transmembrane protein that primarily localizes to the endoplasmic reticulum. It is thought to function as a calcium channel or regulator of a calcium channel.[354,355]

Trauma. The axons of vasopressin-containing magnocellular neurons extend uninterrupted to the posterior pituitary over a distance of approximately 10 mm. Trauma to the base of the brain can cause swelling around or severance of these axons, resulting in either transient or permanent diabetes insipidus.[356] Permanent diabetes

insipidus can occur after seemingly minor trauma. Approximately half of patients with fractures of the sella turcica will develop permanent diabetes insipidus,[357] which may be delayed as long as a month following the trauma, during which time neurons of severed axons may undergo retrograde degeneration.[358] Septic shock[359] and postpartum hemorrhage associated with pituitary infarction (Sheehan syndrome)[360,361] may involve the posterior pituitary with varying degrees of diabetes insipidus. Diabetes insipidus is never associated with cranial irradiation of the hypothalamic-pituitary region, although this treatment can cause deficits in all of the hypothalamic releasing hormones carried by the portal-hypophyseal system to the anterior pituitary (see the discussion of growth hormone in Chapter 10). This occurs because vasopressin is carried directly to the posterior pituitary via magnocellular axonal transport, whereas radiation affects hypothalamic releasing hormone function by interrupting the portal-hypophyseal circulation, which is absent from the vasopressin circuitry.

Neurosurgical Intervention. One of the most common causes of central diabetes insipidus is the neurosurgical destruction of vasopressin neurons following pituitary-hypothalamic surgery. It is important to distinguish polyuria associated with the onset of acute postsurgical central diabetes insipidus from polyuria due to the normal diuresis of fluids given during surgery. In both cases, the urine may be very dilute and of high volume, exceeding 200 mL/m^2/hr. However, in the former case, serum osmolality will be high, whereas in the latter case it will be normal. A careful examination of the intraoperative record should also help distinguish between these two possibilities. Vasopressin axons traveling from the hypothalamus to the posterior pituitary terminate at various levels within the stalk and gland (see Figure 11-4). Because surgical interruption of these axons can result in retrograde degeneration of hypothalamic neurons, lesions closer to the hypothalamus will affect more neurons and cause greater permanent loss of hormone secretion. Not infrequently, a "triple phase" response is seen.[362] Although the exact incidence of this phenomenon remains unknown, in a small study nearly one in three children who underwent surgery for a craniopharyngioma developed it.[363] Following surgery, an initial phase of transient diabetes insipidus is observed, lasting a half-day to 2 days, and possibly due to edema in the area interfering with normal vasopressin secretion. If significant vasopressin cell destruction has occurred, this is often followed by a second phase of SIAD, which may last up to 10 days, and is due to the unregulated release of vasopressin by dying neurons. A third phase of permanent diabetes insipidus may follow if more than 90% of vasopressin cells are destroyed. Usually, a marked degree of SIAD in the second phase portends significant permanent diabetes insipidus in the final phase of this response. In patients with coexisting vasopressin and cortisol deficits (e.g., in combined anterior and posterior hypopituitarism following neurosurgical treatment of craniopharyngioma), symptoms of diabetes insipidus may be masked because cortisol deficiency impairs renal free water clearance, as discussed subsequently. In such cases, the institution of glucocorticoid therapy alone may precipitate polyuria, leading to the diagnosis of diabetes insipidus.

Congenital Anatomic Defects. Midline brain anatomic abnormalities such as septo-optic dysplasia with agenesis of the corpus callosum,[364] the Kabuki syndrome,[365] holoprosencephaly,[366] and familial pituitary hypoplasia with absent stalk[367] may be associated with central diabetes insipidus. These patients need not have external evidence of craniofacial abnormalities.[366] Central diabetes insipidus due to midline brain abnormalities is often accompanied by defects in thirst perception,[364] suggesting that a common osmosensor may control both vasopressin release and thirst perception. Some patients with suspected defects in osmosensor function but with intact vasopressin neurons may have recumbent diabetes insipidus, with a baroreceptor-mediated release of vasopressin while upright and vasopressin-deficient polyuria while supine.[368]

Neoplasms. Several important clinical implications follow from knowledge of the anatomy of the vasopressin system. Because hypothalamic vasopressin neurons are distributed over a large area within the hypothalamus, tumors that cause diabetes insipidus must either be very large or infiltrative or be strategically located at the point of convergence of the hypothalamoneurohypophyseal axonal tract in the infundibulum. Germinomas and pinealomas typically arise near the base of the hypothalamus where vasopressin axons converge before they enter the posterior pituitary and for this reason are among the most common primary brain tumors associated with diabetes insipidus. Germinomas causing the disease can be very small[369,370] and undetectable by magnetic resonance imaging (MRI) for several years following the onset of polyuria.[371] For this reason, quantitative measurement of the β-subunit of human chorionic gonadotropin, often secreted by germinomas and pinealomas, and regularly repeated MRI scans should be performed in children with idiopathic or unexplained diabetes insipidus. Empty sella syndrome, possibly due to unrecognized pituitary infarction, can be associated with diabetes insipidus in children.[372] Craniopharyngiomas and optic gliomas can also cause central diabetes insipidus when very large, although this is more often a postoperative complication of the treatment for these tumors. Hematologic malignancies can cause diabetes insipidus. In some cases, such as with acute myelocytic leukemia, the cause is infiltration of the pituitary stalk and sella.[373-375] However, more than 30 patients with monosomy or deletion of chromosome 7 associated with acute blast transformation of myelodysplastic syndrome presented with central diabetes insipidus[376-379] without evidence of infiltration of the posterior pituitary by neoplastic cells, leaving the cause of the diabetes insipidus unresolved.

Infiltrative, Autoimmune, and Infectious Diseases. Langerhans cell histiocytosis and lymphocytic hypophysitis are the most common types of infiltrative disorders causing central diabetes insipidus. Approximately 10% of patients with histiocytosis will have diabetes insipidus. These patients tend to have more serious, multisystem disease for longer periods of time than those without diabetes insipidus,[380,381] and anterior pituitary deficits often accompany posterior pituitary deficiency.[382] MRI characteristically

shows thickening of the pituitary stalk.[383] One report suggests that in patients with Langerhans cell histiocytosis, radiation treatment to the pituitary region within 14 days of the onset of symptoms of diabetes insipidus may result in return of vasopressin function in more than one third of affected patients.[384]

Lymphocytic infundibuloneurohypophysitis may account for more than a third of patients with "idiopathic" central diabetes insipidus.[385] This entity may be associated with other autoimmune diseases.[386] Image analysis discloses an enlarged pituitary and thickened stalk,[385,387] and biopsy of the posterior pituitary reveals lymphocytic infiltration of the gland, stalk, and magnocellular hypothalamic nuclei.[388] A necrotizing form of this entity has been described, which also causes anterior pituitary failure and responds to steroid treatment.[389] Diabetes insipidus can also be associated with pulmonary granulomatous diseases[390] including sarcoidosis.[391]

Whether antibody-mediated destruction of vasopressin cells occurs is controversial. More than half of patients with central diabetes insipidus of a nontraumatic cause have antibodies directed against vasopressin-containing cells,[392] and patients with other autoimmune diseases have such antibodies without evidence of diabetes insipidus.[393] Many patients with central diabetes insipidus also have antivasopressin peptide antibodies, although their appearance usually follows the institution of vasopressin treatment.[394] It is possible that antibodies directed against vasopressin-containing cells or vasopressin are not pathogenetic but instead are markers of prior neuronal cell destruction.

Infections involving the base of the brain, such as meningococcal,[395] cryptococcal, listeria,[396] and toxoplasmosis[397] meningitis, congenital cytomegalovirus infection,[398] and nonspecific inflammatory disease of the brain,[399] can cause central diabetes insipidus. The disease is often transient, suggesting that it is due to inflammation rather than destruction of vasopressin-containing neurons.

Brain Death. Central diabetes insipidus can appear in the setting of hypoxic brain death.[400] Although its presence has been suggested as a marker for brain death in children,[401] in some studies only a minority of patients with brain death manifest the disorder,[402] and up to 15% of patients with cerebral insults and diabetes insipidus ultimately recover brain function.[403] Polyuria in the setting of brain death can be accompanied by high concentrations of plasma vasopressin,[404] suggesting that some cases mistaken for diabetes insipidus are actually due to other causes, such as cerebral salt wasting with polyuria, as discussed subsequently.

Increased Metabolism of Vasopressin. The metabolic clearance rate of vasopressin increases fourfold during pregnancy due to the elaboration of a vasopressinase by the placenta.[72] If the mother cannot respond with a concomitant increase in vasopressin action because of preexisting subclinical central or nephrogenic diabetes insipidus,[73] overt, transient disease will appear, usually early in the third trimester and resolve within a week of delivery.[76,405] Even without prior defects, vasopressin function—an extreme elevation in vasopressinase concentrations in primigravidas with either preeclampsia, liver dysfunction, or multiple gestation[74,75,78,406-408]—may result in the development of the syndrome.

Drugs. The most common agent associated with the inhibition of vasopressin release and impaired urine concentrating ability is ethanol.[409] Because the inhibition of vasopressin release by ethanol can be overcome in the setting of concurrent hypovolemia, clinically important diabetes insipidus due to ethanol ingestion is uncommon.[410] Phenytoin, opiate antagonists, halothane, and β-adrenergic agents have also been associated with impaired vasopressin release.[411,412]

Children with Primary Enuresis. Although normal children have a nocturnal rise in plasma vasopressin associated with an increase in urine osmolality and a decrease in urine volume, those with primary enuresis have a blunted or absent rise in vasopressin and excrete a higher urine volume of lower tonicity.[413,414] This has suggested that enuretic children have a primary deficiency in vasopressin secretion, although the same outcome could be caused solely by excessive water intake in these children. The use of the V2 agonist dDAVP is highly effective in abolishing bed-wetting episodes, although relapse is high once therapy ends.[415-417] Fluid intake must be limited while a child is exposed to the antidiuretic action of dDAVP to guard against water intoxication.

Treatment of Central Diabetes Insipidus

Fluid Therapy. Patients with otherwise untreated diabetes insipidus crave cold fluids, especially water. With complete central diabetes insipidus, maximum urine concentrating ability is approximately 100 mOsm/kg. Because 5 L of urine would be required to excrete an average daily solute load of 500 mOsm/m^2, fluid intake must match this to maintain normal plasma tonicity. With an intact thirst mechanism and free access to oral fluids, a person with complete diabetes insipidus can maintain plasma osmolality and sodium in the high normal range, although at great inconvenience. Furthermore, long-standing intake of these volumes of fluid in children can lead to hydroureter,[418] and even hyperfluorosis in communities that provide fluoridated water.[419] With fluid management alone or with the use of medications other than vasopressin or its analogs, these children can develop nonobstructive hydroureteronephrosis, bladder wall thickening and trabeculation, overflow incontinence, and impaired renal function requiring a drainage procedure.[420] These complications are more likely to occur in children with nephrogenic as opposed to central diabetes insipidus because treatment with vasopressin analogs is used most of the time in children with central diabetes mellitus.

There are two situations in which central diabetes insipidus may be treated solely with high levels of fluid intake, without vasopressin. Vasopressin therapy coupled with excessive fluid intake (usually greater than 1 L/m^2/day, as discussed subsequently) can result in unwanted hyponatremia. Because neonates and young infants receive all of their nutrition in liquid form, the obligatory high oral fluid requirements for this age (3 L/m^2/day) combined with vasopressin treatment are likely to lead to this dangerous complication.[316] In infants, oral tablet and intranasal liquid desmopressin are not only difficult to administer accurately, but also their use is associated with significant fluctuations in the serum sodium levels.

Such neonates may be better managed with fluid therapy alone. A reduced solute load diet will aid in this regard. Human milk is best for this purpose (75 mOsm/kg H_2O), whereas cow's milk is the worst option (230 mOsm/kg H_2O). For example, in an infant with diabetes insipidus with a fixed urine osmolality of 100 mOsm/kg H_2O, 300 mL of urine per day is required to excrete the amount of solute consumed in human milk, whereas 900 mL of urine per day is required to excrete the higher amount of solute consumed in cow's milk. The Similac PM 60/40 formula has a renal solute load of 92 mOsm/kg H_2O. Additionally, supplementation free water may be needed depending on the severity of the diabetes insipidus. Options such as 20 to 30 mL of supplemental free water for every 120 to 160 mL of formula or dilution of the formula with free water have been used. Although children managed with such a regimen may be chronically thirsty, parents may have difficulty keeping up with the voluminous fluid intake and urine output, and poor growth may occur if adequate calories are not provided along with water,[238] these problems are more easily addressed than is life-threatening hyponatremia. Alternatively, thiazide (chlorothiazide, 5 to 10 mg/kg/dose, twice or thrice daily [421]) or amiloride diuretics may be added to facilitate renal proximal tubular sodium and water reabsorption[422] and thereby decrease oral fluid requirements. This therapy may be accompanied by a mild degree of dehydration. More recently, parenteral desmopressin (0.02 to 0.08 μg/dose given once or twice daily) has been administered subcutaneously in infants with good results, although this method has not been approved by the Food and Drug Administration (FDA).[423] Parenteral desmopressin was originally formulated at a concentration of 4 μg/mL, to be used at a 0.3-μg/kg/dose to treat bleeding diatheses such as hemophilia A and von Willebrand disease type 1. Thus, care must be taken if it is used at one fortieth to one fourth of this dose to treat infants with diabetes insipidus. In older children on more calorie-dense solid diets, the use of short-acting agents such as arginine vasopressin (Pitressin) or lysine vasopressin (Diapid; lypressin), or longer-acting desmopressin will decrease fluid needs while minimizing the possible occurrence of hyponatremia (see "Vasopressin and Vasopressin Analogs," presented later in the chapter).

In the acute postoperative management of central diabetes insipidus occurring after neurosurgery in children, vasopressin therapy may be successfully employed,[424,425] [426] but extreme caution must be exerted with its use. While under the full antidiuretic effect of vasopressin, a patient will have a urine osmolality of approximately 1000 mOsm/kg and become hyponatremic if she or he receives an excessive amount of fluids, depending on the solute load and nonrenal water losses. With a solute excretion of 500 mOsm/m^2/day, normal renal function, and nonrenal fluid losses of 500 mL/m^2/day, fluid intake of greater than 1 L/m^2/day (two thirds of the normal maintenance fluid requirement) will result in hyponatremia. In addition, vasopressin therapy will mask the emergence of the SIAD phase of the triple phase neurohypophyseal response to neurosurgical injury (as has been discussed).

Because of the concerns associated with perioperative vasopressin administration, two different approaches to managing central diabetes insipidus in the surgical patient have been employed. The first approach may be particularly useful for managing acute postoperative diabetes insipidus in young children. It employs fluids alone and avoids the use of vasopressin.[427] This method consists of matching input and output hourly using between 1 to 3 L/m^2/day (40 to 120 mL/m^2/hr). If intravenous therapy is used, a basal 40 mL/m^2/hr should be given as 5% dextrose (D5) in one-fourth normal saline (normal saline = 0.9% sodium chloride) and the remainder, depending on the urine output, as 5% dextrose in water. Potassium chloride (40 mEq/L) may be added if oral intake is to be delayed for several days. No additional fluid should be administered for hourly urine volumes less than 40 mL/m^2/hr. For hourly urine volumes above 40 mL/m^2/hr, the additional volume should be replaced with 5% dextrose up to a total maximum of 120 mL/m^2/hr. For example, in a child with a surface area of 1 m^2 (approximately 30 kg), the basal infusion rate would be 40 mL/hr of 5% dextrose in one-fourth normal saline. For an hourly urine output of 60 mL, an additional 20 mL/hr 5% dextrose would be given, for a total infusion rate of 60 mL/hr. For urine outputs above 120 mL/hr, the total infusion rate would be 120 mL/hr. In the presence of diabetes insipidus, this will result in a serum sodium concentration in the 150 mEq/L range and a mildly volume contracted state, which will allow one to assess both thirst sensation as well as the return of normal vasopressin function or the emergence of SIAD. Patients may become mildly hyperglycemic with this regimen, particularly if they are also receiving postoperative glucocorticoids. However, because it does not use vasopressin, this fluid management protocol prevents any chance of hyponatremia.

Vasopressin and Vasopressin Analogs. Evidence suggests that perioperative use of intravenous vasopressin in children with central diabetes insipidus may be the treatment modality of choice in most situations, resulting in excursions of serum sodium of smaller magnitude and few adverse sequelae.[426] Although intravenous therapy with synthetic aqueous vasopressin (Pitressin) had been shown to be useful in the management central diabetes insipidus of acute onset,[424,425] concern existed as to the safety of its administration in the complex, rapidly changing course of the child recovering from hypothalamic/pituitary surgery. If continuous vasopressin is administered, fluid intake must be limited to 1 L/m^2/day or two-thirds maintenance fluid administration (assuming normal solute intake and nonrenal water losses as described). The potency of synthetic vasopressin is still measured using a bioassay and is expressed in bioactive units, with 1 milliunit (mU) equivalent to approximately 2.5 ng of vasopressin. For intravenous vasopressin therapy, 1.5 mU/kg/hr results in a blood vasopressin concentration of approximately 10 pg/mL,[428] twice that needed for full antidiuretic activity.[429] Vasopressin's effect is maximal within 2 hours of the start of infusion,[429] and one must beware of it sticking to intravenous bottles and tubing. Occasionally following hypothalamic (but not trans-sphenoidal) surgery, higher initial concentrations of vasopressin are required to treat acute diabetes insipidus, which may be attributable to the release of a

substance related to vasopressin from the damaged hypothalamoneurohypophyseal system, which acts as an antagonist to normal vasopressin activity.[430] Much higher rates of vasopressin infusion, resulting in plasma concentrations above 1000 pg/mL, should be avoided, as they may cause cutaneous necrosis,[431] rhabdomyolysis,[431,432] and cardiac rhythm disturbances.[433]

In light of the considerations described in the previous paragraph, an effective and safe algorithm for the management of perioperative central diabetes insipidus with central diabetes insipidus has been utilized with encouraging results.[426] This algorithm starts with the child receiving an intravenous infusion of normal saline at two-thirds maintenance or 1 L/m^2/day. A tentative diagnosis of intraoperative or postoperative central diabetes insipidus is made by documenting a serum sodium concentration of greater than 145 mEq/L along with urine output of greater than 4 mL/kg/hr. Additional confirmatory evidence includes plasma osmolarity above 300 mOsm/kg H_2O and a relatively hypotonic urine. When documentation of parameters consistent with central diabetes insipidus is obtained, an intravenous infusion of aqueous vasopressin begins at 0.5 mU/kg/hr with no change in intravenous fluid administration. The dose of vasopressin is titrated upward in 0.5-mU/kg/hr increments to establish a urine output rate of less than 2 mL/kg/hr at approximately 10-minute intervals. The vasopressin and intravenous fluid administration then remain stable at these rates, with additional normal saline, or equivalent volume expanding solutions, given only to replace ongoing blood loss or to maintain hemodynamic stability. Postoperatively this management paradigm requires intensive care unit monitoring, with frequent assessment of electrolytes (hourly initially), urine output and osmolarity/specific gravity, and vital signs. This management scheme can also be followed for patients with established central diabetes insipidus requiring general surgery and prolonged restriction of oral intake. In this situation, the usual dose of chronic, long-acting vasopressin is withheld or reduced immediately prior to surgery depending on the timing of surgery in relation to the usual administration times. In preparation for surgery, normal saline is infused at two-thirds maintenance (1 L/m^2/day). When measures consistent with emergence of central diabetes insipidus due to termination of efficacy of the presurgical dose are obtained, the intravenous vasopressin is initiated and titrated as described previously.

Patients treated with vasopressin for post-neurosurgical diabetes insipidus should be switched from intravenous to oral fluid intake at the earliest opportunity, because thirst sensation, if intact, will help regulate blood osmolality, as discussed. Intravenous dDAVP (desmopressin) should not be used in the acute management of postoperative central diabetes insipidus, for it offers no advantage over vasopressin, and its long half-life (8 to 12 hours) compared with that of vasopressin (5 to 10 minutes) is a distinct disadvantage, as it may increase the chance of water intoxication.[225] In fact, the use of intravenous dDAVP, 0.3 μg/kg, to shorten the bleeding time in a variety of bleeding disorders (as has been discussed) has been associated with water intoxication,[317] particularly in young children who have high obligate oral fluid needs.

A special problem arises when a patient with established central diabetes insipidus must receive a high volume of fluid for therapeutic reasons (e.g., accompanying cancer chemotherapy). Such patients can be managed by discontinuing antidiuretic therapy and increasing fluid intake to 3 to 5 L/m^2/day (rendering the patient moderately hypernatremic). Although 5 L/m^2/day is typically adequate to maintain serum sodium concentration in the range of 150 mEq/L in children with central diabetes insipidus, this rate may not be adequate in the setting of chemotherapy administration when solute excretion increases due to cell death and the release of cellular contents. By using a low dose of intravenous vasopressin (0.08 to 0.1 mU/kg/hr, approximately one eighth of the full antidiuretic dose, titrated upward as needed), a partial antidiuretic effect allows the administration of higher amounts of fluid without causing hyponatremia.[434] Data suggest that excretion of a hypotonic urine, as would occur in patients with diabetes insipidus management with fluids alone, increases the risk of developing nephrotoxicity during therapy with platinum-based antineoplastic agents.[435] By allowing the administration of 0.45 normal saline at rates of approximately 3 L/m^2/day, the low-dose vasopressin infusion yields a urine osmolarity higher than that achievable with fluids alone[434] and may have the additional benefit of conferring renal protection.

In the outpatient setting, treatment of central diabetes insipidus in older children should begin with oral (discussed later) or intranasal dDAVP (10 μg/0.1 mL), 0.025 mL (2.5 μg) given by rhinal tube at bedtime and the dose increased to the lowest amount that gives an antidiuretic effect. If the dose is effective but has too short a duration, it should be increased further or a second, morning dose should be added. Patients should escape from the antidiuretic effect for at least an hour before the next dose to ensure that any excessive water will be excreted. Otherwise, water intoxication may occur. dDAVP is also available as a nasal spray in the same concentration, with each spray delivering 10 μg (0.1 mL). This is the standard preparation used to treat primary enuresis. Oral dDAVP tablets have come into widespread use and have largely replaced intranasal therapy. Although when given orally dDAVP is at least 20-fold less potent than when given via the intranasal route, oral dDAVP in doses of 25 to 300 μg every 8 to 12 hours is reported to be highly effective and safe in children.[436-438] Lysine vasopressin (Diapid) nasal spray (50 U/mL) may be used if a duration less than that of dDAVP is desired. One spray delivers 2 U (0.04 mL), with a duration of action between 2 and 8 hours.

As noted previously, cortisol deficiency may cause decreased free water clearance by stimulating a nitric oxide–mediated pathway, which results in the insertion of aquaporin-2 channels into the apical membranes of collecting duct cells, in a vasopressin-independent fashion.[247,248] Conversely, it is possible that excessive amounts of cortisol, due to endogenous release during stress or to treatment with exogenous drug, may inhibit the insertion of water channels. This may explain why patients with central diabetes insipidus treated with desmopressin

become "resistant," and require an increased dosage, during times of stress or treatment with glucocorticoids.

In addition to polyuria and polydipsia, decreased bone mineral density has been reported in patients with central diabetes insipidus.[439] The decreased bone density was not corrected by vasopressin analog treatment alone, suggesting that the institution of bisphosphonate or other therapies designed to prevent bone loss may be of long-term benefit in the treatment of diabetes insipidus.

Nephrogenic Diabetes Insipidus

Causes of Nephrogenic Diabetes Insipidus. Nephrogenic (vasopressin-resistant) diabetes insipidus can be the result of genetic or acquired causes. Genetic causes are less common but more severe than acquired forms of the disease, although genetic etiologies are more common in children than in adults.

Genetic Causes.

Congenital, X-Linked Diabetes Insipidus: V2 Receptor Mutations. Congenital, X-linked nephrogenic diabetes insipidus is caused by inactivating mutations of the vasopressin V2 receptor. Due to its mode of transmission, it is a disease of males, although rarely females may be affected, presumably due to extreme lyonization during X chromosome inactivation.[440] In keeping with a germline, as opposed to somatic, mutation in the V2 receptor, these patients are deficient in all systemic V2 receptor-mediated actions[441,442] and have intact V1-receptor-mediated responses.[443,444] As expected, the V2 receptor defect is proximal to the activation of renal adenylate cyclase.[445,446] Unlike the function of other G protein-coupled seven transmembrane receptors such as the parathyroid hormone (PTH) and thyroid-stimulating hormone (TSH) receptors, that of the V2 receptor is unaffected in patients with pseudohypoparathyroidism, who have inactivating mutations in the alpha subunit of G_s.[447]

Because of vasopressin resistance in congenital nephrogenic diabetes insipidus, the kidney elaborates large volumes of hypotonic urine with osmolality ranging between 50 and 100 mOsm/kg. Manifestations of the disease are usually present within the first several weeks of life,[448] but they may only become apparent after weaning from the breast. The predominant symptoms are polyuria and polydipsia. Thirst may be more difficult to satisfy than in central diabetes insipidus. Many infants initially present with fever, vomiting, and dehydration, often leading to an evaluation for infection. Growth failure in the untreated child may be secondary to the ingestion of large amounts of water, which the child may prefer over milk and other higher-caloric substances.[449] Mental retardation of variable severity may result from repeated episodes of dehydration.[450] Intracerebral calcification of the frontal lobes and basal ganglia is not uncommon in children with X-linked nephrogenic diabetes insipidus.[451-454] Because this appears early and is not seen in children with central diabetes insipidus of equivalent severity, cerebral calcification is probably unrelated to the level of dehydration or therapeutic intervention. It is possible that elevated vasopressin concentrations, acting via intact V1 or V3 receptors, contribute to some of the unique manifestations of X-linked nephrogenic diabetes insipidus, such as cerebral calcification, intense thirst, vomiting, and growth failure. Older children may present with enuresis or nocturia. They may learn to reduce food intake (and therefore solute load) to decrease polyuria, which may contribute to growth failure. After long-standing ingestion and excretion of large volumes of water, patients may develop nonobstructive hydronephrosis, hydroureter, and megabladder.[418]

Although one founder (arriving in North America from Scotland in 1761 on the ship *Hopewell*) was initially postulated to be the ancestor of most North American subjects with congenital, X-linked nephrogenic diabetes insipidus,[455] more than 209 mutations in the V2 receptor have been found, with some appearing to have arisen independently more than once[456-469] (Figure 11-14). These are mostly single base mutations that result in either amino acid substitutions, translational frame shifts, or termination of peptide synthesis, and are distributed fairly evenly throughout the receptor protein (www.hgmd.cf.ac.uk/ac/gene.php?gene5AVPR2). Mutations may affect vasopressin binding, cyclic AMP generation, or possibly transcriptional regulation.[470-474] Patients with different mutations will likely be found to exhibit phenotypic heterogeneity, including in severity of disease and response to treatment. Genetic heterogeneity may underlie the variable response of patients with X-linked diabetes insipidus to dDAVP treatment. In a family with a known mutation, prenatal or early postnatal DNA screening can unambiguously identify affected males, allowing the institution of appropriate therapy.[226]

Congenital, Autosomal Nephrogenic Diabetes Insipidus: Aquaporin-2 Mutations. After the initial description of X-linked nephrogenic diabetes insipidus,[475] several patients were reported with similar clinical findings except for autosomal recessive transmission of the disease[476] or normal V2 receptor function outside of the kidney.[477] With the cloning of the complementary DNA for the renal water channel, aquaporin-2, many patients with autosomal recessive nephrogenic diabetes insipidus have been reported who have a total of 51 mutations different in this gene (www.hgmd.cf.ac.uk/ac/gene.php?gene5AQP2).[471] Most are missense mutations, although four are nonsense or frameshift mutations. They are scattered throughout the molecule, including within four of the five transmembrane domains, two of three extracellular domains, and two of four intracellular domains (see Figure 11-10). An autosomal dominant mode of inheritance for nephrogenic diabetes insipidus has been described, associated with mutations in aquaporin-2. One of these dominant mutations results in mixed tetramers of the wild-type and mutant alleles being retained in the Golgi apparatus.[478] Aquaporin-2 mutations impair the ability of the luminal membrane to undergo an increase in water permeability following signaling through the V2 receptor. They could include patients previously described who had a normal rise in urinary cyclic AMP in response to vasopressin, without a concomitant increase in urine osmolality.[445] Aquaporin-2 protein has been shown to be excreted in the urine in both soluble and membrane-bound forms. Aquaporin-2 excretion is low in untreated central and nephrogenic diabetes insipidus, but following dDAVP

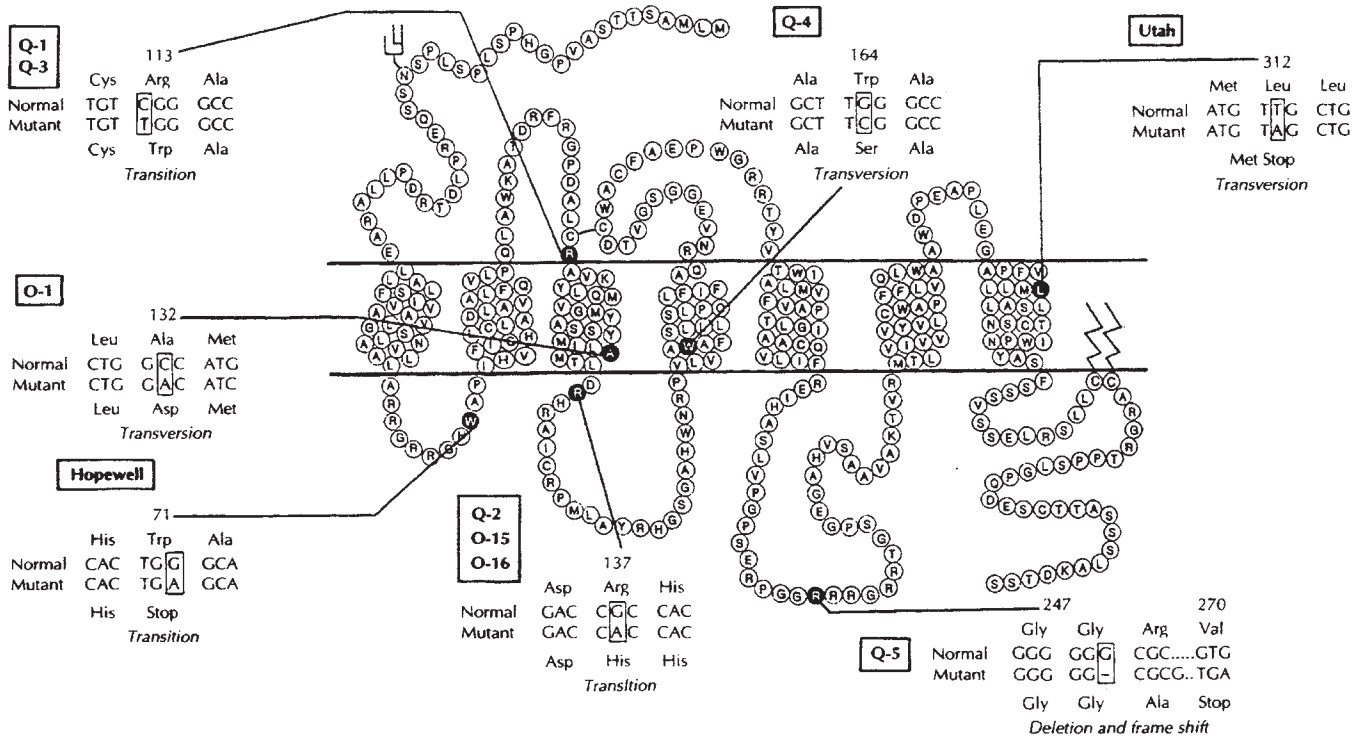


FIGURE 11-14 ■ Schematic representation of seven of the more than 180 V2 receptor mutations in families with X-linked nephrogenic diabetes insipidus. Q, families from Quebec; F, families from France; O, other families. (Reproduced with permission from Bichet, D. G. (1995). The posterior pituitary. In S. Melmed (Ed.), *The pituitary* (p. 277). Cambridge: Blackwell Science.)

administration it increases markedly in the former, but not latter, disease.[119] For this reason, its measurement in urine has been suggested as an aid in the differential diagnosis of diabetes insipidus.[119]

Acquired Cause. Acquired causes of nephrogenic diabetes insipidus are more common and less severe than genetic causes. Nephrogenic diabetes insipidus may be caused by drugs such as lithium and demeclocycline, both of which are thought to interfere with vasopressin-stimulated cyclic AMP generation or action. Approximately 50% of patients receiving lithium have impaired urinary concentrating ability, although only 10% to 20% of them develop symptomatic nephrogenic diabetes insipidus, which is almost always accompanied by a reduction in the glomerular filtration rate.[479,480] The risk increases with the duration of therapy. Lithium impairs the ability of vasopressin to stimulate adenylate cyclase,[481] resulting in a 90% fall in aquaporin-2 messenger RNA expression in the renal collecting duct[482] and which may be the basis for its causing nephrogenic diabetes insipidus.

Demeclocycline treatment causes nephrogenic diabetes insipidus by inhibiting transepithelial water transport.[483] For this reason, it is useful in the treatment of dilutional hyponatremia associated with the inappropriate secretion of vasopressin as discussed previously. Other agents that cause nephrogenic diabetes insipidus include hypercalcemia, hyperkalemia, and therapy with foscarnet (used in the treatment of cytomegalovirus infection in immunosuppressed patients),[484,485] clozapine,[485] amphotericin,[486] methicillin,[487] or rifampin.[488] Whether any of these agents cause nephrogenic diabetes insipidus by interfering with the expression or insertion into apical collecting duct membranes of aquaporin-2 water channels is not yet known. Ureteral obstruction,[489] chronic renal failure, polycystic kidney disease, medullary cystic disease, Sjögren syndrome[490] and sickle cell disease can also impair renal concentrating ability. Osmotic diuresis due to glycosuria in diabetes mellitus, or to sodium excretion with diuretic therapy, will interfere with renal water conservation. Primary polydipsia can result in secondary nephrogenic diabetes insipidus because the chronic excretion of a dilute urine lowers the osmolality of the hypertonic renal interstitium, thus decreasing renal concentrating ability. Finally, decreased protein or sodium intake also can lead to diminished tonicity of the renal medullary interstitium and nephrogenic diabetes insipidus.

Treatment of Nephrogenic Diabetes Insipidus. The treatment of acquired nephrogenic diabetes insipidus focuses on elimination, if possible, of the underlying disorder, such as offending drugs, hypercalcemia, hypokalemia, or ureteral obstruction. Congenital nephrogenic diabetes insipidus is often difficult to treat. The main goals should be to ensure the intake of adequate calories for growth and to avoid severe dehydration. Foods with the highest ratio of caloric content to osmotic load should be ingested to maximize growth and minimize the urine volume required to excrete urine solute. However, even with the early institution of therapy, growth and mental retardation are not uncommon.[491]

Thiazide diuretics in combination with amiloride or indomethacin are the most useful pharmacologic agents in the treatment of nephrogenic diabetes insipidus. Thiazides work both by enhancing sodium excretion at the expense of water, as well as by causing a fall in glomerular filtration rate, which results in proximal tubular sodium and water reabsorption.[422,492] Indomethacin, 2 mg/kg/day, further enhances proximal tubular sodium and water reabsorption,[422,493,494] although this effect is not mediated by inhibition of cyclo-oxygenase.[495] The combination of thiazide and amiloride diuretics is the most commonly used regimen for the treatment of congenital, X-linked nephrogenic diabetes insipidus, because amiloride counteracts thiazide-induced hypokalemia,[448] avoids the nephrotoxicity associated with indomethacin therapy, and is well tolerated, even in infants.[496] In addition, amiloride decreases the uptake of lithium by renal epithelial cells, and for this additional reason it has been proposed in combination with thiazide as treatment for lithium-induced nephrogenic diabetes insipidus.[497] High-dose dDAVP therapy, in combination with indomethacin, has been reported to be helpful in treating some subjects with nephrogenic diabetes insipidus.[498] This treatment may prove to be useful in patients with genetic defects in the V2 receptor, which reduce the binding affinity for vasopressin.

A therapy that has thus far only been employed in mice and not yet available for humans, in which abnormal stop codons are bypassed,[499] may prove useful to treat patients with nonsense mutations in AVPR2 and AQP2.

CONCLUDING REMARKS

Precise regulation of water balance is necessary for the proper function of multiple cellular pathways. Vasopressin released from the posterior pituitary, stimulated by both hyperosmolar and nonosmotic factors, acts via the kidney V2 vasopressin receptor to stimulate both an increase in aquaporin-2 expression and its insertion into collecting duct luminal membrane, thereby enhancing renal water reabsorption to minimize subsequent water loss. Thirst controls the second major physiologic response to hyperosmolality and results in increased water intake to make up for past water loss. The renin-angiotensin-aldosterone and atrial natriuretic peptide systems also make important contributions to water and volume regulation by modulating sodium intake and output.

The proper diagnosis of disorders caused by deficient and excessive action of vasopressin requires a thorough understanding of the physiologic regulation of this hormone. Advances in molecular medicine have revealed mutations in the vasopressin gene and the V2 receptor or aquaporin-2 genes, responsible for familial central and nephrogenic diabetes insipidus, respectively. Molecular methods allow the diagnosis of these disorders in the prenatal or early postnatal periods. Nevertheless, the most frequent cause of central diabetes insipidus remains a destructive lesion of the central nervous system caused by tumor or neurosurgical insult, and pharmacologic toxicity remains the most common cause of nephrogenic diabetes insipidus.

Hyponatremia is a common occurrence in childhood but is rarely due to a primary increase in vasopressin secretion or an increase in intrinsic activity of the V2 receptor (SIAD). It is more commonly caused by hypovolemia (either primary or secondary to decreased effective vascular volume), salt loss, excessive ingestion of hypotonic fluids, or cortisol deficiency. Hyponatremia due to increased vasopressin action is most commonly caused by excessive vasopressin administration during the treatment of central diabetes insipidus or coagulopathies.

Central diabetes insipidus is best treated in infants with fluid therapy, which avoids the administration of vasopressin or its V2 receptor analog, dDAVP, whereas in older children, dDAVP is the drug of choice. Nephrogenic diabetes insipidus remains a therapeutic challenge. Hyponatremia due to SIAD is best managed by restricting water intake, whereas salt and water replacement is indicated when hyponatremia is due to hypovolemia or excessive secretion of atrial natriuretic peptide, as occurs in cerebral salt wasting. Hyponatremia causing central nervous system dysfunction is a medical emergency. Blood sodium must be raised promptly, but at a rate not greater than 0.5 mEq/L/hr to avoid the occurrence of central pontine myelinolysis.

REFERENCES

1. Strange, K. (1992). Regulation of solute and water balance and cell volume in the central nervous system [editorial]. [Review]. *J Am Soc Nephrol*, *3*, 12-27.
2. Vokes, T. J., & Robertson, G. L. (1988). Disorders of antidiuretic hormone. [Review]. *Endocrinol Metab Clin North Am*, *17*, 281-299.
3. Acher, R. (1980). Molecular evolution of biologically active peptides. *Proc R Soc*, *210*, 21-43.
4. Gorbman, A., Dickoff, W. W., Vigna, S. R., et al (1983). Comparative endocrinology. New York: John Wiley & Sons.
5. Turner, R. A., Pierce, J. G., & Du Vigneaud, V. (1951). The purification and amino acid content of vasopressin preparation. *J Biol Chem*, *191*, 21-28.
6. Du Vigneaud, V. (1954). Hormones of the posterior pituitary gland: oxytocin and vasopressin. *Harvey Lect*, *50*, 1-26.
7. Oliver, G., Schafer, E. A. (1895). On the action of extracts of pituitary body and certain other glandular organs. *J Physiol* (London), *18*, 277-279.
8. Du Vigneaud, V., Gish, D. T., & Katsoyannis, P. G. (1954). A synthetic preparation possessing biological properties associated with arginine-vasopressin. *J Am Chem Soc*, *76*, 4751-4752.
9. Du Vigneaud, V., Ressler, C., Swan, J. M., et al. (1953). The synthesis of an octapeptide amide with the hormonal activity of oxytocin. *J Am Chem Soc*, *75*, 4879-4880.
10. Vavra, I., Machova, A., Holecek, V., et al. (1968). Effect of a synthetic analogue of vasopressin in animals and in patients with diabetes insipidus. *Lancet*, *1*, 948-952.
11. Osborne, W. A., & Vincent, S. (1900). A contribution to the study of the pituitary body. *Br Med J*, *1*, 502-503.
12. Acher, R., & Fromagest, C. (1957). The relationship of oxytocin and vasopressin to active proteins of posterior pituitary origin. In H. Heller (Ed.), *The neurohypophysis* (pp. 39-50). London: Butterworths.
13. Pickering, B. T., & Jones, C. W. (1978). The neurophysins. *Horm Proteins Peptides*, *5*, 103-158.
14. Chen, L., Rose, J. P., Breslow, E., et al (1991). Crystal structure of a bovine neurophysin II dipeptide complex at 2.8A determined from the single-wave length anomalous scattering signal of an incorporated iodine atom. *Proc Natl Acad Sci USA*, *88*, 4240-4244.
15. Rose, J. P., Breslow, E., Huang, H. B., & Wang, B. C. (1991). Crystallographic analysis of the neurophysin-oxytocin complex: A preliminary report. *J Mol Biol*, *221*, 43-45.

16. Sachs, H., Fawcett, P., Takabatake, Y., et al. (1969). Biosynthesis and release of vasopressin and neurophysin. *Recent Prog Horm Res*, *25*, 447-484.
17. Sachs, H., & Takabatake, Y. (1964). Evidence for a precursor in vasopressin biosynthesis. *Endocrinology*, *75*, 943-948.
18. Brownstein, M. J., Russell, J. T., & Gainer, H. (1980). Synthesis, transport, and release of posterior pituitary hormones. *Science*, *207*, 373-378.
19. Takabatake, Y., & Sachs, S. (1964). Vasopressin biosynthesis. III. In vitro studies. *Endocrinology*, *75*, 934-942.
20. Hara, Y., Battey, J., Gainer, H. (1990). Structure of the mouse vasopressin and oxytocin genes. *Brain Res Mol Brain Res*, *8*, 319-324.
21. Ivell, R., & Richter, D. (1984). Structure and comparison of the oxytocin and vasopressin genes from rat. *Proc Natl Acad Sci USA*, *81*, 2006-2010.
22. Summar, M. L., Phillips, J. A., Battey, J., et al. (1990). Linkage relationships of human arginine vasopressin-neurophysin-II and oxytocin-neurophysin-I to prodynorphin and other loci on chromosome 20. *Mol Endocrinol*, *4*, 947-950.
23. Ruppert, S. D., Schere, G., & Schutz, G. (1984). Recent gene conversion involving bovine vasopressin and oxytocin precursor genes suggested by nucleotide sequence. *Nature*, *308*, 554-557.
24. Vandesande, F., & Dierickx, K. (1975). Identification of the vasopressin producing and of the oxytocin producing neurons in the hypothalamic magnocellular neurosecretory system of the rat. *Cell Tissue Res*, *164*, 153-162.
25. Sawchenko, P. E., Swanson, L. W., & Vale, W. W. (1984). Co-expression of corticotropin-releasing factor and vasopressin immunoreactivity in parvocellular neurosecretory neurons of the adrenalectomized rat. *Proc Natl Acad Sci USA*, *81*, 1883-1887.
26. Whitnall, M. H., Mezey, E., & Gainer, H. (1985). Co-localization of corticotropin-releasing factor and vasopressin in median eminence neurosecretory vesicles. *Nature*, *317*, 248-252.
27. Majzoub, J. A., & Herman, M. (2002). Adrenocorticotropin. In S. Melmed (Ed.), *The pituitary* (2nd ed.). Cambridge, England: Blackwell Scientific.
28. Verney, E. B. (1947). The antidiuretic hormone and the factors which determine its release. *Proc R Soc*, *135*, 25-105.
29. Hammer, M., Ladefoged, J., & Olgaard, K. (1980). Relationship between plasma osmolality and plasma vasopressin in human subjects. *Am J Physiol*, *238*, E313-E317.
30. Robertson, G. L., Shelton, R. L., & Athar, S. (1976). The osmoregulation of vasopressin. *Kidney Int*, *10*, 25-37.
31. Leng, G. (1980). Rat supraoptic neurones: the effects of locally applied hypertonic saline. *J Physiol* (London), *304*, 405-414.
32. Bealer, S. L., Crofton, J. T., & Share, L. (1983). Hypothalamic knife cuts alter fluid regulation, vasopressin secretion and natriuresis during water deprivation. *Neuroendocrinology*, *36*, 364-370.
33. Thrasher, T. N., Keil, L. C., & Ramsay, D. J. (1982). Lesions of the organum vasculosum of the lamin terminalis (OVLT) attenuate osmotically-induced drinking and vasopressin secretion in the dog. *Endocrinology*, *110*, 1837-1839.
34. McKinley, M. J., Denton, D. A., & Weisinger, R. S. (1978). Sensors for antidiuresis and thirst-osmoreceptors or CSF sodium detectors? *Brain Res*, *141*, 89-103.
35. Phillips, M. I. (1987). Functions of angiotensin in the central nervous system. *Ann Rev Physiol*, *49*, 413-435.
36. Simpson, J. B. (1981). The circumventricular organs and the central action of angiotensin. *Neuroendocrinology*, *32*, 248-256.
37. Yamaguchi, K., Koike, M., & Hama, H. (1985). Plasma vasopressin response to peripheral administration of angiotensin in conscious rats. *Am J Physiol*, *248*, R249-R256.
38. Robertson, G. L. (1977). The regulation of vasopressin function in health and disease. *Recent Prog Horm Res*, *33*, 333-385.
39. Davison, J. M., Gilmore, E. A., Durr, J., et al. (1984). Altered osmotic thresholds for vasopressin secretion and thirst in human pregnancy. *Am J Physiol*, *246*, F105-F109.
40. Boykin, J., DeTorrente, A., Erickson, A., et al. (1978). Role of plasma vasopressin in impaired water excretion of glucocorticoid deficiency. *J Clin Invest*, *62*, 738-744.
41. Linas, S. L., Berl, T., Robertson, G. L., et al. (1980). Role of vasopressin in the impaired water excretion of glucocorticoid deficiency. *Kidney Int*, *18*, 58-67.
42. Lindheimer, M. D., Barron, W. M., & Davison, J. M. (1989). Osmoregulation of thirst and vasopressin release in pregnancy [published erratum appears in Am J Physiol 1989 257(4 Pt 2): preceding F503]. [Review]. *Am J Physiol*, *257*, F159-F169.
43. Spruce, B. A., Baylis, P. H., Burd, J., & Watson, M. J. (1985). Variation in osmoregulation of arginine vasopressin during the human menstrual cycle. *Clin Endocrinol*, *22*, 37-42.
44. Vokes, T. J., Weiss, N. M., Schreiber, J., et al. (1988). Osmoregulation of thirst and vasopressin during normal menstrual cycle. *Am J Physiol*, *254*, R641-R647.
45. Davison, J. M., Shiells, E. A., Philips, P. R., & Lindheimer, M. D. (1988). Serial evaluation of vasopressin release and thirst in human pregnancy: role of human chorionic gonadotrophin in the osmoregulatory changes of gestation. *J Clin Invest*, *81*, 798-806.
46. Kucharczyk, J., & Morgenson, G. J. (1975). Separate lateral hypothalamic pathways for extracellular and intracellular thirst. *Am J Physiol*, *228*, 295-301.
47. Phillips, P. A., Rolls, B. J., Ledingham, J. G., et al. (1985). Angiotensin II-induced thirst and vasopressin release in man. *Clin Sci*, *68*, 669-674.
48. Gruber, K. A., Wilkin, L. D., & Johnson, A. K. (1986). Neurohypophyseal hormone release and biosynthesis in rats with lesions of the anteroventral third ventricle (AV3V) region. *Brain Res*, *378*, 115-119.
49. Thompson, C. J., Burd, J. M., & Baylis, P. H. (1987). Acute suppression of plasma vasopressin and thirst after drinking in hypernatremic humans. *Am J Physiol*, *252*, R1138-R1142.
50. Salata, R. A., Verbalis, J. G., & Robinson, A. G. (1987). Cold water stimulation of oropharyngeal receptors in man inhibits release of vasopressin. *J Clin Endocrinol Metab*, *65*, 561-567.
51. Williams, T. D., Seckl, J. R., & Lightman, S. L. (1989). Dependent effect of drinking volume on vasopressin but not atrial peptide in humans. *Am J Physiol*, *257*, R762-R764.
52. Gauer, O. H., & Henry, J. P. (1963). Circulatory basis of fluid volume control. *Physiol Rev*, *43*, 423-481.
53. Thrasher, T. N. (1994). Baroreceptor regulation of vasopressin and renin secretion: low-pressure versus high-pressure receptors. [Review]. *Front Neuroendocrinol*, *15*, 157-196.
54. Cunningham, E. T., Jr., & Sawchenko, P. E. (1991). Reflex control of magnocellular vasopressin and oxytocin secretion. [Review]. *Trends Neurosci*, *14*, 406-411.
55. Sawchenko, P. E., & Swanson, L. W. (1981). Central noradrenergic pathways for the integration of hypothalamic neuroendocrine and autonomic responses. *Science*, *214*, 685.
56. Blessing, W. W., Sved, A. F., & Reis, D. J. (1983). Destruction of noradrenergic neurons in rabbit brainstem. *Science*, *217*, 661.
57. Schrier, R. W., Berl, T., & Harbottle, J. A. (1972). Mechanism of the antidiuretic effect associated with interruption of parasympathetic pathways. *J Clin Invest*, *51*, 2613.
58. Goetz, K. L., Bond, G. C., & Smith W. E. (1974). Effect of moderate hemorrhage in humans on plasma ADH and renin. *Proc Soc Exp Biol Med*, *145*, 277-280.
59. Robertson, G. L. (1976). The regulation of vasopressin function in health and disease. [Review]. *Recent Prog Horm Res*, *33*, 333-385.
60. Robertson, G. L., & Athar, S. (1976). The interaction of blood osmolality and blood volume in regulating plasma vasopressin in man. *J Clin Endocrinol Metab*, *42*, 613-620.
61. Arnauld, E., Czernichow, P., Fumoux, F., & Vincent, J. D. (1977). The effects of hypotension and hypovolaemia on the liberation of vasopressin during haemorrhage in the unanaesthetized monkey (Macaca mulatta). *Pflugers Arch*, *371*, 193-200.
62. Murase, T., & Yoshida, S. (1971). Effect of hypotension on levels of antidiuretic hormone in plasma in dogs. *Endocrinol Jpn*, *18*, 215-219.
63. Wiggins, R. C., Basar, I., Slater, J. D., et al. (1977). Vasovagal hypotension and vasopressin release. *Clin Endocrinol*, *6*, 387-393.
64. Hirsch, A. T., Majzoub, J. A., Ren, C. J., et al. (1993). Contribution of vasopressin to blood pressure regulation during hypovolemic hypotension in humans. *J Appl Physiol*, *75*, 1984-1988.
65. Feldman, M., Samson, W. K., & O'Dorisio, T. M. (1988). Apomorphine-induced nausea in humans: Release of vasopressin and pancreatic polypeptide. *Gastroenterology*, *95*, 721-726.
66. Koch, K. L., Summy-Long, J., Bingaman, S., et al. (1990). Vasopressin and oxytocin responses to illusory self-motion and nausea in man. *J Clin Endocrinol Metab*, *71*, 1269-1275.

67. Seckl, J. R., Johnson, M., Shakespear, C., & Lightman, S. L. (1988). Endogenous opioids inhibit oxytocin release during nicotine-stimulated secretion of vasopressin in man. *Clin Endocrinol*, *28*, 509-514.
68. Ishikawa, S., Fujisawa, G., Tsuboi, Y., et al. (1991). Role of antidiuretic hormone in hyponatremia in patients with isolated adrenocorticotropic hormone deficiency. *Endocrinol Jpn*, *38*, 325-330.
69. Oelkers, W. (1989). Hyponatremia and inappropriate secretion of vasopressin (antidiuretic hormone) in patients with hypopituitarism [see comments]. *N Engl J Med*, *321*, 492-496.
70. Green, H. H., Harrington, A. R., & Valtin, H. (1970). On the role of antidiuretic hormone in the inhibition of acute water diuresis in adrenal insufficiency and the effects of gluco- and mineralocorticoids in reversing the inhibition. *J Clin Invest*, *49*, 1724-1736.
71. Viinamaki, O., Erkkola, R., & Kanto, J. (1986). Plasma vasopressin concentrations and serum vasopressinase activity in pregnant and nonpregnant women. *Biol Res Pregnancy Perinatol*, *7*, 17-19.
72. Davison, J. M., Sheills, E. A., Barron, W. M., et al. (1989). Changes in the metabolic clearance of vasopressin and in plasma vasopressinase throughout human pregnancy. *J Clin Invest*, *83*, 1313-1318.
73. Iwasaki, Y., Oiso, Y., Kondo, K., et al. (1991). Aggravation of subclinical diabetes insipidus during pregnancy. *N Engl J Med*, *324*, 522-526.
74. Kennedy, S., Hall, P. M., Seymour, A. E., & Hague, W. M. (1994). Transient diabetes insipidus and acute fatty liver of pregnancy. *Br J Obstet Gynaecol*, *101*, 387-391.
75. Katz, V. L., & Bowes, W. A., Jr. (1987). Transient diabetes insipidus and preeclampsia. *South Med J*, *80*, 524-545.
76. Durr, J. A., Hoggard, J. G., Hunt, J. M., & Schrier, R. W. (1987). Diabetes insipidus in pregnancy associated with abnormally high circulating vasopressinase activity. *N Engl J Med*, *316*, 1070-1074.
77. Davison, J. M., Sheills, E. A., Philips, P. R., et al. (1993). Metabolic clearance of vasopressin and an analogue resistant to vasopressinase in human pregnancy. *Am J Physiol*, *264*, F348-F353.
78. Krege, J., Katz, V. L., & Bowes, W. A., Jr. (1989). Transient diabetes insipidus of pregnancy. [Review]. *Obstet Gynecol Surv*, *44*, 789-795.
79. Jard, S. (1983). Vasopressin isoreceptors in mammals: Relation to cyclic-AMP dependent and cyclic-AMP-independent transducer mechanisms. *Curr Top Membr Transp*, *18*, 225-285.
80. Hirasawa, A., Shibata, K., Kotosai, K., & Tsujimoto, G. (1994). Cloning, functional expression and tissue distribution of human cDNA for the vascular-type vasopressin receptor. *Biochem Biophys Res Commun*, *203*, 72-79.
81. Morel, A., O'Carroll, A. M., Brownstein, M. J., & Lolait, S. J. (1992). Molecular cloning and expression of a rat V1a arginine vasopressin receptor. *Nature*, *356*, 523-526.
82. Ostrowski, N. L., Young, W. S., Knepper, M. A., & Lolait, S. J. (1993). Expression of vasopressin V1a and V2 receptor messenger ribonucleic acid in the liver and kidney of embryonic, developing, and adult rats. *Endocrinology*, *133*, 1849-1859.
83. Thibonnier, M., Auzan, C., Madhun, Z., et al. (1994). Molecular cloning, sequencing, and functional expression of a cDNA encoding the human V1a vasopressin receptor. *J Biol Chem*, *269*, 3304-2210.
84. Ostrowski, N. L., Lolait, S. J., Bradley, D. J., et al. (1992). Distribution of V1a and V2 vasopressin receptor messenger ribonucleic acids in rat liver, kidney, pituitary and brain. *Endocrinology*, *131*, 533-535.
85. Johnson, E. M., Theler, J. M., Capponi, A. M., & Vallotton, M. B. (1991). Characterization of oscillations in cytosolic free Ca2+ concentration and measurement of cytosolic Na+ concentration changes evoked by angiotensin II and vasopressin in individual rat aortic smooth muscle cells: Use of microfluorometry and digital imaging. *J Biol Chem*, *266*, 12618-12626.
86. Takeuchi, K., Abe, K., Yasujima, M., et al. (1992). Phosphoinositide hydrolysis and calcium mobilization induced by vasopressin and angiotensin II in cultured vascular smooth muscle cells. *Tohoku J Exp Med*, *166*, 107-122.
87. Spruce, B. A., McCulloch, A. J., Burd, J, et al. (1985). The effect of vasopressin infusion on glucose metabolism in man. *Clin Endocrinol*, *22*, 463-468.
88. Inaba, K., Umeda, Y., Yamane, Y., et al. (1988). Characterization of human platelet vasopressin receptor and the relation between vasopressin-induced platelet aggregation and vasopressin binding to platelets. *Clin Endocrinol*, *29*, 377-386.
89. Briley, E. M., Lolait, S. J., Axelrod, J., & Felder, C. C. (1994). The cloned vasopressin V1a receptor stimulates phospholipase A2, phospholipase C, and phospholipase D through activation of receptor-operated calcium channels. *Neuropeptides*, *27*, 63-74.
90. Montani, J. P., Liard, J. F., Schoun, J., & Mohring, J. (1984). Hemodynamic effects of exogenous and endogenous vasopressin at low plasma concentrations in conscious dogs. *Circ Res*, *47*, 346-355.
91. Altura, B. M., & Altura, B. T. (1984). Actions of vasopressin, oxytocin, and synthetic analogs on vascular smooth muscle. *Fed Proc*, *43*, 80-86.
92. Kimura, T., Tanizawa, O., Mori, K., et al. (1992). Structure and expression of a human oxytocin receptor. *Nature*, *356*, 526-529.
93. Rozen, F., Russo, C., Banville, D., & Zingg, H. H. (1995). Structure, characterization, and expression of the rat oxytocin receptor gene. *Proc Natl Acad Sci USA*, *92*, 200-204.
94. Aoyagi, T., Birumachi, J., Hiroyama, M., et al. (2007). Alteration of glucose homeostasis in V1a vasopressin receptor-deficient mice. *Endocrinology*, *148*, 2075-2084.
95. Baertschi, A. J., & Friedli, M. (1985). A novel type of vasopressin receptor on anterior pituitary corticotrophs. *Endocrinology*, *116*, 499-502.
96. de Keyzer, Y., Auzan, C., Lenne, F., et al. (1994). Cloning and characterization of the human V3 pituitary vasopressin receptor. *FEBS Lett*, *356*, 215-220.
97. Sugimoto, T., Saito, M., Mochizuki, S., et al. (1994). Molecular cloning and functional expression of a cDNA encoding the human V1b vasopressin receptor. *J Biol Chem*, *269*, 27088-27092.
98. Lolait, S. J., Stewart, L. Q., Jessop, D. S., et al. (2007). The hypothalamic-pituitary-adrenal axis response to stress in mice lacking functional vasopressin V1b receptors. *Endocrinology*, *148*, 849-856.
99. Roberts, E. M., Pope, G. R., Newson, M. J., et al. (2011). The vasopressin V1b receptor modulates plasma corticosterone responses to dehydration-induced stress. *J Neuroendocrinol*, *23*, 12-19.
100. Wersinger, S. R., Kelliher, K. R., Zufall, F., et al. (2004). Social motivation is reduced in vasopressin 1b receptor null mice despite normal performance in an olfactory discrimination task. *Horm Behav*, *46*, 638-645.
101. Lolait, S. J., O'Carroll, A. M., McBride, O. W., et al. (1992). Cloning and characterization of a vasopressin V2 receptor and possible link to nephrogenic diabetes insipidus. *Nature*, *357*, 336-339.
102. Hirsch, A. T., Dzau, V. J., Majzoub, J. A., & Creager, M. A. (1989). Vasopressin-mediated forearm vasodilation in normal humans: Evidence for a vascular vasopressin V2 receptor. *J Clin Invest*, *84*, 418-426.
103. Tagawa, T., Imaizumi, T., Endo, T., et al. (1993). Vasodilatory effect of arginine vasopressin is mediated by nitric oxide in human forearm vessels. *J Clin Invest*, *92*, 1483-1490.
104. Kobrinsky, N. L., Israels, E. D., Gerrard, J. M., et al. (1984). Shortening of bleeding time by 1-deamino-8-D-arginine vasopressin in various bleeding disorders. *Lancet*, *1*, 1145-1148.
105. Birnbaumer, M., Seibold, A., Gilbert, S., et al. (1992). Molecular cloning of the receptor for human antidiuretic hormone. *Nature*, 357, 333-335.
106. Frattini, A., Zucchi, I., Villa, A., et al. (1993). Type 2 vasopressin receptor gene, the gene responsible nephrogenic diabetes insipidus, maps to Xq28 close to the LICAM gene. *Biochem Biophys Res Commun*, *193*, 864-871.
107. Seibold, A., Brabet, P., Rosenthal, W., & Birnbaumer, M. (1992). Structure and chromosomal localization of the human antidiuretic hormone receptor gene. *Am J Hum Genet*, *51*, 1078-1083.
108. Yun, J., Schoneberg, T., Liu, J., et al. (2000). Generation and phenotype of mice harboring a nonsense mutation in the V2 vasopressin receptor gene. *J Clin Invest*, *106*, 1361-1371.
109. Harris, H. W., Paredes, A., & Zeidel, M. L. (1993). The molecular structure of the antidiuretic hormone elicited water channel. [Review]. *Pediatr Nephrol*, *7*, 680-684.
110. Harris, H. W., Strange, K., & Zeidel, M. L. (1991). Current understanding of the cellular biology and molecular structure of the antidiuretic hormone-stimulated water transport pathway. *J Clin Invest*, *88*, 1-8.
111. Nielsen, S., Marples, D., Birn, H., et al. (1995). Expression of VAMP2-like protein in kidney collecting duct intracellular vesicles. *J Clin Invest*, *96*, 1834-1844.

112. Mandon, B., Chou, C. L., Nielsen, S., & Knepper, M. A. (1996). Syntaxin-4 is localized to the apical plasma membrane of rat renal collecting duct cells: possible role in aquaporin-2 trafficking. *J Clin Invest*, *98*, 906-913.
113. Knepper, M. A. (1994). The aquaporin family of molecular water channels. *Proc Natl Acad Sci* U S A, *91*, 6255-6258.
114. Sasaki, S., Fushimi, K., Saito, H., et al. (1994). Cloning, characterization, and chromosomal mapping of human aquaporin of collecting duct. *J Clin Invest*, *93*, 1250-1256.
115. Deen, P. M., Verdijk, M. A., Knoers, N. V., et al. (1994). Requirement of human renal water channel aquaporin-2 for vasopressin-dependent concentration of urine. *Science*, *264*, 92-95.
116. Stevens, A. L., Breton, S., Gustafson, C. E., et al. (2000). Aquaporin 2 is a vasopressin-independent, constitutive apical membrane protein in rat vas deferens. *Am J Physiol*, *278*, C791-C802.
117. Fushimi, K., Sasaki, S., & Marumo, F. (1997). Phosphorylation of serine 256 is required for cAMP-dependent regulatory exocytosis of the aquaporin-2 water channel. *J Biol Chem*, *272*, 14800-14804.
118. Valenti, G., Procino, G., Liebenhoff, U., et al. (1998). A heterotrimeric G protein of the Gi family is required for cAMP-triggered trafficking of aquaporin 2 in kidney epithelial cells. *J Biol Chem*, 273, 22627-22634.
119. Kanno, K., Sasaki, S., Hirata, Y., et al. (1995). Urinary excretion of aquaporin-2 in patients with diabetes insipidus. *N Engl J Med*, *332*, 1540-1545.
120. Yang, B., Gillespie, A., Carlson, E. J., et al. (2001). Neonatal mortality in an aquaporin-2 knock-in mouse model of recessive nephrogenic diabetes insipidus. *J Biol Chem*, *276*, 2775-2779.
121. Ma, T., Yang, B., Gillespie, A., et al. (1997). Generation and phenotype of a transgenic knockout mouse lacking the mercurial-insensitive water channel aquaporin-4. *J Clin Invest*, *100*, 957-962.
122. Ma, T., Song, Y., Yang, B., et al. (2000). Nephrogenic diabetes insipidus in mice lacking aquaporin-3 water channels. *Proc Natl Acad Sci USA*, *97*, 4386-4391.
123. Schnermann, J., Chou, C. L., Ma, T., et al. (1998). Defective proximal tubular fluid reabsorption in transgenic aquaporin-1 null mice. *Proc Natl Acad Sci USA*, *95*, 9660-9664.
124. Hori, K., Nagai, T., Izumi, Y., et al. (2012). Vasopressin V1a receptor is required for nucleocytoplasmic transport of mineralocorticoid receptor. *Am J Physiol Renal Physiol*, *303*, F1080-F1088.
125. Gibbons, G. H., Dzau, V. J., Farhi, E. R., & Barger, A. C. (1984). Interaction of signals influencing renin release. [Review]. *Ann Rev Physiol*, *46*, 291-308.
126. Quinn, S. J., & Williams, G. H. (1988). Regulation of aldosterone secretion. *Ann Rev Physiol*, *50*, 409-426.
127. Balla, T., Baukal, A. J., Eng, S., & Catt, K. J. (1991). Angiotensin II receptor subtypes and biological responses in the adrenal cortex and medulla. *Mol Pharmacol*, *40*, 401-406.
128. Murphy, T. J., Alexander, R. W., Griendling, K. K., et al. (1991). Isolation of a cDNA encoding the vascular type-1 angiotensin II receptor. *Nature*, *351*, 233-236.
129. Murphy, T. J., Takeuchi, K., & Alexander, R. W. (1992). Molecular cloning of AT1 angiotensin receptors. [Review]. *Am J Hypertens*, *5*, 236S-242S.
130. Stromberg, C., Tsutsumi, K., Viswanathan, M., & Saavedra, J. M. (1991). Angiotensin II AT1 receptors in rat superior cervical ganglia: characterization and stimulation of phosphoinositide hydrolysis. *Eur J Pharmacol*, *208*, 331-336.
131. Tsutsumi, K., Stromberg, C., Viswanathan, M., & Saavedra, J. M. (1991). Angiotensin-II receptor subtypes in fetal tissue of the rat: autoradiography, guanine nucleotide sensitivity, and association with phosphoinositide hydrolysis. *Endocrinology*, *129*, 1075-1082.
132. Aguilera, G., & Catt, K. J. (1979). Loci of action of regulators of aldosterone biosynthesis in isolated glomerulosa cells. *Endocrinology*, *104*, 1046-1052.
133. Aguilera, G., Menard, R. H., & Catt, K. J. (1980). Regulatory actions of angiotensin II on receptors and steroidogenic enzymes in adrenal glomerulosa cells. *Endocrinology*, *107*, 55-60.
134. Catt, K. J., Aguilera, G., Capponi, A., et al. (1979). Angiotensin II receptors and aldosterone secretion. [Review]. *J Endocrinol*, *81*, 37P-48P.
135. Kramer, R. E., Gallant, S., Brownie, A. C., et al. (1990). Actions of angiotensin II on aldosterone biosynthesis in the rat adrenal cortex: effects on cytochrome P-450 enzymes of the early and late pathway discrimination of two angiotensin II receptor subtypes with a selective agonist analogue of angiotensin II, p-aminophenylalanine6 angiotensin II. *J Biol Chem Biochem Biophys Res Commun*, *169*, 997-1006.
136. Bottari, S. P., de Gasparo, M., Steckelings, U. M., & Levens, N. R. (1993). Angiotensin II receptor subtypes: characterization, signalling mechanisms, and possible physiological implications. [Review]. *Front Neuroendocrinol*, *14*, 123-271.
137. Bottari, S. P., Taylor, V., King, I. N., et al. (1991). Angiotensin II AT2 receptors do not interact with guanine nucleotide binding proteins. *Eur J Pharmacol*, *207*, 157-163.
138. Speth, R. C., & Kim, K. H. (1990). Discrimination of two angiotensin II receptor subtypes with a selective agonist analogue of angiotensin II, p-aminophenylalanine6 angiotensin II. *Biochem Biophys Res Commun*, *169*, 997-1006.
139. Morris, D. J. (1981). The metabolism and mechanism of action of aldosterone. [Review]. *Endocr Rev*, *2*, 234-247.
140. Dzau, V. J. (1988). Molecular and physiological aspects of tissue renin-angiotensin system: emphasis on cardiovascular control. [Review]. *J Hypertens* (Suppl. 2), *6*, S7-S12.
141. Chartier, L., & Schiffrin, E. L. (1987). Role of calcium in effects of atrial natriuretic peptide on aldosterone production in adrenal glomerulosa cells. *Am J Physiol*, *252*, E485-E491.
142. Chartier, L., Schiffrin, E., Thibault, G., & Garcia, R. (1984). Atrial natriuretic factor inhibits the stimulation of aldosterone secretion by angiotensin II, ACTH and potassium in vitro and angiotensin II-induced steroidogenesis in vivo. *Endocrinology*, *115*, 2026-2028.
143. Chartier, L., & Schiffrin, E. L. (1987). Atrial natriuretic peptide inhibits the effect of endogenous angiotensin II on plasma aldosterone in conscious sodium-depleted rats. *Clin Sci*, *72*, 31-35.
144. Hausdorff, W. P., Aguilera, G., & Catt, K. J. (1989). Inhibitory actions of somatostatin on cyclic AMP and aldosterone production in agonist-stimulated adrenal glomerulosa cells. *Cell Signal*, *1*, 377-386.
145. Rebuffat, P., Mazzocchi, G., Gottardo, G., & Nussdorfer, G. G. (1989). Further studies on the involvement of dopamine and somatostatin in the inhibitory control of the growth and steroidogenic capacity of rat adrenal zona glomerulosa. *Exp Clin Endocrinol*, *93*, 73-81.
146. Robba, C., Mazzocchi, G., & Nussdorfer, G. G. (1986). Further studies on the inhibitory effects of somatostatin on the growth and steroidogenic capacity of rat adrenal zona glomerulosa. *Exp Pathol*, *29*, 77-82.
147. Gallo-Payet, N., Chouinard, L., Balestre, M. N., & Guillon, G. (1990). Dual effects of dopamine in rat adrenal glomerulosa cells. *Biochem Biophys Res Commun*, *172*, 1100-1108.
148. Missale, C., Lombardi, C., Sigala, S., & Spano, P. F. (1990). Dopaminergic regulation of aldosterone secretion: biochemical mechanisms and pharmacology. [Review]. *Am J Hypertens*, *3*, 93S-95S.
149. Dzau, V. J., & Hirsch, A. T. (1990). Emerging role of the tissue renin-angiotensin systems in congestive heart failure. [Review]. *Eur Heart J* (Suppl. B), *11*, 65-71.
150. Ganten, D., Paul, M., Deboben, A., et al. (1984). The brain renin angiotensin system in central cardiovascular control. [Review]. *Contrib Nephrol*, *43*, 114-128.
151. Hirsch, A. T., Dzau, V. J., & Creager, M. A. (1987). Baroreceptor function in congestive heart failure: effect on neurohumoral activation and regional vascular resistance. [Review]. *Circulation*, *75*, IV36-IV48.
152. Tang, S. S., Stevenson, L., & Dzau, V. J. (1990). Endothelial renin-angiotensin pathway. Adrenergic regulation of angiotensin secretion. *Circ Res*, *66*, 103-108.
153. Unger, T., Badoer, E., Ganten, D., et al. (1988). Brain angiotensin: pathways and pharmacology. [Review]. *Circulation*, *77*, I40-I54.
154. Saavedra, J. M. (1992). Brain and pituitary angiotensin. [Review]. *Endocr Rev*, *13*, 329-380.
155. Van Houten, M., & Posner, B. I. (1983). Circumventricular organs: receptors and mediators of direct peptide hormone action on brain. [Review]. *Adv Metab Disord*, *10*, 269-289.
156. Van Houten, M., Schiffrin, E. L., Mann, J. F., et al. (1980). Radioautographic localization of specific binding sites for blood-borne angiotensin II in the rat brain. *Brain Res*, *186*, 480-485.
157. Stornetta, R. L., Hawelu-Johnson, C. L., Guyenet, P. G., & Lynch, K. R. (1988). Astrocytes synthesize angiotensinogen in brain. *Science*, *242*, 1444-1446.

158. Paul, M., Printz, M. P., Harms, E., et al. (1985). Localization of renin (EC 3.4.23) and converting enzyme (EC 3.4.15.1) in nerve endings of rat brain. *Brain Res*, *334*, 315-324.
159. Correa, F. M., Guilhaume, S. S., & Saavedra, J. M. (1990). Autoradiography of angiotensin-converting enzyme in fixed and unfixed rat brain using the specific enzyme inhibitor [125I]351A or a polyclonal antibody and [125I]staphylococcal protein A. *Neurosci Lett*, *110*, 244-248.
160. Correa, F. M., Plunkett, L. M., & Saavedra, J. M. (1986). Quantitative distribution of angiotensin-converting enzyme (kininase II) in discrete areas of the rat brain by autoradiography with computerized microdensitometry. *Brain Res*, *375*, 259-266.
161. Plunkett, L. M., Correa, F. M., & Saavedra, J. M. (1985). Quantitative autoradiographic determination of angiotensin-converting enzyme binding in rat pituitary and adrenal glands with 125I-351A, a specific inhibitor. *Regul Pept*, *12*, 263-272.
162. Pickel, V. M., Chan, J., & Ganten, D. (1986). Dual peroxidase and colloidal gold-labeling study of angiotensin converting enzyme and angiotensin-like immunoreactivity in the rat subfornical organ. *J Neurosci*, *6*, 2457-2469.
163. Castren, E., & Saavedra, J. M. (1989). Angiotensin II receptors in paraventricular nucleus, subfornical organ, and pituitary gland of hypophysectomized, adrenalectomized, and vasopressin-deficient rats. *Proc Natl Acad Sci USA*, *86*, 725-729.
164. Andersson, B., Leksell, L. G., & Lishajko, F. (1975). Perturbations in fluid balance induced by medially placed forebrain lesions. *Brain Res*, *99*, 261-275.
165. Saavedra, J. M., Castren, E., Gutkind, J. S., & Nazarali, A. J. (1989). Regulation of brain atrial natriuretic peptide and angiotensin receptors: quantitative autoradiographic studies. *Int Rev Neurobiol*, *31*, 257-296.
166. Saper, C. B., & Levisohn, D. (1983). Afferent connections of the median preoptic nucleus in the rat: anatomical evidence for a cardiovascular integrative mechanism in the anteroventral third ventricular (AV3V) region. *Brain Res*, *288*, 21-31.
167. Saper, C. B., Loewy, A. D., Swanson, L. W., & Cowan, W. M. (1976). Direct hypothalamo-autonomic connections. *Brain Res*, *117*, 305-312.
168. Sawchenko, P. E. (1983). Central connections of the sensory and motor nuclei of the vagus nerve. *J Auton Nerv Syst*, *9*, 13-26.
169. Sawchenko, P. E., & Swanson, L. W. (1983). The organization of forebrain afferents to the paraventricular and supraoptic nuclei of the rat. *J Comp Neurol*, *218*, 121-144.
170. Swanson, L. W., & Sawchenko, P. E. (1983). Hypothalamic integration: organization of the paraventricular and supraoptic nuclei. *Ann Rev Neurosci*, *6*, 269-324.
171. Ishikawa, S., Saito, T., & Yoshida, S. (1980). Effects of glucose and sodium chloride on the release of vasopressin in response to angiotensin II from the guinea pig. *Neuroendocrinology*, *31*, 365-368.
172. Simon-Oppermann, C., Gray, D. A., & Simon, E. (1986). Independent osmoregulatory control of central and systemic angiotensin II concentrations in dogs. *Am J Physiol*, *250*, R918-R925.
173. de Bold, A. J. (1982). Tissue fractionation studies on the relationship between an atrial natriuretic factor and specific atrial granules. *Can J Physiol Pharmacol*, *60*, 324-330.
174. de Bold, A. J., Borenstein, H. B., Veress, A. T., & Sonnenberg, H. (1981). A rapid and potent natriuretic response to intravenous injection of atrial myocardial extract in rats. *Life Sci*, *28*, 89-94.
175. Yandle, T. G. (1994). Biochemistry of natriuretic peptides. [Review]. *J Intern Med*, *235*, 561-576.
176. Hamid, Q., Wharton, J., Terenghi, G., et al. (1987). Localization of atrial natriuretic peptide mRNA and immunoreactivity in the rat heart and human atrial appendage. *Proc Natl Acad Sci USA*, *84*, 6760-6764.
177. Imura, H., Nakao, K., & Itoh, H. (1992). The natriuretic peptide system in the brain: implications in the central control of cardiovascular and neuroendocrine functions. *Front Neuroendocrinol*, *13*, 217-249.
178. Inagaki, S., Kubota, Y., Kito, S., et al. (1986). Atrial natriuretic peptide-like immunoreactivity in the rat pituitary: light and electron microscopic studies. *Regul Pept*, *14*, 101-111.
179. Inagaki, S., Kubota, Y., Kito, S., et al. (1986). Immunoreactive atrial natriuretic polypeptide in the adrenal medulla and sympathetic ganglia. *Regul Pept*, *15*, 249-260.
180. Ritter, D., Chao, J., Needeleman, P., et al. (1992). Localization, synthetic regulation, and biology of renal atriopeptin-like prohormone *Am J Physiol*, *263*, F503-F509.
181. Grundlach, A. L., & Knobe, K. E. (1992). Distribution of preproatrial natriuretic peptide mRNA in rat brain detected by in situ hybridization of DNA oligonucleotides: enrichment in hypothalamic and limbic regions. *J Neurochem*, *59*, 758-761.
182. Herman, J. P., Langub, M. C. J., & Watson, R. E. J. (1993). Localization of C-type natriuretic peptide mRNA in rat hypothalamus. *Endocrinology*, *133*, 1903-1906.
183. Lewicki, J. A., Greenberg, B., Yamanaka, M., et al. (1986). Cloning, sequence analysis, and processing of the rat and human atrial natriuretic peptide precursors. [Review]. *Fed Proc*, *45*, 2086-2090.
184. Sudoh, T., Kanagawa, K., Minamino, N., & Matsuo, H. (1988). A new natriuretic peptide in porcine brain. *Nature*, *332*, 78-81.
185. Hasegawa, K., Fujiwara, H., Doyama, K., et al. (1993). Ventricular expression of brain natriuretic peptide in hypertrophic cardiomyopathy. *Circulation*, *88*, 372-380.
186. Hasegawa, K., Fujiwara, H., Doyama, K., et al. (1993). Ventricular expression of atrial and brain natriuretic peptides in dilated cardiomyopathy: an immunohistocytochemical study of the endomyocardial biopsy specimens using specific monoclonal antibodies. *Am J Pathol*, *142*, 107-116.
187. Yoshimura, M., Yasue, H., Okumura, K., et al. (1993). Different secretion patterns of atrial natriuretic peptide and brain natriuretic peptide in patients with congestive heart failure. *Circulation*, *87*, 464-469.
188. Sudoh, T., Maekawa, K., Kojima, M., et al. (1989). Cloning and sequence analysis of cDNA encoding a precursor for human brain natriuretic peptide. *Biochem Biophys Res Commun*, *159*, 1427-1434.
189. Sudoh, T., Minamino, N., Kanagawa, K., & Matsuo, H. (1990). C-type natriuretic peptide (CNP): a new member of natriuretic peptide family identified in porcine brain. *Biochem Biophys Res Commun*, *168*, 863-870.
190. Cuneo, R. C., Espiner, E. A., Nicholls, M. G., et al. (1986). Renal, hemodynamic, and hormonal responses to atrial natriuretic peptide infusions in normal man, and effect of sodium intake. *J Clin Endocrinol Metab*, *63*, 946-953.
191. Espiner, E. A. (1994). Physiology of natriuretic peptides. [Review]. *J Intern Med*, *235*, 527-541.
192. Nakao, K., Ogawa, Y., Suga, S., & Imura, H. (1992). Molecular biology and biochemistry of the natriuretic peptide system. I: natriuretic peptides. [Review]. *J Hypertens*, *10*, 907-912.
193. Garbers, D. L., Koesling, D., & Schultz, G. (1994). Guanylyl cyclase receptors. [Review]. *Mol Biol Cell*, *5*, 1-5.
194. Schulz, S., Singh, S., Bellet, R. A., et al. (1989). The primary structure of a plasma membrane guanylate cyclase demonstrates diversity within this new receptor family. *Cell*, *58*, 1155-1162.
195. Yamaguchi, M., Rutledge, L. J., & Garbers, D. L. (1990). The primary structure of the rat guanylyl cyclase A/atrial natriuretic peptide receptor gene. *J Biol Chem*, *265*, 20414-20420.
196. Koller, K. J., Lowe, D. G., Bennett, G. L., et al. (1991). Selective activation of the B natriuretic peptide receptor by C-type natriuretic peptide (CNP). *Science*, *252*, 120-123.
197. Fuller, F., Porter, J. G., Arfsten, A. E., et al. (1982). Atrial natriuretic peptide clearance receptor: complete sequence and functional expression of cDNA clones. *J Biol Chem*, *263*, 9395-9401.
198. Maack, T. (1992). Receptors of atrial natriuretic factor. *Ann Rev Physiol*, *54*, 11-27.
199. Wilcox, J. N., Augustine, A., Goeddel, D. V., & Lowe, D. G. (1991). Differential regional expression of three natriuretic peptide genes within primate tissues. *Mol Cell Biol*, *11*, 3454-3462.
200. Tallerico-Melnyk, T., Yip, C. C., Watt, V. M. (1992). Widespread colocalization of messenger RNAs encoding the guanylate cyclase-coupled natriuretic peptide receptors in rat tissues. *Biochem Biophys Res Commun*, *189*, 610-616.
201. Espiner, E. A., Nicholls, M. G., Yandle, T. G., et al. (1986). Studies on the secretion, metabolism and action of atrial natriuretic peptide in man. *J Hypertens* (Suppl.), *4*, S85-S91.
202. Crozier, I. G., Nicholls, M. G., Ikram, H., et al. (1986). Atrial natriuretic peptide in humans: production and clearance by various tissues. *Hypertension*, *8*, II11-II15.

203. Yandle, T. G., Espiner, E. A., Nicholls, M. G., & Duff, H. (1986). Radioimmunoassay and characterization of atrial natriuretic peptide in human plasma. *J Clin Endocrinol Metab*, *63*, 72-79.
204. Fujii, T., Kojima, S., Imanishi, M., et al. (1991). Different mechanisms of polyuria and natriuresis associated with paroxysmal supraventricular tachycardia. *Am J Cardiol*, *68*, 343.
205. Fujii, T., Kojima, S., Ohe, T., et al. (1991). Dominance of blood pressure in natriuresis associated with supraventricular tachycardia. *Nephron*, *57*, 262.
206. Kaye, G. C., Bayliss, P., Lowry, P. J., et al. (1992). Effect of induced supraventricular tachycardias on changes in urine and plasma hormone levels in man. *Clin Sci*, *82*, 33.
207. Cuneo, R. C., Espiner, E. A., Nicholls, M. G., et al. (1987). Effect of physiological levels of atrial natriuretic peptide on hormone secretion: inhibition of angiotensin-induced aldosterone secretion and renin release in normal man. *J Clin Endocrinol Metab*, *65*, 765-772.
208. Kimura, T., Abe, K., Shoji, M., et al. (1986). Effects of human atrial natriuretic peptide on renal function and vasopressin release. *Am J Physiol*, *250*, R789-R794.
209. Thrasher, T. N., & Ramsay, D. J. (1993). Interactions between vasopressin and atrial natriuretic peptides. [Review]. *Ann N Y Acad Sci*, *689*, 426-437.
210. Williams, T. D., Walsh, K. P., Lightman, S. L., & Sutton, R. (1988). Atrial natriuretic peptide inhibits postural release of renin and vasopressin in humans. *Am J Physiol*, *255*, R368-R372.
211. Bahr, V., Sander-Bahr, C., Ardevol, R., et al. (1993). Effects of atrial natriuretic factor on the renin-aldosterone system: in vivo and in vitro studies. [Review]. *J Steroid Biochem Mol Biol*, *45*, 173-178.
212. Florkowski, C. M., Richards, A. M., Espiner, E. A., et al. (1994). Renal, endocrine, and hemodynamic interactions of atrial and brain natriuretic peptides in normal men. *Am J Physiol*, *266*, R1244-R1250.
213. Wittert, G. A., Espiner, E. A., Richards, A. M., et al. (1993). Atrial natriuretic factor reduces vasopressin and angiotensin II but not the ACTH response to acute hypoglycaemic stress in normal men. *Clin Endocrinol*, *38*, 183-189.
214. Levin, E. R., Mills, S., & Weber, M. A. (1989). Central nervous system mediated vasodepressor action of atrial natriuretic factor. *Life Sci*, *44*, 1617-1624.
215. Yeung, V. T., Lai, C. K., Cockram, C. S., et al. (1991). Atrial natriuretic peptide in the central nervous system. [Review]. *Neuroendocrinology* (Suppl. 1), *53*, 18-24.
216. Bordicchia, M., Liu, D., Amri, E. Z., et al. (2012). Cardiac natriuretic peptides act via p38 MAPK to induce the brown fat thermogenic program in mouse and human adipocytes. *J Clin Invest*, *122*, 1022-1036.
217. Wei, C., Aarhus, L. L., Miller, V. M., & Burnett, J. C. (1993). Action of C-type natriuretic peptide in isolated canine arteries and veins. *Am J Physiol*, *264*, H71-H73.
218. Clavell, A., Stingo, A., Wei, C., et al. (1993). C-type natriuretic peptide: a selective cardiovascular peptide. *Am J Physiol*, *264*, R290-R295.
219. Charles, C. J., Richards, A. M., & Espiner, E. A. (1992). Central C-type natriuretic peptide but not atrial natriuretic factor lowers blood pressure and adrenocortical secretion in normal conscious sheep. *Endocrinology*, *131*, 1721-1726.
220. Samson, W. K., Skala, K. D., & Huang, F. L. S. (1991). CNP-22 stimulates, rather than inhibits, water drinking in the rat: evidence for a unique biological action of the C-type natriuretic peptides. *Brain Res*, *568*, 285-288.
221. Assadi, F. K., & John, E. G. (1985). Hypouricemia in neonates with syndrome of inappropriate secretion of antidiuretic hormone. *Pediatr Res*, *19*, 424-427.
222. Ganong, C. A., & Kappy, M. S. (1993). Cerebral salt wasting in children. The need for recognition and treatment [published erratum appears in *Am J Dis Child* (1993). *147*, 369]. *Am J Dis Child*, *147*, 167-169.
223. Frasier, S. D., Kutnik, L. A., Schmidt, R. T., & Smith, F. G., Jr. (1967). A water deprivation test for the diagnosis of diabetes insipidus in children. *Am J Dis Child*, *114*, 157-160.
224. Richman, R. A., Post, E. M., Notman, D. D., et al. (1981). Simplifying the diagnosis of diabetes insipidus in children. *Am J Dis Child*, *135*, 839-841.
225. Koskimies, O., & Pylkkanen, J. (1984). Water intoxication in infants caused by the urine concentration test with the vasopressin analogue (DDAVP). *Acta Paediatr Scand*, *73*, 131-132.
226. Bichet, D. G. (1994). Molecular and cellular biology of vasopressin and oxytocin receptors and action in the kidney. [Review]. *Curr Opin Nephrol Hypertens*, *3*, 46-53.
227. Milles, J. J., Spruce, B., & Baylis, P. H. (1983). A comparison of diagnostic methods to differentiate diabetes insipidus from primary polyuria: a review of 21 patients. *Acta Endocrinol*, *104*, 410-416.
228. Zerbe, R. L., & Robertson, G. L. (1981). A comparison of plasma vasopressin measurements with a standard indirect test in the differential diagnosis of polyuria. *N Engl J Med*, *305*, 1539-1546.
229. Andersson, B., Leksell, L. G., & Rundgren, M. (1982). Regulation of water intake. [Review]. *Ann Rev nutr*, *2*, 73-89.
230. Fenske, W., & Allolio, B. (2012). Current state and future perspectives in the diagnosis of diabetes insipidus: a clinical review. *J Clin Endocrinol Metab*, *97*, 3426-3437.
231. Papapostolou, C., Mantzoros, C. S., Evagelopoulou, C., et al. (1995). Imaging of the sella in the syndrome of inappropriate secretion of antidiuretic hormone. *J Intern Med*, *237*, 181-185.
232. Moses, A. M., Clayton, B., & Hochhauser, L. (1992). Use of T1-weighted MR imaging to differentiate between primary polydipsia and central diabetes insipidus [comment] [see comments]. *AJNR Am J Neuroradiol*, *13*, 1273-1277.
233. Halimi, P., Sigal, R., Doyon, D., et al. (1988). Post-traumatic diabetes insipidus: MR demonstration of pituitary stalk rupture. *J Comput Assist Tomogr*, 12, 135-137.
234. Maghnie, M., Villa, A., Arico, M., et al. (1992). Correlation between magnetic resonance imaging of posterior pituitary and neurohypophyseal function in children with diabetes insipidus. *J Clin Endocrinol Metab*, *74*, 795-800.
235. Maghnie, M., Genovese, E., Arico, M., et al. (1994). Evolving pituitary hormone deficiency is associated with pituitary vasculopathy: dynamic MR study in children with hypopituitarism, diabetes insipidus, and Langerhans cell histiocytosis. *Radiology*, *193*, 493-499.
236. Sato, N., Ishizaka, H., Yagi, H., Matsumoto, M., & Endo, K. (1993). Posterior lobe of the pituitary in diabetes insipidus: dynamic MR imaging. *Radiology*, *186*, 357-360.
237. Medani, C. R. (1987). Seizures and hypothermia due to dietary water intoxication in infants. *South Med J*, *80*, 421-425.
238. Davidson, S., Frand, M., & Rotem, Y. (1978). Primary polydipsia in infancy: a benign disorder simulating diabetes insipidus. *Clin Pediatr*, *17*, 419-420.
239. Kovacs, L., Sulyok, E., Lichardus, B., et al. (1986). Renal response to arginine vasopressin in premature infants with late hyponatraemia. *Arch Dis Child*, *61*, 1030-1032.
240. Robertson, G. L. (1987). Dipsogenic diabetes insipidus: a newly recognized syndrome caused by a selective defect in the osmoregulation of thirst. *Trans Assoc Am Physicians*, *100*, 241-249.
241. Ahmed, A. B., George, B. C., Gonzalez-Auvert, C., & Dingman, J. F. (1967). Increased plasma arginine vasopressin in clinical adrenocortical insufficiency and its inhibition by glucosteroids. *J Clin Invest*, *46*, 111-123.
242. Dingman, J. F., & Despointes, R. H. (1960). Adrenal steroid inhibition of vasopressin release from the neurohypophysis of normal subjects and patients with Addison's disease . *J Clin Invest*, *39*, 1851-1863.
243. Iwasaki, Y., Oiso, Y., Saito, H., & Majzoub, J. A. (1997). Positive and negative regulation of the rat vasopressin gene promoter. *Endocrinology*, *138*, 5266-5274.
244. Kleeman, C. R., Czaczkes, J. W., & Cutler, R. (1964). Mechanisms of impaired water excretion in adrenal and pituitary insufficiency. IV. Antidiuretic hormone in primary and secondary adrenal insufficiency. *J Clin Invest*, *43*, 1641-1648.
245. Kamoi, K., Tamura, T., Tanaka, K., et al. (1993). Hyponatremia and osmoregulation of thirst and vasopressin secretion in patients with adrenal insufficiency. *J Clin Endocrinol Metab*, 77, 1584-1588.
246. Laczi, F., Janaky, T., Ivanyi, T., et al. (1987). Osmoregulation of arginine-8-vasopressin secretion in primary hypothyroidism and in Addison's disease. *Acta Endocrinologica*, *114*, 389-395.
247. Bouley, R., Breton, S., Sun, T., et al. (2000). Nitric oxide and atrial natriuretic factor stimulate cGMP-dependent membrane

insertion of aquaporin 2 in renal epithelial cells. *J Clin Invest*, *106*, 1115-1126.
248. Wallerath, T., Witte, K., Schafer, S. C., et al. (1999). Down-regulation of the expression of endothelial NO synthase is likely to contribute to glucocorticoid-mediated hypertension. *Proc Natl Acad Sci USA*, *96*, 13357-13362.
249. Schwartz, M. J., & Kokko, J. P. (1980). Urinary concentrating defect of adrenal insufficiency: permissive role of adrenal steroids on the hydroosmotic response across the rabbit cortical collecting tubule. *J Clin Invest*, *66*, 234-242.
250. Schrier, R. W., & Berl, T. (1975). Nonosmolar factors affecting renal water excretion (first of two parts). [Review]. *N Engl J Med*, *292*, 81-88.
251. Iwasaki, Y., Oiso, Y., Yamauchi, K., et al. (1990). Osmoregulation of plasma vasopressin in myxedema. *J Clin Endocrinol Metab*, *70*, 534-539.
252. Discala, V. A., & Kinney, M. J. (1971). Effects of myxedema in the renal diluting and concentrating mechanism. *Am J Med*, *50*, 325-335.
253. Michael, U. F., Barenberg, R. L., Chavez, R., et al. (1972).Renal handling of sodium and water in the hypothyroid rat: clearance and micropuncture studies. *J Clin Invest*, *51*, 1405-1412.
254. Yount, E., & Little, J. M. (1955). Renal clearance in patients with myxedema. *J Clin Endocrinol Metab*, *15*, 343-346.
255. Iwasaki, Y., Kondo, K., Hasegawa, H., & Oiso, Y. (1997). Osmoregulation of plasma vasopressin in three cases with adrenal insufficiency of diverse etiologies. *Horm Res*, *47*, 38-44.
256. Yamada, K., Tamura, Y., & Yoshida, S. (1989). Effect of administration of corticotropin-releasing hormone and glucocorticoid on arginine vasopressin response to osmotic stimulus in normal subjects and patients with hypocorticotropinism without overt diabetes insipidus. *J Clin Endocrinol Metab*, *69*, 396-401.
257. Kamiyama, T., Iseki, K., Kawazoe, N., et al. (1993). Carbamazepine-induced hyponatremia in a patient with partial central diabetes insipidus. *Nephron*, *64*, 142-145.
258. Van Amelsvoort, T., Bakshi, R., Devaux, C. B., & Schwabe, S. (1994). Hyponatremia associated with carbamazepine and oxcarbazepine therapy: a review. [Review]. *Epilepsia*, *35*,181-188.
259. Lozada, E. S., Gouaux, J., Franki, N., et al. (1972). Studies of the mode of action of the sulfonylureas and phenylacetamides in enhancing the effect of vasopressin. *J Clin Endocrinol Metab*, *34*, 704-712.
260. Moses, A. M., Numann, P., & Miller, M. (1973). Mechanism of chlorpropamide-induced antidiuresis in man: evidence for release of ADH and enhancement of peripheral action. *Metabolism*, *22*, 59-66.
261. Bressler, R. B., & Huston, D. P. (1985). Water intoxication following moderate-dose intravenous cyclophosphamide. *Arch Intern Med*, *145*, 548-549.
262. Harlow, P. J., DeClerck, Y. A., Shore, N. A., et al. (1979). A fatal case of inappropriate ADH secretion induced by cyclophosphamide therapy. *Cancer*, *44*, 896-898.
263. Larose, P., Ong, H., & du Souich, P. (1987). The effect of cyclophosphamide on arginine vasopressin and the atrial natriuretic factor. *Biochem Biophys Res Commun*, *143*, 140-44.
264. Bode, U., Seif, S. M., & Levine, A. S. (1980). Studies on the antidiuretic effect of cyclophosphamide: vasopressin release and sodium excretion. *Med Pediatr Oncol*, *8*, 295-303.
265. Zavagli, G., Ricci, G., Tataranni, G., et al. (1988). Life-threatening hyponatremia caused by vinblastine. *Med Oncol Tumor Pharmacother*, *5*, 67-69.
266. Hutchison, F. N., Perez, E. A., Gandara, D. R., et al. (1988). Renal salt wasting in patients treated with cisplatin. *Ann Intern Med*, *108*, 21-25.
267. Ritch, P. S. (1988). Cis-dichlorodiammineplatinum II-induced syndrome of inappropriate secretion of antidiuretic hormone. *Cancer*, *61*, 448-450.
268. Gerigk, M., Bald, M., Feth, F., & Rascher, W. (1993). Clinical settings and vasopressin function in hyponatraemic children. *Eur J Pediatr*, *152*, 301-305.
269. Judd, B. A., Haycock, G. B., Dalton, N., & Chantler, C. (1987). Hyponatraemia in premature babies and following surgery in older children. *Acta Paediatr Scand*, *76*, 385-493.
270. Anderson, R. J., Chung, H. M., Kluge, R., & Schrier, R. W. (1985). Hyponatremia: a prospective analysis of its epidemiology and the pathogenetic role of vasopressin. *Ann Intern Med*, *102*, 164-168.
271. Kanakriyeh, M., Carvajal, H. F., & Vallone, A. M. (1987). Initial fluid therapy for children with meningitis with consideration of the syndrome of inappropriate anti-diuretic hormone. *Clin Pediatr*, *26*, 126-130.
272. Padilla, G., Ervin, M. G., Ross, M. G., & Leake, R. D. (1991). Vasopressin levels in infants during the course of aseptic and bacterial meningitis. *Am J Dis Child*, *145*, 991-993.
273. Powell, K. R., Sugarman, L. I., Eskenazi, A. E., et al. (1990). Normalization of plasma arginine vasopressin concentrations when children with meningitis are given maintenance plus replacement fluid therapy. *J Pediatr*, *117*, 515-522.
274. Vingerhoets, F., & de Tribolet, N. (1988). Hyponatremia hypo-osmolarity in neurosurgical patients: "appropriate secretion of ADH" and "cerebral salt wasting syndrome." *Acta Neurochirurgica*, *91*, 50-54.
275. Neville, K. A., Verge, C. F., O'Meara, M. W., & Walker, J. L. (2005). High antidiuretic hormone levels and hyponatremia in children with gastroenteritis. *Pediatrics*, *116*, 1401-1407.
276. Neville, K. A., Verge, C. F., Rosenberg, A. R., et al. (2006). Isotonic is better than hypotonic saline for intravenous rehydration of children with gastroenteritis: a prospective randomised study. *Arch Dis Child*, *91*, 226-232.
277. Choong, K., Kho, M. E., Menon, K., & Bohn, D. (2006). Hypotonic versus isotonic saline in hospitalised children: a systematic review. *Arch Dis Child*, *91*, 828-835.
278. Zanardo, V., Ronconi, M., Ferri, N., & Zacchello, G. (1989). Plasma arginine vasopressin, diuresis, and neonatal respiratory distress syndrome. *Padiatr Padol*, *24*, 297-302.
279. Potts, F. L., & May R. B. (1986). Early syndrome of inappropriate secretion of antidiuretic hormone in a child with burn injury. *Ann Emerg Med*, *15*, 834-835.
280. Kojima, T., Fukuda, Y., Hirata, Y., et al. (1990). Changes in vasopressin, atrial natriuretic factor, and water homeostasis in the early stage of bronchopulmonary dysplasia. *Pediatr Res*, *27*, 260-263.
281. Rao, M., Eid, N., Herrod, L., et al. (1986). Antidiuretic hormone response in children with bronchopulmonary dysplasia during episodes of acute respiratory distress. *Am J Dis Child*, *140*, 825-882.
282. Sulyok, E., Kovacs, L., Lichardus, B., et al (1985). Late hyponatremia in premature infants: role of aldosterone and arginine vasopressin. *J Pediatr*, *106*, 990-994.
283. Cohen, L. F., di Sant'Agnese, P. A., Taylor, A., & Gill, J. R., Jr. (1977). The syndrome of inappropriate antidiuretic hormone secretion as a cause of hyponatremia in cystic fibrosis. *J Pediatr*, *90*, 574-578.
284. Stegner, H., Caspers, S., Niggemann, B., & Commentz, J. (1986). Urinary arginine-vasopressin (AVP) excretion in cystic fibrosis (CF). *Acta Endocrinol* (Suppl.), *279*, 448-451.
285. Arisaka, O., Shimura, N., Hosaka, A., et al. (1998). Water intoxication in asthma assessed by urinary arginine vasopressin. *Eur J Pediatr*, *148*, 167-169.
286. Iikura, Y., Odajima, Y., Akazawa, A., et al. (1989). Antidiuretic hormone in acute asthma in children: effects of medication on serum levels and clinical course. *Allergy Proc*, *10*, 197-201.
287. Schrier, R. W., & Berl, T. (1975). Nonosmolar factors affecting renal water excretion (second of two parts). [Review]. *N Engl J Med*, *292*, 141-145.
288. O'Rahilly, S. (1985). Secretion of antidiuretic hormone in hyponatraemia: not always "inappropriate." *Br Med J* (Clin Res Ed), *290*,1803-1804.
289. Kamel, K. S., & Bear, R. A. (1993). Treatment of hyponatremia: a quantitative analysis. [Review]. *Am J Kidney Dis*, *21*, 439-443.
290. Schrier, R. W. (1985). Treatment of hyponatremia [Editorial]. *N Engl J Med*, *312*, 1121-1123.
291. Videen, J. S., Michaelis, T., Pinto, P., & Ross, B. D. (1995). Human cerebral osmolytes during chronic hyponatremia: a proton magnetic resonance spectroscopy study. *J Clin Invest*, *95*, 788-793.
292. Ayus, J. C., & Arieff, A. I. (1993). Pathogenesis and prevention of hyponatremic encephalopathy. [Review]. *Endocrinol Metab Clin North Am*, *22*, 425-446.
293. Sterns, R. H., Riggs, J. E., & Schochet, S. S., Jr. (1986). Osmotic demyelination syndrome following correction of hyponatremia. *N Engl J Med*, *314*, 1535-1542.

294. Ayus, J. C., Krothapalli, R. K., & Arieff, A. I. (1985). Changing concepts in treatment of severe symptomatic hyponatremia: rapid correction and possible relation to central pontine myelinolysis. *Am J Med*, *78*, 897-902.
295. Ayus, J. C., Krothapalli, R. K., & Arieff, A. I. (1987). Treatment of symptomatic hyponatremia and its relation to brain damage: a prospective study. [Review]. *N Engl J Med*, *317*, 1190-1195.
296. Sklar, C., Fertig, A., & David, R. (1985). Chronic syndrome of inappropriate secretion of antidiuretic hormone in childhood. *Am J Dis Child*, *139*, 733-735.
297. Tang, T. T., Whelan, H. T., Meyer, G. A., et al. (1991). Optic chiasm glioma associated with inappropriate secretion of antidiuretic hormone, cerebral ischemia, nonobstructive hydrocephalus and chronic ascites following ventriculoperitoneal shunting. *Childs Nerv Syst*, *7*, 458-461.
298. Padilla, G., Leake, J. A., Castro, R., et al. (1989). Vasopressin levels and pediatric head trauma. *Pediatrics*, *83*, 700-705.
299. Goldman, M. B., Luchins, D. J., & Robertson, G. L. (1988). Mechanisms of altered water metabolism in psychotic patients with polydipsia and hyponatremia. *N Engl J Med*, *318*, 397-403.
300. Meierkord, H., Shorvon, S., & Lightman, S. L. (1994). Plasma concentrations of prolactin, noradrenaline, vasopressin and oxytocin during and after a prolonged epileptic seizure *Acta Neurol Scand*, *90*, 73-77.
301. Edwards, C. M., Carmichael, J., Baylis, P. H., & Harris, A. L. (1989). Arginine vasopressin: a mediator of chemotherapy induced emesis? *Br J Cancer*, *59*, 467-470.
302. Coslovsky, R., Bruck, R., & Estrov, Z. (1984). Hypo-osmolal syndrome due to prolonged nausea. *Arch Intern Med*, *144*, 191-192.
303. Dhawan, A., Narang, A., & Singhi, S. (1992). Hyponatraemia and the inappropriate ADH syndrome in pneumonia. *Ann Trop Paediatr*, *12*, 455-462.
304. van Steensel-Moll, H. A., Hazelzet, J. A., van der Voort, E., & Neijens, H. J. (1990). Excessive secretion of antidiuretic hormone in infections with respiratory syncytial virus. *Arch Dis Child*, *65*, 1237-1239.
305. Tang, W.W., Kaptein, E. M., Feinstein, E. I., & Massry, S. G. (1993). Hyponatremia in hospitalized patients with the acquired immunodeficiency syndrome (AIDS) and the AIDS-related complex. *Am J Med*, *94*, 169-174.
306. Weissman, P. N., Shenkman, L., & Gregerman, R. I. (1971). Chlorpropamide hyponatremia: drug-induced inappropriate antidiuretic-hormone activity. *N Engl J Med*, *284*, 65-71.
307. Escuro, R. S., Adelstein, D. J., & Carter, S. G. (1992). Syndrome of inappropriate secretion of antidiuretic hormone after infusional vincristine. *Cleve Clin J Med*, *59*, 643-644.
308. Liskin, B., Walsh, B. T., Roose, S. P., & Jackson, W. (1984). Imipramine-induced inappropriate ADH secretion. *J Clin Psychopharmacol*, *4*, 146-147.
309. Parker, W. A. (1984). Imipramine-induced syndrome of inappropriate antidiuretic hormone secretion. *Drug Intell Clin Pharm*, *18*, 890-894.
310. Moses, A. M., Howanitz, J., & Miller, M.(1973). Diuretic action of three sulfonylurea drugs. *Ann Intern Med*, *78*, 541-544.
311. Cotton, M. F., Donald, P. R., Schoeman, J. F., et al. (1991). Plasma arginine vasopressin and the syndrome of inappropriate antidiuretic hormone secretion in tuberculous meningitis. *Pediatr Infect Dis J*, 10, 837-842.
312. Cotton, M. F., Donald, P. R., Schoeman, J. F., et al. (1993). Raised intracranial pressure, the syndrome of inappropriate antidiuretic hormone secretion, and arginine vasopressin in tuberculous meningitis. *Childs Nerv Syst*, *9*, 10-15, Discussion 15-16.
313. Hill, A. R., Uribarri, J., Mann, J., & Berl, T. (1990). Altered water metabolism in tuberculosis: role of vasopressin. *Am J Med*, *88*, 357-364.
314. Olson, B. R., Rubino, D., Gumowski, J., & Oldfield, E. H. (1995). Isolated hyponatremia after transsphenoidal pituitary surgery. *J Clin Endocrinol Metab*, *80*, 85-91.
315. Sane, T., Rantakari, K., Poranen, A., et al. (1994). Hyponatremia after transsphenoidal surgery for pituitary tumors. *J Clin Endocrinol Metab*, *79*, 1395-1398.
316. Crigler, J. F., Jr. (1976). Commentary: On the use of pitressin in infants with neurogenic diabetes insipidus. *J Pediatr*, *88*, 295-296.
317. Smith, T. J., Gill, J. C., Ambruso, D. R., & Hathaway, W. E. (1989). Hyponatremia and seizures in young children given DDAVP. *Am J Hematol*, *31*, 199-202.
318. Feldman, B. J., Rosenthal, S. M., Vargas, G. A., et al. (2005). Nephrogenic syndrome of inappropriate antidiuresis. *N Engl J Med*, *352*, 1884-1890.
319. Morello, J. P., & Bichet, D. G. (2001). Nephrogenic diabetes insipidus. *Annu Rev Physiol*, *63*, 607-630.
320. Vandergheynst, F., Brachet, C., Heinrichs, C., & Decaux, G. (2012). Long-term treatment of hyponatremic patients with nephrogenic syndrome of inappropriate antidiuresis: personal experience and review of published case reports. *Nephron Clin Pract*, *120*, c168-c172.
321. Robertson, G. L. (2011). Vaptans for the treatment of hyponatremia. *Nat Rev Endocrinol*, *7*, 151-161.
322. Decaux, G., Soupart, A., & Vassart, G. (2008). Non-peptide arginine-vasopressin antagonists: the vaptans. *Lancet*, *371*, 1624-1632.
323. Soupart, A., Gross, P., Legros, J. J., et al (2006). Successful long-term treatment of hyponatremia in syndrome of inappropriate antidiuretic hormone secretion with satavaptan (SR121463B), an orally active nonpeptide vasopressin V2-receptor antagonist. *Clin J Am Soc Nephrol*, *1*, 1154-1160.
324. Rianthavorn, P., Cain, J. P., & Turman, M. A. (2008). Use of conivaptan to allow aggressive hydration to prevent tumor lysis syndrome in a pediatric patient with large-cell lymphoma and SIADH. *Pediatr Nephrol*, *23*, 1367-1370.
325. Decaux, G. (2007). V2-antagonists for the treatment of hyponatraemia. *Nephrol Dial Transplant*, *22*, 1853-1855.
326. Kamoi, K., Ebe, T., Kobayashi, O., et al. (1990). Atrial natriuretic peptide in patients with the syndrome of inappropriate antidiuretic hormone secretion and with diabetes insipidus. *J Clin Endocrinol Metab*, *70*, 1385-1390.
327. Manoogian, C., Pandian, M., Ehrlich, L., et al. (1988). Plasma atrial natriuretic hormone levels in patients with the syndrome of inappropriate antidiuretic hormone secretion. *J Clin Endocrinol Metab*, *67*, 571-575.
328. Kojima, T., Hirata, Y., Umeda, Y., et al. (1989). Role of atrial natriuretic peptide in the diuresis of a newborn infant with the syndrome of inappropriate antidiuretic hormone secretion. *Acta Paediatr Scand*, *78*, 793-796.
329. Wijdicks, E. F., Ropper, A. H., Hunnicutt, E. J., et al. (1991). Atrial natriuretic factor and salt wasting after aneurysmal subarachnoid hemorrhage. *Stroke*, *22*, 1519-1524.
330. Isotani, E., Suzuki, R., Tomita, K., et al. (1994). Alterations in plasma concentrations of natriuretic peptides and antidiuretic hormone after subarachnoid hemorrhage. *Stroke*, *25*, 2198-2203.
331. Diringer, M., Ladenson, P. W., Borel, C., et al. (1989). Sodium and water regulation in a patient with cerebral salt wasting. *Arch Neurol*, *46*, 928-930.
332. Rivkees, S. A. (2008). Differentiating appropriate antidiuretic hormone secretion, inappropriate antidiuretic hormone secretion and cerebral salt wasting: the common, uncommon, and misnamed. *Curr Opin Pediatr*, *20*, 448-452.
333. Brimioulle, S., Orellana-Jimenez, C., Aminian, A., & Vincent, J. L. (2008). Hyponatremia in neurological patients: cerebral salt wasting versus inappropriate antidiuretic hormone secretion. *Intensive Care Med*, *34*, 125-131.
334. Vokes, T. P., Aycinena, P. R., & Robertson, G. L. (1987). Effect of insulin on osmoregulation of vasopressin. *Am J Physiol*, *252*, E538-E548.
335. Zerbe, R. L., Vinicor, F., & Robertson, G. L. (1979). Plasma vasopressin in uncontrolled diabetes mellitus. *Diabetes*, *28*, 503-508.
336. Ishikawa, S., Saito, T., Okada, K., et al. (1990). Prompt recovery of plasma arginine vasopressin in diabetic coma after intravenous infusion of a small dose of insulin and a large amount of fluid. *Acta Endocrinol*, *122*, 455-461.
337. Tulassay, T., Rascher, W., Korner, A., & Miltenyi, M. (1987). Atrial natriuretic peptide and other vasoactive hormones during treatment of severe diabetic ketoacidosis in children. *J Pediatr*, *111*, 329-334.
338. Zerbe, R. L., Vinicor, F., & Robertson, G. L. (1985). Regulation of plasma vasopressin in insulin-dependent diabetes mellitus. *Am J Physiol*, *249*, E317-E325.

339. Greger, N. G., Kirkland, R. T., Clayton, G. W., & Kirkland, J. L. (1986). Central diabetes insipidus. 22 years' experience. *Am J Dis Child*, *140*, 551-554.
340. Wang, L. C., Cohen, M. E., & Duffner, P. K. (1994). Etiologies of central diabetes insipidus in children. *Pediatr Neurol*, *11*, 273-277.
341. Richards, G. E., Thomsett, M. J., Boston, B. A., et al. (2011). Natural history of idiopathic diabetes insipidus. *J Pediatr*, *159*, 566-570.
342. Pedersen, E. B., Lamm, L. U., Albertsen, K., et al. (1985). Familial cranial diabetes insipidus: a report of five families: Genetic, diagnostic and therapeutic aspects. *Q J Med*, *57*, 883-896.
343. Os, I., Aakesson, I., & Enger, E. (1985). Plasma vasopressin in hereditary cranial diabetes insipidus. *Acta Med Scand*, *217*, 429-434.
344. Toth, E. L., Bowen, P. A., & Crockford, P. M. (1984). Hereditary central diabetes insipidus: plasma levels of antidiuretic hormone in a family with a possible osmoreceptor defect. *CMAJ*, *131*, 1237-1241.
345. Nagai, I., Li, C. H., Hsieh, S. M., et al. (1984). Two cases of hereditary diabetes insipidus, with an autopsy finding in one. *Acta Endocrinol*, *105*, 318-323.
346. Bergeron, C., Kovacs, K., Ezrin, C., & Mizzen, C. (1991). Hereditary diabetes insipidus: an immunohistochemical study of the hypothalamus and pituitary gland. *Acta Neuropathol*, *81*, 345-348.
347. Willcutts, M. D., Felner, E., & White, P. C. (1999). Autosomal recessive familial neurohypophyseal diabetes insipidus with continued secretion of mutant weakly active vasopressin. *Hum Mol Genet*, *8*, 1303-1307.
348. Ito, M., Yu, R. N., & Jameson, J. L. (1999). Mutant vasopressin precursors that cause autosomal dominant neurohypophyseal diabetes insipidus retain dimerization and impair the secretion of wild-type proteins. *J Biol Chem*, *274*, 9029-9037.
349. Ito, M., & Jameson, J. L. (1997). Molecular basis of autosomal dominant neurohypophyseal diabetes insipidus: cellular toxicity caused by the accumulation of mutant vasopressin precursors within the endoplasmic reticulum. *J Clin Invest*, *99*, 1897-1905.
350. Russell, T. A., Ito, M., Ito, M., et al. (2003). A murine model of autosomal dominant neurohypophyseal diabetes insipidus reveals progressive loss of vasopressin-producing neurons. *J Clin Invest*, *112*, 1697-1706.
351. Russell, T. A., Ito, M., Yu, R. N., et al. (2003). A murine model of autosomal dominant neurohypophyseal diabetes insipidus reveals progressive loss of vasopressin-producing neurons. *J Clin Invest*, *112*, 1697-1706.
352. Thompson, C. J., Charlton, J., Walford, S., et al. (1989).Vasopressin secretion in the DIDMOAD (Wolfram) syndrome. *Q J Med*, 71, 333-345.
353. Grosse Aldenhovel, H. B., Gallenkamp, U., & Sulemana, C. A. (1991). Juvenile onset diabetes mellitus, central diabetes insipidus and optic atrophy (Wolfram syndrome): neurological findings and prognostic implications. *Neuropediatrics*, *22*, 103-106.
354. Osman, A. A., Saito, M., Makepeace, C., et al. (2003). Wolframin expression induces novel ion channel activity in endoplasmic reticulum membranes and increases intracellular calcium. *J Biol Chem*, *278*, 52755-52762.
355. Riggs, A. C., Bernal-Mizrachi, E., Ohsugi, M., et al. (2005). Mice conditionally lacking the Wolfram gene in pancreatic islet beta cells exhibit diabetes as a result of enhanced endoplasmic reticulum stress and apoptosis. *Diabetologia*, *48*, 2313-2321.
356. Labib, M., McPhate, G., & Marks, V. (1987). Post-traumatic diabetes insipidus combined with primary polydipsia. *Postgrad Med J*, *63*, 33-35.
357. Defoer, F., Mahler, C., Dua, G., & Appel, B. (1987). Posttraumatic diabetes insipidus. *Acta Anaesthesiol Bel*, *38*, 397-399.
358. Hadani, M., Findler, G., Shaked, I., & Sahar, A. (1985). Unusual delayed onset of diabetes insipidus following closed head trauma: case report. *J Neurosurg*, *63*, 456-458.
359. Jenkins, H. R., Hughes, I. A., & Gray, O. P. (1988). Cranial diabetes insipidus in early infancy. *Arch Dis Child*, *63*, 434-435.
360. Piech, J. J., Thieblot, P., Haberer, J. P., et al. (1985). [Twin pregnancy with acute hepatic steatosis followed by antehypophyseal insufficiency and diabetes insipidus]. *Presse Med*, *14*, 1421-1423 (in French).
361. Iwasaki, Y., Oiso, Y., Yamauchi, K., et al. (1989). Neurohypophyseal function in postpartum hypopituitarism: impaired plasma vasopressin response to osmotic stimuli. *J Clin Endocrinol Metab*, *68*, 560-565.
362. Seckl, J. R., Dunger, D. B., & Lightman, S. L. (1987). Neurohypophyseal peptide function during early postoperative diabetes insipidus. *Brain*, *110*, 737-746.
363. Finken, M. J., Zwaveling-Soonawala, N., Walenkamp, M. J., et al. (2011). Frequent occurrence of the triphasic response (diabetes insipidus/hyponatremia/diabetes insipidus) after surgery for craniopharyngioma in childhood. *Horm Res Paediatr*, *76*, 22-26.
364. Masera, N., Grant, D. B., Stanhope, R., & Preece, M. A. (1994). Diabetes insipidus with impaired osmotic regulation in septo-optic dysplasia and agenesis of the corpus callosum. *Arch Dis Child*, *70*, 51-53.
365. Tawa, R., Kaino, Y., Ito, T., et al. (1994). A case of Kabuki make-up syndrome with central diabetes insipidus and growth hormone neurosecretory dysfunction. *Acta Paediatr Jpn*, *36*, 412-415.
366. Van Gool, S., de Zegher, F., de Vries, L. S., et al. (1990). Alobar holoprosencephaly, diabetes insipidus and coloboma without craniofacial abnormalities: a case report. *Eur J Pediatr*, *149*, 621-622.
367. Yagi, H., Nagashima, K., Miyake, H., et al. (1994). Familial congenital hypopituitarism with central diabetes insipidus. *J Clin Endocrinol Metab*, *78*, 884-889.
368. Villadsen, A. B., & Pedersen, E. B. (1997). Recumbent cranial diabetes insipidus. Studies in a patient with adipsia, hypernatremia, poikilothermia and polyphagia. *Acta Paediatr Scand*, *76*, 179-183.
369. Ono, N., Kakegawa, T., Zama, A., et al. (1992). Suprasellar germinomas: relationship between tumour size and diabetes insipidus. *Acta Neurochir*, *114*, 26-32.
370. Tarng, D. C., & Huang, T. P. (1995). Diabetes insipidus as an early sign of pineal tumor. *Am J Nephrol*, *15*, 161-164.
371. Appignani, B., Landy, H., & Barnes, P. (1993). MR in idiopathic central diabetes insipidus of childhood. *AJNR Am J Neuroradiol*, *14*, 1407-1410.
372. Hung, W., & Fitz, C. R. (1992). The primary empty-sella syndrome and diabetes insipidus in a child. *Acta Paediatrica*, *81*, 459-461.
373. Eichhorn, P., Rhyner, K., Haller, D., et al. (1988). [Diabetes insipidus in chronic myeloid leukemia: remission of hypophyseal infiltration during busulfan treatment]. *Schweiz Med Wochenschr*, *118*, 275-279 (in German).
374. Puolakka, K., Korhonen, T., & Lahtinen, R. (1984). Diabetes insipidus in preleukaemic phase of acute myeloid leukaemia in 2 patients with empty sella turcica: a report of 2 cases. *Scand J Haematol*, *32*, 364-366.
375. Foresti, V., Casati, O., Villa, A., et al. (1992). Central diabetes insipidus due to acute monocytic leukemia: case report and review of the literature. [Review]. *J Endocrinol Invest*, *15*, 127-130.
376. de la Chapelle, A., & Lahtinen, R. (1987). Monosomy 7 predisposes to diabetes insipidus in leukaemia and myelodysplastic syndrome. [Review]. *Eur J Haematol*, *39*, 404-411.
377. Kanabar, D. J., Betts, D. R., Gibbons, B., et al. (1994). Monosomy 7, diabetes insipidus and acute myeloid leukemia in childhood. *Pediatr Hematol Oncol*, *11*, 111-114.
378. La Starza, R., Falzetti, D., Fania, C., et al. (1994). 3q aberration and monosomy 7 in ANLL presenting with high platelet count and diabetes insipidus. *Haematologica*, *79*, 356-359.
379. Ra'anani, P., Shpilberg, O., Berezin, M., & Ben-Bassat, I. (1994). Acute leukemia relapse presenting as central diabetes insipidus. [Review]. *Cancer*, *73*, 2312-2316.
380. Dunger, D. B., Broadbent, V., Yeoman, E., et al. (1989). The frequency and natural history of diabetes insipidus in children with Langerhans-cell histiocytosis. *N Engl J Med*, *321*, 1157-1162.
381. Grois, N., Flucher-Wolfram, B., Heitger, A., et al. (1995). Diabetes insipidus in Langerhans cell histiocytosis: results from the DAL-HX 83 study. *Med Pediatr Oncol*, *24*, 248-256.
382. Broadbent, V., Dunger, D. B., Yeomans, E., & Kendall, B. (1993). Anterior pituitary function and computed tomography/magnetic resonance imaging in patients with Langerhans cell histiocytosis and diabetes insipidus. *Medic Pediatr Oncol*, *21*, 649-654.
383. Tien, R. D., Newton, T. H., McDermott, M. W., et al. (1990). Thickened pituitary stalk on MR images in patients with diabetes insipidus and Langerhans cell histiocytosis. *AJNR Am J Neuroradiol*, *11*, 703-708.

384. Minehan, K. J., Chen, M. G., Zimmerman, D., et al. (1992). Radiation therapy for diabetes insipidus caused by Langerhans cell histiocytosis. *Intl J Radiat Oncol Biol Phys*, *23*, 519-524.
385. Imura, H., Nakao, K., Shimatsu, A., et al. (1993). Lymphocytic infundibuloneurohypophysitis as a cause of central diabetes insipidus. *N Engl J Med*, *329*, 683-689.
386. Paja, M., Estrada, J., Ojeda, A., et al. (1994). Lymphocytic hypophysitis causing hypopituitarism and diabetes insipidus, and associated with autoimmune thyroiditis, in a non-pregnant woman. *Postgrad Med J*, *70*, 220-224.
387. Koshiyama, H., Sato, H., Yorita, S., et al. (1994). Lymphocytic hypophysitis presenting with diabetes insipidus: case report and literature review. [Review]. *Endocr J*, *41*, 93-97.
388. Kojima, H., Nojima, T., Nagashima, K., et al. (1989). Diabetes insipidus caused by lymphocytic infundibuloneurohypophysitis. *Arch Pathol Lab Med*, *113*, 1399-1401.
389. Ahmed, S. R., Aiello, D. P., Page, R., et al. (1993). Necrotizing infundibulo-hypophysitis: a unique syndrome of diabetes insipidus and hypopituitarism. *J Clin Endocrinol Metab*, *76*, 1499-1504.
390. Rossi, G. P., Pavan, E., Chiesura-Corona, M., et al. (1994). Bronchocentric granulomatosis and central diabetes insipidus successfully treated with corticosteroids. *Eur Respir J*, *7*, 1893-1898.
391. Lewis, R., Wilson, J., & Smith, F. W. (1987). Diabetes insipidus secondary to intracranial sarcoidosis confirmed by low-field magnetic resonance imaging. *Magn Reson Med*, *5*, 466-470.
392. Scherbaum, W. A., Wass, J. A., Besser, G. M., et al. (1986). Autoimmune cranial diabetes insipidus: its association with other endocrine diseases and with histiocytosis X. *Clin Endocrinol*, *25*, 411-420.
393. De Bellis, A., Bizzarro, A., Amoresano Paglionico, V., et al. (1994). Detection of vasopressin cell antibodies in some patients with autoimmune endocrine diseases without overt diabetes insipidus. *Clin Endocrinol*, *40*, 173-177.
394. Vokes, T. J., Gaskill, M. B., & Robertson, G. L. (1988). Antibodies to vasopressin in patients with diabetes insipidus: implications for diagnosis and therapy. *Ann Intern Med*, *108*, 190-195.
395. Christensen, C., & Bank, A. (1988). Meningococcal meningitis and diabetes insipidus. *Scand J Infect Dis*, *20*, 341-343.
396. Sloane, A. E. (1989). Transient diabetes insipidus following listeria meningitis. *Ir Med J*, *82*, 132-134.
397. Brandle, M., Vernazza, P. L., Oesterle, M., & Galeazzi, R. L. (1995). [Cerebral toxoplasmosis with central diabetes insipidus and panhypopituitarism in a patient with AIDS]. *Schweiz Med Wochenschr* (Journal Suisse de Med), *125*, 684-687 (in German).
398. Mena, W., Royal, S., Pass, R. F., et al. (1993). Diabetes insipidus associated with symptomatic congenital cytomegalovirus infection. *J Pediatr*, *122*, 911-913.
399. Watanabe, A., Ishii, R., Hirano, K., et al. (1994). Central diabetes insipidus caused by nonspecific chronic inflammation of the hypothalamus: case report. *Surg Neurol*, *42*, 70-73.
400. Arisaka, O., Arisaka, M., Ikebe, A., et al. (1992). Central diabetes insipidus in hypoxic brain damage. *Childs Nerv Syst*, *8*, 81-82.
401. Outwater, K. M., & Rockoff, M. A. (1984). Diabetes insipidus accompanying brain death in children. *Neurology*, *34*, 1243-1246.
402. Fiser, D. H., Jimenez, J. F., Wrape, V., & Woody, R. (1987). Diabetes insipidus in children with brain death. *Crit Care Med*, *15*, 551-553.
403. Barzilay, Z., & Somekh, E. (1988). Diabetes insipidus in severely brain damaged children. *J Med*, *19*, 47-64.
404. Hohenegger, M., Vermes, M., Mauritz, W., et al. (1990). Serum vasopressin (AVP) levels in polyuric brain-dead organ donors. *Eur Arch Psychiatry Clin Neurosci*, *239*, 267-269.
405. Durr, J. A. (1967). Diabetes insipidus in pregnancy. [Review]. *Am J Kidney Dis*, *9*, 276-283.
406. Hadi, H. A., Mashini, I. S., & Devoe, L. D. (1985). Diabetes insipidus during pregnancy complicated by preeclampsia: a case report. *J Reprod Med*, *30*, 206-208.
407. Harper, M., Hatjis, C. G., Appel, R. G., & Austin, W. E. (1987). Vasopressin-resistant diabetes insipidus, liver dysfunction, hyperuricemia and decreased renal function: a case report. J Reprod Med, *32*, 862-865.
408. Frenzer, A., Gyr, T., Schaer, H. M., et al. (1994). [Triplet pregnancy with HELLP syndrome and transient diabetes insipidus]. *Schweiz Med Wochenschr* (Journal Suisse de Med), *124*, 687-691 (in German).
409. Kleeman, C. R., Rubini, M. E., Lamdin, E., et al. (1955). Studies on alcohol diuresis. II. The evaluation of ethyl alcohol as an inhibitor of the neurohypophysis. *J Clin Invest*, *34*, 448-455.
410. Tata, P. S., & Buzalkov, R. (1966). Vasopressin studies in the rat. III. Inability of ethanol anesthesia to prevent ADH secretion due to pain and hemorrhage. *Pfluegers Arch*, *290*, 294-297.
411. Miller, M., & Moses, A. M. (1977). Clincial states due to alteration of ADH release and action. In A. M. Moses, & L. Share (Eds.), *Neurohypophysis* (pp. 153-366). Basel: Karger.
412. Sklar, A. H., & Schrier, R. W. (1983). Central nervous system mediators of vasopressin release. *Physiol Rev*, *63*, 1243-1280.
413. Rittig, S., Knudsen, U. B., Norgaard, J. P., et al. (1989). Abnormal diurnal rhythm of plasma vasopressin and urinary output in patients with enuresis. *Am J Physiol*, *256*, F664-F671.
414. Wille, S., Aili, M., Harris, A., & Aronson, S. (1994). Plasma and urinary levels of vasopressin in enuretic and non-enuretic children. *Scand J Urol Nephrol*, *28*, 119-122.
415. Terho, P., & Kekomaki, M. (1984). Management of nocturnal enuresis with a vasopressin analogue. *J Urol*, *131*, 925-927.
416. Evans, J. H., & Meadow, S. R. (1992). Desmopressin for bed wetting: length of treatment, vasopressin secretion, and response. *Arch Dis Child*, *67*, 184-188.
417. Steffens, J., Netzer, M., Isenberg, E., et al. (1993). Vasopressin deficiency in primary nocturnal enuresis: results of a controlled prospective study. *Eur Urol*, *24*, 366-370.
418. Uribarri, J., & Kaskas, M. (1993). Hereditary nephrogenic diabetes insipidus and bilateral nonobstructive hydronephrosis. [Review]. *Nephron*, *65*, 346-349.
419. Seow, W. K., & Thomsett, M. J. (1994). Dental fluorosis as a complication of hereditary diabetes insipidus: studies of six affected patients. *Pediatr Dent*, *16*, 128-132.
420. Colliver, D., Storey, R., Dickens, H., & Subramaniam, R. (2012). Nonobstructive urinary tract dilatation in children with diabetes insipidus. *J Pediatr Surg*, *47*, 752-755.
421. Rivkees, S. A., Dunbar, N., & Wilson, T. (2007). The management of central diabetes insipidus in infancy: desmopressin, low renal solute load formula, thiazide diuretics. J Pediatr Endocrinol Metab, 20, 459-469.
422. Jakobsson, B., & Berg, U. (1994). Effect of hydrochlorothiazide and indomethacin treatment on renal function in nephrogenic diabetes insipidus. *Acta Paediatr*, *83*, 522-525.
423. Blanco, E. J., Lane, A. H., Aijaz, N., et al. (2006). Use of subcutaneous DDAVP in infants with central diabetes insipidus. *J Pediatr Endocrinol Metab*, *19*, 919-925.
424. McDonald, J. A., Martha, P. M., Jr., Kerrigan, J., et al. (1989). Treatment of the young child with postoperative central diabetes insipidus. *Am J Dis Child*, *143*, 201-204.
425. Ralston, C., & Butt, W. (1990). Continuous vasopressin replacement in diabetes insipidus. *Arch Dis Child*, *65*, 896-807.
426. Wise-Faberowski, L., Soriano, S. G., Ferrari, L., et al. (2004). Perioperative management of diabetes insipidus in children [corrected]. *J Neurosurg Anesthesiol*, *16*, 14-19.
427. Muglia, L. J., & Majzoub, J. A. (1993). Diabetes insipidus. In F. Burg, J. Inglefinger, & E. Wald (Eds.), *Gellis and Kagan's Current Pediatric Therapy* (Vol. 14) (pp. 318-319). Philadelphia: W. B. Saunders.
428. Aylward, P. E., Floras, J. S., Leimbach, W. N., Jr., & Abboud, F. M. (1986). Effects of vasopressin on the circulation and its baroreflex control in healthy men. *Circulation*, *73*, 1145-1154.
429. Andersen, L. J., Andersen, J. L., Schutten, H. J., et al. (1990). Antidiuretic effect of subnormal levels of arginine vasopressin in normal humans. *Am J Physiol*, *259*, R53-R60.
430. Seckl, J. R., Dunger, D. B., Bevan, J. S., et al. (1990). Vasopressin antagonist in early postoperative diabetes insipidus. *Lancet*, *335*, 1353-1356.
431. Moreno-Sanchez, D., Casis, B., Martin, A., et al. (1991). Rhabdomyolysis and cutaneous necrosis following intravenous vasopressin infusion. [Review]. *Gastroenterology*, *101*, 529-532.
432. Pierce, S. T., & Nickl, N. (1993). Rhabdomyolysis associated with the use of intravenous vasopressin. *Am J Gastroenterol*, *88*, 424-427.
433. Mauro, V. F., Bingle, J. F., Ginn, S. M., & Jafri, F. M. (1988). Torsade de pointes in a patient receiving intravenous vasopressin. *Crit Care Med*, *16*, 200-201.

434. Bryant, W. P., O'Marcaigh, A. S., Ledger, G. A., & Zimmerman, D. (1994). Aqueous vasopressin infusion during chemotherapy in patients with diabetes insipidus. *Cancer, 74*, 2589-2592.
435. Polycarpe, E., Arnould, L., Schmitt, E., et al. (2004). Low urine osmolarity as a determinant of cisplatin-induced nephrotoxicity. *Int J Cancer, 111*, 131-137.
436. Williams, T. D., Dunger, D. B., Lyon, C. C., et al. (1986). Antidiuretic effect and pharmacokinetics of oral 1-desamino-8-D-arginine vasopressin. 1. Studies in adults and children. *J Clin Endocrinol Metab, 63*, 129-132.
437. Cunnah, D., Ross, G., Besser GM (1986). Management of cranial diabetes insipidus with oral desmopressin (DDAVP). *Clin Endocrinol 24*:253-257.
438. Stick SM, Betts PR (1987). Oral desmopressin in neonatal diabetes insipidus. Arch Dis Child 62:1177-1178.
439. Pivonello R, Colao A, Di Somma C, et al. (1998). Impairment of bone status in patients with central diabetes insipidus. *J Clin Endocrinol Metab 83*:2275-2280.
440. Moses AM, Sangani G, Miller JL (1995). Proposed cause of marked vasopressin resistance in a female with an X-linked recessive V2 receptor abnormality. *J Clin Endocrinol Metab 80*:1184-1186.
441. Kobrinsky NL, Doyle JJ, Israels ED, et al. (1985). Absent factor VIII response to synthetic vasopressin analogue (DDAVP) in nephrogenic diabetes insipidus. Lancet 1:1293-1294.
442. Bichet DG, Razi M, Lonergan M, et al. (1988). Hemodynamic and coagulation responses to 1-desamino[8-D-arginine] vasopressin in patients with congenital nephrogenic diabetes insipidus. *N Engl J Med 318*:881-887.
443. Knoers VV, Janssens PM, Goertz J, Monnens LA (1992). Evidence for intact V1-vasopressin receptors in congenital nephrogenic diabetes insipidus. *Eur J Pediatr 151*:381-383.
444. Brink HS, Derkx FH, Boomsma F, et al. (1993). 1-Desamino-8-D-arginine vasopressin (DDAVP) in patients with congenital nephrogenic diabetes insipidus. *Netherlands J Med 43*:5-12.
445. Ohzeki T (1985). Urinary adenosine 3',5'-monophosphate (cAMP) response to antidiuretic hormone in diabetes insipidus (DI): comparison between congenital nephrogenic DI type 1 and 2, and vasopressin sensitive DI. *Acta Endocrinol 108*:485-490.
446. Bichet DG, Razi M, Arthus MF, et al. (1989). Epinephrine and dDAVP administration in patients with congenital nephrogenic diabetes insipidus: evidence for a pre-cyclic AMP V2 receptor defective mechanism. *Kidney Int 36*:859-866.
447. Moses AM, Weinstock RS, Levine MA, Breslau NA (1986). Evidence for normal antidiuretic responses to endogenous and exogenous arginine vasopressin in patients with guanine nucleotide-binding stimulatory protein-deficient pseudohypoparathyroidism. *J Clin Endocrinol Metab 62*:221-224.
448. Knoers N, Monnens LA (1992). Nephrogenic diabetes insipidus: clinical symptoms, pathogenesis, genetics and treatment. [Review]. *Pediatr Nephrol 6*:476-482.
449. Vest M, Talbot NB, Crawford JD (1963). Hypocaloric dwarfism and hydronephrosis in diabetes insipidus. *Am J Dis Child 105*:175-181.
450. Macaulay D, Watson M (1967). Hypernatremia in infants as a cause of brain damage. *Arch Dis Child 42*:485-491.
451. Freycon MT, Lavocat MP, Freycon F (1988). [Familial nephrogenic diabetes insipidus with chronic hypernatremia and cerebral calcifications]. [Review] [French]. *Pediatrie 43*:409-413.
452. Nozue T, Uemasu F, Endoh H, et al. (1993). Intracranial calcifications associated with nephrogenic diabetes insipidus [see comments]. *Pediatr Nephrol 7*:74-76.
453. Schofer O, Beetz R, Kruse K, et al. (1990). Nephrogenic diabetes insipidus and intracerebral calcification. *Arch Dis Child 65*:885-887.
454. Tohyama J, Inagaki M, Koeda T, et al. (1993). Intracranial calcification in siblings with nephrogenic diabetes insipidus: CT and MRI. *Neuroradiology 35*:553-555.
455. Bode HH, Crawford JD (1969). Nephrogenic diabetes insipidus in North America: the Hopewell hypothesis. *N Engl J Med 280*:750-754.
456. Rosenthal W, Seibold A, Antaramian A, et al. (1992). Molecular identification of the gene responsible for congenital nephrogenic diabetes insipidus. *Nature 359*:233-235.
457. Bichet DG, Birnbaumer M, Lonergan M, et al. (1994). Nature and recurrence of AVPR2 mutations in X-linked nephrogenic diabetes insipidus. *Am J Hum Genet 55*:278-286.
458. Bichet DG, Hendy GN, Lonergan M, et al. (1992). X-linked nephrogenic diabetes insipidus: from the ship Hopewell to RFLP studies. *Am J Hum Genet 51*:1089-1102.
459. Birnbaumer M, Gilbert S, Rosenthal W (1994). An extracellular congenital nephrogenic diabetes insipidus mutation of the vasopressin receptor reduces cell surface expression, affinity for ligand, and coupling to the Gs/adenylyl cyclase system. *Mol Endocrinol 8*:886-894.
460. Holtzman EJ, Kolakowski LF Jr, Geifman-Holtzman O, et al. (1994). Mutations in the vasopressin V2 receptor gene in two families with nephrogenic diabetes insipidus. *J Am Soc Nephrol 5*:169-176.
461. Holtzman EJ, Kolakowski LF Jr, O'Brien D, et al. (1993). A null mutation in the vasopressin V2 receptor gene (AVPR2) associated with nephrogenic diabetes insipidus in the Hopewell kindred. *Hum Mol Genet 2*:1201-1204.
462. Knoers NV, van den Ouweland AM, Verdijk M, et al. (1994). Inheritance of mutations in the V2 receptor gene in thirteen families with nephrogenic diabetes insipidus. *Kidney Int 46*: 170-176.
463. Yuasa H, Ito M, Oiso Y, et al. (1994). Novel mutations in the V2 vasopressin receptor gene in two pedigrees with congenital nephrogenic diabetes insipidus. *J Clin Endocrinol Metab 79*: 361-365.
464. Oksche A, Dickson J, Schulein R, et al. (1994). Two novel mutations in the vasopressin V2 receptor gene in patients with congenital nephrogenic diabetes insipidus [published erratum appears in Biochem Biophys Res Commun 1995;207:1059]. *Biochem Biophys Res Commun 205*:552-557.
465. Pan Y, Metzenberg A, Das S, et al. (1992). Mutations in the V2 vasopressin receptor gene are associated with X-linked nephrogenic diabetes insipidus. *Nat Genet 2*:103-106.
466. Tsukaguchi H, Matsubara H, Aritaki S, et al. (1993). Two novel mutations in the vasopressin V2 receptor gene in unrelated Japanese kindreds with nephrogenic diabetes insipidus. *Biochem Biophys Res Commun 197*:1000-1010.
467. van den Ouweland AM, Dreesen JC, Verdijk M, et al. (1992). Mutations in the vasopressin type 2 receptor gene (AVPR2) associated with nephrogenic diabetes insipidus. *Nat Genet 2*:99-102.
468. Wenkert D, Merendino JJ Jr, Shenker A, et al. (1994). Novel mutations in the V2 vasopressin receptor gene of patients with X-linked nephrogenic diabetes insipidus. *Hum Mol Gene 3*:1429-1430.
469. Wildin RS, Antush MJ, Bennett RL, et al. (1994). Heterogeneous AVPR2 gene mutations in congenital nephrogenic diabetes insipidus. *Am J Hum Genet 55*:266-277.
470. Friedman E, Bale AE, Carson E, et al. (1994). Nephrogenic diabetes insipidus: an X chromosome-linked dominant inheritance pattern with a vasopressin type 2 receptor gene that is structurally normal. *Proc Natl Acad Sci U S A 91*:8457-84561.
471. Oksche A, Rosenthal W (1998). The molecular basis of nephrogenic diabetes insipidus. J Mol Med 76:326-337.
472. Pan Y, Wilson P, Gitschier J (1994). The effect of eight V2 vasopressin receptor mutations on stimulation of adenylyl cyclase and binding to vasopressin. *J Biol Chem 269*:31933-31937.
473. Rosenthal W, Antaramian A, Gilbert S, Birnbaumer M (1993). Nephrogenic diabetes insipidus: a V2 vasopressin receptor unable to stimulate adenylyl cyclase. *J Biol Chem 268*: 13030-13033.
474. Rosenthal W, Seibold A, Antaramian A, et al. (1994). Mutations in the vasopressin V2 receptor gene in families with nephrogenic diabetes insipidus and functional expression of the Q-2 mutant. *Cell Mol Biol 40*:429-436.
475. Waring AJ, Kajdi L, Tappan V (1945). A congenital defect of water metabolism. *Am J Dis Child 69*:323.
476. Langley JM, Balfe JW, Selander T, et al. (1991). Autosomal recessive inheritance of vasopressin-resistant diabetes insipidus. *Am J Med Genet 38*:90-94.
477. Knoers N, Monnens LA (1991). A variant of nephrogenic diabetes insipidus: V2 receptor abnormality restricted to the kidney. *Eur J Pediatr 150*:370-373.
478. Mulders SM, Bichet DG, Rijss JP, et al. (1998). An aquaporin-2 water channel mutant which causes autosomal dominant nephrogenic diabetes insipidus is retained in the Golgi complex. *J Clin Invest 102*:57-66.

479. Boton R, Gaviria M, Batlle DC (1987). Prevalence, pathogenesis, and treatment of renal dysfunction associated with chronic lithium therapy. [Review]. *Am J Kidney Dis 10*:329-345.
480. Bendz H, Aurell M, Balldin J, et al. (1994). Kidney damage in long-term lithium patients: a cross-sectional study of patients with 15 years or more on lithium. *Nephrol Dial Transplant 9*:1250-1254.
481. Yamaki M, Kusano E, Tetsuka T, et al. (1991). Cellular mechanism of lithium-induced nephrogenic diabetes insipidus in rats. *Am J Physiol 261*:F505-F511.
482. Marples D, Christensen S, Christensen EI, et al. (1995). Lithium-induced downregulation of aquaporin-2 water channel expression in rat kidney medulla. *J Clin Invest 95*:1838-1845.
483. Hirji MR, Mucklow JC (1991). Transepithelial water movement in response to carbamazepine, chlorpropamide and demeclocycline in toad urinary bladder. *Br J Pharmacol 104*:550-553.
484. Navarro JF, Quereda C, Quereda C, et al. (1996). Nephrogenic diabetes insipidus and renal tubular acidosis secondary to foscarnet therapy. *Am J Kidney Dis 27*:431-434.
485. Bendz H, Aurell M (1999). Drug-induced diabetes insipidus: incidence, prevention and management. *Drug Saf 21*:449-546.
486. Hohler T, Teuber G, Wanitschke R, Meyer zum Buschenfeld KH (1994). Indomethacin treatment in amphotericin B induced nephrogenic diabetes insipidus. *Clin Investig 72*:769-771.
487. Vigeral P, Kanfer A, Kenouch S, et al. (1987). Nephrogenic diabetes insipidus and distal tubular acidosis in methicillin-induced interstitial nephritis. *Adv Exp Med Biol 212*:129-134.
488. Quinn BP, Wall BM (1989). Nephrogenic diabetes insipidus and tubulointerstitial nephritis during continuous therapy with rifampin. *Am J Kidney Dis 14*:217-220.
489. Kato A, Hishida A, Ishibashi R, et al. (1994). Nephrogenic diabetes insipidus associated with bilateral ureteral obstruction. *Intern Med 33*:231-233.
490. Nagayama Y, Shigeno M, Nakagawa Y, et al. (1994). Acquired nephrogenic diabetes insipidus secondary to distal renal tubular acidosis and nephrocalcinosis associated with Sjogren's syndrome. *J Endocrinol Invest 17*:659-663.
491. Hartenberg MA, Cory M, Chan JC (1985). Nephrogenic diabetes insipidus. Radiological and clinical features. *Intl J Pediatr Nephrol 6*:281-286.
492. Alon U, Chan JC (1985). Hydrochlorothiazide-amiloride in the treatment of congenital nephrogenic diabetes insipidus. *Am J Nephrol 5*:9-13.
493. Libber S, Harrison H, Spector D (1986). Treatment of nephrogenic diabetes insipidus with prostaglandin synthesis inhibitors. *J Pediatr 108*:305-311.
494. Rascher W, Rosendahl W, Henrichs IA, et al. (1987). Congenital nephrogenic diabetes insipidus-vasopressin and prostaglandins in response to treatment with hydrochlorothiazide and indomethacin. *Pediatr Nephrol 1*:485-490.
495. Vierhapper H, Jorg J, Favre L, et al. (1984). Comparative therapeutic benefit of indomethacin, hydrochlorothiazide, and acetyl-salicylic acid in a patient with nephrogenic diabetes insipidus. *Acta Endocrinol 106*:311-316.
496. Uyeki TM, Barry FL, Rosenthal SM, Mathias RS (1993). Successful treatment with hydrochlorothiazide and amiloride in an infant with congenital nephrogenic diabetes insipidus. *Pediatr Nephrol* 7:554-556.
497. Batlle DC, von Riotte AB, Gaviria M, Grupp M (1985). Amelioration of polyuria by amiloride in patients receiving long-term lithium therapy. *N Engl J Med 312*:408-414.
498. Weinstock RS, Moses AM (1990). Desmopressin and indomethacin therapy for nephrogenic diabetes insipidus in patients receiving lithium carbonate. *South Med J 83*:1475-1477.
499. Welch EM, Barton ER, Zhuo J, et al. (2007). PTC124 targets genetic disorders caused by nonsense mutations. *Nature 447*: 87-91.
500. Liu BA, Mittman N, Knowles SR, Shear NH (1996). Hyponatremia and the syndrome of inappropriate secretion of antidiuretic hormone associated with the use of selective serotonin reuptake inhibitors: a review of spontaneous reports. *Can Med Assoc J 155*:519-527.
501. Branten AJ, Wetzels JF, Weber AM, Koene RA (1998). Hyponatremia due to sodium valproate. *Ann Neurol 43*:265-267.
502. Jojart I, Laczi F, Laszlo FA, et al. (1987). Hyponatremia and increased secretion of vasopressin induced by vincristine administration in rat. *Exp Clin Endocrinol 90*:213-220.
503. Rider JM, Mauger TF, Jameson JP, Notman DD (1995). Water handling in patients receiving haloperidol decanoate. *Ann Pharmacother 29*:663-666.
504. Settle ECJ (1998). Antidepressant drugs: disturbing and potentially dangerous adverse effects. *J Clin Psychiatry 59*:25-60.
505. Shaikh ZHA, Taylor HC, Maroo PV, Llerena LA (2000). Syndrome of inappropriate antidiuretic hormone secretion associated with lisinopril. *Ann Pharmacother 34*:176-179.
506. van Laar T, Lammers G-J, Roos RAC, et al. (1998). Antiparkinsonian drugs causing inappropriate antidiuretic hormone secretion. *Movement disorders 13*:176-178.
507. Baylis PH. Vasopressin and its neurophysin (1989). In Degroot LJ (ed.), *Endocrinology*, 2nd ed. Philadelphia: WB Saunders, 213.
508. Dunn FL, Brennan TJ, Nelson AE, et al. (1973). The role of blood osmolality and volume regulating vasopressin secretion in the rat. *J Clin Invest 52*:3212.
509. Robertson GL (1985). Regulation of vasopressin secretion. In Seldin DW, Giebisch G (eds.), *The kidney, physiology and pathophysiology*. New York: Raven Press, 869.
510. Bichet DG (1995). The posterior pituitary. In Melmed S (ed.), *The pituitary.* Cambridge, UK: Blackwell Science, 277.
511. Reeves WB, Andreoli TE (1985). Nephrogenic diabetes insipidus. In Scriver CR, Beaudet AL, Sly WS (eds.), *The metabolic basis of inherited disease.* New York: McGraw-Hill, 1985.

QUESTIONS

1. What is the relationship between vasopressin and neurophysin?
   a. They are produced by different genes and bind together in the bloodstream.
   b. They are produced by the same gene and bind together in the vasopressin neuron.
   c. They are made from a common precursor protein, separated by copeptin.

Answer: b

2. Mutations in the vasopressin (AVP) gene
   a. Cause autosomal dominant disease
   b. Cause central diabetes insipidus that has its onset in the neonatal period
   c. Cause nephrogenic diabetes insipidus

Answer: a

3. A child develops central diabetes insipidus and is otherwise well. A brain MRI is normal. You should be concerned that she may have which of the following conditions? (Choose one best answer.)
   a. A small germinoma
   b. An elevated beta hCG level in the CNS
   c. A prolactinoma
   d. An elevated prolactin level in the blood
   e. a and b
   f. c and d

Answer: e

4. A patient with polydipsia and polyuria is given a water deprivation test. After several hours the urine osmolality plateaus at 400 mOsm/kg H_2O and rises no further with vasopressin administration. This is compatible with
   a. Partial central DI
   b. Primary polydipsia
   c. Partial nephrogenic DI
   d. a or b
   e. b or c
   f. a or c

Answer: e

5. Rapid correction of severe hyponatremia (Na $<$ 120 mEq/L) is warranted if
   a. The hyponatremia has been present for several days
   b. The patient is having an acute generalized seizure
   c. The patient has congestive heart failure
   d. The patient is hyperglycemic

Answer: b

6. A neonate with central DI should not be treated with
   a. A low-salt diet
   b. Oral desamino-D arginine vasopressin (dDAVP)
   c. A thiazide diuretic

Answer: b

CHAPTER 12

Thyroid Disorders in Children and Adolescents

Scott A. Rivkees, MD

CHAPTER OUTLINE

INTRODUCTION

Thyroid disease can present with overt symptoms, insidiously, or with isolated thyromegaly. Thyroid disease in children can encompass isolated biochemical abnormalities that have little or no physiologic consequence or with overt clinical symptoms. Clinically, hypothyroidism occurs more commonly than hyperthyroidism. Thyroid nodules and masses occur much less commonly than functional disorders, but they can portend the presence of thyroid cancer. This chapter focuses on the most common conditions that affect the thyroid gland of children and adolescents.

Box 12-1 provides a classification of thyroid disorders in children. Thyroid system development, fetal thyroid physiology, thyroid dysfunction in premature infants, and congenital thyroid disorders (including inborn defects in thyroid hormone synthesis, metabolism, and action and thyroid binding protein abnormalities) are discussed in Chapter 6.

Thyroid Hormones and Their Action

Few hormones exert as profound and essential role in human physiology as thyroid hormones.[1,2] The major hormones released by the thyroid gland include tetraiodothyronine, or thyroxine (T4), and triiodothyroine (T3).[1] The production of these hormones involves several discrete biochemical steps that are depicted in Figure 12-1. Of these hormones, T3 plays the pivotal role in affecting physiology, being the molecule that principally binds to the thyroid hormone receptor (TRs). The thyroid hormone nuclear receptor belongs to the steroid hormone–retinoic acid receptor superfamily and is a regulator of DNA transcription.[1,3,4] Two genes encode the TR; one on chromosome 17 designated alpha (TRa) and one on chromosome 3 designated beta (TRb).[3,4] The TRs can exist as monomers or homodimers, and they can dimerize with other members of the family of nuclear receptors.[3,4] After T3 binding to the TR, gene transcription is regulated in many tissues.[4]

BOX 12-1 Thyroid Disorders in Childhood and Adolescence

Autoimmune Thyroid Disease
- Hashimoto thyroiditis, juvenile acquired hypothyroidism
- Stimulating antibody, Graves disease
- Blocking antibody, hypothyroidism

Infectious Thyroiditis
- Suppurative thyroiditis
- Subacute thyroiditis

Binding Protein Abnormalities
- Complete TBG deficiency
- Partial TBG deficiency
- TBG excess
- Transthyretin variants

TSH Receptor Mutations
- Loss-of-function hypothyroidism
- Gain-of-function hyperthyroidism

Thyroid Hormone Resistance Syndromes
- Thyroid hormone beta receptor (TRβj) mutations
- Peripheral tissue resistance syndrome
- Pituitary resistance syndrome
- Thyroid hormone membrane transport defects

Iodine Deficiency Syndromes
- Goiter
- Mental impairment
- Cretinism

Diffuse Nontoxic Goiter
- Nonthyroidal illness
- Thyroid neoplasia
  - Adenoma
  - Nonfunctional
  - Functional
  - Papillary-follicular carcinoma
  - Medullary carcinoma
  - MEN 2A, 2B, Ret mutations
  - Sporadic
  - Undifferentiated
  - Metastatic

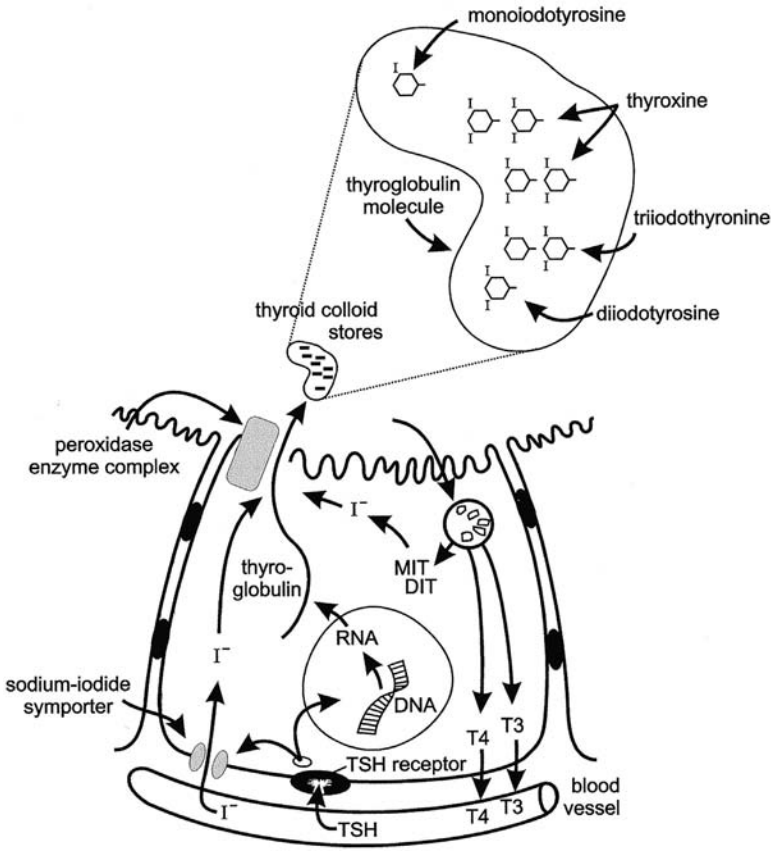


FIGURE 12-1 ■ Illustration of thyroid hormone synthesis and secretion. TSH regulates the process via the G–protein-linked plasma membrane TSH receptor. TSH binding stimulates thyroglobulin synthesis and sodium-iodide symporter (iodide transporter) uptake of circulating iodide. Iodide diffuses in the cytosol to the apical membrane and is transported to the apical lumen by Pendrin, an anion-bicarbonate family exchanger, making iodide available to the enzyme organification complex (Pendrin; thyroid peroxidase, TPO, THOX). The tyrosine residues of thyroglobulin are iodinated at the apical cell membrane and are catalyzed by thyroid peroxidase, the organification enzyme. The resulting monoiodotyrosine (MIT) and diiodotyrosine (DIT) residues couple to form the iodothyronines thyroxine (T4) and triiodothyronine (T3) within the stored thyroglobulin molecule. TSH stimulates micropinocytosis of colloid droplets and progressive thyroglobulin proteolysis within the resulting phagolysosomes. T4 and T3 are secreted into the circulation. The uncoupled MIT and DIT are deiodinated by iodotyrosine deiodinase (DEHAL) to release iodide, which is largely recycled within the follicular cell. (From Fisher and Greuters [2008] Thyroid disorders in childhood and adolescence. In *Pediatric Endocrinology*, (3rd ed.) (pp 227-253). Philadelphia: Saunders.)

T4 is the predominant hormone released from thyroid follicular cells. After release it circulates in protein-bound and free states at a ratio of about 1000 to 1. Thyroid hormone-binding proteins in the blood include thyroxine-binding globulin (TBG), prealbumin or transthyretin, and albumin.[5-7] TBG is the predominant carrier protein for T4; TBG and albumin also carry T3.[5-7] In the euthyroid steady, the circulating concentration of free T4 (FT4) and free T3 are about 0.03% and 0.30%, respectively, of total hormone concentrations.

It is important to recognize that circulating levels of thyroid hormones and carrier proteins change with age (Tables 12-1 and 12-2). Absolute mean free T4 and free T3 concentrations are about 10 and 4 pg/mL, respectively, and differ according to age. In adolescents and adults, the plasma concentrations of the several binding proteins are 1 to 3 mg/dL for TBG, 20 to 30 mg/dL for TBPA, and 2 to 5 g/dL for albumin.[2,5-7] TBG concentrations are greater in children than in adults, and they decline to adult levels during adolescence.[2] Because the thyroid-hormone binding proteins are produced in the liver, they are acute phase reactants, with concentrations increasing during acute illness[2,5-7]; they also increase in response to estrogen exposure.

Conversion of T4 to T3 involves the deiodination of T4 (Figure 12-2). Monodeiodination of the beta or outer ring by monodeiodinase (MD) type II produces T3.[8,9] Monodeiodination of the alpha or inner-ring produces reverseT3 (rT3), which is inactive metabolically. Under normal circumstances, T3 and rT3 are produced at similar rates. About 70% to 90% of circulating T3 is derived from peripheral conversion of T4, and 10% to 30% of circulating T3 is from the thyroid gland.[2] Reflecting the age-related changes in the hormones that regulate T4 stability, the clearance of T4 generally decreases from infancy to adulthood (Table 12-3).

TABLE 12-1 **Changes with Age in Serum Concentrations of T4, TSH, TBG, and Thyroglobulin (Tg)**

Age	TSH* (μU/mL)	T4† (μg/dL)	TBG† (mg/dL)	Tg† (ng/mL)
Cord blood	1-20	6.6-15	0.8-5.2	15-101
1-7 days	1-39	11-22	0.8-5.2	1-110
1-4 weeks	0.5-6.5	8.2-17	0.6-5	11-92
1-12 months	0.5-6.5	5.9-16	1.6-3.6	12-113
1-5 years	0.6-8	7.3-15	1.4-2.8	5-72
6-10 years	0.6-8	6.4-13	1.4-2.8	3-40
11-15 years	0.6-8	5.5-12	1.4-2.8	3-40
16-20 years	0.5-6	4.2-12	1.4-2.8	2- 36
21-50 years	0.5-6	4.3 -12	1.2-2.6	2-35

*Mean and 95 % range.
†Mean and two standard deviations (SD) range.
Compiled from Fisher, D. A., & Vanderschueren-Lodeweycky, M. (1985). Laboratory tests for thyroid diagnosis in infants and children. In F. Delange, D. A. Fisher (Eds.), Pediatric thyroidology *(pp. 127–142). Basel: Karger; Walfish, P. G., & Tseng, K. H. (1989). Thyroid physiology and pathology. In R. Collu, J. R. Ducharme, H. Guyda (Eds.),* Pediatric endocrinology *(pp. 367–448). New York: Raven; Delange, F., Dahlem, A., Bourdoux, P., et al (1984). Increased risk of primary hypothyroidism in preterm infants.* Pediatrics, 105, *462; Pazzino, V., Filetti, S., Belfiore, A., et al. (1981). Serum thyroglobulin levels in the newborn.* J Clin Endocrinol Metab, 52, *3634; Delange, F. (1993). Thyroid hormones: biochemistry and physiology. In J. Bertrang, R. Rappaport, P. C. Sizonenko (Eds.),* Pediatric endocrinology *(pp. 242–251). Baltimore: Williams and Wilkins; Lazar, L., Frumkin, R. B., Battat, E., et al. (2009).* J Clin Endocrinol Metab, 94, *1678–1682.*

TABLE 12-2 **Changes with Age in Serum Concentrations of T3, rT3, Free T4, and Free T3**

	T3* (ng/dL)	rT3 * (ng/dL)	Free T4** (ng/dL)	Free T3** (pg/mL)
Cord blood	14-86	100-501	1.2-2.2	—
4-7 days	36-316	34-258	2.2-5.3	1.3-6.1
1-4 weeks	105-345	26-290	0.9-2.3	2.2-8
1-12 months	105-245	11-129	0.8-2.1	2.5-7
1-5 years	105-269	15-71	0.8-2	2.8-5.2
6-10 years	94-241	17-79	0.8-2	2.8-5.2
11-15 years	83-213	19-88	0.8-2	2.9-5.6
16-20 years	80-210	25-80	0.8-2	2.4-5
21-50 years	70-204	30-80	0.9-2.5	−4.4

*Geometric mean and range.
†Two standard deviations (SD) range, by tracer dialysis.
Compiled from Delange, F. (1993). Thyroid hormones: biochemistry and physiology. In J. Bertrang, R. Rappaport, P. C. Sizonenko (Eds.), Pediatric endocrinology *(pp. 242–251). Baltimore: Williams and Wilkins; Lucas, C., Carayan, P., Bellhilehi, J., & Giraud, F. (1980). Changes in levels of free thyroid hormones in children from 1 to 16 years: comparison with other thyroid indices.* Pediatric, 35, *197; Nelson, J. C., Clark, S. J., Borut, D. L., et al. (1993). Age related changes in serum free thyroxine during childhood adolescence.* J Pediatr, 123, *899.*

Regulation of Thyroid Function

The production of T4 and T3 within the thyroid gland is regulated by the thyroid stimulating hormone (TSH; also called thyrotropin), which is released from the anterior pituitary gland (Figure 12-3).[10,11] TSH receptors are present on thyroid follicular cells and are G protein–coupled receptors with a large extracellular amino terminus.[12] Mutations of the TSH receptor can result in constitutive activation of the receptor with severe hyperthyroidism, whereas inactivating mutations result in TSH unresponsiveness and hence hypothyroidism.[12]

TSH receptor activation stimulates adenylate cyclase accumulation within follicular cells, which in turn causes accumulation of cyclic adenosine monophosphate (cAMP). Increased cellular concentrations of cAMP promote iodide trapping, iodotyrosine synthesis, thyroglobulin (Tg) synthesis, and hormone release.

TSH release is regulated by the hypothalamic hormone *thyrotropin releasing hormone* (TRH) (see Figure 12-3).[13,14] This peptide hormone is produced in medial neurons of the paraventricular nucleus of the hypothalamus and is released into the portal circulation of the pituitary gland.[13,14] Several different neurotransmitters have been observed to influence THRH release.[15,16]

In addition to normal regulation of TSH receptor activity by TSH, thyroid function can be adversely affected by antibodies that can either stimulate or block

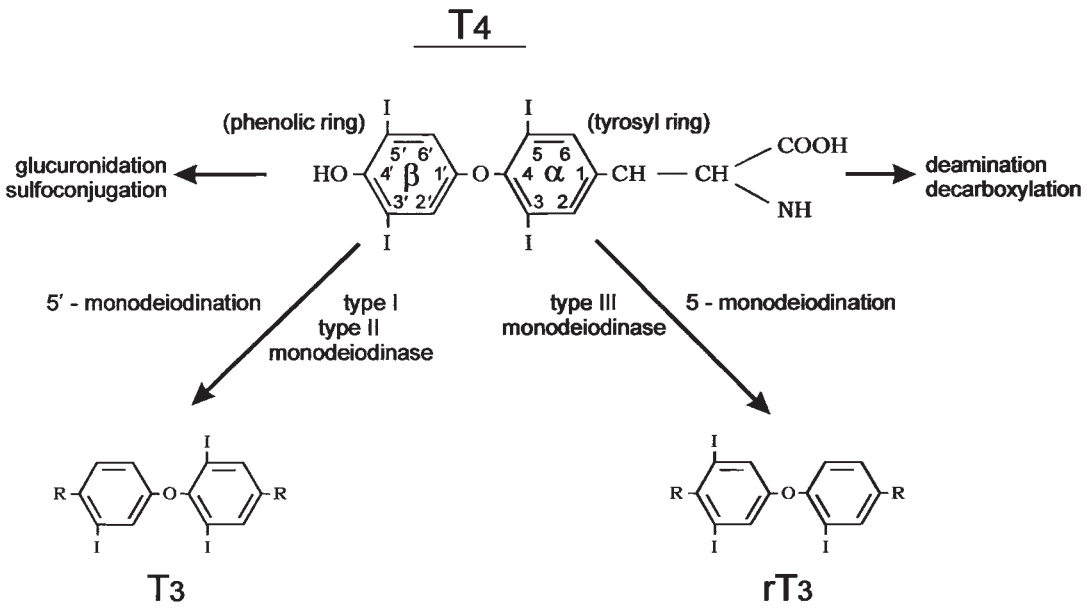


FIGURE 12-2 ■ The metabolism of thyroxine (tetraiodothyronine). The major metabolic pathway is progressive monodeiodination mediated by the three iodothyronine monodeiodinase enzymes type I, type II, and type III. Outer (phenolic) ring 5′monodeiodination produces active 3,5,3′ triiodothyronine. Inner (tyrosyl) ring 5′monodeiodination produces inactive reverse 3,3,5′ triiodothyronine. Type I deiodinase is also capable of inner-ring monodeiodination. The alanine side chain of the tyrosyl ring is also subject to degradative reactions, including deamination and decarboxylation. Sulfoconjugation and glucuronide conjugation reactions at the 4′ phenolic ring site occur largely in liver tissue. (From Fisher and Greuters [2008] Thyroid disorders in childhood and adolescence. In *Pediatric Endocrinology*, (3rd ed.) (pp 227-253). Philadelphia: Saunders.)

TABLE 12-3 **Variation in Peripheral Thyroxin Metabolism with Age***

Thyroxine Kinetic Parameter	Children (3-9 years)	Adolescents (10-16 years)	Adults (23-26 years)
Half-life (d)	5 (0.13)	6 (0.35)	6.7 (0.30)
Fractional clearance†	0.14 (0.005)	0.12 (0.008)	0.11 (0.004)
Distribution volume (L/kg)	0.16 (0.008)	0.16 (0.014)	0.12 (0.005)
Thyroxine turnover (μg/kg/d)	1.9 (0.09)	1.5 (0.07)	1.1 (0.06)

*Data are means and SE.
†Fraction of extrathyroid pool per day.
Data from Beckers, C., Malvaux, C., & De Visscher, M. (1966). Quantitative aspects of the secretion and degradation of thyroid hormones during adolescence. J Clin Endocrinol Metab, 26, *202–306; Sterling, K., & Chodos, R. (1956). Radiothyroxine turnover studies in myxedema, thyrotoxicosis and hypermetabolism without endocrine disease.* J Clin Invest, 35, *806–813.*

TSH action. TSH receptor–stimulating immunoglobulin antibodies (TSI), or thyroid receptor antibodies (TRAb), are present in the circulation of individuals with Graves disease and activate TSH receptors.[17-19] Conversely, TSH receptor–blocking immunoglobulins (TBI) antagonize TSH action and can lead to hypothyroidism.[17-19]

CLINICAL AND BIOCHEMICAL ASSESSMENT OF THYROID STATUS

Clinical Evaluation of Thyroid Function

Thyroid disease can present with overt symptoms, insidiously, or with isolated thyromegaly. Thus, evaluation of the thyroid gland should be included in the routine examination of children. The thyroid gland can be visualized by having the patient look to the ceiling and swallow. As the thyroid moves, the margins of the gland are viewed to estimate size and symmetry. The thyroid should be palpated to assess size, consistency, and symmetry. This is best performed with the clinician standing behind the patient and palpating the neck with the fingertips. The texture of the thyroid can be assessed to determine if it is smooth or irregular, and the texture may feel firm or soft if nodules are present. If any asymmetry or abnormal thyroid fullness is noted, ultrasonographic evaluation is recommended, as pathologic thyroid nodules may feel like normal tissue.

To assess gland size, one may estimate the size of each thyroid lobe relative to that of a teaspoon (5 g) or a

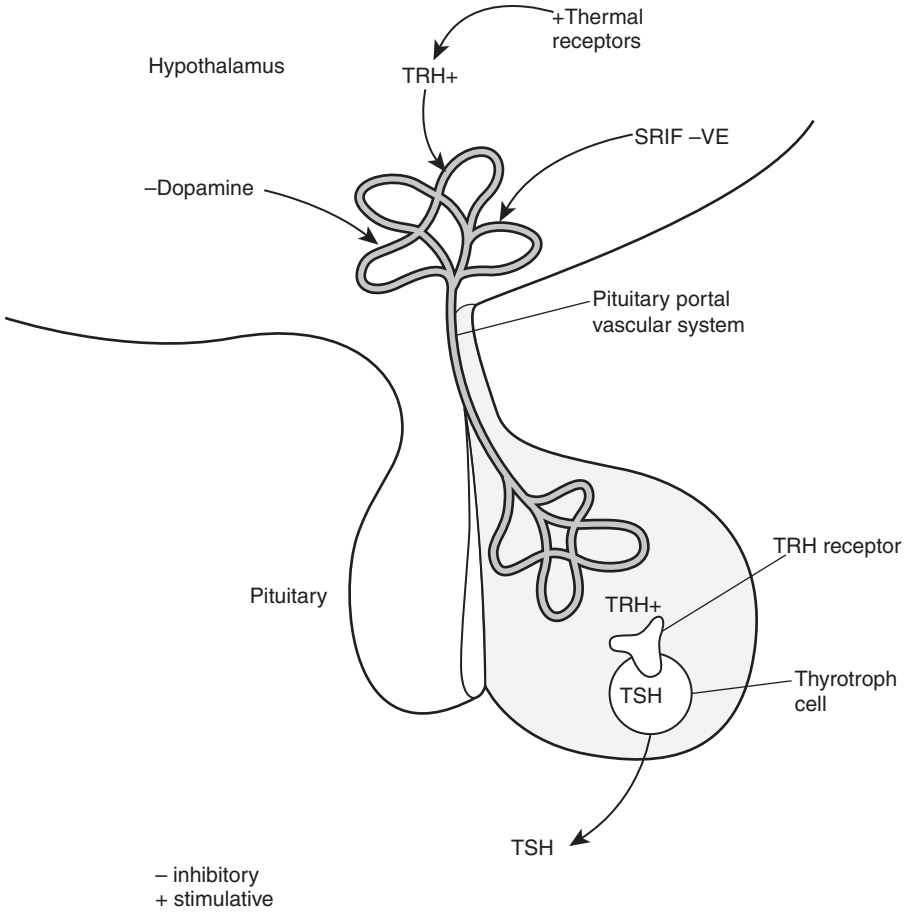


FIGURE 12-3 ■ The hypothalamic-pituitary TSH axis. Thyrotropin-releasing hormone (TRH) secreted into the pituitary portal vascular system stimulates TSH synthesis and secretion from the pituitary thyrotroph cell. TRH secretion is modulated by central and peripheral thermal sensors. Dopamine or somatostatin (SRIF) can inhibit TSH release. (From Fisher and Greuters [2008] Thyroid disorders in childhood and adolescence. In *Pediatric Endocrinology*, (3rd ed.) (pp 227-253). Philadelphia: Saunders.)

tablespoon (15 g). Generally, until the end of puberty, gland size (in grams) approximates the patient's age in years times 0.5 to 0.7.[20] Thus, each thyroid lobe of a 10-year-old is approximately one half of a teaspoon for a total gland size of 5 to 7 g.[20] For teens and adults, each lobe of the thyroid may reach one teaspoon in size for an approximate total gland size of about 10 g.[20]

In newborns and young infants, the thyroid can be examined by placing the infant supine on the parent's lap, with the head toward the parent's knees. The head can then be gently lowered backward to expose the neck, which facilitates thyroid palpation. If the examiner can palpate each ring of the trachea from the sternal notch to above the larynx, then the absence of pretracheal thyroid tissue is suggested with a lingual thyroid gland or complete absence (athyreosis). Failure to detect pretracheal thyroid tissue in older children warrants visual examination of the base of the tongue for ectopic thyroid tissue.

When a sublingual thyroid gland is discovered late in childhood or in adolescence, the tissue should be palpated with a gloved finger during regular office visits, because nodules and malignancies may develop in ectopic thyroid glands.[21] In contrast, when an ectopic thyroid is detected in infancy and replacement therapy is initiated, the residual thyroid tissue becomes atrophic and does not present long-term problems.

Biochemical Evaluation of Thyroid Function

Thyroid function can be assessed by measurement of total T4 and T3 levels, along with indices that reflect thyroid hormone-binding proteins (T3 or T4 resin uptake).[22] The levels of estimated free (unbound) T4 (FT4) are measured to assess thyroid hormone status without the confounding influences of carrier proteins.

Several conditions occur in which thyroid hormone levels are abnormal, yet the individual is euthyroid. Because of their confusing nature, these conditions may result in the patient being erroneously diagnosed or treated for hypothyroidism or hyperthyroidism. When FT4 values are normal yet total T4 values are high, familial dysalbuminemic hyperthyroxinemia needs to be considered as the cause.[23,24] In the United States, this autosomal dominant disorder is most commonly seen in Hispanic individuals and can be diagnosed by thyroid hormone–binding protein electrophoresis. If FT4 values are normal but total T4 values are low, the possibility of TBG deficiency must be entertained. TBG deficiency is an X-linked disorder that may be associated with color blindness.[25] In these and other conditions affecting thyroid hormone binding, treatment is not needed and the patient should be educated

about the condition to avoid unnecessary treatment by unsuspecting practitioners.

As indicated previously, T4 is much more abundant in the circulation, but T3 is the more metabolically active thyroid hormone. The majority of T3 is produced peripherally from T4, but some (10% to 30%) is also secreted by the thyroid gland. A metabolically inactive form of T3, reverse T3, also is produced, and its concentration is elevated in conditions such as euthyroid sick syndrome[26] (see Figure 12-2). Ultrasensitive thyrotropin or thyroid stimulating (TSH) assays have been developed, and assessment of TSH has greatly improved the evaluation of thyroid status.[27] TSH concentrations help to distinguish many thyroid disorders that present with either low or high T4 concentration. TSH values within the normal range for the assay are indicative of a euthyroid state if the hypothalamic pituitary axis is intact. Elevations of TSH above the normal levels for age generally indicate primary thyroid hypofunction; suppressed or undetectable TSH values generally indicate hyperthyroidism, providing that there are no substances in the circulation such as drugs or endogenous antibodies that interfere with the assay. When both FT4 and TSH levels are elevated, TSH-producing pituitary adenomas or thyroid hormone resistance needs to be considered.

Critical in the interpretation of thyroid hormone concentration is the recognition that concentrations of T4, T3, and TSH vary with age (see Box 12-1). TSH values in children differ from those in adults, defined by an upper limit value of about 4 μU/mL or less.[28-31] In comprehensive studies of this issue, the upper limit of TSH values in healthy children and adolescents without thyroid disease is about 7 μU/mL.[32-34] The application of an adult reference range to children results in the erroneous diagnosis of subclinical hypothyroidism and the unnecessary referral of children for subspecialty care by primary care providers.

HYPOTHYROIDISM

Disorders of the thyroid lead to hypothyroidism much more commonly than to hyperthyroidism. Hypothyroidism may be present at birth, acquired during childhood or adolescence, present with or without symptoms, or be present after long-standing onset or acutely.

The public and many practitioners commonly believe that hypothyroidism is associated with and is a cause of obesity, yet there is little support for the notion that the hypothyroid state contributes to obesity.[35,36] It is also important to note that TSH levels are slightly higher in obese individuals than nonobese individuals.[37-40] With weight loss, TSH levels normalize in these children.[37-39,41] Thus, slight elevations in TSH in obese individuals are physiologic, reflecting an attempt by the body to increase metabolism and limit adipose tissue deposition and, hence, do not warrant therapy.

Hypothyroidism signs may be elusive, with symptoms elicited only in retrospect. In the extreme, hypothyroidism can be associated with cold intolerance, bradycardia, carotenemia, coarse and brittle hair, dry skin, pallor, and myxedema. These symptoms may not be distressing, which allows prolonged hypothyroidism to escape detection.

The most common causes of hypothyroidism in children are autoimmune processes resulting in Hashimoto thyroiditis.[42,43] Autoimmune thyroiditis also leads to juvenile acquired hypothyroidism that can present with growth failure when chronically present.[44] Hypothyroidism in children can be caused by iodine exposure or hypothalamic-pituitary dysfunction. Other causes of hypothyroidism include exogenous goitrogens,[45,46] cystinosis,[47,48] acute and subacute thyroiditis,[49] and thyroid irradiation during cancer treatment.[50] Hypothyroidism in the newborn is a serious health concern and is detected by newborn screening programs as detailed in Chapter 7

Hashimoto or Autoimmune Thyroiditis

Autoimmune thyroiditis with thyroid enlargement is one of the most common presentations of childhood thyroid disease.[43,51] It is associated with antibodies against thyroglobulin and thyroperoxidase and is characterized by lymphocytic infiltration of the thyroid gland, which results in thyromegaly.[43,51] Depending on the nature of the antithyroid antibodies, Hashimoto disease may be associated with a euthyroid state, hypothyroidism, or transient hyperthyroidism.[43,51] Damage to the thyroid gland reflects both antibody-mediated and cell-mediated injury.

Hashimoto thyroiditis may rarely occur in very young infants[52] but typically presents in adolescents, affecting females three to five times more commonly than males.[43] The thyroid gland is usually diffusely enlarged and may seem to have an irregular, cobblestone texture on palpation. Asymmetric thyroid enlargement, mimicking a thyroid nodule, may be noted. The presence of antithyroid antibodies and the absence of nodules on ultrasonography can distinguish inflammation from other pathologic processes.

Importantly, the presence of antithyroid antibodies does not portend the development of complete or partial thyroid failure that will warrant therapy. In the healthy adult population, up to 5% of individuals had circulating antithyroid antibodies present.[53] Less than 10% of these individuals (i.e., only 0.5% of those with antibodies) will develop hypothyroidism, and those who have elevated antithyroperoxidase (TOP) antibodies are much more at risk than those who have antithyroglobulin (TG) antibodies.[53]

In children, the incidence of antithyroid antibodies in the population is not defined. Of those children with antithyroid antibodies, about 20% are reported to develop hypothyroidism that requires treatment with exogenous thyroid hormone,[54,55] and these children often have very high antithyroid antibody titers. If a child is found to have low concentrations of antithyroid antibodies, it is reasonable to assess thyroid indices every 6 to 12 months and initiate therapy when the TSH rises above the upper limit of normal for children. If high titers are present at presentation, it is reasonable to initiate therapy at that time.

In some children, untreated Hashimoto thyroiditis can result in progressive thyromegaly and hypothyroidism.[43] Treatment with levothyroxine prevents hypothyroidism

and the TSH elevations that stimulate gland enlargement. When T4 levels are modestly depressed (< 5 µg/dL) or normal, treatment can be initiated with 1 to 2 µg/kg/day of levothyroxine. If profound hypothyroidism is present, pseudotumor cerebri may develop when children are treated with conventional doses.[56] Thus, treatment is initiated with one third to one half of the usual dose of levothyroxine. After 2 to 4 weeks, the patient can be advanced to conventional doses. However, children with profound hypothyroidism can develop pseudotumor cerebri even if treatment is initiated with low doses of levothyroxine.[57-59]

It has been reported that some children with profound TSH elevations (> 250 mIU/mL) and those with severe hypothyroidism may experience complete spontaneous resolution of the hypothyroid state without treatment and restoration of a euthyroid state occurs.[60] Based on the experience of others, this is very uncommon.

Although it has been reported that there are some differences in the oral bioavailability of different levothyroxine preparations,[61-63] from a practical vantage these differences are minor.[62-64] Thus, the routine use of less expensive generic compounds versus more expensive brand-name products is justified.

The timing of levothyroxine ingestion has been the subject of study. In contrast to the standard recommendation that thyroid replacement be taken on an "empty stomach," taking the medication at bedtime is associated with higher T4 levels and lower TSH levels over the course of the day.[65,66] This is believed to be related to better gastrointestinal absorption in the evening than during the day.[65,66]

The suggestion has also been made that hypothyroidism in adolescents can be treated with a single dose given weekly.[67] This approach is not recommended, because thyroid hormone levels are high shortly after the dose is administered and are low by the week's end.[67] Treatment of congenital hypothyroidism with weekly doses of levothyroxine can result in mental retardation.[68] It is also recognized that excessive soy intake, iron tablets, and excessive fiber can interfere with the absorption of levothyroxine.[69-71]

Other potential therapies that may theoretically alter the autoimmune process have been tested. Among the proposed remedies, no benefit has been observed in patients who have taken selenium.[72]

Hashitoxicosis

Uncommonly, patients may present with hashitoxicosis, in which the immunologic destruction of thyroid tissue results in the release of preformed thyroid hormone, leading to elevated T4 levels in the circulation with symptoms and signs of hyperthyroidism.[73] In contrast to Graves disease, hyperthyroidism is transient, eye findings are absent, radionuclide uptake is low, and levels of thyroid-stimulating immunoglobulins are not elevated.[73]

Hashimoto thyroiditis may be associated with other autoimmune diseases, including diabetes mellitus, adrenal insufficiency, vitiligo, and hypoparathyroidism.[74] Autoimmune thyroiditis is also seen in patients with inflammatory bowel disease and juvenile arthritis.[75,76] Annual surveillance of thyroid gland size and TSH levels should thus be considered for children with other autoimmune problems, and clinicians should be vigilant for signs of hyperthyroidism or hypothyroidism. Conversely, children with autoimmune thyroiditis should be observed for signs of diabetes mellitus and Addison disease (see Chapter 20 for a discussion of autoimmune polyglandular syndromes).

The incidence of coexisting celiac disease in the setting of Hashimoto thyroiditis is about 1%.[77] If patients manifest abdominal discomfort, weight loss, or gastrointestinal symptoms, celiac disease screening should be performed, but does not need to be done routinely in children with thyroid disease. We have found a 1% incidence of autoimmune liver disease in children with autoimmune thyroid disease. Because such liver disease can be occult, we annually assess circulating transaminase values (alanine amino transferase [ALT] and aspartate amino transferase [AST]). If values are elevated, evaluation of possible autoimmune liver disease is initiated and level of antineutrophil antibodies (ANA) as well as smooth muscle and liver-kidney microsomal antibodies obtained.[78]

Several groups of children are at risk for autoimmune thyroiditis. Because girls with Turner syndrome are predisposed to autoimmune thyroiditis,[79] TSH levels should be assessed annually. Turner syndrome should be considered in girls with hypothyroidism, especially if the child is prepubertal at presentation.[80,81] Children with Down syndrome warrant annual screening for hypothyroidism because they are more prone to develop autoimmune disorders.[82,83]

Subclinical Hypothyroidism

Subclinical hypothyroidism refers to a situation where circulating T4 and T3 concentrations are normal but TSH values are elevated.[36,84] As noted previously, many children are erroneously diagnosed with this condition when TSH concentrations are found to be elevated relative to adult reference range values.[33] However, if appropriate pediatric-based TSH values are applied, the majority of children so diagnosed will not have hypothyroidism. Thus, some experts have questioned if subclinical hypothyroidism is a real entity in children.[36,84]

Studies of children with mild TSH elevations (5 to 10 µU/mL) reveal that only a small fraction will progress to TSH elevations > 10 µU/mL.[36,84] Data also show that treatment of children with TSH values of 5 to 10 µU/mL do not exhibit somatic or other benefits when treated with levothyroxine.[36,84] Thus, treatment of children with TSH levels < 10 µU/mL is not needed. For children with TSH levels > 10 µU/mL, treatment with low doses of levothyroxine is indicated.

Juvenile Acquired Hypothyroidism

When autoimmune thyroiditis occurs during childhood, it is referred to as juvenile acquired hypothyroidism. In children, severe hypothyroidism can be well tolerated. Thus, prolonged hypothyroidism may not be detected until growth failure occurs.[44,85]

Because untreated infantile hypothyroidism is associated with mental retardation, the assumption is often

made that juvenile hypothyroidism is associated with learning problems and poor academic performance. This notion is not correct, as children with juvenile hypothyroidism can be successful academically and do not manifest overt learning problems or cognitive impairment related to the hypothyroid state,

Children with severe hypothyroidism may manifest cold intolerance, decreased frequency of bowel movements, and decreased physical activity.[44,85] Bradycardia, facial puffiness, delayed reflexes, and carotenemia may be present. In comparison with Hashimoto thyroiditis, the thyroid gland is either small or only modestly enlarged. [44,85] Antithyroid antibodies usually are present.[44,85] These patients are generally not obese, and body mass index values are similar before and after treatment.[35,86] The development of slipped capital femoral epiphyses may antedate the detection of hypothyroidism.[87]

Some children with juvenile hypothyroidism may present with signs of puberty but without pubic hair.[88-90] Boys may present with testicular enlargement and girls may present with menarche, with or without breast development.[91] With treatment of the hypothyroid state, these characteristics may regress.[88-90] Available evidence suggests that the hypothyroid state leads to increased gonadotropin secretion, which triggers gonadal activity.[88,91] Alternatively, it has been suggested that extreme high levels of TSH cross-react with the follicle-stimulating hormone (FSH) receptor in the gonads. This entity is referred to as the "overlap" or Van Wyk–Grumbach syndrome.[92,93] The very high TSH values reflect pituitary hyperplasia of thyrotrophs and may be associated with an appearance of an enlarged pituitary on imaging studies that might be mistaken for a pituitary TSH secreting adenoma.[94] However, these findings resolve with treatment of the hypothyroid state. In some children, true puberty may develop within a year or two of treatment onset, which may limit catch-up growth due to earlier closure of epiphyses induced by sex steroids.[44]

Juvenile hypothyroidism may not be recognized until a sizable statural deficit is present, and the lost height is usually not recovered.[44] Children with juvenile hypothyroidism who present with growth failure manifest very low T4 values that are often less than 2 μg/dL and profoundly elevated TSH levels that are higher than 250 μU/mL.[44] Hypercholesterolemia and anemia may be present.[44]

The magnitude of the height deficit is proportional to the duration of hypothyroidism, which can be estimated as the difference between the chronologic and bone age.[44] When the individual is treated with conventional doses of levothyroxine, accelerated skeletal maturation is observed, with the skeletal age advancing disproportionately faster than gains in height.[44] Thus, predicted heights fall, and genetic growth potential is not achieved.

Because of the potential poor growth outcomes of patients with hypothyroidism, we have treated these patients with low doses of levothyroxine (0.25 to 0.5 μg/kg/day; e.g., 50 μg for a 10-year-old). With this regimen we find that, with low-dose levothyroxine therapy, T4 values normalize (6 to 7 μg/dL) within 2 months, and TSH levels normalize or remain only modestly elevated. Moreover, serial bone age determinations do not show the disproportionate advancement of skeletal age seen with conventional therapy. However, we do not know if this approach leads to more favorable height outcomes.[95] Some physicians have also suggested that treating these children with gonadotropin-releasing hormone analogs to delay puberty will lead to improved long-term growth.[96-100] However, we have found that catch-up growth slows markedly in some hypothyroid children receiving gonadotropin-releasing hormone analog therapy so that predicted adult heights is less and others have not observed added benefit.[101] Because the loss in adult height is proportional to the duration of hypothyroidism,[44] early detection of this disorder is the best intervention for preventing statural deficits.

Iodine-Induced Hypothyroidism

Sixty percent of the weight of T4 is iodine, and iodine is the rate-limiting substrate for synthesis of thyroid hormones[2] (see Figure 12-1). Iodine is present in small amounts (15 to 20 mg) in humans. The recommended dietary allowance of iodine is 100 μg/day for adolescents and adults, and 150 μg/day for pregnant and lactating women. It is 60 to 100 μg/day for children aged 1 to 10 years, 40 μg/day for infants aged 6 to 12 months, and 30 μg/day for infants 6 months of age or younger. In areas of low iodine intake, the recommended dietary intake should be 90 μg for infants aged less than 1 year.[2]

Although modest iodine intake is essential for thyroid function, high-level iodine exposure results in an acute block in the release of preformed thyroid hormone and impaired thyroid hormone synthesis, a phenomenon referred to as the Wolff-Chaikoff effect.[102] When iodine-induced hypothyroidism is suspected, it can be diagnosed by the detection of high iodine levels in urine samples.[103]

In children, iodine can be absorbed through the skin, and iodine-induced hypothyroidism has been observed after cutaneous iodine or Betadine use.[103-105] Iodine-induced suppression of thyroid hormone production has also been observed in children with central intravenous lines when regular cleansing of the insertion site with iodine was included in central line care. Neonatal hypothyroidism has also been associated with maternal povidone (iodine) exposure at the time of delivery.[104]

In preterm infants, iodine-induced hypothyroidism warrants special attention, because the suggestion has been made that cutaneous iodine exposure is a major cause of hypothyroidism in premature infants.[106] Studies show that iodine-induced hypothyroidism is infrequent in the United States.[107]

Significant iodine exposure also occurs from amiodarone, an antiarrhythmic drug that contains 37% iodine.[108] Hypothyroidism occurs in 10% of individuals treated with this compound.[109] Amiodarone can also reach the fetus by transplacental passage and induce fetal hypothyroidism.[108]

In addition to iodine excess, iodine deficiency also leads to hypothyroidism. Estimates indicate that more than 1 billion people worldwide are at risk for iodine deficiency.[110] Clinically, iodine deficiency is associated with goiter, hypothyroidism,[110] and endemic cretinism.[111] Endemic cretinism is classified into neurologic or

myxedematous type,[112] with severe mental retardation, mutism, and cerebral diplegia found in neurologic type. Children with the myxedematous type, have less severe mental retardation and have severe growth retardation and myxedema.[112]

Geographic areas of iodine deficiency exist, even in the United States.[113,114] With the prevalent use of iodized salt, however, the incidence of iodine deficiency has been markedly reduced, and hypothyroidism and goiter due to iodine deficiency are rare in the developed world.[113,114] Iodine intake in the United States has declined, an issue that may have future clinical implications.[113,114] In Australia, a reduction in iodine intake has been reported, with potential implications for pregnant and lactating women,[115] as reduced iodine intake may predispose to maternal and child hypothyroidism predisposing to hypothyroidism or developmental delay.[112] The exclusive use of deiodized salt, which includes sea salt, is thus not recommended.

Hypothalamic-Pituitary Dysfunction

Central hypothyroidism should be considered in children with a history of head trauma, brain tumors, meningitis, central nervous system irradiation, or congenital nervous system malformations. Central hypothyroidism has also been associated with the use of retinoid X receptor-selective ligands in the treatment of lymphomas.[116]

In contrast to primary hypothyroidism, the diagnosis of hypothyroidism secondary to hypothalamic-pituitary dysfunction may be difficult to establish. Often, circulating concentrations of T4 are in the low-normal range, and TSH may be low, normal, or elevated.[117,118] FT4 values, however, are usually low.

Whereas congenital central hypothyroidism will be diagnosed in states that perform T4 screening of newborns, neonatal screening programs that rely on TSH determinations will not detect this condition. Central hypothyroidism should therefore be suspected in infants with cholestasis, poor growth, hypoglycemia, structural nervous system problems, or pituitary insufficiency.[119] When neonatal T4 values are interpreted, care should also be taken to use infant thyroid hormone values for comparison, because infantile T4 levels are higher than those seen in adults[120] (see Tables 12-1 and 12-2; also see Chapter 7).

Importantly, up to 30% of children who will develop central hypothyroidism may have normal T4 and TSH levels at birth.[121] Thus, all children with evidence of hypopituitarism should be regularly monitored for central hypothyroidism onset.

When central hypothyroidism is suspected, the thyrotropin releasing hormone (TRH) test helps distinguish pituitary (secondary) and hypothalamic (tertiary) hypothyroidism.[118,120,122] Typically, there is a minimal rise in serum TSH levels in response to TRH in patients with pituitary disease, whereas there is a delayed response (> 60 minutes) in patients with hypothalamic disease. However, responses to this test are variable, making it difficult to distinguish between pituitary and hypothalamic hypothyroidism.[118,120,122] Central nervous system imaging should be performed to look for congenital malformations or hypothalamic-pituitary lesions. Care should be taken to search for other pituitary hormone deficiencies, especially abnormalities of the hypothalamic-pituitary adrenal and growth hormone axes, as gene defects that adversely affect hypothalamic pituitary dysfunction, include mutations in the LIM/homeobox protein 3 (LHX3) and 4, prophet of pit-1 (PROP1), and pituitary transcription factor (PIT).[123-125]

Treatment consists of replacement therapy with levothyroxine. Some children with central hypothyroidism require doses lower than those used to treat primary hypothyroidism.[126] Because TSH values are not helpful in guiding treatment, measurement of FT4 levels is recommended.[126] Furthermore, a dose of 1.6 µg/kg of levothyroxine is recommended to maintain the FT4 levels in the upper half of the reference range.[127]

Giant Hemangiomas

Hypothyroidism has been associated with giant hemangiomas.[128] In some infantile hemangiomas, the endothelium of these vascular structures produces type 3 iodothyronine deiodinase, which degrades circulating T4 (see Figure 12-2). Treatment of hypothyroidism in this setting requires high doses of levothyroxine.[128]

Hypothyroidism in Cancer Survivors

It is well recognized that children who are cancer survivors and who have had head and neck irradiation are at increased risk for differentiated thyroid cancer.[129,130] More common, though, is the development of mild hypothyroidism.[131-133] Up to 30% of children who have had head and neck irradiation will develop primary hypothyroidism.[133] Thus, annual TSH screening is suggested. In addition, ultrasound studies are recommended beginning 5 years after radiation exposure.

Practitioners who argue that palpation alone is sufficient for the follow-up of individuals who have had head and neck irradiation need to recognize that ultrasonography will detect thyroid nodules well before palpation.[130,134] It must also be recognized that earlier rather than later recognition of thyroid cancer can lead to less extensive surgery, lower administered activities of ^{131}I, and a better chance of cure.[135]

THYROID HORMONE RESISTANCE

Thyroid hormones exert their effects by binding to a specific nuclear receptors to regulate cellular gene expression.[136] When the thyroid hormone receptor is mutated, impaired tissue responsiveness results, leading to thyroid enlargement, elevated levels of T4 and T3, tachycardia, and behavioral problems.[137-140] Unlike Graves disease, TSH values are normal or slightly elevated.

The most common forms of thyroid hormone resistance are caused by mutations of the thyroid hormone receptor beta gene.[138-140] More than 100 mutations have been identified that result in impaired affinity for T3.[138] Mutant thyroid hormone receptors also block the function of normal thyroid hormone receptors.[138] Thus,

thyroid hormone resistance is a dominant-negative mutation, and inheritance is autosomal dominant.[138] Detection of thyroid hormone resistance in the index case may therefore lead to diagnosis of the condition in other family members. In up to 50% of children with thyroid hormone resistance, the mutations are spontaneous.

Most individuals with resistance to thyroid hormone have generalized thyroid hormone resistance.[138] These individuals are eumetabolic and asymptomatic, with TSH levels in the normal range.

In contrast, some individuals have isolated pituitary thyroid hormone resistance. These individuals have symptoms of hyperthyroidism, because they are sensitive to the effects of increased circulating thyroid hormone in the periphery, where the thyroid receptors are functionally intact.[141] Resistance to thyroid hormone can be associated with central nervous system problems. Approximately 50% of individuals with resistance to thyroid hormone have attention deficit hyperactivity disorder and a minority have mental retardation.[142]

Because individuals compensate for thyroid hormone resistance by secreting more thyroid hormone, treatment is generally not necessary.[138,143] However, some patients with thyroid hormone resistance may be improperly diagnosed as having Graves disease and undergo ablation of the thyroid. In this situation, replacement therapy with appropriate doses of exogenous thyroid hormone is needed. With the earlier recognition of resistance to thyroid hormone due to newborn screening, the issue of whether children with resistance to thyroid hormone should be treated prenatally or during infancy has been raised.[138,143] Treatment is generally reserved for infants who show elevated TSH levels, growth failure, seizures, and developmental delay.[138,143]

In some cases, TSH secretion may be profound, leading to massive thyromegaly, which may adversely impact upper airway function.[144,145] These cases are associated with severe loss of function mutations. Treatment with high doses of triiodiothyroine every other day has been shown to be somewhat effective in this setting.[144,145] In other cases, thyroidectomy is needed to prevent airway compromise.[144,145]

HYPERTHYROIDISM

Hyperthyroidism occurs less commonly in children than hypothyroidism, yet is far more symptomatic.[146-150] Graves disease is the most common cause of childhood thyrotoxicosis and is characterized by diffuse goiter, hyperthyroidism, and occasionally ophthalmopathy. Other causes of hyperthyroidism in children include autonomously functioning thyroid nodules, neonatal thyrotoxicosis, and infections of the thyroid. Hyperthyroidism also results from thyroid hormone ingestion, McCune-Albright syndrome, struma ovarii, and TSH-producing pituitary adenomas. Epidemic hyperthyroidism has also been seen when thyroid tissue has been inadvertently included in meat products.[151] In contrast to these disorders, thyroid hormone resistance may appear similar to hyperthyroidism yet is best left untreated.

Graves Disease

Graves disease is the most common cause of hyperthyroidism in children and adults and occurs when the thyroid gland is stimulated by immunoglobulins.[51,148,152] In children, the incidence of Graves disease is about 1:10,000.[153] Current treatment approaches for Graves disease include the antithyroid drugs (ATDs) propylthiouracil (PTU) or methimazole (MMI), surgery, and radioactive iodine (RAI; ^{131}I), therapies that have been used since the 1960s.[148,154-157]

Medical Therapy

PTU and MMI reduce thyroid hormone synthesis by inhibiting the oxidation and organic binding of thyroid iodide.[158] Importantly, these medications are not curative. Rather, they palliate the hyperthyroid state until it spontaneously resolves or definitive treatment is rendered. In 2008, serious complications related to the use of PTU in children were noted and a review of adverse events related to ATD use in the pediatric population was reported.[153] The risk of PTU-induced liver failure leading to transplantation was estimated to be 1 in 2000 children. The number of children developing PTU-induced liver injury that was reversible was estimated to be at least 10-fold greater than the number of children who develop liver failure requiring transplantation. Because PTU-induced liver injury is of rapid onset and can be rapidly progressive, biochemical monitoring of liver function tests and transaminase levels is not useful in managing the hepatotoxicity risk in a PTU-treated patient.[153] Considering the risk of PTU-related hepatotoxicity in children, it is now recommended that the use of PTU be stopped, and children taking the medication should be considered for alternative treatments.[159]

Although PTU use should be avoided in favor of MMI, there is a role for the limited use of PTU. PTU use should be considered in circumstances when neither prompt surgery nor ^{131}I treatments are readily available options, or when a toxic reaction to MMI has occurred but therapy for Graves disease is necessary. In this situation, PTU use should be used for the short term only. Because of potential teratogenic effects of MMI,[160] PTU also is the drug of choice over the first trimester of pregnancy.[161] In the United States and many other countries, MMI (or carbimazole) is available and is the drug of choice for Graves disease. The typical MMI dose is 0.2 to 0.5 mg/kg/day, with a range from 0.1 to 1 mg/kg/day.[152] Although many practitioners give MMI in divided administered doses, data do not support a need for such titration.[162]

Overall MMI has a better safety profile than PTU, but MMI can be associated with minor adverse events;[163] major adverse events include agranulocytosis and allergic reactions. Agranulocytosis has been reported in about 0.3% of adult patients taking MMI or PTU.[156,164,165] Agranulocytosis is dose dependent with MMI and rarely occurs at low doses.[156,164,165] When it develops, agranulocytosis occurs over the first 100 days of therapy in 95% of individuals.[156,164,165] If patients taking MMI develop fever, pharyngitis, or feel ill, the medication should be

immediately discontinued by the patient, a physician contacted, and a white blood cell count obtained.

The issue of how long anti-thyroid drugs (ATDs) should be used in children before considering radioactive iodine or surgery is a topic of controversy and warrants further study. Prospective studies in adults show that if remission does not occur within 18 months, there is little chance of remission with prolonged therapy.[166] In children, when ATDs are used for 1 to 2 years, remission rates are generally 20% to 30%.[167-169] The chance of remission after 2 years of ATD use will be low if the thyroid gland is large (> 2.5 normal size for age),[170] the child is young (< 12 years)[168,169,171] not Caucasian, initial serum TRAb levels are high, or FT4 levels are high at diagnosis (> 4 ng/dL; 50 pmol/l). [169]

Studies of large cohorts of pediatric patients with Graves disease treated with ATDs for extended periods[170,172] have revealed low remission rates that are comparable to those seen with 2 years of therapy. In view of these data, many practitioners will consider a trial of MMI for 1 or 2 years and proceed to surgery or ^{131}I therapy if remission does not occur. Practitioners may also elect to continue ATDs for many years, as long as toxic reactions and progressive thyromegaly do not occur. Most recently, higher remission rates for children treat with antithyroid medications have been reported.[173] Yet, these studies are at variance with most other studies[148] and warrant additional follow-up.

Radioactive Iodine Therapy

The use of RAI has been reported in more than 1200 children.[157] Patients as young as 1 year of age have been treated with ^{131}I with excellent outcomes.[157] Overall studies of ^{131}I use in children report remission rates that exceed 95%.[157,174,175] The goal of ^{131}I therapy for Graves disease is to induce hypothyroidism. ^{131}I doses are typically calculated to deliver the desired amount of radiation based on gland size and 24-hour ^{123}I uptake. Some centers administer all patients the same fixed dose of ^{131}I with excellent outcome.[176] To achieve thyroid ablation or hypothyroidism, more than 150 uCi of ^{131}I per gram of thyroid tissue should be administered.[177,178] With larger glands (30 to 80 g), higher administered doses of ^{131}I (200 to 300 uCi of ^{131}I per g) may be needed.[177] To assess gland size when the thyroid is large, ultrasonography is recommended with gland size determined by the formula, lobe size = [length × width × depth × 0.6] and the volumes of each lobe summed. Radioactive iodine is often not effective with large glands (> 80 g).[179] Thus, surgery may be preferable to ^{131}I in these patients, although patients can be given a repeat RAI treatment.

Some centers administer a fixed dose of about 15 mCi ^{131}I to all children[176] rather than providing individually calculated doses. One potential advantage of calculated versus fixed dosing, though, is that it may be possible to administer lower doses of ^{131}I when the administered dose is calculated, especially when uptake is high. Less than 10% of children complain of mild tenderness over the thyroid in the first week after therapy, which can be treated effectively with acetaminophen or nonsteroidal anti-inflammatory agents for 24 to 48 hours[175,177] There are rare reports of pediatric patients with severe hyperthyroidism who have developed thyroid storm after receiving ^{131}I.[180] Thyroid storm in this setting is believed to reflect progression of the uncontrolled hyperthyroid state. Thus, if T4 values are > 20 μg/dL or freeT4 values are > 5 ng/dL (60 pmol/l), children should be treated with MMI until T4 or free T4 levels normalize before proceeding with ^{131}I therapy.[177]

Hypothyroidism typically develops by 2 to 3 months posttreatment.[176,177] When administered doses > 150 uCi of ^{131}I per gram of thyroid tissue are administered, hypothyroidism rates are about 95%.[152,157,181] If hyperthyroidism persists 4 to 6 months after therapy, retreatment with ^{131}I is indicated.

The thyroid gland is unique in its developmental sensitivity to malignancy following low-level radiation exposure.[182,183] When individuals are younger than 20 years at the time of exposure to low-level thyroid irradiation, thyroid cancer risks increase the younger the patient is at the time of exposure.[182,183]

Detractors of ^{131}I therapy point to the increased rates of thyroid cancer and thyroid nodules observed in young children exposed to radiation from nuclear fallout at Hiroshima or after the explosion at the Chernobyl nuclear reactor.[183,184] The risk of thyroid neoplasms, however, is greatest with exposure to low level external radiation (0.1 to 25 Gy; ~0.09 to 30 uCi/g),[182-185] not with the higher doses used to treat Graves disease. We are not aware of cases of thyroid cancer developing in patients treated with > 150 uCi of ^{131}I per gram of thyroid tissue for childhood Graves disease attributable to RAI therapy.

Although RAI is being used in progressively younger ages, we do not know if there is an age below which high-dose ^{131}I therapy should be avoided. Risks of thyroid cancer after external irradiation are highest in children less than 5 years of age and progressively decline with advancing age.[174,182,183,186] If there is residual thyroid tissue in young children after RAI treatment, there is a theoretic risk of thyroid cancer.

In addition to thyroid cancer risks, potential influences of ^{131}I therapy on other cancers need to be considered. This issue has been examined in several large cohorts of adults in the United States and other countries. These studies have not revealed increased cancer incidence or mortality in adults treated with ^{131}I for Graves disease.[187-193]

In comparison with the studies in adults, few studies have focused on populations exposed to ^{131}I in childhood for the treatment of Graves disease. The longest follow-up study of pediatric patients involved 36-year outcomes of 116 patients who were less than 20 years of age when treated with ^{131}I between 1953 and 1973.[174] This group did not have an increased cancer rate.

The total-body radiation dose after ^{131}I varies with age, and the same absolute dose of ^{131}I will result in more radiation exposure to a young child than to an adolescent or adult.[194,195] At present, we do not have dosimetry information regarding ^{131}I use in children with Graves disease to assess total body exposure in children. But, based on theoretic calculations, we feel it is prudent to avoid radioactive iodine therapy in very young children (< 5 years) and to avoid > 10 mCi in patients younger

than 10 years of age. It is important to recognize that there may be circumstances in which ^{131}I therapy is required in young children. This situation may occur when a child has developed a reaction to antithyroid medications, proper surgical expertise is not available, or the patient is not a suitable surgical candidate. In this situation, ^{131}I therapy is needed.

Surgery

Surgery is an acceptable form of therapy for Graves disease in children.[196] When performed, near total or total thyroidectomy is recommended, as subtotal thyroidectomy is associated with a higher relapse rate.[197] Surgery is preferred in young children (< 5 years) when definitive therapy is required and can be performed by an experienced thyroid surgeon. In individuals with large thyroid glands (> 80 g), the response to ^{131}I may be poor,[179,198] and surgery is recommended for these patients.

Data in adults show that acute complications following thyroidectomy include hypocalcemia (40%), hematoma (2%), and recurrent laryngeal nerve paresis (2%).[199,200] Children aged 0 to 6 years had complication rates of 22%, whereas those aged 7 to 12 years had complication rates of 11%, and those aged 13 to 17 years had complication rates of 11%. These rates are higher than those seen in adults. Thus, surgery may not be an optimal option for some pediatric patients with Graves disease, especially in young children. In circumstances where local pediatric thyroid surgery expertise is not available, referral of a child with Graves disease to a high-volume thyroid surgery center of excellence that also has pediatric experience should be considered.

Stratification of Treatment

Based on what is known about the risks of different treatments and the pathogenesis of Graves disease, we can be more selective in our approach to therapy.[148] To reduce treatment risks and expedite cure, the treatment options can be guided by the patient's age and the nature of the intrinsic autoimmune disease (Figure 12-4).

In determining if drug therapy is likely to be successful, TRAb levels and thyroid size may be predictive of remission rates. The presence of low TRAb levels and a small thyroid suggests the possibility of remission on medical therapy. Yet if TRAb levels are high and the thyroid is large, the odds of spontaneous remission are low.[201,202] However, TRAb levels and thyroid size may not always be indicative of the likelihood for remission.

For children younger than 5 years of age, we consider MMI as a first-line therapy. Because young children are less likely to have remission than older children on drug treatment,[168,171] prolonged drug therapy may be needed. If there are no toxic effects, continuing MMI is reasonable until the child is considered old enough for radioactive iodine therapy. Alternatively, thyroidectomy or ablative radioactive iodine therapy can be considered if reactions to medications develop or there is the desire to avoid prolonged drug use.

Fifteen percent of children with Graves disease will present between 6 and 10 years of age.[147] MMI therapy as a first-line measure for this age group is reasonable. Yet as the child approaches 10 years of age, either radioactive iodine or drug therapy can be considered as initial therapy.

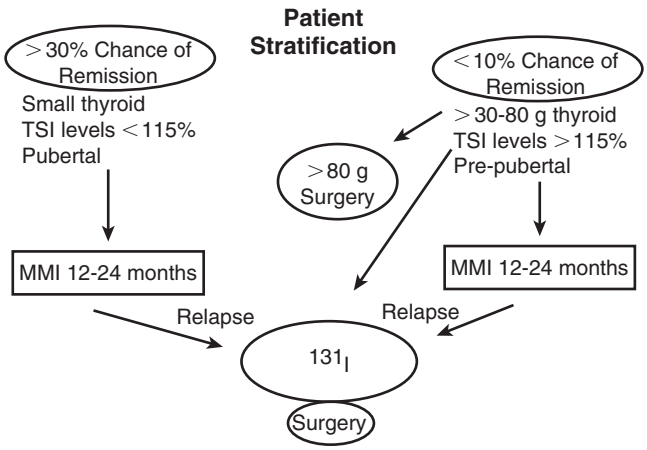


FIGURE 12-4 ■ Graves disease therapy in children: Stratification by clinical characteristics. Patients can be grouped into those with a better or worse chance of remission based on age, TSI levels, and thyroid size. For very large glands (> 80 g), surgery is the treatment of choice. Patients can be treated with antithyroid drugs for 12 to 24 months and the drug withdrawn to see if remission is achieved. If there is no remission, either surgery of radioactive iodine can be administered. Alternatively, the patient can be restarted on antithyroid medication. Only methimazole or carbimazole should be used.

Children 10 years of age and older account for 80% of the pediatric cases of Graves disease. For this age group, radioactive iodine or MMI can be considered as first-line treatment options.

Finally, irrespective of the treatment option selected, careful follow-up is needed for all patients treated for Graves disease. Long-term follow-up should include regular examination of the thyroid gland and measurement of circulating levels of thyroid hormones once or twice a year. All newly appearing thyroid nodules should be biopsied or excised.

In addition to assessing thyroid function in children with Graves disease, attention also needs to focus on body composition and weight change in children after treatment onset. Data now show that Graves disease therapy can be associated with a significant increase in body weight.[86,203]

OTHER CAUSES OF HYPERTHYROIDISM

Neonatal Thyrotoxicosis

Thyrotoxicosis in the neonate is a severe and life-threatening condition that call be associated with lasting neurologic problems.[204,205] If a mother has Graves disease, the chance is 1 in 80 that TSI (or TRAbs) will be transferred to the fetus, which will result in intrauterine or neonatal hyperthyroidism.[206] Rarely, neonatal thyrotoxicosis will persist, like the Graves disease seen in older children.[205] In other rare cases, persistent neonatal thyrotoxicosis is caused by the activation of the TSH receptor.[207,208]

The fetal thyroid gland is responsive to maternal TSI, which, if present at elevated levels, may result in

hyperthyroidism.[209-212] Fetal hyperthyroidism manifests during the second half of gestation, as transfer of TSI from the mother to the fetus increases with the progression of pregnancy.[209-211]

The risk of fetal hyperthyroidism and neonatal Graves disease is proportional to the magnitude of elevation of TSI levels.[209-211] Fetal hyperthyroidism is generally associated with levels of TSI more than two- to fourfold greater than the upper limit of normal for assay.[209-211] Because the fetus is at risk for hyperthyroidism when there is active or past maternal Graves disease, fetal growth and heart rate should be regularly assessed from midpregnancy onward.[209-211] Excessive fetal heart rate (> 160 beats per minute after 20 weeks) and the presence of a fetal goiter suggest hyperthyroidism in the fetus. In addition, accelerated maturation of the femoral ossification center is seen with fetal hyperthyroidism.[210]

If a mother with Graves disease is taking antithyroid medications during pregnancy, fetal thyroid hormone synthesis will be inhibited, which will prevent the development of intrauterine hyperthyroidism.[213] However, the infant may be born with a goiter and hypothyroidism.[165] At birth, circulating levels of T4 may be low and TSH levels elevated. In most cases, the effects of antithyroid drugs wane, and thyroid function normalizes within a week.[206] If significant transplacental passage of TSI has occurred, however, thyrotoxicosis will develop.[206,214]

If a mother with a history of Graves disease is not taking antithyroid drugs during pregnancy, the fetus may develop intrauterine hyperthyroidism.[205] If the condition is not recognized, it may result in profound intrauterine thyrotoxicosis and growth retardation.[205] Such infants have prematurely fused cranial sutures, advanced skeletal age, long-term learning problems, and mental retardation.[205,215] If hyperthyroidism is recognized prenatally because of fetal tachycardia (heart rate higher than 160 beats per minute after 22 weeks), treatment of the mother with antithyroid drugs will reduce the severity of intrauterine thyrotoxicosis.[213,216,217]

Treatment of thyrotoxic infants consists of administration of antithyroid medications (PTU 5 to 10 mg/kg/day or MMI 0.5 to 1 mg/kg/day) and beta-blockers (propranolol 1 mg/kg/day). Lugol solution or saturated potassium iodide may be given (one to two drops every 8 hours) for 7 to 10 days to more rapidly control biochemical hyperthyroidism. After approximately 2 weeks of antithyroid drug therapy, thyroid hormone levels will decline. When thyroid hormone levels fall below normal (8 μg/dL), supplementary levothyroxine (37.5 μg/day for full-term infants) is added to prevent hypothyroidism. As TRAbs are cleared from the infant's circulation, the half-life of IgG being approximately 3 weeks, spontaneous recovery usually begins within 3 months and is usually complete by 6 months.[205,214] Thus, the infant can be weaned from treatment after 3 months. Monitoring of the infant's TSI levels is also a useful predictor of when antithyroid medication can be tapered.[218,219]

Infectious Thyroiditis

Occasionally a child presents with hyperthyroidism, tenderness over the thyroid gland, and fever due to bacterial infection of the thyroid, a condition called acute thyroiditis.[220] Acute thyroiditis can be associated with the presence of a fistula connecting the piriform sinus on the left side of the pharynx to the thyroid.[220] Fevers can be high and erythrocyte sedimentation rates and white counts elevated. Ultrasonography may reveal a local abscess. In contrast to Graves disease, uptake of technetium 99-pertechnetate or radioiodine is reduced when thyroid scanning is performed.

The offending bacteria include *Haemophilus influenza* and *group A streptococci.*[220] Thus, treatment with an antibiotic resistant to disruption by beta-lactamase is recommended. In severe cases, hospitalization and intravenous antibiotic administration is indicated, because lymphatic drainage into the mediastinal region may occur. Surgical drainage is needed if a localized abscess develops and the response to antibiotics is poor.[220]

Because the infectious process results in destruction of thyroid tissue, release of preformed thyroid hormone and clinical hyperthyroidism may occur during infection. The hyperthyroid state is usually transient, and treatment with antithyroid drugs is not indicated.[220] If the patient becomes symptomatic, beta-blockers may be used.

After the child has recovered, pharyngography is indicated to test for a patent piriform sinus tract. Occasionally, the tract may close as the result of the infection. If the tract persists, however, and acute thyroiditis recurs, resection is needed.

Subacute Thyroiditis

Viral infections of the thyroid may occur and result in subacute thyroiditis.[221] In comparison with acute thyroiditis, subacute thyroiditis may be less severe.[221,222] Fever, thyroid tenderness, and hyperthyroidism may be present and may last for several weeks.[223] Because clinically distinguishing between bacterial and viral thyroid infections is difficult, antibiotic treatment is indicated when infectious thyroiditis is suspected.

Hyperfunctioning Nodules

Warm or hot nodules lead to excessive production of thyroid hormone and can be associated with clinical and biochemical hyperthyroidism.[224] Activating mutations of the TSH receptor and Gs have been discovered in hyperfunctioning nodules.[225,226] Although hyperfunctioning nodules may be ablated with radioiodine, surgical excision of hyperfunctioning nodules is recommended in children and adolescents, because radiation-exposed normal thyroid tissue will remain after the hyperfunctioning nodule is ablated. Although the risk of malignancy in hyperfunctioning nodules is low, thyroid cancers have been described in warm nodules.[227,228]

Toxic Multinodular Goiters

Multinodular goiters are uncommon in children, but patients with this condition can develop thyrotoxicosis, which is usually related to the time the goiter has been present and goiter size. In this setting, hyperthyroidism

develops as a single nodule in the thyroid, becomes overly active, and functions autonomously.[229,230] Forty-six percent of patients may have T3 thyrotoxicosis, and nodules are 3 cm or more in diameter.[229,230]

In adults, ^{131}I is routinely used in the treatment of isolated toxic adenomas and toxic multinodular goiters.[229,230] The use of radioiodine to treat these conditions in children is uncommon, however, and few follow-up data are available. Although strong justification exists for the use of radioiodine in the treatment of childhood Graves disease, especially when appropriate doses are used, we recommend that radioiodine be avoided in children with toxic adenomas or multinodular goiters.

When a toxic nodule is present, either as an isolated nodule or in the setting of a multinodular goiter, thyroid function is suppressed in the nontoxic regions. When radioiodine is given, uptake will be limited to the autonomously functioning tissue, and if large doses are administered, the remaining thyroid tissue will receive external irradiation. Because the risk of thyroid cancer following external radiation is very low after 20 years of age,[231,232] the use of radioiodine for toxic nodule ablation in adults is not associated with increased thyroid cancer risks. In the child or adolescent treated with ^{131}I for toxic nodules, however, low-level irradiation to the remaining thyroid tissue may be associated with an increased thyroid cancer risk.

THYROID NODULES AND THYROID CANCER

Nodule Evaluation

Thyroid nodules in children are uncommon and encompass nonfunctional and functional lesions and benign and malignant tumors.[135,233,234] Thyroid cancer needs to be suspected when thyroid nodules are detected in children and adolescents. In a compilation of 16 studies that examined the malignancy rate of thyroid nodules in children, 299 of 1134 nodules were malignant for an overall rate of 26%.[235]

When thyroid nodules are detected, serum TSH, estimated FT4 or T4, and a neck ultrasound should be obtained. Calcitonin levels should be assessed to screen for medullary thyroid cancer, which accounts for 3% to 5% of pediatric thyroid cancers.[236,237] If the TSH is suppressed, a radionuclide scan may identify a hyperfunctioning nodule.

Ultrasound characteristics suggestive of malignancy include microcalcifications, indistinct margins, and a variable echotexture.[130,234,238] Ultrasound can determine the intrathyroidal location of nodules, identify additional nodules, and assess if there is lymph node involvement.[130,234,238] After the Chernobyl disaster, the most reliable ultrasound diagnostic criteria for malignancy in children were an irregular, subcapsular location and an increased intranodular vascularization by Doppler technique.[239] Ultrasonographic appearance alone, though, cannot reliably distinguish between benign and malignant lesions. Thus, fine-needle aspiration (FNA) is indicated for children with thyroid nodules.[234]

FNA is the most accurate means to evaluate if a thyroid nodule is malignant.[234] Reports of FNAs performed in children describe similar specificity and sensitivity as adults.[240-242] Difficulty arises when the FNA is nondiagnostic or the cytology is "indeterminate," as malignancy can be present up to 50% of the time with such cytologic features.[243] If this occurs, the clinician may repeat the ultrasound study and FNA within 3 to 6 months. The nodule size at which point FNA should be performed in children is a matter for discussion.[244] In adults, recommendations suggest that FNA be performed when nodule diameter is or exceeds 1 cm.[234] However, as about 30% of pediatric thyroid nodules are malignant, and FNA can be performed in nodules smaller than 1 cm, it is reasonable to biopsy smaller lesions in children if such capabilities are available, especially for nodules 0.5 to 1 cm. Ultrasound-guided FNA is recommended especially in children because of the difficulty to biopsy small nodules, which rarely can be palpated.[245] When FNA is performed in children, because this is an uncommon procedure, special expertise outside of pediatric departments may be needed.

Thyroid Cancer

Pediatric thyroid cancer is a rare and treatable disease with an excellent prognosis.[135,236] Compared with adults, epithelial-derived differentiated thyroid cancer (DTC), which includes papillary thyroid cancer (PTC) and follicular thyroid cancer (FTC), presents at more advanced stages of disease in children and is associated with higher rates of recurrence. Thyroid cancer in the pediatric population is rare, as a total of 1753 patients with malignant thyroid neoplasms were identified with an age-adjusted annual incidence of 0.54 cases per 100,000 persons. The thyroid cancer types in children in the United States are PTC in 60%, follicular variant of papillary in 23%, FTC in 10% (FTC), and medullary in 5% (MTC)[236] (Box 12-2). Algorithm for the evaluation and follow-up of DTC are presented in Figures 12-5 and 12-6.

Compared with adults, children with DTC present with more extensive disease.[246-255] Lymph node involvement at diagnosis is seen in 40% to 90% of children[246-256] compared with 20% to 50% of adults.[257] The prevalence

BOX 12-2 Thyroid Neoplasia in Childhood

Tumors of the Follicular Epithelium

- Follicular adenoma
- Papillary carcinoma
- Follicular carcinoma
- Anaplastic carcinoma

Tumors of Nonfollicular Origin

- Medullary carcinoma
- Metastatic tumors
- Teratoma
- Lymphoma
- Other

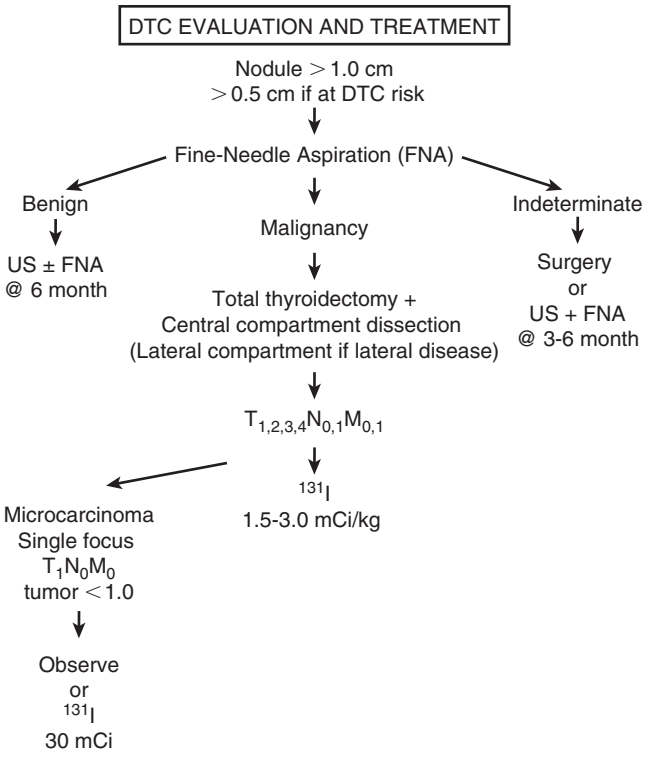


FIGURE 12-5 ■ Algorithm for the evaluation and treatment of differentiated thyroid cancer (DTC) in children.

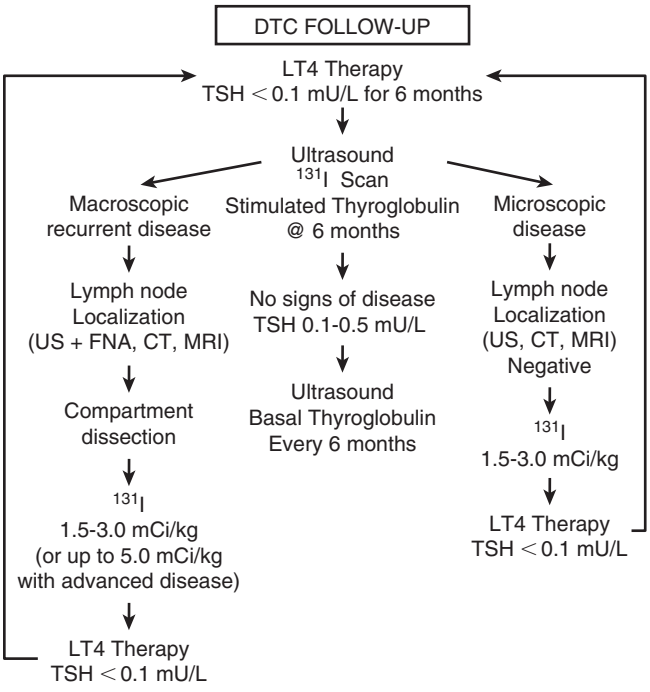


FIGURE 12-6 ■ Algorithm for the follow-up of differentiated thyroid cancer (DTC) in children.

of distant metastases, most commonly lung, is 20% to 30% in children versus 2% in adults.[246-255,258] Multifocal disease is more common in children than adults and is seen in about 40% of childhood PTC cases.

In general, DTC that first presents when a person is younger than 10 years of age appears to have higher recurrence and mortality rates than when presentation occurs at older ages.[258,259] DTC that first presents when a person is older than 10 years of age behaves similar to that in young adults.[258] DTC is generally more widespread at presentation and more likely to recur in younger than older children.[260] Other investigators, though, have found that DTC has similar biologic properties in younger children and adolescents.[261]

Even in the presence of metastatic disease, long-term follow-up data show 30-year survival rates of 90% to 99% for children with DTC (see Figure 12-2).[251,252,262] Even with distant metastases, mortality rates are more favorable in children than adults,[263] and pulmonary metastases can remain stable for extended periods.[264] The favorable prognosis reflects the fact that most young patients have well-differentiated tumor types, few have bone metastasis, and most tumors respond well to RAI therapy.

In most cases, specific risk factors for DTC cannot be identified in children; however, risk factors are found in a subset of patients. Exposure to low-level head and neck irradiation has been recognized for more than six decades as predisposing to DTC.[265,266] Low-level radiation doses to the thyroid of less than 30 Gy (3000 cGy or Rad) increase the risk for cancer, with the risk being higher at progressively younger ages.[183-185] Above 20 years of age, the risk of thyroid cancer following low-level irradiation is either very low or undetectable.[183,185] The latency period between the time of radiation exposure and cancer onset in children is typically 10 to 20 years.[183,185,266]

Thyroid cancer in children can also be observed in families. Familial nonmedullary differentiated thyroid cancer (FNMTC) is diagnosed when three or more individuals in the family have DTC.[267-270] Although studies are under way to identify specific genes leading to cancer risk in FNMTC, specific common molecular signatures have yet to be identified.[271-273] When FNMTC is present, it is recommended that thyroid ultrasonography be performed every year or 2 beginning at 8 years as part of tumor surveillance. DTC in children with FNMTC has been identified in children as young as 8 years of age, and the age of cancer occurrence is generally younger in the second than in the first generation.[274]

Other rare genetic syndromes are associated with thyroid cancer. Cowden syndrome is caused by mutation in the PTEN gene and is a rare autosomal dominant disorder associated with hamartomas of mucosal surfaces and DTC.[275-277] Cancer in Cowden syndrome has been identified in children as young as 8 years.[278] Gardner syndrome (familial colorectal polyposis) is a genetically transmitted, autosomal dominant condition associated with multiple polyps in the colon and other tumors including DTC.[279-281] Gardner syndrome is caused by mutation in the APC gene located on chromosome 5q21.[279,280] Werner syndrome, caused by a mutation in the WRN gene, a DNA helicase, is a very rare autosomal recessive disorder characterized by premature aging.[277] The syndrome is associated with DTC, melanomas, and sarcomas.[277] Certain forms of congenital hypothyroidism, caused by mutations in the TPO gene, can lead to thyroid nodules goiter[282] and rarely DTC.[283] Mutations in the RET protooncogene typically lead to medullary

carcinoma of the thyroid in MEN2a, MEN2b, and isolated familial medullary carcinoma of the thyroid, which are not forms of DTC.[284] (See Chapter 14 for a discussion of MEN syndromes.)

Recognizing that thyroid cancer is rare in children, studies reporting outcomes of more than 100 children are few,[246,285-287] and there are a small number of reports with sample sizes between 25 and 100 patients.[251,253,260,288,289] Outcomes of some of these studies* were recently independently tabulated by Thompson and Luster.[250,253] In general, the following observations about pediatric DTC can be made: (1) about 70% of children present with disease that is metastatic to lymph nodes. (2) About 15% of children present with distant metastatic disease, most commonly to the lungs. In more than 50% of these cases, lung tumors are micrometastases that are not apparent with chest radiographs or computerized tomography (CT) scanning but are apparent with RAI scans.[258] (3) Recurrence rates over 5 to 20 years are as high as 30%. (4) About 10% to 20% of children will have complications related to surgery. (5) A standardized approach to the care of children with DTC is lacking. Some children are routinely treated with RAI, whereas others are not. Some children are treated by lobectomy, and others are treated by total thyroidectomy and central compartment lymph node dissection. Collectively, these studies show that DTC recurrence risk can be reduced by performing total thyroidectomy versus lobectomy, performing compartment lymph node dissection versus selective or no lymph node dissection, and administering RAI.

Surgical Options

The preoperative evaluation of pediatric patients with thyroid disease involves both a general examination to rule out comorbid conditions and a thyroid focused evaluation.[233,234,304] A neck ultrasound using a high-resolution probe (7.5 MHz or higher) should be performed to examine the contralateral thyroid lobe and the central and lateral neck compartments.[305-308] Most children with PTC will have metastatic cervical lymph node disease.[246,256,285,301,309-313] FNA prior to surgery of suspicious lymph nodes should be performed prior to surgery. Ultrasonography may not detect the full of lymph node involvement.[314,315] When further delineation of potential neck disease is needed, imaging using contrast-enhanced CT or magnetic resonance imaging (MRI) may be considered.

The extent of lymph node surgery has been the subject of attention.[316,317] Cancer recurrence most commonly takes place in lymph nodes in the laryngotracheal region.[318] In children and adults, the greater the lymph node involvement in recurrent disease, the greater is the risk of distant metastasis and mortality.[236,250,253-255] Lymph node metastasis is a pervasive component of DTC in children, as up to 90% of children with DTC will have nodal disease. Importantly, in up to 50% of cases, DTC involvement of lymph nodes is not detectable by preoperative ultrasonography.[319,320]

Pediatric data summarized by Thompson and Luster revealed complication rates ranging from 0 to 40% for recurrent laryngeal nerve injury and 0 to 32% for permanent hypoparathyroidism.[250,253] A database analysis of thyroidectomy complications in the United States showed that children aged 0 to 6 years have higher complication rates (22%) than older children (15% for those aged 7 to 12 years and 11% for those aged 13 to17 years).[199] Children had higher endocrine-specific complication rates than adults after thyroidectomy (9.1% versus 6.3%).[199] Importantly, surgical outcome was significantly optimized when surgeries were performed by high-volume surgeons, defined as those performing 30 or more thyroid operations per year.[199] Yet, even when surgery is performed by high-volume thyroid surgeons, complication rates reach 6%.[199]

Recognizing data from children and adults, as such, for children with DTC, we recommend total or near-total thyroidectomy along with central compartment lymph node dissection as part of the initial operation. In addition, lateral compartment dissection with lymph node removal is indicated when lymph node involvement is localized preoperatively by imaging studies or FNA. To minimize the risk of complications, high-volume thyroid surgeons should perform the surgery.

Radioactive Iodine Therapy

Radioactive iodine (RAI, ^{131}I, also referred to as radioiodine) was observed to kill thyroid tumor cells more than 60 years ago.[321,322] Based on available evidence from studies of adults,[233,234,323-335] the following conclusions can be tendered: (1) RAI treatment leads to a reduced risk of recurrence and mortality in patients with DTC with postsurgical residual disease. (2) RAI benefit is clearly demonstrated in adult patients with stage 2 and 3 disease, but not in stage 1. (3) RAI treatment of remnant tissue results in lower rates of DTC recurrence and metastasis. (4) Relatively high cumulative activities of RAI (> 300 mCi; 11 GBq) may be associated with an increased risk of second primary malignancies (SPM). (5) Different approaches have been used to determine activities including empiric dosaging, blood-based upper-limit dosimetry, and tumor dosimetry. (6) RAI efficacy is dependent on factors that include tumor biology and the radiation dose to the tumor. (7) Six decades after the introduction of DTC therapy, RAI use is still under refinement and viewed with controversy.

The overwhelming majority of pediatric patients will have nodal involvement.[246-250,336] In this setting, based on studies showing the potential extent of lymph node spread,[337-339] it must be assumed that there will be residual lymph tissue containing micrometastases following compartment dissection. Lymph node spread is associated with increased mortality and distant metastatic spread,[334,336,340,341] and adjunctive RAI will reduce recurrence risk.[285,286] Thus, RAI is favored in children with DTC.

Administered ^{131}I activities to be applied should range from 100 to 200 mCi (3.7 to 7.4 GBq) in physically mature

*See references 246, 256, 259, 263, 264, 285, and 290-303.

children and may be corrected for body weight 1.35 to 2.7 mCi/kg (50 to 100 MBq/kg) in younger children. New analyses show that treatment with at least 200 MBq/kg (5.4 mCi/kg), and in most patients even much higher activities, is possible without a risk of exceeding bone marrow tolerance limits.[342]

For the uncommon low-risk pediatric patient with microcarcinoma (tumor < 1 cm) and no lymph node involvement, treatment with 30 mCi (1.2 GBq) for the purposes of remnant ablation may be administered and additional courses given if there is Tg persistence. Based on studies in adults,[343-345] about 10% of patients administered ^{131}I for remnant ablation will have biochemical evidence of remaining thyroid tissue and will require retreatment. Alternatively, as suggested,[233] RAI may be withheld for the child with microcarcinoma, and the patient monitored for disease persistence and recurrence via concentrations of Tg and ultrasonography. If Tg levels rise in the absence of gross disease, RAI can be later administered.

Levothyroxine Therapy

It is standard practice to treat thyroid cancer patients with levothyroxine postoperatively, as it is well recognized that TSH suppression can reduce rates of recurrence.[346,347] The optimal degree of TSH suppression is debated in low-risk patients, as it is not clear if complete suppression of TSH secretion confers benefit.

In adults, the long-term impact of supraphysiologic doses of thyroid hormone on bone mineral density and cardiovascular risks is well recognized.[348,349] In children, high levels of thyroid hormones can have effects on growth and profoundly impact on behavior and learning ability.[350] On the other hand, children generally need considerably higher doses of levothyroxine to completely suppress TSH as compared to adults. To date, studies of the effects of treatment resulting in subclinical hyperthyroidism in children treated for DTC have yet to be performed to assess impact.

In adults with low-risk disease, it is recommended to maintain TSH levels in the low normal range (0.5 to 2.5 μU/mL).[351,352] The American Thyroid Association Taskforce recommends more aggressive suppression (TSH 0.1 to 0.5 μU/mL).[234] For high-risk adult patients, TSH values should be suppressed to < 0.1 μU/mL.[234]

One scheme proposed for children is to initially suppress TSH levels to < 0.1 μU/mL and then allow the TSH to rise to 0.5 μU/mL once the patient enters remission.[353] These recommendations seem appropriate for children when one considers that most recurrent DTC develops 5 years after initial treatment.[354]

In pediatrics it is well recognized that medical compliance can be a major problem, especially for teens and young adults, including those with serious medical conditions.[355-358] Although TSH suppression is desirable, clinicians must recognize that TSH-suppression may be difficult to enforce in the pediatric population. As such, TSH-suppressive therapy cannot be practically considered to be a mainstay of therapy, supporting surgical and RAI approaches that minimize recurrence risk in children.

Follow-up

Follow-up care of the child with DTC involves the regular assessment of circulating thyroid hormone levels, ultrasonography of the neck, measurement of Tg, and whole-body radioiodine scans[135,258] (see Figure 12-6).

A very pertinent issue is the criteria used to assess if a patient is disease free. With more sensitive Tg assays, one can aim for an undetectable Tg level as indicative of a disease-free state, rather than a Tg of < 2 μg/L, which had been standard practice. Although an ^{131}I uptake of < 0.1% is considered an indication of being disease free, small metastases or thyroid remnants may be present even with such low uptake values; modern gamma cameras can reliably detect thyroid remnants with an uptake as low as 0.01%.

In general, a follow-up ultrasound and a TSH-suppressed Tg level assessment are performed 6 months after initial therapy, and at least annually thereafter, although following patients every 6 months for at least 5 years after diagnosis may be preferred for patients with more advanced initial or metastatic DTC. TSH-stimulated Tg levels are assessed 6 months after initial therapy, and 6 to 12 months thereafter, based on the suspicion of residual disease. Assessment of T4, T3, and TSH levels is indicated every 6 months and 1 to 2 months after dose changes.[234,359]

Overall, ample evidence suggests that more extensive surgery is associated with lower rates of recurrence.[135] Surgery is associated with clear and definable rates of complications that can be minimized when surgery is performed by high-volume thyroid surgeons. Evidence shows that, properly administered, RAI is associated with lower recurrence rates. Evidence also shows that DTC is associated with an increase secondary primary malignancy (SPM) risk, which reflects intrinsic factors related to having DTC itself. Evidence also suggests that relatively high doses of ^{131}I may contribute to an increased risk of SPM. Thus, the proven benefit of ^{131}I in preventing cancer recurrence and cancer-related deaths needs to be weighed against potential long-term risks. Long-term follow-up for the child with DTC is essential, as disease can recur decades after initial diagnosis and therapy.

MEDULLARY THYROID CARCINOMA

Medullary thyroid carcinoma (MTC) accounts for about 5% of thyroid cancer in children.[360] In comparison with differentiated thyroid cancer, MTC arises from the parafollicular or C cells of the thyroid gland that produce calcitonin.[360] Hereditary forms of MTC account for about 30% of cases and include multiple endocrine neoplasia type 2A (MEN2A), MEN2B, and familial MTC (FMTC).[361] However, most patients with MTC have sporadic disease. MEN 2A includes MTC, pheochromocytomas, and hyperparathyroidism.[362] MEN 2B includes MTC, pheochromocytomas, and multiple mucosal neuromas[362] (Table 12-4). Mucosal neuromas usually have a distinctive appearance, and children with MEN2B may have thick and bumpy lips, a prominent jaw and a marfanoid habitus.[362] FMTC is said to be present when four

TABLE 12-4 **Classification of Hereditary Medullary Thyroid Cancer**

Phenotype	MTC	MN	PCT	HPT
MEN2A	100%	100%	50%	20%
MEN2B	100%	N	50%	N
FMTC	100%	N	N	N

MTC, medullary thyroid carcinoma; MN, mucosal neuromata; PCT, pheochromocytoma; HPT, hyperparathyroidism; N = not present.

or more family members have MTC. In comparison with MEN 2A and 2B, FMTC occurs at later ages, between 20 to 40 years of age.[360]

MTC is caused by autosomal-dominant gain-of-function mutations in the RET protooncogene on chromosome 10.[362] The RET gene includes 21 exons and encodes a plasma membrane–bound tyrosine kinase enzyme.[362] RET is expressed in neuroendocrine and neural cells, including thyroid C cells, adrenal medullary cells, parathyroid cells, and colonic ganglion cells.[362] There are three RET isoforms with 9,43 or 51 distinct amino acids in the intracellular C-terminal tail. These isoforms have different roles in kidney differentiation, in sympathetic neuronal growth and function, and in neuronal signaling.[362] Activating RET mutations may also be associated with short segment Hirschsprung disease, and inactivating RET mutations are associated with congenital megacolon. Activating gain-of-function germline mutations are responsible for FMTC and its variants (see Chapter 14 for a discussion of pheochromocytoma and multiple endocrine neoplasia syndromes).

Numerous mutations of the RET protooncogene have been identified and found to correlate with disease type and tumor aggressiveness.[363,364] Mutation at codon 634 is present in 85% of MEN2A patients, and mutations in codons 609, 611, 618, and 620 in 10% to 15%. A single point mutation at codon 918 of the intracellular tyrosine kinase domain of the RET oncogene is present in 95% of patients with MEN2B. In cases of sporadic MEN2A, MEN2B, and FMTC, new mutations in the RET gene are found in most cases in the paternal allele, and somatic RET mutations are also found in sporadic MTC. These most frequently occur in codon 918, but a variety of mutations, including multiple mutations, have been characterized.[363,364]

Serum calcitonin is the most reliable marker of MTC. In children, calcitonin levels usually do not exceed 20 pg/mL.[365,366] When the compound pentagastrin was available, the pentagastrin test was used to distinguish C-cell hyperplasia from MTC, with stimulated calcitonin concentrations between 30 and 100 pg/mL associated with C-cell hyperplasia and levels in the 100- to 1000-pg/mL associated with microscopic MTC localized to the thyroid gland.[367-369] In contrast, stimulated calcitonin levels greater than 10,000 pg/mL are associated with macroscopic MTC, and 20% of these patients have distant metastases.[367-369] Because pentagastrin is not available, basal levels of calcitonin are used to assess disease.[370,371] The doubling rate of circulating calcitonin levels is also of prognostic significance.[372,373]

Genetic testing of the RET protooncogene is commercially available, and the treatment is based on the mutation present. The goal of mutation analysis is to optimize the timing of surgery so that surgery can be performed before the onset of malignancy. MTC risk based on mutation analysis is divided into three categories called levels 1 through 3, with 3 being highest risk.[362,374-376] For individuals in the highest-risk group (mutated exons 883 or 918 in MEN2B patients), thyroidectomy is recommended before age 6 months (or preferably within the first month of life).[362,374-376] In the case of risk level 2, thyroidectomy before age 5 is indicated. With level 1 mutations, it is recommended that thyroidectomy be performed between 5 and 10 years of age[362,374-376] (Table 12-5). When surgery is performed, it is important that it be performed by a "high-volume" thyroid surgeon.[200,377]

TABLE 12-5 **Laboratory Tests for the Management of MEN2 and FMTC**

Test Name	Patient Preparation	Specimen Requirements
Calcitonin (ICMA), post calcium-pentagastrin stimulation	NPO after midnight; with the patient supine, give 20 mg/kg calcium gluconate (2 mg elemental calcium) intravenously over 1 minute, followed by pentagastrin 0.5 μg/kg as a bolus over 5 seconds; draw blood at 0, 1, 2, 5, and 10 minutes	3 mL frozen serum (red top tube)
Catecholamines	Avoid alcohol, coffee, tea, tobacco, and strenuous exercise prior to specimen collection	—
Plasma		4 mL frozen plasma (green top tube)
Random urine		10 mL refrigerated aliquot of 24-hour urine preserved in 25 mL of 6N HCl; record 24-hour urine volume
Random urine		10 mL refrigerated aliquot of random urine specimen
Calcium, total, serum	Overnight fasting preferred	1 mL refrigerated serum (red top tube)
PTH, intact (ICMA)	None	2 mL refrigerated serum (red top tube) Centrifuge and separate immediately

Before surgery, patients should be screened for pheochromocytomas. Pheochromocytomas in MEN patients are diagnosed after MTC in 90% of patients. Pheochromocytomas in MEN2 patients are typically intra-adrenal and are rare in the first decade of life.[378,379] Screening can be performed by assessment of circulating catecholamine levels or via timed urinary collections.[378,379] Direct visualization of the adrenal gland is also indicated when a tumor is suspected by either CT or MRI methods.[378,379]

Patients should also be screened for hypercalcemia. Parathyroid hyperplasia is associated most commonly with codon 634 mutations and less frequently with codon 609, 611, 618, 620, 790, and 791 mutations. Assessment of circulating PTH concentrations relative to calcium concentration is needed when the diagnosis is considered.

SYNOPSIS

Whereas many thyroid disorders are common in children and adults, the diagnosis and treatment of pediatric thyroid disease require a specialized approach. Clinicians need to recognize that normative thyroid hormone values for adults may not apply to children, especially for TSH values that trend higher for children than adults. Children with autoimmune thyroid disease need individualized treatment to help maximize their growth when they present with growth delay due to hypothyroidism, and they need individually tailored regimens to minimize the risks of therapy when they present with Graves disease. Because of the rarity of thyroid cancer, children need to be cared for in centers with proven expertise in the care of patients with thyroid cancer. We are now in an era where the benefits of personalized medicine are recognized. The opportunity before us is to recognize the nature of the thyroid disorder affecting the child and to best optimize that child's treatment.

REFERENCES

1. Stathatos, N. (2012). Thyroid physiology. *Med Clin North Am*, *96*, 165–173.
2. Fisher, D. A., & Grueters, A. (2008). Thyroid disorders in childhood and adolescence. In M. A. Sperling (Ed.), *Pediatric endocrinology.* Philadelphia: Saunders, 227–253.
3. Flamant, F., Baxter, J. D., Forrest, D., et al. (2006). International Union of Pharmacology. LIX. The pharmacology and classification of the nuclear receptor superfamily: thyroid hormone receptors. *Pharmacol Rev*, *58*, 705–711.
4. Cheng, S. Y., Leonard, J. L., & Davis, P. J. (2010). Molecular aspects of thyroid hormone actions. *Endocr Rev*, *31*, 139–170.
5. Schussler, G. C. (2000). The thyroxine-binding proteins. *Thyroid*, *10*, 141–149.
6. Bartalena, L., & Robbins, J. (1993). Thyroid hormone transport proteins. *Clini Lab Med*, *13*, 583–598.
7. Robbins, J. (1976). Thyroxine-binding proteins. *Prog Clin Biol Res*, *5*, 331–355.
8. Dentice, M., & Salvatore, D. (2011). Deiodinases: the balance of thyroid hormone: local impact of thyroid hormone inactivation. *J Endocrinol*, *209*, 273–282.
9. Courtin, F., Zrouri, H., Lamirand, A., et al. (2005). Thyroid hormone deiodinases in the central and peripheral nervous system. *Thyroid*, *15*, 931–942.
10. Zoeller, R. T., Tan, S. W., & Tyl, R. W. (2007). General background on the hypothalamic-pituitary-thyroid (HPT) axis. *Crit Rev Toxicol*, *37*, 11–53.
11. Fliers, E., Alkemade, A., Wiersinga, W. M., & Swaab, D. F. (2006). Hypothalamic thyroid hormone feedback in health and disease. *Prog Brain Res*, *153*, 189–207.
12. Kopp, P. (2001). The TSH receptor and its role in thyroid disease. *Cell Mol life Sci*, *58*, 1301–1322.
13. Wilber, J. F., & Xu, A. H. (1998). The thyrotropin-releasing hormone gene 1998: cloning, characterization, and transcriptional regulation in the central nervous system, heart, and testis. *Thyroid*, *8*, 897–901.
14. Gershengorn, M. C. (1993). Thyrotropin-releasing hormone receptor: cloning and regulation of its expression. *Recent Prog Horm Res*, *48*, 341–363.
15. Annunziato, L., Di Renzo, G., Quattrone, A., et al. (1981). Brain neurotransmitters regulating TRH producing neurons. *Pharmacol Res Commun*, *13*, 1–10.
16. Frohman, L. A. (1972). Clinical neuropharmacology of hypothalamic releasing factors. *N Engl J Med*, *286*, 1391–1397.
17. Sinclair, D. (2006). Clinical and laboratory aspects of thyroid autoantibodies. *Ann Clin Biochem*, *43*, 173–183.
18. Kohn, L. D., & Harii, N. (2003). Thyrotropin receptor autoantibodies (TSHRAbs): epitopes, origins and clinical significance. *Autoimmunity*, *36*, 331–337.
19. Orgiazzi, J., Madec, A. M., & Ducottet, X. (2003). The role of stimulating, function-blocking and growth-blocking anti-TSH receptor antibodies (TRAbs) in GD, Hashimoto's disease and in atrophic thyroiditis. *Annales d'endocrinologie*, *64*, 31–36.
20. Ueda, D. (1990). Normal volume of the thyroid gland in children. *J Clin Ultrasound*, *18*, 455–462.
21. Hopwood, N. J., Carroll, R. G., Kenny, F. M., & Foley, T. P., Jr. (1976). Functioning thyroid masses in childhood and adolescence: clinical, surgical, and pathologic correlations. *J Pediatr*, *89*, 710–718.
22. Kaplan, M. M. (1999). Clinical perspectives in the diagnosis of thyroid disease. *Clin Chem*, *45*, 1377–1383.
23. Farror, C., Wellby, M. L., & Beng, C. (1987). Familial dysalbuminaemic hyperthyroxinaemia and other causes of euthyroid hyperthyroxinaemia. *J R Soci Med*, *80*, 750–752.
24. Ruiz, M., Rajatanavin, R., Young, R. A., et al. (1982). Familial dysalbuminemic hyperthyroxinemia: a syndrome that can be confused with thyrotoxicosis. *N Engl J Med*, *306*, 635–659.
25. Nikolai, T. F., & Seal, U. S. (1967). X-chromosome linked inheritance of thyroxine-binding globulin deficiency. *J Clin Endocrinol Metab*, *27*, 1515–1520.
26. De Groot, L. J. (1999). Dangerous dogmas in medicine: the nonthyroidal illness syndrome. *J Clin Endocrinol Metab*, *84*, 151–164.
27. Ross, D. S. (2001). Serum thyroid-stimulating hormone measurement for assessment of thyroid function and disease. *Endocrinol Metab Clin North Am*, *30*, 245–264, vii.
28. Spencer, C. A., Hollowell, J. G., Kazarosyan, M., Braverman, L. E. (2007). National Health and Nutrition Examination Survey III thyroid-stimulating hormone (TSH)-thyroperoxidase antibody relationships demonstrate that TSH upper reference limits may be skewed by occult thyroid dysfunction. *J Clin Endocrinol Metab*, *92*, 4236–4240.
29. Zarkovic, M., Ciric, J., Beleslin, B., et al. (2011). Further studies on delineating thyroid-stimulating hormone (TSH) reference range. *Horm Metab Res* = Hormon-und Stoffwechselforschung = Hormones et metabolisme, *43*, 970–976.
30. Volzke, H., Schmidt, C. O., John, U., et al. (2010). Reference levels for serum thyroid function tests of diagnostic and prognostic significance. *Horm Metab Res* = Hormon- und Stoffwechselforschung = Hormones et metabolisme, *42*, 809–814.
31. Surks, M. I., Goswami, G., & Daniels, G. H. (2005). The thyrotropin reference range should remain unchanged. *J Clin Endocrinol Metab*, *90*, 5489–5496.
32. Verburg, F. A., Kirchgassner, C., Hebestreit, H., et al. (2011). Reference ranges for analytes of thyroid function in children. *Horm Metab Res* = Hormon- und Stoffwechselforschung = Hormones et metabolisme, *43*, 422–426.
33. Lazar, L., Frumkin, R. B., Battat, E., et al. (2009). Natural history of thyroid function tests over 5 years in a large pediatric cohort. *J Clin Endocrinol Metab*, *94*, 1678–1682.
34. Brabant, G., Beck-Peccoz, P., Jarzab, B., et al. (2006). Is there a need to redefine the upper normal limit of TSH? *Eur J Endocrinol*, *154*, 633–637.

35. Kaplowitz, P. B. (2010). Subclinical hypothyroidism in children: normal variation or sign of a failing thyroid gland? *Int J Pediatr Endocrinol*, *2010*, 281453.
36. Rapa, A., Monzani, A., Moia, S., et al. (2009). Subclinical hypothyroidism in children and adolescents: a wide range of clinical, biochemical, and genetic factors involved. *J Clin Endocrinol Metab*, *94*, 2414–2420.
37. Reinehr, T. (2011). Thyroid function in the nutritionally obese child and adolescent. *Curr Opin Pediatr*, *23*, 415–420.
38. Dekelbab, B. H., Abou Ouf, H. A., & Jain, I. (2010). Prevalence of elevated thyroid-stimulating hormone levels in obese children and adolescents. *Endocr Pract*, *16*, 187–190.
39. Reinehr, T., Isa, A., de Sousa, G., et al. (2008). Thyroid hormones and their relation to weight status. *Horm Res*, *70*, 51–57.
40. Radetti, G., Kleon, W., Buzi, F., et al. (2008). Thyroid function and structure are affected in childhood obesity. *J Clin Endocrinol Metab*, *93*, 4749–4754.
41. Reinehr, T., de Sousa, G., & Andler, W. (2006). Hyperthyrotropinemia in obese children is reversible after weight loss and is not related to lipids. *J Clin Endocrinol Metab*, *91*, 3088–3091.
42. Lafranchi, S. (1992). Thyroiditis and acquired hypothyroidism. *Pediatr Ann*, *21*, 29, 32–39.
43. Rapoport, B. (1991). Pathophysiology of Hashimoto's thyroiditis and hypothyroidism. *Ann Rev Med*, *42*, 91–96.
44. Rivkees, S. A., Bode, H. H., & Crawford, J. D. (1998). Long-term growth in juvenile acquired hypothyroidism: the failure to achieve normal adult stature. *N Engl J Med*, *318*, 599–602.
45. Gaitan, E, (1988). Goitrogens. *Baillieres Clin Endocrinol Metab*, *2*, 683–702.
46. McLaren, E. H., & Alexander, W. D. (1979). Goitrogens. *Clin Endocrinol Metab*, *8*, 129–144.
47. Rezvani, I., DiGeorge, A. M., & Cote, M. L. (1977). Primary hypothyroidism in cystinosis. *J Pediatr*, *91*, 340–341.
48. Wassner, S. J., Buckingham, B. A., Kershnar, A. J., et al. (1977). Thyroid function in children with chronic renal failure. *Nephron*, *19*, 236–241.
49. Singer, P. A. (1991). Thyroiditis: acute, subacute, and chronic. *Med Clin North Am*, *75*, 61–77.
50. Wu, Y. H., Wang, H. M., Chen, H. H., et al. (2010). Hypothyroidism after radiotherapy for nasopharyngeal cancer patients. *Int J Radiat Oncol Biol Phys*, *76*, 1133–1139.
51. LeFranchi, S., & Mandel, S. H. (1995). *Graves disease in the neonatal period and childhood* (pp. 1237–1246). Philadelphia: JB Lippincott.
52. Foley, T. P., Jr., Abbassi, V., Copeland, K. C., & Draznin, M. B. (1994). Brief report: hypothyroidism caused by chronic autoimmune thyroiditis in very young infants. *N Engl J Med*, *330*, 466–468.
53. Wells, B. J., & Hueston, W. J. (2005). Are thyroid peroxidase antibodies associated with cardiovascular disease risk in patients with subclinical hypothyroidism? *Clin Endocrinol* (Oxf), *62*, 580–584.
54. Radetti, G., Maselli, M., Buzi, F., et al. (2012). The natural history of the normal/mild elevated TSH serum levels in children and adolescents with Hashimoto's thyroiditis and isolated hyperthyrotropinaemia: a 3-year follow-up. *Clin Endocrinol* (Oxf), *76*, 394–398.
55. Radetti, G., Gottardi, E., Bona, G., et al. (2006). The natural history of euthyroid Hashimoto's thyroiditis in children. *J Pediatr*, *149*, 827–832.
56. Van Dop, C., Conte, F. A., Koch, T. K., et al. (1983). Pseudotumor cerebri associated with initiation of levothyroxine therapy for juvenile hypothyroidism. *New Engl J Med*, *308*, 1076–1080.
57. Raghavan, S., DiMartino-Nardi, J., Saenger, P., & Linder, B. (1997). Pseudotumor cerebri in an infant after L-thyroxine therapy for transient neonatal hypothyroidism. *J Pediatr*, *130*, 478–480.
58. Campos, S. P., & Olitsky, S. (1995). Idiopathic intracranial hypertension after L-thyroxine therapy for acquired primary hypothyroidism. *Clin Pediatr* (Phila), *34*, 334–337.
59. Rohn, R. (1985). Pseudotumor cerebri following treatment of hypothyroidism. *Am J Dis Child*, *139*, 752.
60. Kaplowitz, P. B. (2012). Case report: rapid spontaneous recovery from severe hypothyroidism in 2 teenage girls. *Int J Pediatr Endocrinol*, *2012*, 2019.
61. Dong, B. J., & Brown, C. H. (1991). Hypothyroidism resulting from generic levothyroxine failure. *J Am Board Fam Med*, *4*, 167–170.
62. Bolton, S. (2005). Bioequivalence studies for levothyroxine. *AAPS J*, *7*, E47–E53.
63. Fischer, M. A., & Avorn, J. (2003). Economic consequences of underuse of generic drugs: evidence from Medicaid and implications for prescription drug benefit plans. *Health Serv Res*, *38*, 1051–1063.
64. Dong, B. J., Hauck, W. W., Gambertoglio, J. G., et al. (1997). Bioequivalence of generic and brand-name levothyroxine products in the treatment of hypothyroidism. *JAMA*, *277*, 1205–1213.
65. Bolk, N., Visser, T. J., Nijman, J., et al. (2010). Effects of evening vs morning levothyroxine intake: a randomized double-blind crossover trial. *Arch Intern Med*, *170*, 1996–2003.
66. Bolk, N., Visser, T. J., Kalsbeek, A., et al. (2007). Effects of evening vs morning thyroxine ingestion on serum thyroid hormone profiles in hypothyroid patients. *Clin Endocrinol* (Oxf), *66*, 43–48.
67. Sekadde, C. B., Slaunwhite, W. R. Jr., et al. (1994). Administration of thyroxin once a week. *J Clin Endocrinol Metab*, *39*, 759–764.
68. Rivkees, S. A., & Hardin, D. S. (1994). Cretinism after weekly dosing with levothyroxine for treatment of congenital hypothyroidism. *Journal Pediatr*, *125*, 147–149.
69. Bell, D. S., & Ovalle, F. (2001). Use of soy protein supplement and resultant need for increased dose of levothyroxine. *Endocr Pract*, *7*, 193–194.
70. Jabbar, M. A., Larrea, J., & Shaw, R. A. (1997). Abnormal thyroid function tests in infants with congenital hypothyroidism: the influence of soy-based formula. *J Am Coll Nutr*, *16*, 280–282.
71. Chorazy, P. A., Himelhoch, S., Hopwood, N. J., et al. (1995). Persistent hypothyroidism in an infant receiving a soy formula: case report and review of the literature. *Pediatrics*, *96*, 148–150.
72. Karanikas, G., Schuetz, M., Kontur, S., et al. (2008). No immunological benefit of selenium in consecutive patients with autoimmune thyroiditis. *Thyroid*, *18*, 7–12.
73. Fisher, D. A., Pandian, M. R., & Carlton, E. (1987). Autoimmune thyroid disease: an expanding spectrum. *Pediatr Clin North Am*, *34*, 907–918.
74. Ahonen, P., Myllarniemi, S., Sipila, I., & Perheentupa, J. (1990). Clinical variation of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) in a series of 68 patients. *N Engl J Med*, *322*, 1829–1836.
75. Mihailova, D., Grigorova, R., Vassileva, B., et al. (1999). Autoimmune thyroid disorders in juvenile chronic arthritis and systemic lupus erythematosus. *Adv Exp Med Biol*, *455*, 55–60.
76. Jarnerot, G., Azad Khan, A. K., & Truelove, S. C. (1975). The thyroid in ulcerative colitis and Crohn's disease. II. Thyroid enlargement and hyperthyroidism in ulcerative colitis. *Acta Med Scand*, *197*, 83–87.
77. Sattar, N., Lazare, F., Kacer, M., et al. (2011). Celiac disease in children, adolescents, and young adults with autoimmune thyroid disease. *J Pediatr*, *158*, 272–275, e1.
78. Maheshwari, A., & Thuluvath, P. J. (2011). Endocrine diseases and the liver. *Clin Liver Dis*, *15*, 55–67.
79. Ranke, M. B., & Saenger, P. (2001). Turner's syndrome. *Lancet*, *358*, 309–314.
80. Gawlik, A., Gawlik, T., Januszek-Trzciakowska, A., et al. (2011). Incidence and dynamics of thyroid dysfunction and thyroid autoimmunity in girls with Turner's syndrome: a long-term follow-up study. *Horm Res Paediatr*, *76*, 314–320.
81. Livadas, S., Xekouki, P., Fouka, F., et al. (2005). Prevalence of thyroid dysfunction in Turner's syndrome: a long-term follow-up study and brief literature review. *Thyroid*, *15*, 1061–1066.
82. Murphy, J., Philip, M., Macken, S., et al. (2008). Thyroid dysfunction in Down's syndrome and screening for hypothyroidism in children and adolescents using capillary TSH measurement. *J Pediatr Endocrinol Metab*, *21*, 155–163.
83. Fort, P., Lifshitz, F., Bellisario, R., et al. (1984). Abnormalities of thyroid function in infants with Down syndrome. *J Pediatri*, *104*, 545–549.
84. Wasniewska, M., Salerno, M., Cassio, A., et al. (2009). Prospective evaluation of the natural course of idiopathic subclinical hypothyroidism in childhood and adolescence. *Eur J Endocrinol*, *160*, 417–421.
85. Sklar, C. A., Qazi, R., & David, R. (1986). Juvenile autoimmune thyroiditis: hormonal status at presentation and after long-term follow-up. *Am J Dis Child*, *140*, 877–880.

86. Crocker, M. K., & Kaplowitz, P. (2010). Treatment of paediatric hyperthyroidism but not hypothyroidism has a significant effect on weight. *Clin Endocrinol (Oxf)*, *73*, 752–759.
87. Guiral, J., Fisac, R., Martin-Herraez, A., & Garcia-Velazquez, J. (1994). Slipped capital femoral epiphysis in primary juvenile hypothyroidism. *Acta Orthop Belg*, *60*, 343–345.
88. Anasti, J. N., Flack, M. R., Froehlich, J., et al. (1995). A potential novel mechanism for precocious puberty in juvenile hypothyroidism *J Clin Endocrinol Metab*, *80*, 276–279.
89. Rao, N. S., Sriprakash, M. L., & Dash, R. J. (1987). Primary juvenile hypothyroidism with precocious puberty, galactorrhoea and multicystic ovaries. *J Assoc Physicians India*, *35*, 161–163.
90. Piziak, V. K., Hahn, H. B., Jr. (1984). Isolated menarche in juvenile hypothyroidism. *Clin Pediatr*, *23*, 177–179.
91. Pringle, P. J., Stanhope, R., Hindmarsh, P., & Brook, C. G. (1988). Abnormal pubertal development in primary hypothyroidism. *Clin Endocrinol*, *28*, 479–486.
92. Durbin, K. L., Diaz-Montes, T., & Loveless, M. B. (2011). Van Wyk and Grumbach syndrome: an unusual case and review of the literature. Pediatr Adolesc Gynecol, 24, e93–e96.
93. Browne, L. P., Boswell, H. B., Crotty, E. J., et al. (2008). Van Wyk and Grumbach syndrome revisited: imaging and clinical findings in pre- and postpubertal girls. *Pediatr Radiol*, *38*, 538–542.
94. Rivkees, S. A. (2006). Thyrotrope hyperplasia. *J Pediatr Endocrinol Metab*, *19*, 1395.
95. Nebesio, T. D., Wise, M. D., Perkins, S. M., & Eugster, E. A. (2011). Does clinical management impact height potential in children with severe acquired hypothyroidism? *J Pediatr Endocrinol Metab*, *24*, 893–896.
96. Watanabe, T., Minamitani, K., Minagawa, M., et al. (1998). Severe juvenile hypothyroidism: treatment with GH and GnRH agonist in addition to thyroxine. *Endocr J*, *45*(Suppl.), S159-S162.
97. Minamitani, K., Murata, A., Ohnishi, H., et al. (1994). Attainment of normal height in severe juvenile hypothyroidism. *Arch Dis Child*, *70*, 429–430; discussion 430–431.
98. Quintos, J. B., & Salas, M. (2005). Use of growth hormone and gonadotropin releasing hormone agonist in addition to L-thyroxine to attain normal adult height in two patients with severe Hashimoto's thyroiditis. *J Pediatr Endocrinol Metab*, *18*, 515–521.
99. Teng, L., Bui, H., Bachrach, L., et al. (2004). Catch-up growth in severe juvenile hypothyroidism: treatment with a GnRH analog. *J Pediatr Endocrinol Metab*, *17*, 345–354.
100. Watanabe, T., Minamitani, K., Minagawa, M., et al. (1998). Severe juvenile hypothyroidism: treatment with GH and GnRH agonist in addition to thyroxine. *Endocr J*, *45*(Suppl.), S-59-S162.
101. Miyazaki, R., Yanagawa, N., Higashino, H., & Kobayashi, Y. (2000). LHRH analogue and growth hormone did not improve the final height of a patient with juvenile hypothyroidism accompanied by precocious puberty. Arch Dis Child, *83*, 87.
102. Woeber, K. A. (1991). Iodine and thyroid disease. *Med Clin North Am*, *75*, 169–178.
103. Bona, G., Chiorboli, E., Rapa, A., et al. (1998). Measurement of urinary iodine excretion to reveal iodine excess in neonatal transient hypothyroidism. *J Pediatr Endocrinol Metab*, *11*, 739–743.
104. Koga, Y., Sano, H., Kikukawa, Y., et al. (1995). Effect on neonatal thyroid function of povidone-iodine used on mothers during perinatal period. *J Obstet Gynaecol*, *21*, 581–585.
105. Gordon, C. M., Rowitch, D. H., Mitchell, M. L., & Kohane, I. S. (1995). Topical iodine and neonatal hypothyroidism. *Arch Pediatr Adolesc Med*, *149*, 1336–1339.
106. L'Allemand, D., Gruters, A., Beyer, P., & Weber, B. (1987). Iodine in contrast agents and skin disinfectants is the major cause for hypothyroidism in premature infants during intensive care. *Horm Res*, *28*, 42–49.
107. Brown, R. S., Bloomfield. S., Bednarek, F. J., et al. (1997). Routine skin cleansing with povidone-iodine is not a common cause of transient neonatal hypothyroidism in North America: a prospective controlled study. *Thyroid*, *7*, 395–400.
108. Grosso, S., Berardi, R., Cioni, M., & Morgese, G. (1998). Transient neonatal hypothyroidism after gestational exposure to amiodarone: a follow-up of two cases. *J Endocrinol Investigat*, *21*, 699–702.
109. Costigan, D. C., Holland, F. J., Daneman, D., et al. (1986). Amiodarone therapy effects on childhood thyroid function. *Pediatrics*, *77*, 703–708.
110. Delange, F. (1994). The disorders induced by iodine deficiency. *Thyroid*, *4*, 107–228.
111. Boyages, S. C., & Halpern, J. P. (1993). Endemic cretinism: toward a unifying hypothesis. *Thyroid*, *3*, 59–69.
112. Halpern, J. P., Boyages, S. C., Maberly, G. F., et al. (1991). The neurology of endemic cretinism: a study of two endemias. *Brain*, *114*(Pt. 2), 825–841.
113. Matovinovic, I., Trowbridge, F. L., Nichaman, M. Z., & Child, M. A. (1973). Iodine nutriture and prevalence of endemic goiter in USA. *Acta Endocrinol Suppl*, *179*, 17–18.
114. Lee, K., Bradley, R., Dwyer, J., & Lee, S. L. (1999). Too much versus too little: the implications of current iodine intake in the United States. *Nutri Rev*, *57*, 177–181.
115. Gallego, G., Goodall, S., & Eastman, C. J. (2010). Iodine deficiency in Australia: is iodine supplementation for pregnant and lactating women warranted? *Med J Aust*, *192*, 461–463.
116. Sherman, S. I., Gopal, J., Haugen, B. R., et al. (1999). Central hypothyroidism associated with retinoid X receptor-selective ligands. *N Engl J Med*, *340*, 1075–1079.
117. Samuels, M. H., Lillehei, K., Kleinschmidt-Demasters, B. K., et al. (1990). Patterns of pulsatile pituitary glycoprotein secretion in central hypothyroidism and hypogonadism. *J Clin Endocrinol Metab*, *70*, 391–395.
118. Trejbal, D., Sulla, I., Trejbalova, L., et al. (1994). Central hypothyroidism: various types of TSH responses to TRH stimulation. *Endocr Regul*, *28*, 35–40.
119. Hanna, C. E., Krainz, P. L., Skeels, M. R., et al. (1986). Detection of congenital hypopituitary hypothyroidism: ten-year experience in the Northwest Regional Screening Program. *J Pediatr*, *109*, 959–964.
120. Adams, L. M., Emery, J. R., Clark, S. J., et al. (1995). Reference ranges for newer thyroid function tests in premature infants. *J Pediatr*, *126*, 122–127.
121. Nebesio, T. D., McKenna, M. P., Nabhan, Z. M., & Eugster, E. A. (2010). Newborn screening results in children with central hypothyroidism. *J Pediatr*, *156*, 990–993.
122. Persani, L. (1998). Hypothalamic thyrotropin-releasing hormone and thyrotropin biological activity. *Thyroid*, *8*, 941–946.
123. Cohen, L. E. (2012). Genetic disorders of the pituitary. *Curr Opin Endocrinol Diabetes Obes*, *19*, 33–39.
124. Pfaffle, R., & Klammt, J. (2011). Pituitary transcription factors in the aetiology of combined pituitary hormone deficiency. *Best Pract Res Clin Endocrinol Metab*, *25*, 43–60.
125. Turki, Z. M., Hamaoui, R. B., & Ben Slama, C. (2077). Molecular basis of pituitary development defects. *La Tunisie Medicale*, *85*, 999–1003.
126. Carrozza, V., Csako, G., Yanovski, J. A., et al (1999). Levothyroxine replacement therapy in central hypothyroidism: a practice report. *Pharmacotherapy*, *19*, 349–355.
127. Slawik, M., Klawitter, B., Meiser, E., et al (2007). Thyroid hormone replacement for central hypothyroidism: a randomized controlled trial comparing two doses of thyroxine (T4) with a combination of T4 and triiodothyronine. *J Clin Endocrinol Metab*, *92*, 4115–4122.
128. Huang, S. A., Tu, H. M., Harney, J. W., et al. (2000). Severe hypothyroidism caused by type 3 iodothyronine deiodinase in infantile hemangiomas. *N Engl J Med*, *343*, 185–189.
129. Boice, J. D., Jr. (2005). Radiation-induced thyroid cancer—what's new? *J Natl Cancer Inst*, *97*, 703–705.
130. Brignardello, E., Corrias, A., Isolato, G., et al. (2008). Ultrasound screening for thyroid carcinoma in childhood cancer survivors: a case series. *J Clin Endocrinol Metab*, *93*, 4840–4843.
131. Madanat, L. M., Lahteenmaki, P. M., Hurme, S., et al. (2008). Hypothyroidism among pediatric cancer patients: a nationwide, registry-based study. *Int J Cancer Suppl*, *122*, 1868–1872.
132. Rivkees, S. A. (2008). The evolving field of "onco-endocrinology." *J Pediatr Endocrinol Metab*, *21*, 915–916.
133. Bonato, C., Severino, R. F., & Elnecave, R. H. (2008). Reduced thyroid volume and hypothyroidism in survivors of childhood cancer treated with radiotherapy. *J Pediatr Endocrinol Metab*, *21*, 943–949.

134. Topliss, D. (2004). Thyroid incidentaloma: the ignorant in pursuit of the impalpable. *Clinical endocrinology*, *60*, 18–20.
135. Rivkees, S. A., Mazzaferri, E. L., Verburg, F. A., et al. (2011). The treatment of differentiated thyroid cancer in children: emphasis on surgical approach and radioactive iodine therapy. *Endocr Rev*, *32*, 798–826.
136. Brent, G. A. (1994). The molecular basis of thyroid hormone action. *N Engl J Med*, *331*, 847–853.
137. Chatterjee, V. K. (1997). Resistance to thyroid hormone. *Horm Res*, *48*, 43–46.
138. Refetoff, S. (1997). Resistance to thyroid hormone. *Curr Ther Endocrinol Metab*, *6*, 132–134.
139. Refetoff, S., & Dumitrescu, A. M. (2007). Syndromes of reduced sensitivity to thyroid hormone: genetic defects in hormone receptors, cell transporters and deiodination. *Best Pract Res Clin Endocrinol Metab*, *21*, 277–305.
140. Refetoff, S. (2003). The syndrome of resistance to thyroid stimulating hormone. *J Chin Med Assoc*, *66*, 441–452.
141. Beck-Peccoz, P., Forloni, F., Cortelazzi, D., et al. (1992). Pituitary resistance to thyroid hormones. *Horm Res*, *38*, 66–72.
142. Stein, M. A., Weiss, R. E., & Refetoff, S. (1995). Neurocognitive characteristics of individuals with resistance to thyroid hormone: comparisons with individuals with attention-deficit hyperactivity disorder. *J Dev Behav Pediatr*, *16*, 406–411.
143. Weiss, R. E., & Refetoff, S. (1999). Treatment of resistance to thyroid hormone—primum non nocere. *J Clin Endocrinol Metab*, *84*, 401–404.
144. Canadas, K. T., Rivkees, S. A., Udelsman, R., & Breuer, C. K. (2011). Resistance to thyroid hormone associated with a novel mutation of the thyroid beta receptor gene in a four-year-old female. *Int J Pediatr Endocrinol*, *2011*, 3.
145. Anselmo, J., & Refetoff, S. (2004). Regression of a large goiter in a patient with resistance to thyroid hormone by every other day treatment with triiodothyronine. *Thyroid*, *14*, 71–74.
146. Talbot, N. B., Sobel, E. H., McArthur, J. W., & Crawford, J. D. (1952). *Functional endocrinology: from birth to adolescence* (pp. 1–51). Cambridge, MA: Harvard University Press.
147. Wilkins, L. (1965). *The diagnosis and treatment of endocrine disorders in children and adolescence* (pp. 141–150). Springfield, IL: Charles Thomas.
148. Rivkees, S. A. (2010). Pediatric Graves disease: controversies in management. *Horm Res Paediatr*, *74*, 305–311.
149. Bahn, R. S., Burch, H. B., Cooper, D. S., et al. (2011). Hyperthyroidism and other causes of thyrotoxicosis: management guidelines of the American Thyroid Association and American Association of Clinical Endocrinologists. *Endocr Pract*, *17*, 456–520.
150. Bahn Chair, R. S., Burch, H. B., Cooper, D. S., et al. (2011). Hyperthyroidism and other causes of thyrotoxicosis: management guidelines of the American Thyroid Association and American Association of Clinical Endocrinologists. *Thyroid*, *21*, 593–646.
151. Hedberg, C. W., Fishbein, D. B., Janssen, R. S., et al. (1987). An outbreak of thyrotoxicosis caused by the consumption of bovine thyroid gland in ground beef. *N Engl J Med*, *316*, 993–998.
152. Rivkees, S. A. (2006). The treatment of Graves disease in children. *J Pediatr Endocrinol Metab*, *19*, 1095–1111.
153. *Hepatic Toxicity Following Treatment for Pediatric Graves disease*. (2008, October 28). Eunice Kennedy Shriver National Institute of Child Health and Human Development. Accessed January 14, 2009, from http://bpca.nichd.nih.gov/outreach/index.cfm.
154. Weetman, A. P. (2000). Graves disease. *N Engl J Med*, *343*, 1236–1248.
155. Chapman, E. M. (1983). History of the discovery and early use of radioactive iodine. *JAMA*, *250*, 2042–2044.
156. Cooper, D. S. (2005). Antithyroid drugs. *N Engl J Med*, *352*, 905–917.
157. Rivkees, S. A., Sklar, C., & Freemark, M. (1998). Clinical review 99: the management of Graves disease in children, with special emphasis on radioiodine treatment *J Clin Endocrinol Metab*, *83*, 3767–3776.
158. Cooper, D. S. (1998). Antithyroid drugs for the treatment of hyperthyroidism caused by Graves disease. *Endocrinol Metab Clin North Am*, *27*, 225–247.
159. Rivkees, S. A., & Mattison, D. R. (2009). Ending propylthiouracil-induced liver failure in children. *N Engl J Med*, *360*, 1574–1575.
160. Abalovich, M., Amino, N., Barbour, L. A., et al. (2007). Management of thyroid dysfunction during pregnancy and postpartum: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*, *92*, S1–S47.
161. Bahn, R. S., Burch, H. S., Cooper, D. S., et al. (2009). The role of propylthiouracil in the management of Graves disease in adults: report of a meeting jointly sponsored by the American Thyroid Association and the Food and Drug Administration. *Thyroid*, *19*, 673–674.
162. Nakamura, H., Noh, J. Y., Itoh, K., et al. (2007). Comparison of methimazole and propylthiouracil in patients with hyperthyroidism caused by Graves disease. *J Clin Endocrinol Metab*, *92*, 2157–2162.
163. Rivkees, S. A., Stephenson, K., & Dinauer, C. (2010). Adverse events associated with methimazole therapy of graves disease in children. *Int J Pediatr Endocrinol*, *2010*, 176970.
164. Tajiri, J., Noguchi, S. (2005). Antithyroid drug-induced agranulocytosis: how has granulocyte colony-stimulating factor changed therapy? *Thyroid*, *15*, 292–297.
165. Cooper, D. S., Goldminz, D., Levin, A. A., et al. (1983). Agranulocytosis associated with antithyroid drugs: effects of patient age and drug dose. *Ann Intern Med*, *98*, 26–29.
166. Weetman, A. P. (2006). Graves' hyperthyroidism: how long should antithyroid drug therapy be continued to achieve remission? *Nat Clin Pract Endocrinol Metab*, *2*, 2–3.
167. Glaser, N. S., & Styne, D. M. (2008). Predicting the likelihood of remission in children with Graves disease: a prospective, multicenter study. *Pediatrics*, *121*, e481–e488.
168. Shulman, D. I., Muhar, I., Jorgensen, E. V., et al. (1997). Autoimmune hyperthyroidism in prepubertal children and adolescents: comparison of clinical and biochemical features at diagnosis and responses to medical therapy. *Thyroid*, *7*, 755–760.
169. Kaguelidou, F., Alberti, C., Castanet, M., et al. (2008). Predictors of autoimmune hyperthyroidism relapse in children after discontinuation of antithyroid drug treatment. *J Clin Endocrinol Metab*, *93*, 3817–3826.
170. Glaser, N. S., & Styne, D. M. (1997). Predictors of early remission of hyperthyroidism in children. *J Clin Endocrinol Metab*, *82*, 1719–1726.
171. Lazar, L., Kalter-Leibovici, O., Pertzelan, A., et al. (2000). Thyrotoxicosis in prepubertal children compared with pubertal and postpubertal patients. *J Clin Endocrinol Metab*, *85*, 3678–3682.
172. Hamburger, J. I. (1985). Management of hyperthyroidism in children and adolescents. *J Clin Endocrinol Metab*, *60*, 1019–1024.
173. Leger, J., Gelwane, G., Kaguelidou, F., et al. (2012). Positive impact of long-term antithyroid drug treatment on the outcome of children with Graves disease: national long-term cohort study. *J Clin Endocrinol Metab*, *97*, 110–119.
174. Read, C. H., Jr., Tansey, M. J., & Menda, Y. (2004). A thirty-six year retrospective analysis of the efficacy and safety of radioactive iodine in treating young Graves' patients. *J Clin Endocrinol Metab*, *89*, 4229–4233.
175. Levy, W. J., Schumacher, O. P., & Gupta, M. (1988). Treatment of childhood Graves disease: a review with emphasis on radioiodine treatment. *Cleve Clin J Med*, *55*, 373–382.
176. Nebesio, T. D., Siddiqui, A. R., Pescovitz, O. H., & Eugster, E. A. (2002). Time course to hypothyroidism after fixed-dose radioablation therapy of Graves disease in children. *J Pediatr*, *141*, 99–103.
177. Rivkees, S. A., & Cornelius, E. A. (2003). Influence of iodine-131 dose on the outcome of hyperthyroidism in children. *Pediatrics*, *111*, 745–749.
178. Rivkees, S. A., & Dinauer, C. (2007). An optimal treatment for pediatric Graves disease is radioiodine. *J Clin Endocrinol Metab*, *92*, 797–800.
179. Peters, H., Fischer, C., Bogner, U., et al. (1997). Treatment of Graves' hyperthyroidism with radioiodine: results of a prospective randomized study. *Thyroid*, *7*, 247–251.
180. Kadmon, P. M., Noto, R. B., Boney, C. M., et al. (2001). Thyroid storm in a child following radioactive iodine (RAI) therapy: a consequence of RAI versus withdrawal of antithyroid medication. *J Clin Endocrinol Metab*, *86*, 1865–1867.
181. Rivkees, S. A. (2007). Graves disease therapy in children: truth and inevitable consequences. *J Clin Endocrinol Metab*, *20*, 953–955.
182. Boice, J. D., Jr. (2006). Thyroid disease 60 years after Hiroshima and 20 years after Chernobyl. *JAMA*, *295*, 1060–1062.

183. Dolphin, G. W. (1968). The risk of thyroid cancers following irradiation. *Health Phys*, *15*, 219–228.
184. Sigurdson, A. J., Ronckers, C. M., Mertens, A. C., et al. (2005). Primary thyroid cancer after a first tumour in childhood (the Childhood Cancer Survivor Study): a nested case-control study. *Lancet*, *365*, 2014–2023.
185. Ron, E., Lubin, J., Shore, R.E., et al. (1995). Thyroid cancer after exposure to external radiation: a pooled analysis of seven studies. *Radiat Res*, *141*, 259–277.
186. Boice, J. D., Jr. (1998). Radiation and thyroid cancer-what more can be learned? *ACTA Oncol*, *34*, 321–324.
187. Flynn, R. W., Macdonald, T. M., Jung, R. T., et al. (2006). Mortality and vascular outcomes in patients treated for thyroid dysfunction. *J Clin Endocrinol Metab*, *91*, 2159–2164.
188. Franklyn, J. A., Maisonneuve, P., Sheppard, M. C., et al. (1998). Mortality after the treatment of hyperthyroidism with radioactive iodine. *N Engl J Med*, *338*, 712–718.
189. Franklyn, J. A., Sheppard, M. C., & Maisonneuve, P. (2005). Thyroid function and mortality in patients treated for hyperthyroidism. *JAMA*, *294*, 71–80.
190. Goldman, M. B., Monson, R. R., & Maloof, F. (1990). Cancer mortality in women with thyroid disease. *Cancer Res*, *50*, 2283–2289.
191. Holm, L. E., Hall, P., Wiklund, K., et al. (1991). Cancer risk after iodine-131 therapy for hyperthyroidism. *J Natl Cancer Inst*, *83*, 1072–1077.
192. Metso, S., Auvinen, A., Huhtala, H., et al. (2007). Increased cancer incidence after radioiodine treatment for hyperthyroidism. *Cancer*, *109*, 1972–1979.
193. Ron, E., Doody, M.M., Becker D. V., et al. (1998). Cancer mortality following treatment for adult hyperthyroidism. Cooperative Thyrotoxicosis Therapy Follow-up Study Group. *JAMA*, *280*, 347–55.
194. Toohey, R. E., Stabin, M. G., & Watson, E. E. (2000). The AAPM/RSNA physics tutorial for residents: internal radiation dosimetry: principles and applications. *Radiographics*, *20*, 533–546.
195. Toohey, R. E., & Stabin, M. G. (1996). *Comparative analysis of dosimetry parameters for nuclear medicine* (pp. 532–551). ORISE Report 99–1064, 1999. In Proceedings of the Sixth International Radiopharmaceutical Dosimetry Symposium, Gatlinburg, TN.
196. Lee, J. A., Grumbach, M. M., & Clark, O. H. (2007). The optimal treatment for pediatric Graves disease is surgery. *J Clin Endocrinol Metab*, *92*, 801–803.
197. Miccoli, P., Vitti, P., Rago, T., et al. (1996). Surgical treatment of Graves disease: subtotal or total thyroidectomy? *Surgery*, *120*, 1020–1024; discussion 1024–1025.
198. Peters, H., Fischer, C., Bogner, U., et al. (1996). Reduction in thyroid volume after radioiodine therapy of Graves' hyperthyroidism: results of a prospective, randomized, multicentre study. *Eur J Clin Invest*, *26*, 59–63.
199. Sosa, J. A., Tuggle, C. T., Wang, T. S., et al. (2008). Clinical and economic outcomes of thyroid and parathyroid surgery in children. *J Clin Endocrinol Metab*, *93*, 3058–3065.
200. Sosa, J. A., Bowman, H. M., Tielsch, J. M., et al. (1998). The importance of surgeon experience for clinical and economic outcomes from thyroidectomy. *Ann Surg*, *228*, 320–330.
201. Vitti, P., Rago, T., Chiovato, L., et al. (1997). Clinical features of patients with Graves disease undergoing remission after antithyroid drug treatment. *Thyroid*, *7*, 69–375.
202. Davies, T., Roti, E., Braverman, L. E., & De Groot, L. J. (1998). Thyroid controversy—stimulating antibodies. *J Clin Endocrinol Metab*, *83*, 3777–3785.
203. van Veenendaal, N. R., & Rivkees, S. A. (2011). Treatment of pediatric Graves disease is associated with excessive weight gain. *J Clin Endocrinol Metab*, *96*, 3257–3263.
204. Aslam, M., & Inayat, M. (2008). Fetal and neonatal Graves disease: a case report and review of the literature. *Southern Med J*, *101*, 840–841.
205. Zimmerman, D. (1999). Fetal and neonatal hyperthyroidism. *Thyroid*, *9*, 727–733.
206. Skuza, K. A., Sills, I. N., Stene, M., & Rapaport, R. (1996). Prediction of neonatal hyperthyroidism in infants born to mothers with Graves disease. *J Pediatr*, *128*, 264–268.
207. Chester, J., Rotenstein, D., Ringkananont, U., et al. (2008). Congenital neonatal hyperthyroidism caused by germline mutations in the TSH receptor gene. *J Pediatr Endocrinol Metab*, *21*, 479–486.
208. Watkins, M. G., Dejkhamron, P., Huo, J., et al. (2008). Persistent neonatal thyrotoxicosis in a neonate secondary to a rare thyroid-stimulating hormone receptor activating mutation: case report and literature review. *Endocr Pract*, *14*, 479–483.
209. Van Vliet, G., Polak, M., & Ritzen, E. M. (2008). Treating fetal thyroid and adrenal disorders through the mother. *Nat Clin Pract Endocrinol Metab*, *4*, 675–682.
210. Polak, M., Legac, I., Vuillard, E., et al. (2006). Congenital hyperthyroidism: the fetus as a patient. *Horm Res*, *65*, 235–242.
211. Luton, D., Le Gac, I., Vuillard, E., et al. (2005). Management of Graves disease during pregnancy: The key role of fetal thyroid gland monitoring. *J Clin Endocrinol Metab*, *90*, 6093–6098.
212. Rivkees, S. A., & Mandel, S. J. (2011). Thyroid disease in pregnancy. *Horm Res Paediatr*, *76*(Suppl. 1), 91–96.
213. Check, J. H., Rezvani, I., Goodner, D., & Hopper, B. (1982). Prenatal treatment of thyrotoxicosis to prevent intrauterine growth retardation. *Obstet Gynecol*, *60*, 122–124.
214. McKenzie, J. M., & Zakarija, M. (1992). Fetal and neonatal hyperthyroidism and hypothyroidism due to maternal TSH receptor antibodies. *Thyroid*, *2*, 155–159.
215. Daneman, D., & Howard, N. J. (1980). Neonatal thyrotoxicosis: intellectual impairment and craniosynostosis in later years. *J pediatr*, *97*, 257–259.
216. Bruinse, H. W., Vermeulen-Meiners, C., & Wit, J. M. (1988). Fetal treatment for thyrotoxicosis in non-thyrotoxic pregnant women. *Fetal Ther*, *3*, 152–157.
217. Momotani, N., Noh, J., Oyanagi, H., (1986). Antithyroid drug therapy for Graves disease during pregnancy: optimal regimen for fetal thyroid status. *N Engl J Med*, *315*, 24–28.
218. Tamaki, H., Amino, N., Iwatani, Y., et al. (1989). Evaluation of TSH receptor antibody by "natural in vivo human assay" in neonates born to mothers with Graves disease. *Clin Endocrinol*, *30*, 493–503.
219. Sunshine, P., Kusumoto, H., & Kriss, J. P. (1965). Survival time of circulating long-acting thyroid stimulator in neonatal thyrotoxicosis: implications for diagnosis and therapy of the disorder. *Pediatrics*, *36*, 869–876.
220. Jeng, L. B., Lin, J. D., & Chen, M. F. (1994). Acute suppurative thyroiditis: a ten-year review in a Taiwanese hospital. *Scand J Infect Dis*, *26*, 297–300.
221. Vitti, P., Rago, T., Barbesino, G., & Chiovato, L. (1999). Thyroiditis: clinical aspects and diagnostic imaging. *Rays*, *24*, 301–314.
222. Szabo, S. M., & Allen, D. B. (1989). Thyroiditis: differentiation of acute suppurative and subacute. Case report and review of the literature. *Clin Pediatr*, *28*, 171–174.
223. Geva, T., & Theodor, R. (1988). Atypical presentation of subacute thyroiditis. *Arch Dis Child*, *63*, 845–846.
224. Lugo-Vicente, H., Ortiz, V. N., Irizarry, H., et al. (1998). Pediatric thyroid nodules: management in the era of fine needle aspiration. *J Pediatr Surg*, *33*, 1302–1305.
225. Tonacchera, M., Vitti, P., Agretti, P., et al. (1998). Activating thyrotropin receptor mutations in histologically heterogeneous hyperfunctioning nodules of multinodular goiter. *Thyroid*, *8*, 559–564.
226. Esapa, C., Foster, S., Johnson, S., et al. (1997). G protein and thyrotropin receptor mutations in thyroid neoplasia. *J Clin Endocrinol Metab*, *82*, 493–496.
227. Vattimo, A., Bertelli, P., Cintorino, M., et al. (1998). Hurthle cell tumor dwelling in hot thyroid nodules: preoperative detection with technetium-99m-MIBI dual-phase scintigraphy . J Nucl Med, *39*, 822–825.
228. David, E., Rosen, I. B., Bain, J., et al. (1995). Management of the hot thyroid nodule. *Am J Surg*, *170*, 481–483.
229. Hamburger, J. I., & Hamburger, S. W. (1985). Diagnosis and management of large toxic multinodular goiters. *J Nucl Med*, *26*, 888–892.
230. Derwahl, M., & Studer, H. (2000). Multinodular goitre: "much more to it than simply iodine deficiency." *Baillieres Best Pract Res Clin Endocrinol Metab*, *14*, 577–600.
231. Nygaard B, Hegedus L, Ulriksen P, et al. (1999). Radioiodine therapy for multinodular toxic goiter. Arch Intern Med 159, 1364–1368.

232. Nygaard, B., Hegedus, L., Nielsen, K. G., et al. (1999). Long-term effect of radioactive iodine on thyroid function and size in patients with solitary autonomously functioning toxic thyroid nodules. *Clin Endocrinol*, *50*, 197–202.
233. Cooper, D. S., Doherty, G. M., Haugen, B. R., et al. (2009). Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer. *Thyroid*, *19*, 1167–1214.
234. Cooper, D. S., Doherty, G. M., Haugen, B. R., et al. (2006). Management guidelines for patients with thyroid nodules and differentiated thyroid cancer. *Thyroid*, *16*, 109–142.
235. Niedziela, M. (2006). Pathogenesis, diagnosis and management of thyroid nodules in children. *Endocr Relat Cancer*, *13*, 427–453.
236. Hogan, A. R., Zhuge, Y, Perez, E. A., et al. (2009). Pediatric thyroid carcinoma: incidence and outcomes in 1753 patients. *J Surg Res*, *156*, 167–172.
237. Cheung, K., Roman, S. A., Wang, T. S., et al. (2008). Calcitonin measurement in the evaluation of thyroid nodules in the United States: a cost-effectiveness and decision analysis. *J Clin Endocrinol Metab*, *93*, 2173–2180.
238. Corrias, A,, Einaudi, S., Chiorboli, E., et al. (2001). Accuracy of fine needle aspiration biopsy of thyroid nodules in detecting malignancy in childhood: comparison with conventional clinical, laboratory, and imaging approaches. *J Clin Endocrinol Metab*, *86*, 4644–4648.
239. Lyshchik, A., Drozd, V., Demidchik, Y., & Reiners, C. (2005). Diagnosis of thyroid cancer in children: value of gray-scale and power Doppler US. *Radiology*, *235*, 604–613.
240. Moslavac, S., Matesa, N., & Kusic, Z. (2010). Thyroid fine needle aspiration cytology in children and adolescents. *Coll Antropol*, *34*, 197–200.
241. Kapila, K., Pathan, S. K., George, S. S., et al. (2010). Fine needle aspiration cytology of the thyroid in children and adolescents: experience with 792 aspirates. *Acta Cytol*, *54*, 569–574.
242. Corrias, A., Mussa, A., Baronio, F., et al. (2010). Diagnostic features of thyroid nodules in pediatrics. *Arch Pediatr Adolesc Med*, *164*, 714–719.
243. Theoharis, C. G., Schofield, K. M., Hammers, L., et al. (2009). The Bethesda thyroid fine-needle aspiration classification system: year 1 at an academic institution. *Thyroid*, *19*, 1215–1223.
244. Mazzaferri, E. L., & Sipos, J. (2008). Should all patients with subcentimeter thyroid nodules undergo fine-needle aspiration biopsy and preoperative neck ultrasonography to define the extent of tumor invasion? *Thyroid*, *18*, 597–602.
245. Izquierdo, R., Shankar, R., Kort, K., & Khurana, K. (2009). Ultrasound-guided fine-needle aspiration in the management of thyroid nodules in children and adolescents. *Thyroid*, *19*, 703–705.
246. Welch Dinauer, C. A., Tuttle, R. M., Robie, D. K., et al. (1998). Clinical features associated with metastasis and recurrence of differentiated thyroid cancer in children, adolescents and young adults. *Clin Endocrinol*, *49*, 619–628.
247. Reiners, C., & Demidchik, Y. E. (2003). Differentiated thyroid cancer in childhood: pathology, diagnosis, therapy. *Pediatric Endocrinol Rev*, *1*(Suppl. 2), 230–235; discussion 235–236.
248. Chaukar, D. A., Rangarajan, V., Nair, N., et al. (2005). Pediatric thyroid cancer. *J Surg Oncol*, *92*, 130–133.
249. Okada, T., Sasaki, F., Takahashi, H., et al. (2006). Management of childhood and adolescent thyroid carcinoma: long-term follow-up and clinical characteristics. *Eur J Pediatr Surg 16*, 8–13.
250. Thompson, G. B., & Hay, I. D. (2004). Current strategies for surgical management and adjuvant treatment of childhood papillary thyroid carcinoma. *World J Surg*, *28*, 1187–1198.
251. O'Gorman, C. S., Hamilton, J., Rachmiel, M., et al. (2010). Thyroid cancer in childhood: a retrospective review of childhood course. *Thyroid*, *20*, 375–380.
252. Rachmiel, M., Charron, M., Gupta, A., et al. (2006). Evidence-based review of treatment and follow up of pediatric patients with differentiated thyroid carcinoma. *J Pediatr Endocrinol Metab*, *19*, 1377–1393.
253. Luster, M., Lassmann, M., Freudenberg, L. S., & Reiners, C. (2007). Thyroid cancer in childhood: management strategy, including dosimetry and long-term results. *Hormones* (Athens), *6*, 269–278.
254. Dinauer, C., & Francis, G. L. (2007). Thyroid cancer in children. *Endocrinol Metab Clin North Am*, *36*, 779–806, vii.
255. Dinauer, C. A., Breuer, C., & Rivkees, S. A. (2008). Differentiated thyroid cancer in children: diagnosis and management. *Curr Opin Oncol*, *20*, 59–65.
256. Zimmerman, D., Hay, I. D., Gough, I. R., et al. (1988). Papillary thyroid carcinoma in children and adults: long-term follow-up of 1039 patients conservatively treated at one institution during three decades. *Surgery*, *104*, 1157–1166.
257. Zaydfudim, V., Feurer, I. D., Griffin, M. R., & Phay, J. E. (2008). The impact of lymph node involvement on survival in patients with papillary and follicular thyroid carcinoma. *Surgery*, *144*, 1070–1077; discussion 1077–1078.
258. Hung, W., & Sarlis, N. J. (2002). Current controversies in the management of pediatric patients with well-differentiated non-medullary thyroid cancer: a review. *Thyroid*, *12*, 683–702.
259. Schlumberger, M., De Vathaire, F., Travagli, J. P., et al. (1987). Differentiated thyroid carcinoma in childhood: long term follow-up of 72 patients. *J Clin Endocrinol Metab*, *65*, 1088–1094.
260. Lazar, L., Lebenthal, Y., Steinmetz, A., et al. (2009). Differentiated thyroid carcinoma in pediatric patients: comparison of presentation and course between pre-pubertal children and adolescents. *J Pediatr*, *154*, 708–714.
261. Machens, A., Lorenz, K., Nguyen Thanh, P., et al. (2010). Papillary thyroid cancer in children and adolescents does not differ in growth pattern and metastatic behavior. *The J Pediatr*, *157*, 648–652.
262. Powers, P. A., Dinauer, C. A., Tuttle, R. M., et al. (2003). Tumor size and extent of disease at diagnosis predict the response to initial therapy for papillary thyroid carcinoma in children and adolescents. *J Pediatr Endocrinol Metab*, *16*, 693–702.
263. Brink, J. S., van Heerden, J. A., McIver, B., et al. (2000). Papillary thyroid cancer with pulmonary metastases in children: long-term prognosis. *Surgery*, *128*, 881–886; discussion 886–887.
264. La Quaglia, M. P., Black, T., Holcomb, G. W., 3rd, et al. (2000). Differentiated thyroid cancer: clinical characteristics, treatment, and outcome in patients under 21 years of age who present with distant metastases. A report from the Surgical Discipline Committee of the Children's Cancer Group. *J Pediatr Surg*, *35*, 955–959; discussion 960.
265. Duffy, B. J., Fitzgerald, P. J. (1950). Cancer of the thyroid in children: a report of twenty-eight cases. *J Clin Endocrinol Metab*, *10*, 1296–1308.
266. Winship, T., & Rosvoll, R. V. (1961). A study of thyroid cancer in children. *Am J Surg*, *102*, 747–752.
267. Malchoff, C. D., & Malchoff, D. M. (2004). Familial papillary thyroid carcinoma. *Cancer treatment and research*, *122*, 381–387.
268. Ozaki, O., Ito, K., Kobayashi, K., et al. (1988). Familial occurrence of differentiated, nonmedullary thyroid carcinoma. *World J Surg*, *12*, 565–571.
269. Korber, C., Geling, M., Werner, E., et al. (2000). [Incidence of familial non-medullary thyroid carcinoma in the patient register of the Clinic and Polyclinic of Nuclear Medicine, University of Wurzburg]. *Nuklearmedizin*, 39, 27–32.
270. Hillenbrand, A., Varhaug, J. E., Brauckhoff, M., et al. (2010). Familial nonmedullary thyroid carcinoma—clinical relevance and prognosis: a European multicenter study. *Langenbecks Arch Surg*, *395*, 851–858.
271. Parameswaran, R., Brooks, S., & Sadler, G. P. (2010). Molecular pathogenesis of follicular cell derived thyroid cancers. *Int J Surg*, *8*, 186–193.
272. Morrison, P. J., & Atkinson, A. B. (2009). Genetic aspects of familial thyroid cancer. *The oncologist*, *14*, 571–577.
273. Tran, T., & Gianoukaki, A. G. (2010). Familial thyroid neoplasia: impact of technological advances on detection and monitoring. *Curr Opin Endocrinol Diabetes Obes*, *17*, 425–431.
274. Capezzone, M., Marchisotta, S., Cantara, S., et al. (2008). Familial non-medullary thyroid carcinoma displays the features of clinical anticipation suggestive of a distinct biological entity. *Endocrine-Related Cancer*, *15*, 1075–1081.
275. Farooq, A., Walker, L. J., Bowling, J., & Audisio, R. A. (2010). Cowden syndrome. *Cancer Treat Rev*, *36*, 577–583.
276. Blumenthal, G. M., & Dennis, P. A. (2008). PTEN hamartoma tumor syndromes. *Eur J Hum Genet*, *16*, 1289–1300.

277. Richards, M. L. (2010). Familial syndromes associated with thyroid cancer in the era of personalized medicine. *Thyroid*, *20*, 707–713.
278. Smith, J.R., Margusee, E., Webb. S., et al. (2011) Thyroid nodules and cancer in children with PTEN hamartoma tumor syndrome, *J Clin Endocrinol Metab*, *96*, 34-7.
279. Vriens, M. R., Suh, I., Moses, W., & Kebebew, E. (2009). Clinical features and genetic predisposition to hereditary nonmedullary thyroid cancer. *Thyroid*, *19*, 1343–1349.
280. Half, E., Bercovich, D., & Rozen, P. (2009). Familial adenomatous polyposis. *Orphanet J Rare Dis*, *4*, 22.
281. Perrier, N. D., van Heerden, J. A., Goellner, J. R., et al. (1998). Thyroid cancer in patients with familial adenomatous polyposis. *World J Surg*, *22*, 738–742; discussion 743.
282. Krohn, K., & Paschke, R. (2001). Loss of heterozygosity at the thyroid peroxidase gene locus in solitary cold thyroid nodules. *Thyroid*, *11*, 741–747.
283. Rastogi, M., Hanna, C., Mandel, S., & LaFranchi, S. (2010). Congenital hypothyroidism associated with goiter: risk of thyroid nodules and cancer. *Abstract. Pediatric Academic Societies.*
284. Landry, C. S., Brock, G., Scoggins, C. R., et al. (2009). A proposed staging system for gastric carcinoid tumors based on an analysis of 1,543 patients. *Ann Surg Oncol*, *16*, 51–60.
285. Demidchik, Y. E., Demidchik, E. P., Reiners, C., et al. (2006). Comprehensive clinical assessment of 740 cases of surgically treated thyroid cancer in children of Belarus. *Ann Surg*, *243*, 525–532.
286. Handkiewicz-Junak, D., Wloch, J., Roskosz, J., et al. (2007). Total thyroidectomy and adjuvant radioiodine treatment independently decrease locoregional recurrence risk in childhood and adolescent differentiated thyroid cancer. *J Nucl Med*, *48*, 879–888.
287. Hay, I. D., Gonzalez-Losada, T., Reinalda, M. S., et al. (2010). Long-term outcome in 215 children and adolescents with papillary thyroid cancer treated during 1940 through 2008. *World J Surg*, *34*, 1192–1202.
288. The Canadian Pediatric Thyroid Nodule Study: an evaluation of current management practices. (2008). *J Pediatr Surg*, *43*, 826–830.
289. Silva, F., Laguna, R., & Nieves-Rivera, F. (2010). Pediatric thyroid cancer with extensive disease in a Hispanic population: outcome and long-term survival. *J Pediatr Endocrinol Metab*, *23*, 59–64.
290. Haveman, J. W., van Tol, K. M., Rouwe, C. W., et al. (2003). Surgical experience in children with differentiated thyroid carcinoma. *Ann Surg Oncol*, *10*, 15–20.
291. Kowalski, L. P., Goncalves Filho, J., Pinto, C. A., et al. (2003). Long-term survival rates in young patients with thyroid carcinoma. *Arch Otolaryngol Head Neck Surg*, *129*, 746–749.
292. Giuffrida, D., Scollo, C., Pellegriti, G., et al. (2002). Differentiated thyroid cancer in children and adolescents. *J Endocrinol Invest*, *25*, 18–24.
293. Grigsby, P. W., Gal-or, A., Michalski, J. M., & Doherty, G. M. (2002). Childhood and adolescent thyroid carcinoma. *Cancer*, *95*, 724–729.
294. Ben Arush, M. W., Stein, M. E., Perez Nahum, M., et al. (2000). Pediatric thyroid carcinoma: 22 years of experience at the Northern Israel Oncology Center (1973–1995). *Pediatr Hematol Oncol*, *17*, 85–92.
295. Jarzab, B., Handkiewicz Junak, D., Wloch, J., et al. (2000). Multivariate analysis of prognostic factors for differentiated thyroid carcinoma in children. *Eur J Nucl Med*, *27*, 833–841.
296. Landau, D., Vini, L., A'Hern, R., & Harmer, C. (2000). Thyroid cancer in children: the Royal Marsden Hospital experience. *Eur J Cancer*, *36*, 214–220.
297. Rybakov, S. J., Komissarenko, I. V., Tronko, N. D., et al. (2000). Thyroid cancer in children of Ukraine after the Chernobyl accident. *World J Surg*, *24*, 1446–1449.
298. Newman, K. D., Black, T., Heller, G., et al. (1998). Differentiated thyroid cancer: determinants of disease progression in patients < 21 years of age at diagnosis: a report from the Surgical Discipline Committee of the Children's Cancer Group. *Ann Surg*, *227*, 533–541.
299. Vassilopoulou-Sellin, R., Goepfert, H., Raney, B., & Schultz, P. N. (1998). Differentiated thyroid cancer in children and adolescents: clinical outcome and mortality after long-term follow-up. *Head Neck*, *20*, 549–555.
300. Vassilopoulou-Sellin, R., Schultz, P. N., & Haynie, T. P. (1996). Clinical outcome of patients with papillary thyroid carcinoma who have recurrence after initial radioactive iodine therapy. *Cancer*, *78*, 493–501.
301. Harness, J. K., Thompson, N. W., McLeod, M. K., et al. (1992). Differentiated thyroid carcinoma in children and adolescents. *World J Surg*, *16*, 547–553; discussion 553–554.
302. Ceccarelli, C., Pacini, F., Lippi, F., et al. (1998). Thyroid cancer in children and adolescents. *Surgery*, *104*, 1143–1148.
303. La Quaglia, M. P., Corbally, M. T., Heller, G., et al. (1998). Recurrence and morbidity in differentiated thyroid carcinoma in children. *Surgery*, *104*, 1149–11456.
304. Carty, S. E., Cooper, D. S., Doherty, G. M., et al. (2009). Consensus statement on the terminology and classification of central neck dissection for thyroid cancer. *Thyroid*, *19*, 1153–1158.
305. Kouvaraki, M. A., Shapiro, S. E., Fornage, B. D., et al. (2003). Role of preoperative ultrasonography in the surgical management of patients with thyroid cancer. *Surgery*, *134*, 946–954; discussion 954–955.
306. Solorzano, C. C., Carneiro, D. M., Ramirez, M., et al. (2004). Surgeon-performed ultrasound in the management of thyroid malignancy. *Am Surg*, *70*, 576–580; discussion 580–582.
307. Gonzalez, H. E., Cruz, F., O'Brien, A., et al. (2007). Impact of preoperative ultrasonographic staging of the neck in papillary thyroid carcinoma. *Arch Otolaryngol Head Neck Surg*, *133*, 1258–1262.
308. Stulak, J. M., Grant, C. S., Farley, D. R., et al. (2006). Value of preoperative ultrasonography in the surgical management of initial and reoperative papillary thyroid cancer. *Arch Surg*, *141*, 489–494; discussion 494–496.
309. Frankenthaler, R. A., Sellin, R. V., Cangir, A., & Goepfert, H. (1990). Lymph node metastasis from papillary-follicular thyroid carcinoma in young patients. *Am J Surg*, *160*, 341–343.
310. Vassilopoulou-Sellin, R., Klein, M. J., Smith, T. H., et al. (1993). Pulmonary metastases in children and young adults with differentiated thyroid cancer. *Cancer*, *71*, 1348–1352.
311. Wada, N., Sugino, K., Mimura, T., et al. (2009). Pediatric differentiated thyroid carcinoma in stage I: risk factor analysis for disease free survival. *BMC Cancer*, *9*, 306.
312. Machens, A., Lorenz, K., Nguyen Thanh, P., et al. (2010). Papillary thyroid cancer in children and adolescents does not differ in growth pattern and metastatic behavior. *J Pediatr*, *157*, 648–652.
313. Demidchik, Y. E., Saenko, V. A., & Yamashita, S. (2007). Childhood thyroid cancer in Belarus, Russia, and Ukraine after Chernobyl and at present. *Arquivos brasileiros de endocrinologia e metabologia*, *51*, 748–762.
314. Shimamoto, K., Satake, H., Sawaki, A., et al. (1998). Preoperative staging of thyroid papillary carcinoma with ultrasonography. *Eur J Radiol*, *29*, 4–10.
315. Choi, J. S., Kim, J., Kwak, J. Y., et al. (2009). Preoperative staging of papillary thyroid carcinoma: comparison of ultrasound imaging and CT. *AJR Am J Roentgenol*, *193*, 871–878.
316. Mazzaferri, E. L. (2009). A vision for the surgical management of papillary thyroid carcinoma: extensive lymph node compartmental dissections and selective use of radioiodine. *J Clin Endocrinol Metab*, *94*, 1086–1088.
317. Doherty, G. M. (2009). Prophylactic central lymph node dissection: continued controversy. *Oncology* (Williston Park), *23*, 603, 608.
318. Gimm, O., Rath, F. W., & Dralle, H. (1998). Pattern of lymph node metastases in papillary thyroid carcinoma. *Br J Surg*, *85*, 252–254.
319. Bonnet, S., Hartl, D., Leboulleux, S., et al. (2009). Prophylactic lymph node dissection for papillary thyroid cancer less than 2 cm: implications for radioiodine treatment. *J Clin Endocrinol Metab*, *94*, 1162–1167.
320. Bonnet, S., Hartl, D. M., & Travagli, J. P. (2010). Lymph node dissection for thyroid cancer. *J Visc Surg*, *147*, e155–e1159.
321. Seidlin, S. M., Oshry, E., & Yalow, A. A. (1948). Spontaneous and experimentally induced uptake of radioactive iodine in metastases from thyroid carcinoma: a preliminary report. *J Clin Endocrinol Metab*, *8*, 423–432.
322. Coliez, R. (1954). Results of examination of 85 cases of cancer of the thyroid with radioactive iodine. *J Radiol Electrol Arch Electr Medicale*, *32*, 881–895.
323. Beierwaltes, W. H. (1978). The treatment of thyroid carcinoma with radioactive iodine. *Semin Nucl Med*, *8*, 79–94.
324. Van Nostrand, D. (2009). The benefits and risks of I-131 therapy in patients with well-differentiated thyroid cancer. *Thyroid*, *19*, 1381–1391.

325. Van Nostrand, D., & Wartofsky, L. (2007). Radioiodine in the treatment of thyroid cancer. *Endocrinol Metab Clin North Am*, *36*, 807–822, vii-viii.
326. Bal, C. S., Kumar, A., Chandra, P., et al. (2006). A prospective clinical trial to assess the efficacy of radioiodine ablation as an alternative to completion thyroidectomy in patients with differentiated thyroid cancer undergoing sub-total thyroidectomy. *ACTA Oncol*, *45*, 1067–1072.
327. Maxon, H. R. (1999). Quantitative radioiodine therapy in the treatment of differentiated thyroid cancer. *Q J Nucl Med*, *43*, 313–323.
328. Maxon, H. R., 3rd, Englaro, E. E., Thomas, S. R., et al. (1992). Radioiodine-131 therapy for well-differentiated thyroid cancer—a quantitative radiation dosimetric approach: outcome and validation in 85 patients. *J Nucl Med*, *33*, 1132–1136.
329. Maxon, H. R., 3rd, & Smith, H. S. (1990). Radioiodine-131 in the diagnosis and treatment of metastatic well differentiated thyroid cancer. *Endocrinol Metab Clin North Am*, *19*, 685–718.
330. Beierwaltes, W. H. (1987). Radioiodine therapy of thyroid disease. *Int J Rad Appl Instrum B*, *14*, 177–181.
331. Schlumberger, M., Tubiana, M., De Vathaire, F., et al. (1986). Long-term results of treatment of 283 patients with lung and bone metastases from differentiated thyroid carcinoma. *The J Clin Endocrinol Metab*, *63*, 960–967.
332. Beierwaltes, W. H., Rabbani, R., Dmuchowski, C., et al. (1984). An analysis of "ablation of thyroid remnants" with I-131 in 511 patients from 1947–1984: experience at University of Michigan. *J Nucl Med*, *25*, 1287–1293.
333. Maxon, H. R., Thomas, S. R., Hertzberg, V. S., et al. (1983). Relation between effective radiation dose and outcome of radioiodine therapy for thyroid cancer. *N Engl J Med*, *309*, 937–941.
334. Mazzaferri, E. L., & Young, R. L. (1981). Papillary thyroid carcinoma: a 10 year follow-up report of the impact of therapy in 576 patients. *Am J Med*, *70*, 511–518.
335. Benua, R. S., Cicale, N. R., Sonenberg, M., & Rawson, R. W. (1962). The relation of radioiodine dosimetry to results and complications in the treatment of metastatic thyroid cancer. *Am J Roentgenol Radium Ther Nucl Med*, *87*, 171–182.
336. Scheumann, G. F., Gimm, O., Wegener, G., et al. (1994). Prognostic significance and surgical management of locoregional lymph node metastases in papillary thyroid cancer. *World J Surg*, *18*, 559–567; discussion 67–68.
337. Machens, A., Hauptmann, S., & Dralle, H. (2009). Lymph node dissection in the lateral neck for completion in central node-positive papillary thyroid cancer. *Surgery*, *145*, 176–181.
338. Dralle, H., & Machens, A. (2008). Surgical approaches in thyroid cancer and lymph-node metastases. *Best Pract Res Clin Endocrinol Metab*, *22*, 971–9787.
339. Machens, A., Hinze, R., Thomusch, O., & Dralle, H. (2002). Pattern of nodal metastasis for primary and reoperative thyroid cancer. *World J Surg*, *26*, 22–28.
340. Robbins, J., Merino, M. J., Boice, J. D., Jr., et al. (1991). Thyroid cancer: a lethal endocrine neoplasm. *Annals Int Med. 115*, 133–147.
341. Leboulleux, S., Rubino, C., Baudin, E., et al. (2005). Prognostic factors for persistent or recurrent disease of papillary thyroid carcinoma with neck lymph node metastases and/or tumor extension beyond the thyroid capsule at initial diagnosis. *J Clin Endocrinol Metab*, *90*, 5723–5729.
342. Verburg, E., Biko, J., Diebl, S., et al (2011). I-131 activities as high as safely administrable for the treatment of children and adolescents with advanced differentiated thyroid cancer. *J Clin Endocrinol Metab*, *96*, E1268–E1271.
343. Bal, C., Padhy, A. K., Jana, S., et al. (1996). Prospective randomized clinical trial to evaluate the optimal dose of 131 I for remnant ablation in patients with differentiated thyroid carcinoma. *Cancer*, *77*, 2574–2580.
344. Catargi, B., Borget, I., Deandreis, D., et al. (2010). Comparison of four strategies of radioiodine ablation in patients with thyroid cancer with low-risk recurrence: the randomized, prospective ESTIMABL study on 753 patients. Presented at the 14th International Thyroid Conference, Paris.
345. Mallick, U., Harmer, C., Clarke, S., et al. (2010). HiLo: Multicentre randomised phase III clinical trials of high vs low dose radioiodine, with or without re-combinant human thyroid stimulating hormone (rhTSH), for remnant ablation for differentiated thyroid cancer. Presented at the 14th International Thyroid Congress, Paris.
346. Cooper, D. S., Specker, B., Ho, M., et al. (1998). Thyrotropin suppression and disease progression in patients with differentiated thyroid cancer: results from the National Thyroid Cancer Treatment Cooperative Registry. *Thyroid*, *8*, 737–744.
347. Burmeister, L. A., Goumaz, M. O., Mariash, C. N., & Oppenheimer, J. H. (1992). Levothyroxine dose requirements for thyrotropin suppression in the treatment of differentiated thyroid cancer. *The J Clin Endocrinol Metab*, *75*, 344–350.
348. Batrinos, M. L. (2006). The problem of exogenous subclinical hyperthyroidism. *Hormones* (Athens), *5*, 119–125.
349. Osman, F., Gammage, M. D., & Franklyn, J. A. (2002). Hyperthyroidism and cardiovascular morbidity and mortality. *Thyroid*, *12*, 483–487.
350. Rivkees, S. A. (2010). Pediatric Graves disease: controversies in management. *Horm Res Paediatr*, *74*, 305–311.
351. Biondi, B., & Cooper, D. S. (2010). Benefits of thyrotropin suppression versus the risks of adverse effects in differentiated thyroid cancer. *Thyroid*, *20*, 135–146.
352. Biondi, B., Filetti, S., & Schlumberger, M. (2005). Thyroid-hormone therapy and thyroid cancer: a reassessment. *Nat Clin Pract Endocrinol Metab*, *1*, 32–40.
353. Baudin, E., Do Cao, C., Cailleux, A. F., et al. (2003). Positive predictive value of serum thyroglobulin levels, measured during the first year of follow-up after thyroid hormone withdrawal, in thyroid cancer patients. *J Clin Endocrinol Metab*, *88*, 1107–1111.
354. Verburg, F. A., Stokkel, M. P., Duren, C., et al. (2010). No survival difference after successful (131)I ablation between patients with initially low-risk and high-risk differentiated thyroid cancer. *Eur J Nucl Med*, *37*, 276–283.
355. Sherry, N. A., & Levitsky, L. L. (2008). Management of diabetic ketoacidosis in children and adolescents. *Paediatr Drugs*, *10*, 209–215.
356. Costello, I., Wong, I. C., & Nunn, A. J. (2004). A literature review to identify interventions to improve the use of medicines in children. *Child Care Health Dev*, *30*, 647–665.
357. Falkenstein, K., Flynn, L., Kirkpatrick, B., et al. (2004). Non-compliance in children post-liver transplant. Who are the culprits? *Pediatr Transplant*, *8*, 233–236.
358. Beardon, P. H., McGilchrist, M. M., McKendrick, A. D., et al. (1993). Primary non-compliance with prescribed medication in primary care. *BMJ*, *307*, 846–848.
359. Beck-Peccoz, P., Persani, L., & LaFranchi, S. (2004). Safety of medications and hormones used in the treatment of pediatric thyroid disorders. *Pediatr Endocrinol Rev*, *2*(Suppl. 1), 124–133.
360. Waguespack, S. G., Rich, T. A., Perrier, N. D., et al. (2011). Management of medullary thyroid carcinoma and MEN2 syndromes in childhood. *Nat Rev Endocrinol*, *7*, 596–607.
361. Machens, A., & Dralle, H. (2012). Multiple endocrine neoplasia type 2: achievements and current challenges. *Clinics* (Sao Paulo), *67*(Suppl. 1), 113–118.
362. Szinnai, G., Sarnacki, S., & Polak, M. (2007). Heriditary medullary thyroid carcinoma: how molecular genetics made multiple endocrine neoplasia type 2 a paediatric disease. In G. Van Vliet, M. Polak (Eds.), *Thyroid gland development and function* (pp. 173–817). Basel: Karger.
363. Chen, H., Sippel, R. S., O'Dorisio, M. S., et al. (2010). The North American Neuroendocrine Tumor Society consensus guideline for the diagnosis and management of neuroendocrine tumors: pheochromocytoma, paraganglioma, and medullary thyroid cancer. *Pancreas*, *39*, 775–783.
364. Brandi, M. L., Gagel, R. F., Angeli, A., et al. (2001). Guidelines for diagnosis and therapy of MEN type 1 and type 2. *J Clin Endocrinol Metab*, *86*, 5658–5671.
365. Body, J. J., Chanoine, J. P., Dumon, J. C., & Delange, F. (1993). Circulating calcitonin levels in healthy children and subjects with congenital hypothyroidism from birth to adolescence. *J Clin Endocrinol Metab*, *77*, 565–567.
366. Basuyau, J. P., Mallet, E., Leroy, M., & Brunelle, P. (2004). Reference intervals for serum calcitonin in men, women, and children. *Clin Chem*, *50*, 1828–1830.
367. Chi, D. D., & Moley, J. F. (1998). Medullary thyroid carcinoma: genetic advances, treatment recommendations, and the approach

to the patient with persistent hypercalcitoninemia. *Surg Oncol Clin North Am*, *7*, 681–706.
368. Dralle, H., Gimm, O., Simon, D., et al. (1998). Prophylactic thyroidectomy in 75 children and adolescents with hereditary medullary thyroid carcinoma: German and Austrian experience. *World J Surg*, *22*, 744–750; discussion 750–751.
369. Karges, W., Dralle, H., Raue, F., et al. (2004). Calcitonin measurement to detect medullary thyroid carcinoma in nodular goiter: German evidence-based consensus recommendation. *Exp Clin Endocrinol Diabetes*, *112*, 52–58.
370. Machens, A., & Dralle, H. (2012). Biological relevance of medullary thyroid microcarcinoma. *J Clin Endocrinol Metab*, *97*, 1547–1553.
371. Daniels, G. H. (2011). Screening for medullary thyroid carcinoma with serum calcitonin measurements in patients with thyroid nodules in the United States and Canada. *Thyroid*, *21*, 1199–1207.
372. Meijer, J. A., le Cessie, S., van den Hout, W. B., et al. (2010). Calcitonin and carcinoembryonic antigen doubling times as prognostic factors in medullary thyroid carcinoma: a structured meta-analysis. *Clin Endocrinol*, *72*, 534–542.
373. van Veelen, W., de Groot, J. W., Acton, D. S., et al. (2009). Medullary thyroid carcinoma and biomarkers: past, present and future. *J Intern Med*, *266*, 126–140.
374. Szinnai, G., Sarnacki, S., & Polak, M. (2007). Hereditary medullary thyroid carcinoma: how molecular genetics made multiple endocrine neoplasia type 2 a paediatric disease. *Endocr Dev*, *10*, 173–187.
375. Tuttle, R. M., Ball, D. W., & Byrd, D., et al. (2010). Medullary carcinoma. *J Natl Compr Canc Netw*, *8*, 512–30.
376. Kloos, R. T., Eng, C., Evans, D. B., et al. (2009). Medullary thyroid cancer: management guidelines of the American Thyroid Association. *Thyroid*, *19*, 565–612.
377. Tuggle, C. T., Roman, S. A., Wang, T. S., et al. (2008). Pediatric endocrine surgery: who is operating on our children? *Surgery*, *144*, 869–877; discussion 877.
378. Waguespack, S. G., Rich, T., Grubbs, E., et al. (2010). A current review of the etiology, diagnosis, and treatment of pediatric pheochromocytoma and paraganglioma. *J Clin Endocrinol Metab*, *95*, 2023–2037.
379. Havekes, B., Romijn, J. A., Eisenhofer, G., et al. (2009). Update on pediatric pheochromocytoma. *Pediatr Nephrol*, *24*, 943–950.

QUESTIONS

1. A 12-year-old Hispanic girl presents with a thyroxine level of 19.8 μg/dL, a free T4 level of 2.2 ng/dL, a total T3 level of 125 ng/dL, and a TSH of 1.1 uU/mL. Her mother has been treated for Graves disease. What condition does the patient have?
   a. Graves disease
   b. Thyroid hormone resistance
   c. Subclinical hyperthyroidism
   d. Familial dysalbuminemic hyperthyroxinemia
   e. Thyroid hormone ingestion

Answer: d

2. A 4-year-old girl presents with tachycardia and thyromegaly. The T4 is 12.9 μ/dL, total T3 is 587 ng/dL, and the TSH is 4.7 uU/ml. There is no history of thyroid disease in any family members. What condition does the patient have?
   a. Graves disease
   b. Thyroid hormone resistance
   c. Subclinical hyperthyroidism
   d. Familial dysalbuminemic hyperthyroxinemia
   e. Thyroid hormone ingestion

Answer: b

3. A 16-year-old girl presents with mild thyromegaly, a T4 level of 14 μ/dL, a free T4 level of 3.7 ng/Dl, and a TSH of 0.01 uU/mL What is the likelihood this individual will undergo spontaneous remission of Graves disease after 2 years of antithyroid medication therapy?
   a. 50%
   b. 40%
   c. 30%
   d. 20%
   e. 10%

Answer: d

4. An 8-year-old boy presents with left-sided thyromegaly. A fine-needle aspiration indicates the presence of papillary thyroid carcinoma. A neck ultrasound does not reveal enlarged lymph nodes. What is the most appropriate operation?
   a. Left-hemithyroidectomy
   b. Total thyroidectomy
   c. Total thyroidectomy with central compartment lymph node dissection
   d. Continued observation with serial ultrasound studies

Answer: c

5. A 12-year-old boy presents with hyperthyroidism Graves disease. The child is treated with methimazole for 2 years. What condition is most likely to be associated with this patient?
   a. Excessive weight gain
   b. A 10% risk of celiac disease
   c. A 5% risk of autoimmune hepatitis
   d. Development of antineutrophil cytoplasmic antibodies (ANCA)

Answer: a

6. A 10-year-old girl presents with a total T4 of 10.1 μ/dL, total T3 of 85 ng/dL, a TSH of 6.2 uU/mL, and no antithyroid antibodies are present. What is the most appropriate treatment option?
   a. Treat with low-dose levothyroxine.
   b. Do not treat, but reassess thyroid hormone levels every 6 to 12 months.
   c. Obtain a nuclear medicine scan assess uptake.
   d. Treat with triiodothyronine for thyroid hormone resistance.

Answer: b

CHAPTER 13

Adrenal Cortex and its Disorders

Walter L. Miller, MD • Christa E. Flück, MD

CHAPTER OUTLINE

HISTORY, EMBRYOLOGY, AND ANATOMY

The adrenal cortex produces three principal categories of steroid hormones that regulate a wide variety of physiologic processes from fetal to adult life. *Mineralocorticoids*, principally aldosterone, regulate renal retention of sodium and thus profoundly influence electrolyte balance, intravascular volume, and blood pressure. *Glucocorticoids*, principally cortisol, are named for their carbohydrate-mobilizing activity, but they are ubiquitous physiologic regulators that influence a wide variety of bodily functions. *Adrenal androgens* serve no known physiologic role but do mediate some secondary sexual characteristics in women (e.g., pubic and axillary hair), and their overproduction may result in virilism. Thus, the adrenal cortex is of considerable interest because of the widespread effects of its secretions and because derivatives of these secreted steroids are widely used as pharmacologic agents. Disorders of the adrenal cortex, once thought to be rare, are being recognized with increasing frequency. The severe congenital adrenal hyperplasias affect nearly 1 in 10,000 persons, and the very mild forms may affect as many as 1 in 100 in some

populations. Cushing disease, once regarded as a true rarity in pediatrics, may affect as many children as adults.

History

The history of adrenal research has been reviewed.[1] The adrenal glands apparently were first described in 1563 by the Italian anatomist Bartolomeo Eustaccio, better known for his description of the eustachian tube of the ear. Medical interest in the adrenals as something other than an anatomic curiosity began in the mid-19th century with Addison's classic description of adrenal insufficiency and Brown-Sequard's experimental creation of similar disorders in animals subjected to adrenalectomy. The signs and symptoms of glucocorticoid excess due to adrenal tumors were well known by 1932, when Cushing described the pituitary tumors that cause what is now known as Cushing disease. Effects of adrenalectomy on salt and water metabolism were reported in 1927, and by the late 1930s, Selye had proposed the terms *glucocorticoid* and *mineralocorticoid* to distinguish the two broad categories of actions of adrenal extracts.

Numerous adrenal steroids were painstakingly isolated and their structures determined during the 1930s in the laboratories of Reichstein and Kendall, leading to their sharing the 1950 Nobel Prize in medicine. Many of these steroids were synthesized chemically, providing pure material for experimental purposes. The observation in 1949 that glucocorticoids ameliorated the symptoms of rheumatoid arthritis greatly stimulated interest in synthesizing new pharmacologically active analogs of naturally occurring steroids. The structures of the various adrenal steroids suggested precursor/product relationships, leading in 1950 to the first treatment of congenital adrenal hyperplasia with cortisone by both Wilkins and Bartter. This opened a vigorous era of clinical investigation of the pathways of steroidogenesis in a variety of inherited adrenal and gonadal disorders. The association of cytochrome P450 with 21-hydroxylation was made in 1965, and some of the steroidogenic enzymes were then isolated in the 1970s, but it was not until the genes for most of these enzymes were cloned in the 1980s that it became clear which proteins participated in which steroidal transformations.[2] The identification of these genes (Table 13-1) then led to an understanding of the genetic lesions causing heritable disorders of steroidogenesis. At the same time, studies of steroid hormone action led to the discovery of steroid hormone receptors in the 1960s, but it was not until they were cloned in the 1980s that their biology has begun to be understood.[3]

Embryology

The cells of the adrenal cortex are of mesodermal origin, in contrast to cells of the adrenal medulla, which are derived from the neuroectoderm. In human embryos,

TABLE 13-1 **Physical Characteristics of Human Genes Encoding Steroidogenic Enzymes**

Enzyme	Gene	Gene Size (kb)	Chromosomal Location	Exons (n)	mRNA Size (kb)
StAR	*STAR*	8	8p11.2	8	1.6
P450scc	*CYP11A1*	30	15q23-q24	9	2.0
P450c11β	*CYP11B1*	9.5	8q21-22	9	4.2
P450c11AS	*CYP11B2*	9.5	8q21-22	9	4.2
P450c17	*CYP17A1*	6.6	10q24.3	8	1.9
P450c21	*CYP21A2*	3.4	6p21.1	10	2.0
P450aro	*CYP19A1*	130	15q21.1	10	1.5-4.5
3βHSD1	*HSD3B1*	8	1p13.1	4	1.7
3βHSD2	*HSD3B2*	8	1p13.1	4	1.7
11βHSD1	*HSD11B1*	7	1q32-q41	6	1.6
11βHSD2	*HSD11B2*	6.2	16q22	5	1.6
17βHSD1	*HSD17B1*	3.3	17q11-q21	6	1.4, 2.4
17βHSD2	*HSD17B2*	63	16q24.1-q24.2	5	1.5
17βHSD3	*HSD17B3*	67	9q22	11	1.2
17βHSD6 (RoDH)	*HSD17B6*	24.5	12q13	5	1.6
AKR1C1	*AKR1C1*	14.3	10p14–p15	9	1.2
AKR1C2	*AKR1C2*	13.8	10p14–p15	9	1.3
AKR1C3 (17βHSD5)	*AKR1C3*	13.0	10p14–p15	9	1.2
AKR1C4	*AKR1C4*	22.1	10p14–p15	9	1.2
5α-Reductase 1	*SRD5A1*	36	5p15	5	2.4
5α-Reductase 2	*SRD5A2*	56	2p23	5	2.4
SULT2A1	*SULT2A1*	17	19q13.3	6	2.0
PAPSS2	*PAPSS2*	85	10q24	13	3.9
P450-Oxidoreductase	*POR*	69	7q11.2	16	2.5
Ferredoxin	*FDX1*	35	11q22	5	1.0, 1.4, 1.7, 3.2
Ferredoxin reductase	*FDXR*	11	17q24-q25	12	2.0
Cytochrome b_5	*CYB5A*	32	18q23	5	0.9
H6PDH	*H6PD*	36.5	1p36	5	9.1

adrenogonadal progenitor cells first appear at around the fourth week of gestation as a thickening of the coelomic epithelium (or intermediate mesoderm) between the urogenital ridge and dorsal mesentery[4] (Figure 13-1). These progenitor cells give rise to the steroidogenic cells of the gonads and to the adrenal cortex. The adrenal and gonadal cells then separate, with the adrenal cells migrating retroperitoneally to the cranial pole of the mesonephros and the gonadal cells migrating caudally. Between the seventh and eighth week of development, the adrenal primordium is invaded by sympathetic cells derived from the neural crest that give rise to the adrenal medulla. By the end of the eighth week, the rudimentary adrenal has become encapsulated and is clearly associated with the upper pole of the kidney, which at this time is much smaller than the adrenal.[5]

The fetal adrenal cortex consists of an outer "definitive" zone, the principal site of glucocorticoid and mineralocorticoid synthesis, and a much larger "fetal" zone that makes androgenic precursors (DHEA, DHEAS), which the placenta converts to estriol. A putative "transitional" zone exists between these regions toward the end of fetal development, but its role is unclear. The fetal adrenal glands are huge in proportion to other structures, and continue to grow well into the third trimester (Figure 13-2). At birth, the adrenals weigh 8 to 9 g, about the same size of adult adrenals, and represent approximately 0.4% of total body weight. However, the fetal adrenal zone rapidly involutes following birth and has virtually disappeared by 6 to 12 months of postnatal life. Thereafter, adrenal growth is comparatively slow so that the adrenal glands represent only 0.01% of body weight in the adult.

The complex mechanisms regulating adrenal development are still relatively poorly understood. However, important insight into key factors has been obtained

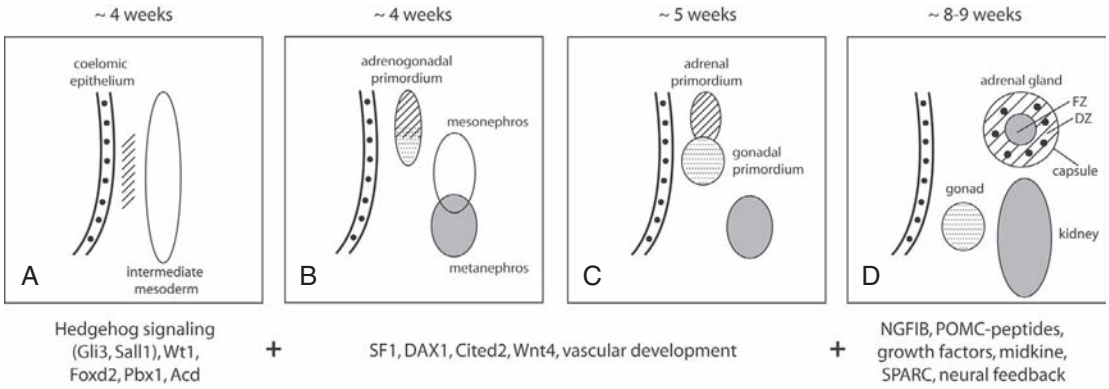


FIGURE 13-1 ■ Overview of human adrenal development. **A-C,** The adrenogonadal primordium develops at around 4 weeks' gestation, after which the adrenal primordium becomes a distinct structure that then migrates retroperitoneally to the cranial pole of the mesonephros. **D,** By 8 to 9 weeks' gestation, the adrenal gland is encapsulated, contains chromaffin cells (black), and has distinct fetal (FZ) and definitive zones (DZ). Some of the signaling molecules, transcription factors and growth factors implicated in adrenal development are shown here, although the exact timing and interaction of many of these factors remains poorly understood at present. (Adapted with permission from Else, T., & Hammer, G. D. (2005). Genetic analysis of adrenal absence: agenesis and aplasia. *Trends Endocrinol Metab, 16,* 458–468.)

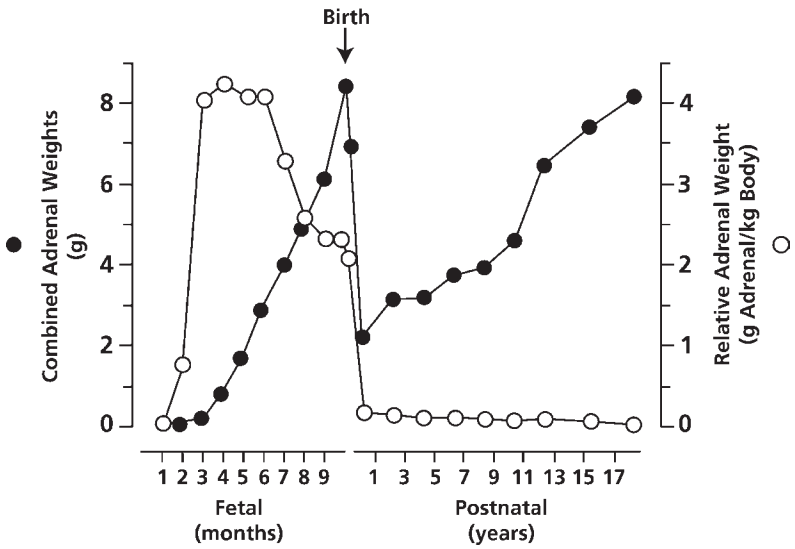


FIGURE 13-2 ■ Combined adrenal weight (filled circles) and relative adrenal weight (open circles) from the first trimester through to early adulthood. (Reprinted with permission from Mesiano, S., & Jaffe, R. B. (1997). Developmental and functional biology of the primate fetal adrenal cortex. *Endocr Rev, 18,* 378–403.)

from studies of transgenic mice and from patients with disorders of adrenal development.[6] For example, the early stages of adrenal differentiation and development involve a number of signaling pathways (hedgehog/GLI3, WNT3/WNT4/WNT11, midkine), transcription factors (SALL1, FOXD2, PBX1, WT1, SF1 [NR5A1], DAX1 [NR0B1]), co-regulators (CITED2), matrix proteins (SPARC), and regulators of telomerase activity (ACD).[7] Subsequent fetal adrenal growth is highly dependent on the tropic effects of adrenocorticotropin (ACTH), its receptor (MC2R), and its downstream signaling pathways, as well as growth factor signaling pathways such as insulin-like growth factor II (IGFII), basic fibroblast growth factor (bFGF), and epidermal growth factor (EGF).

Anatomy

The adrenals, once termed *suprarenal glands*, derive their name from their anatomic location, sitting on top of the upper pole of each kidney. Unlike most other organs, the arteries and veins serving the adrenal do not run in parallel. Arterial blood is provided by several small arteries arising from the renal and phrenic arteries, the aorta, and sometimes the ovarian and left spermatic arteries. The veins are more conventional, with the left adrenal vein draining into the left renal vein and the right adrenal vein draining directly into the vena cava. Arterial blood enters the sinusoidal circulation of the cortex and drains toward the medulla, so that medullary chromaffin cells are bathed in very high concentrations of steroid hormones. High concentrations of cortisol are required for the expression of medullary phenylethanolamine-N-methyltransferase, which converts norepinephrine to epinephrine, linking the adrenal cortical and medullary responses to stress.[8]

The adrenal cortex consists of three histologically recognizable zones: the *glomerulosa* is immediately below the capsule, the *fasciculata* is in the middle, and the *reticularis* lies next to the medulla. The glomerulosa, fasciculata, and reticularis, respectively, constitute about 15%, 75%, and 10% of the adrenal cortex of the older child and adult. These zones appear to be distinct functionally as well as histologically, but considerable overlap exists, and immunocytochemical data show that the zones physically interdigitate. After birth, the large fetal zone begins to involute and disappears by about 3 to 6 months of age. The definitive zone simultaneously enlarges, but two of the adult zones—glomerulosa and fasciculata—are not fully differentiated until about 3 years of age, and the reticularis may not be fully differentiated until about 15 years of age. The origin of the distinct adrenocortical zones and the mechanisms that regulate their proliferation are still poorly understood. One model suggests that a population of undifferentiated stem cells exists between the zona glomerulosa and zona fasciculata, which represents a pool of common precursor cells that can contribute to either the inner or outer zones. In contrast, the "cell-migration" theory proposes that a subcapsular population of stem cells exists. In this model, precursor cells first differentiate within the zona glomerulosa but change their characteristics as they migrate centripetally into the zona fasciculata then zona reticularis.

STEROID HORMONE SYNTHESIS

Early Steps: Cholesterol Uptake, Storage, and Transport

Much is now known about steroid biosynthesis,[9] and the early steps in the intracellular trafficking of cholesterol have been reviewed.[10] The human adrenal can synthesize cholesterol de novo from acetate, but most of its supply of cholesterol comes from plasma low-density lipoproteins (LDL) derived from dietary cholesterol. Rodent adrenals derive most of their cholesterol from high-density lipoproteins via a receptor termed SR-B1, but this pathway appears to play a minor role in human steroidogenesis. Adequate concentrations of LDL will suppress 3-hydroxy-3-methylglutaryl co-enzyme A (HMG CoA) reductase, the rate-limiting enzyme in cholesterol synthesis. ACTH, which stimulates adrenal steroidogenesis, also stimulates the activity of HMG CoA reductase, LDL receptors, and uptake of LDL cholesterol. LDL cholesterol esters are taken up by receptor-mediated endocytosis, then they are stored directly or converted to free cholesterol and used for steroid hormone synthesis. Cholesterol can be esterified by acyl-CoA:cholesterol transferase (ACAT), stored in lipid droplets, and accessed by activation of hormone-sensitive lipase (HSL) and by the so-called NPC proteins, which derive their name from their causative role in Niemann-Pick type C disease. ACTH stimulates HSL and inhibits ACAT, thus increasing the availability of free cholesterol for steroid hormone synthesis.

Steroidogenic Enzymes

Cytochrome P450

Most steroidogenic enzymes are members of the cytochrome P450 group of oxidases.[9] *Cytochrome P450* is a generic term for a group of oxidative enzymes, all of which have about 500 amino acids and contain a single heme group. They are termed P450 (pigment 450) because all absorb light at 450 nm in their reduced states. It is sometimes stated that certain steroidogenic enzymes are "P450-dependent" enzymes. This is a misnomer, as it implies a generic P450 cofactor to a substrate-specific enzyme; however, the P450 binds the steroidal substrate and achieves its catalysis on an active site associated with the heme group. Human beings have genes for 57 cytochrome P450 enzymes, of which 7 are targeted to mitochondria and 50 are targeted to the endoplasmic reticulum, especially in the liver, where they metabolize countless endogenous and exogenous toxins, drugs, xenobiotics, and environmental pollutants. Each P450 enzyme can metabolize multiple substrates, catalyzing a broad array of oxidations. This theme recurs with each adrenal P450 enzyme.

Five distinct P450 enzymes are involved in adrenal steroidogenesis (Figure 13-3). Mitochondrial P450scc (CYP11A1) is the cholesterol side-chain cleavage enzyme catalyzing the series of reactions formerly termed 20,22 desmolase. Two distinct isozymes of P450c11, P450c11β (CYP11B1) and P450c11AS (CYP11B2), also found in mitochondria, catalyze 11β-hydroxylase, 18-hydroxylase,

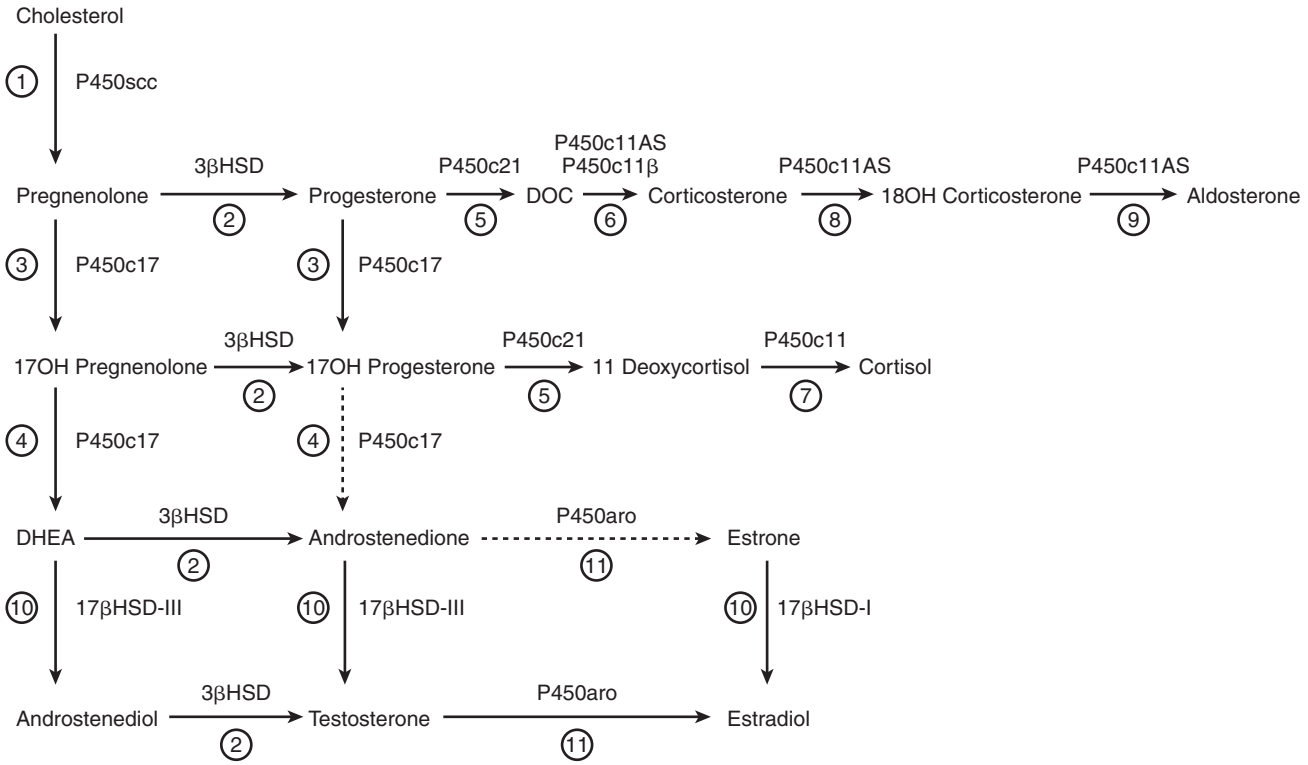


FIGURE 13-3 ■ Principal pathways of human adrenal steroid hormone synthesis. Other quantitatively and physiologically minor steroids are also produced. The names of the enzymes are shown by each reaction, and the traditional names of the enzymatic activities correspond to the *circled numbers.* Reaction 1: Mitochondrial cytochrome P450scc mediates 20α-hydroxylation, 22-hydroxylation, and scission of the C20-22 carbon bond. Reaction 2: 3βHSD mediates 3β-hydroxysteroid dehydrogenase and isomerase activities, converting Δ^5 steroids to Δ^4 steroids. Reaction 3: P450c17 catalyzes the 17α-hydroxylation of pregnenolone to 17OH-pregnenolone and of progesterone to 17OH-progesterone. Reaction 4: The 17,20 lyase activity of P450c17 converts 17OH-pregnenolone to DHEA; only insignificant amounts of 17OH-progesterone are converted to Δ^4 androstenedione by human P450c17, although this reaction occurs in other species. Reaction 5: P450c21 catalyzes the 21-hydroxylation of progesterone to DOC and of 17OH-progesterone to 11-deoxycortisol. Reaction 6: DOC is converted to corticosterone by the 11-hydroxylase activity of P450c11AS in the zona glomerulosa and by P450c11β in the zona fasciculata. Reaction 7: 11-deoxycortisol undergoes 11β-hydroxylation by P450c11β to produce cortisol in the zona fasciculata. Reactions 8 and 9: The 18-hydroxylase and 18-methyl oxidase activities of P450c11AS convert corticosterone to 18OH-corticosterone and aldosterone, respectively, in the zona glomerulosa. Reactions 10 and 11 are found principally in the testes and ovaries. Reaction 10: 17βHSD-III converts DHEA to androstenediol and androstenedione to testosterone, whereas 17βHSD-I converts estrone to estradiol. Reaction 11: Testosterone may be converted to estradiol and androstenedione may be converted to estrone by P450aro.

and 18-methyl oxidase activities. P450c17 (CYP17A1), found in the endoplasmic reticulum, catalyzes both 17α-hydroxylase and 17,20 lyase activities, and P450c21 (CYP21A2) catalyzes the 21-hydroxylation of both glucocorticoids and mineralocorticoids. In the gonads and elsewhere, P450aro (CYP19A1) in the endoplasmic reticulum catalyzes aromatization of androgens to estrogens.

Hydroxysteroid Dehydrogenases

The hydroxysteroid dehydrogenases have molecular masses of about 35 to 45 kilodaltons, do not have heme groups, and require NAD+ or NADP+ as cofactors. Whereas most steroidogenic reactions catalyzed by P450 enzymes are due to the action of a single form of P450, each of the reactions catalyzed by hydroxysteroid dehydrogenases can be catalyzed by at least two, often very different, isozymes. Members of this family include the 3α- and 3β-hydroxysteroid dehydrogenases, the two 11β-hydroxysteroid dehydrogenases, and a series of 17β-hydroxysteroid dehydrogenases; the 5α-reductases are unrelated to this family. Based on their structures, these enzymes fall into two groups: the short-chain dehydrogenase-reductase (SDR) family, characterized by a "Rossman fold," and the aldo-keto reductase (AKR) family, characterized by a triosephosphate isomerase (TIM) barrel motif.[11] The SDR enzymes include 11β-HSDs 1 and 2, and 17β-HSDs 1, 2, 3, and 4; the AKR enzymes include 17β-HSD5, which is important in extraglandular activation of androgenic precursors, and the 3α-hydroxysteroid dehydrogenases that participate in the so-called backdoor pathway of fetal androgen synthesis (discussed later). Based on their activities, it is physiologically more useful to classify them as dehydrogenases or reductases. The dehydrogenases use NAD+ as their cofactor to oxidize hydroxysteroids to ketosteroids, and the reductases mainly use NADPH to reduce ketosteroids to hydroxysteroids. Although these enzymes are typically bidirectional in vitro, they tend to function in only one direction in intact cells, with the direction determined by the cofactor(s) available.[11]

P450scc

Conversion of cholesterol to pregnenolone in mitochondria is the first, rate-limiting, and hormonally regulated step in the synthesis of all steroid hormones.[9,10] This involves three distinct chemical reactions, 20α-hydroxylation, 22-hydroxylation, and scission of the cholesterol side-chain to yield pregnenolone and isocaproic acid. Because 20-hydroxycholesterol, 22-hydroxycholesterol, and 20, 22-hydroxycholesterol could all be isolated from bovine adrenals in significant quantities, it was previously thought that three separate enzymes were involved. However, a single protein, termed P450scc (where SCC refers to the *s*ide *c*hain *c*leavage of cholesterol), encoded by a single gene (*CYP11A1*), on chromosome 15 catalyzes all the steps between cholesterol and pregnenolone. These three reactions occur on a single active site that is in contact with the hydrophobic bilayer membrane. Deletion of the gene for P450scc in the rabbit or mouse eliminates all steroidogenesis, indicating that all steroidogenesis is initiated by this one enzyme.

Transport of Electrons to P450scc: Adrenodoxin Reductase and Adrenodoxin

P450scc functions as the terminal oxidase in a mitochondrial electron transport system.[12] Electrons from NADPH are accepted by a flavoprotein, termed adrenodoxin reductase, that is loosely associated with the inner mitochondrial membrane. Adrenodoxin reductase transfers the electrons to an iron/sulfur protein termed adrenodoxin, which is found in the mitochondrial matrix or loosely adherent to the inner mitochondrial membrane. Adrenodoxin then transfers the electrons to P450scc (Figure 13-4). Adrenodoxin reductase and adrenodoxin serve as generic electron transfer proteins for all mitochondrial P450s, and not just for those involved in steroidogenesis; hence, these proteins are also termed *ferredoxin reductase* and *ferredoxin*. Adrenodoxin forms a 1:1 complex with adrenodoxin reductase, then dissociates, then subsequently reforms an analogous 1:1 complex with a mitochondrial P450 such as P450scc or P450c11, thus functioning as an indiscriminate diffusible electron shuttle mechanism. Adrenodoxin reductase is a membrane-bound mitochondrial flavoprotein that receives electrons from NADPH. The human genes for adrenodoxin reductase and adrenodoxin are expressed in all tissues, indicating they may have other roles. Human mutations in these genes have not been described.

Mitochondrial Cholesterol Uptake: The Steroidogenic Acute Regulatory Protein, StAR

ACTH regulates steroidogenic capacity (chronic regulation) by inducing the transcription of genes for steroidogenic enzymes, but acute regulation, where steroids are released within minutes of a stimulus, is at the level of cholesterol access to P450scc.[13,14] When either steroidogenic cells or intact rats are treated with inhibitors of protein synthesis such as cycloheximide, the acute steroidogenic response is eliminated, suggesting that a short-lived, cycloheximide-sensitive protein acts at the level of the mitochondrion as the specific trigger to the acute steroidogenic response. This factor was first identified as short-lived 30- and 37-kilodalton phosphoproteins that were rapidly synthesized when steroidogenic cells were stimulated with tropic hormones, then cloned from mouse Leydig MA-10 cells and named the steroidogenic acute regulatory protein, StAR.[13,14] The central role of StAR in steroidogenesis was proven by finding that mutations of StAR caused congenital lipoid adrenal hyperplasia.[15,16] Thus, StAR is the acute trigger that is required for the rapid flux of cholesterol from the outer to the inner mitochondrial membrane that is needed for the acute response of aldosterone to angiotensin II, of cortisol to ACTH, and of sex steroids to an LH pulse.

Some adrenal steroidogenesis is independent of StAR; when nonsteroidogenic cells are transfected with StAR

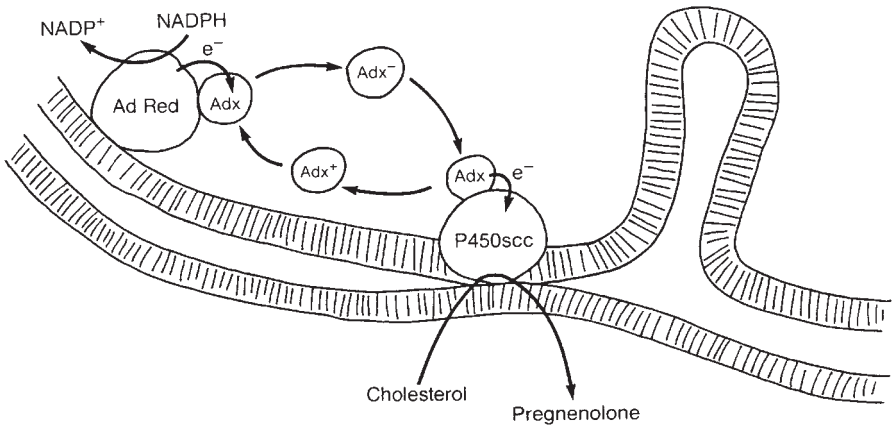


FIGURE 13-4 ■ Electron transport to mitochondrial forms of cytochrome P450. Adrenodoxin reductase (AdRed), a flavoprotein loosely bound to the inner mitochondrial membrane, accepts electrons (e−) from NADPH, converting it to NADP+. These electrons are passed to adrenodoxin (Adx), an iron-sulfur protein in solution in the mitochondrial matrix that functions as a freely diffusible electron shuttle mechanism. Electrons from charged adrenodoxin (Adx−) are accepted by any available cytochrome P450, such as P450c11 or P450scc shown here. The uncharged adrenodoxin (Adx+) may then be again bound to adrenodoxin reductase to receive another pair of electrons. For P450scc, three pairs of electrons must be transported to the P450 to convert cholesterol to pregnenolone. The flow of cholesterol into the mitochondria is facilitated by StAR, which is not shown in this diagram.

and the P450scc system, they convert cholesterol to pregnenolone at about 14% of the StAR-induced rate.[15,16] Furthermore, the placenta utilizes mitochondrial P450scc to initiate steroidogenesis but does not express StAR. The mechanism of StAR-independent steroidogenesis is unclear; it may occur without a triggering protein, or some other protein may exert StAR-like activity to promote cholesterol flux, but without StAR's rapid kinetics. The exact mechanism of StAR's action is unclear, but it is established that StAR acts on the outer mitochondrial membrane, does not need to enter the mitochondria to be active, and undergoes conformational changes on the outer mitochondrial membrane that are required for StAR's activity.[14,17] StAR functions as a component of a molecular machine termed a *transduceosome* on the outer mitochondrial membrane that consists of StAR, TSPO (the translocator protein formerly known as the peripheral benzodiazepine receptor), TSPO-associated protein 7 (PAP7, ACBD3 for acyl-CoA-binding-domain 3), the voltage-dependent anion channel (VDAC-1), and protein kinase A regulatory subunit 1α (PKAR1A).[18] The precise fashion in which these proteins interact and move cholesterol from the OMM to P450scc and the means by which cholesterol is loaded into the OMM remain unclear. It is possible that new disorders of steroidogenesis will be described involving these proteins, but their mutation would be expected to affect many other systems as well.

3β-Hydroxysteroid Dehydrogenase/$\Delta^5 \rightarrow \Delta^4$ Isomerase

Once pregnenolone is produced from cholesterol, it may undergo 17α-hydroxylation by P450c17 to yield 17-hydroxypregnenolone, or it may be converted to progesterone, the first biologically important steroid in the pathway. A single 42-kilodalton microsomal enzyme, 3β-hydroxysteroid dehydrogenase (3βHSD), catalyzes both conversion of the hydroxyl group to a keto group on carbon 3 and the isomerization of the double bond from the B ring (Δ^5 steroids) to the A ring (Δ^4 steroids). [9] Thus, a single enzyme, 3βHSD, converts pregnenolone to progesterone, 17α-hydroxypregnenolone to 17α-hydroxyprogesterone, dehydroepiandrosterone (DHEA) to androstenedione, and androstenediol to testosterone, all with the same enzymologic efficiency (Km and Vmax). As is typical of hydroxysteroid dehydrogenases, there are two isozymes of 3βHSD, encoded by separate genes (*HSD3B1* and *HSD3B2*).[19] These isozymes share 93.5% amino acid sequence identity and are enzymatically very similar. The enzyme catalyzing 3βHSD activity in the adrenals and gonads is the type 2 enzyme, whereas the type 1 enzyme, encoded by a closely linked gene with identical intron/exon organization, catalyzes 3βHSD activity in placenta, breast, and "extraglandular" tissues. Ultrastructural data surprisingly shows that bovine 3βHSD can be found in both the endoplasmic reticulum and in mitochondria. It is not clear if this is also true for human 3βHSD or if this subcellular distribution differs in various types of steroidogenic cells, but this could be a novel point regulating the direction of steroidogenesis.[9]

P450c17

Pregnenolone and progesterone may undergo 17α-hydroxylation to 17α-hydroxypregnenolone and 17α-hydroxyprogesterone (17OHP), respectively. 17OHP may also undergo scission of the C17,20 carbon bond to yield dehydroepiandrosterone (DHEA), but very little 17OHP is converted to androstenedione because the human P450c17 enzyme catalyzes this reaction at only 3% of the rate for conversion of 17α-hydroxypregnenolone to DHEA.[20] These reactions are all mediated by a single enzyme, P450c17 (CYP17A1). This P450 is bound to smooth endoplasmic reticulum, where it accepts electrons from P450 oxidoreductase. As P450c17 has both 17α-hydroxylase activity and 17,20 lyase activity, it is the key branch point in steroid hormone synthesis. Neither activity of P450c17 is present in the adrenal zona glomerulosa, hence pregnenolone is converted to mineralocorticoids; in the zona fasciculata, the 17α-hydroxylase activity is present but 17,20 lyase activity is not, hence pregnenolone is converted to the glucocorticoid cortisol; in the zona reticularis, both activities are present, so that pregnenolone is converted to sex steroids (see Figure 13-3).[9]

17α-Hydroxylase and 17,20 lyase were once thought to be separate enzymes. The adrenals of prepubertal children synthesize ample cortisol but virtually no sex steroids (i.e., have 17α-hydroxylase activity but not 17,20 lyase activity), until adrenarche initiates the production of adrenal androgens (i.e., turns on 17,20 lyase activity). Furthermore, patients had been described lacking 17,20 lyase activity but retaining normal 17α-hydroxylase activity.[21] However, studies of pig P450c17 showed that both 17α-hydroxylase and 17,20 lyase activities reside in a single protein, and cells transfected with a vector expressing P450c17 cDNA acquire both 17α-hydroxylase and 17,20 lyase activities. P450c17 is encoded by a single gene (*CYP17A1*) on chromosome 10q24.3 that is structurally related to the genes for P450c21 (21-hydroxylase).

Thus, the distinction between 17α-hydroxylase and 17,20 lyase is functional and not genetic or structural. Human P450c17 catalyzes 17α-hydroxylation of pregnenolone and progesterone equally well, but the 17,20 lyase activity of human P450c17 strongly prefers 17OH pregnenolone and not 17OH progesterone, consistent with the large amounts of dehydroepiandrosterone (DHEA) secreted by both the fetal and adult adrenal. Furthermore, the 17α-hydroxylase reaction occurs more readily than the 17,20 lyase reaction. The principal factor regulating the 17,20 lyase reaction is electron transport from NADPH.[21]

Electron Transport to P450c17: P450 Oxidoreductase and Cytochrome b_5

P450c17 (and P450c21) receive electrons from a membrane-bound flavoprotein, termed P450 oxidoreductase, which is a different protein from the mitochondrial flavoprotein, adrenodoxin reductase.[12] P450 oxidoreductase receives two electrons from NADPH and transfers them one at a time to the P450. Electron transfer for the lyase reaction is promoted by the action of cytochrome b_5 as an

allosteric factor rather than as an alternate electron donor.[20] 17,20 Lyase activity also requires the phosphorylation of serine residues on P450c17 by a cAMP-dependent protein kinase[21] (Figure 13-5). The availability of electrons determines whether P450c17 performs only 17α-hydroxylation or also performs 17,20 bond scission: increasing the ratio of P450 oxidoreductase or cytochrome b_5 to P450c17 in vitro or in vivo increases the ratio of 17,20 lyase activity to 17α-hydroxylase activity. Competition between P450c17 and P450c21 for available 17-hydroxyprogesterone (17OHP) does not appear to be important in determining whether 17OHP undergoes 21-hydroxylation or 17,20 bond scission. Thus, the regulation of 17,20 lyase activity, and consequently of DHEA production, depends on factors that facilitate the flow of electrons to P450c17: high concentrations of P450 oxidoreductase, the presence of cytochrome b_5, and serine phosphorylation of P450c17.[21]

P450c21

After the synthesis of progesterone and 17-hydroxyprogesterone (17OHP), these steroids are hydroxylated at the 21 position to yield deoxycorticosterone (DOC) and 11-deoxycortisol, respectively[9] (see Figure 13-3). The nature of the 21 hydroxylating step has been of great clinical interest because disordered 21-hydroxylation causes more than 90% of all cases of congenital adrenal hyperplasia. The clinical symptoms associated with this common genetic disease are complex and devastating.[22] Decreased cortisol and aldosterone synthesis often lead to sodium loss, potassium retention, and hypotension, which will lead to cardiovascular collapse and death, usually within a month after birth if not treated appropriately. Decreased synthesis of cortisol in utero leads to overproduction of ACTH and consequent overstimulation of adrenal steroid synthesis; as the 21-hydroxylase step is impaired, 17OHP accumulates because P450c17 converts only miniscule amounts of 17OHP to androstenedione. However, 17-hydroxypregnenolone also accumulates and is converted to DHEA and subsequently to androstenedione and testosterone, resulting in severe prenatal virilization of female fetuses.[22] Congenital adrenal hyperplasia (CAH) has been extensively studied clinically. Variations in the manifestations of the disease, especially the identification of patients without apparent defects in mineralocorticoid activity, suggested that there were two separate 21 hydroxylating enzymes that were differentially expressed in the zones of the adrenal specifically synthesizing aldosterone or cortisol. However, characterization of the P450c21 protein and gene cloning show there is only one 21-hydroxylase encoded by a single functional gene (*CYP21A2*) on chromosome 6p21.[9,23,24] As this gene lies in the middle of the major histocompatibility locus, disorders of adrenal 21-hydroxylation are closely linked to specific HLA types.

Adrenal 21-hydroxylation is mediated by P450c21 found in smooth endoplasmic reticulum. P450c21 employs the same P450 oxidoreductase used by P450c17 to transport electrons from NADPH. 21-hydroxylase activity has also been described in a broad range of adult and fetal extra-adrenal tissues,[25,26] especially in the liver, but is not catalyzed by P450c21.[25] Hepatic 21-hydroxylation is mediated by several enzymes, notably CYP2C19 and CYP3A4, which are principally involved in drug metabolism; these enzymes can 21-hydroxylate progesterone, but not 17OHP, and hence may contribute to the synthesis of mineralocorticoids but not glucocorticoids.[26,27]

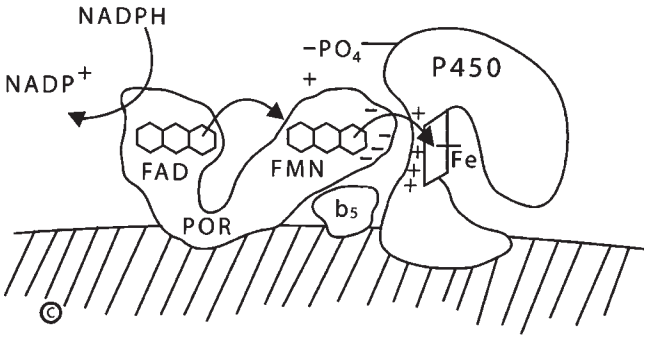


FIGURE 13-5 ■ Electron transport to microsomal forms of cytochrome P450. NADPH interacts with P450 oxidoreductase (POR), bound to the endoplasmic reticulum, and gives up a pair of electrons (e−), which are received by the FAD moiety. Electron receipt elicits a conformational change, permitting the isoalloxazine rings of the FAD and FMN moieties to come close together, so that the electrons pass from the FAD to the FMN. Following another conformational change that returns the protein to its original orientation, the FMN domain of POR interacts with the redox-partner binding site of the P450. Electrons from the FMN domain of POR reach the heme group to mediate catalysis. The interaction of POR and the P450 is coordinated by negatively charged acidic residues on the surface of the FMN domain of POR and positively charged basic residues in the concave redox-partner binding site of the P450. The active site containing the steroid lies on the side of heme ring (Fe) opposite from the redox-partner binding site. In the case of human P450c17, this interaction is facilitated by the allosteric action of cytochrome b_5 and by the serine phosphorylation of P450c17. (Copyright W.L. Miller.)

P450c11β and P450c11AS

Two closely related enzymes, P450c11β and P450c11AS, catalyze the final steps in the synthesis of both glucocorticoids and mineralocorticoids.[9,28,29] These two isozymes have 93% amino acid sequence identity and are encoded by tandemly duplicated genes (*CYP11B1* and *CYP11B2*) on chromosome 8q21-22. Like P450scc, the two forms of P450c11 are found on the inner mitochondrial membrane and use adrenodoxin and adrenodoxin reductase to receive electrons from NADPH. By far the more abundant of the two isozymes is P450c11β, which is the classic 11β-hydroxylase that converts 11-deoxycortisol to cortisol and 11-deoxycorticosterone to corticosterone. The less abundant isozyme, P450c11AS, is found only in the zona glomerulosa, where it has 11β-hydroxylase, 18-hydroxylase, and 18-methyl oxidase (aldosterone synthase) activities; thus, P450c11AS is able to catalyze all the reactions needed to convert DOC to aldosterone.

P450c11β, which is principally involved in the synthesis of cortisol, is encoded by a gene (*CYP11B1*) that is primarily induced by ACTH via cAMP and is suppressed by glucocorticoids. The existence of two distinct functional genes is confirmed by the identification of mutations in each that cause distinct genetic disorders of steroidogenesis. Thus, patients with disorders in P450c11β have classic 11β-hydroxylase deficiency but can still produce

aldosterone, whereas patients with disorders in P450c11AS have rare forms of aldosterone deficiency (so-called corticosterone methyl oxidase deficiency) while retaining the ability to produce cortisol.[9,28,29]

17β-Hydroxysteroid Dehydrogenase

Androstenedione is converted to testosterone, DHEA is converted to androstenediol, and estrone is converted to estradiol by the 17β-hydroxysteroid dehydrogenases (17βHSD; HSD17B), sometimes also termed 17-oxidoreductase or 17-ketosteroid reductase. The terminologies for these enzymes vary, depending on the direction of the reaction being considered.[9,30-32] There is confusion in the literature about the 17βHSDs because (1) there are several different 17βHSDs; (2) some are preferential oxidases whereas others are preferential reductases; (3) they differ in their substrate preference and sites of expression; (4) there is inconsistent nomenclature, especially with the rodent enzymes; and (5) some proteins termed 17βHSD actually have very little 17βHSD activity and are principally involved in other reactions.

Type 1 17βHSD (17βHSD1), also known as estrogenic 17βHSD, is a 34-kilodalton cytosolic reductive SDR enzyme first isolated and cloned from the placenta, where it produces estriol, and is expressed in ovarian granulosa cells, where it produces estradiol.[9,30-32] 17βHSD1 uses NADPH as its cofactor to catalyze reductase activity. It acts as a dimer and only accepts steroid substrates with an aromatic A ring, so that its activity is confined to activating estrogens. The three-dimensional structure of human 17βHSD1 has been determined by x-ray crystallography. No genetic deficiency syndrome for 17βHSD1 has been described.

17βHSD2 is a microsomal oxidase that uses NAD+ to inactivate both estradiol to estrone and testosterone to Δ^4 androstenedione. 17βHSD2 is found in the placenta, liver, small intestine, prostate, secretory endometrium, and ovary. In contrast to 17βHSD1, which is found in placental syncytiotrophoblast cells, 17βHSD2 is expressed in endothelial cells of placental intravillous vessels, consistent with its apparent role in defending the fetal circulation from transplacental passage of maternal estradiol or testosterone.[9,30-32] No deficiency state for 17βHSD2 has been reported.

17βHSD3, the androgenic form of 17βHSD, is a microsomal enzyme that is apparently expressed only in the testis. This is the enzyme that is disordered in the classic syndrome of male pseudohermaphroditism that is often termed 17-ketosteroid reductase deficiency.[9,30-32]

An enzyme termed 17βHSD4 was initially identified as an NAD+-dependent oxidase with activities similar to 17βHSD2, but this peroxisomal protein is primarily an enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase.[9] Deficiency of 17βHSD4 causes a form of Zellweger syndrome, in which bile acid biosynthesis is disturbed but steroidogenesis is not.

17βHSD5, originally cloned as a 3α-hydroxysteroid dehydrogenase, is an AKR enzyme (in contrast to 17βHSD types 1-4, which are SDR enzymes) termed AKR1C3 that catalyzes the reduction of Δ^4 androstenedione to testosterone.[9,33] The 17βHSD activity of 17βHSD5 is quite labile in vitro, and this enzyme catalyzes different activities under different conditions, which has led to confusion in its role in steroidogenesis.[33] The adrenal zona reticularis expresses this enzyme at low levels, accounting for the small amount of testosterone produced by the adrenal.[34]

Steroid Sulfotransferase and Sulfatase

Steroid sulfates may be synthesized directly from cholesterol sulfate or may be formed by sulfation of steroids by cytosolic sulfotransferase (SULT) enzymes.[35,36] At least 44 distinct isoforms of these enzymes have been identified, belonging to five families of SULT genes; many of these genes yield alternately spliced products accounting for the large number of enzymes. The SULT enzymes that sulfonate steroids include SULT1E (estrogens), SULT2A1 (nonaromatic steroids), and SULT2B1 (sterols). SULT2A1 is the principal sulfotransferase expressed in the adrenal, where it sulfates the 3β hydroxyl group of Δ^5 steroids (pregnenolone, 17OH-pregnenolone, DHEA, androsterone) but not of cholesterol. SULT2B1a will also sulfonate pregnenolone but not cholesterol, whereas cholesterol is the principal substrate for SULT2B1b in the skin, liver, and elsewhere. It is not clear whether most steroid sulfates are simply inactivated forms of steroid or if they serve specific hormonal roles. Knockout of the mouse SULT1E1 gene is associated with elevated estrogen levels, increased expression of tissue factor in the placenta, and increased platelet activation, leading to placental thrombi and fetal loss that could be ameliorated by anticoagulant therapy. Mutations ablating the function of human SULT enzymes have not been described, but single nucleotide polymorphisms that alter the amino acid sequences and catalytic activity affecting drug activity are well described. African Americans have a high rate of polymorphisms in SULT2A1 apparently influencing plasma ratios of DHEA:DHEAS, which may correlate with risk of prostatic and other cancers.

To catalyze sulfation, SULT enzymes must receive sulfate in the form of 3'-phosphoadenosine-5'-phosphosulfate (PAPS). The production of PAPS requires PAPS synthase (PAPSS), which first converts ATP and sulfate (SO_4) to adenosine phosphosulfate (APS), and then uses phosphate from another ATP molecule to convert APS to PAPS[36]. There are two human PAPSS genes: *PAPSS1* is expressed ubiquitously, and *PAPSS2* is abundantly expressed in adrenal and liver, where DHEA is sulfated[36]. PAPSS2 deficiency prevents DHEA sulfation so that the adrenal produces more non-sulfated DHEA than normal; this DHEA is converted to excess androgens. Compound heterozygous PAPSS2 deficiency was reported in a 6-year-old girl with premature pubarche and advanced bone age; by age 13 she had acne, hirsutism, and secondary amenorrhea[37]. Her serum concentrations of DHEA were high, DHEAS was very low, and androstendione, testosterone, and DHT were increased. She also had short stature and abnormal bone development, consistent with a role for PAPSS2 in cartilage and bone formation; complete PAPSS2 deficiency causes spondyloepimetaphyseal dysplasia.

Steroid sulfates may also be hydrolyzed to the native steroid-by-steroid sulfatase. Deletions in the steroid sulfatase gene on chromosome Xp22.3 cause X-linked ichthyosis. The fact that males have a single copy of this gene probably accounts for males having higher DHEAS levels than females of the same age. In the fetal adrenal

and placenta, diminished or absent sulfatase deficiency reduces the pool of free DHEA available for placental conversion to estrogen, resulting in low concentrations of estriol in the maternal blood and urine. The accumulation of steroid sulfates in the stratum corneum of the skin causes the ichthyosis.

Aromatase: P450aro

Estrogens are produced by the aromatization of androgens, including adrenal androgens, by a complex series of reactions catalyzed by a single microsomal aromatase, P450aro.[38,39] This typical cytochrome P450 is encoded by a single gene (*CYP19A1*) on chromosome 15q21.1. This gene uses several different promoter sequences, transcriptional start sites, and alternatively chosen first exons to encode aromatase mRNA in different tissues under different hormonal regulation. Aromatase expression in extraglandular tissues, especially adipose tissue, can convert adrenal androgens to estrogens. Aromatase in the epiphyses of growing bone converts testosterone to estradiol. The tall stature, delayed epiphyseal maturation, and osteopenia of males with aromatase deficiency, and their rapid reversal with estrogen replacement indicate that estrogen, not androgen, is responsible for epiphyseal maturation in males. Although it has traditionally been thought that aromatase activity is needed for embryonic and fetal development, infants and adults with genetic disorders in this enzyme have been described, showing that fetoplacental estrogen is not needed for normal fetal development.[39]

5α-Reductase

Testosterone is converted to the more potent androgen, dihydrotestosterone, by 5α-reductase, an enzyme found in testosterone's target tissues. There are two distinct forms of 5α-reductase. The type 1 enzyme, found in the scalp and other peripheral tissues, is encoded by a gene (*SRD5A1*) on chromosome 5; the type 2 enzyme, the predominant form found in male reproductive tissues, is encoded by a structurally related gene (*SRD5A2*) on chromosome 2p23.[40] The syndrome of 5α-reductase deficiency, a disorder of male sexual differentiation, is due to a wide variety of mutations in the gene encoding the type 2 enzyme.[41] The type 1 and 2 genes show an unusual pattern of developmental regulation of expression. The type 1 gene is not expressed in the fetus, then is expressed briefly in the skin of the newborn, and then remains unexpressed until its activity and protein are again found after puberty. The type 2 gene is expressed in fetal genital skin, in the normal prostate, and in prostatic hyperplasia and adenocarcinoma. Thus, the type 1 enzyme may be responsible for the pubertal virilization seen in patients with classic 5α-reductase deficiency, and the type 2 enzyme may be involved in male pattern baldness.[40,41]

11β-Hydroxysteroid Dehydrogenase

Although certain steroids are typically categorized as glucocorticoids or mineralocorticoids, the "mineralocorticoid" (glucocorticoid type 2) receptor has equal affinity for both aldosterone and cortisol. Nevertheless, cortisol does not act as a mineralocorticoid in vivo, even though cortisol concentrations can exceed aldosterone concentrations by 100- to 1000-fold, because mineralocorticoid responsive tissues (such as the kidney) convert cortisol to cortisone, a metabolically inactive steroid. The interconversion of cortisol and cortisone is mediated by two isozymes of 11β-hydroxysteroid dehydrogenase (11βHSD, HSD11B), each of which can catalyze both oxidase and reductase activity, depending on the cofactor available (NADP+ or NADPH).[42-45] The ratio of NADP+ to NADPH is regulated by hexose-6-phosphate dehydrogenase (H6PDH).[46] The type 1 enzyme (11βHSD1; HSD11B1) is expressed mainly in glucocorticoid-responsive tissues such as the liver, testis, lung, and proximal convoluted tubule. 11βHSD1 can catalyze both the oxidation of cortisol to cortisone using NADP+ as its cofactor (K_m 1.6 μM), or the reduction of cortisone to cortisol using NADPH as its cofactor (K_m 0.14 μM); the reaction catalyzed depends on which cofactor is available, but the enzyme can only function with high (micromolar) concentrations of steroid. 11βHSD2 (HSD11B2) catalyzes only the oxidation of cortisol to cortisone using NADH and can function with low (nanomolar) concentrations of steroid (K_m 10 to 100 nM). 11βHSD2 is expressed in mineralocorticoid-responsive tissues and thus serves to "defend" the mineralocorticoid receptor by inactivating cortisol to cortisone, so that only "true" mineralocorticoids, such as aldosterone or deoxycorticosterone, can exert a mineralocorticoid effect. Thus, 11βHSD2 prevents cortisol from overwhelming renal mineralocorticoid receptors, and in the placenta and other fetal tissues 11βHSD2 also inactivates cortisol. The placenta also has abundant NADP+ favoring the oxidative action of 11βHSD1, so that in placenta both enzymes protect the fetus from high maternal concentrations of cortisol, but not from maternally administered betamethasone or dexamethasone. 11βHSD1 is located on the luminal side of the endoplasmic reticulum, and hence is not in contact with the cytoplasm. In this unusual cellular location, 11βHSD1 receives NADPH provided by H6PDH.[44] This links 11βHSD1 to the pentose monophosphate shunt, providing a direct paracrine link between local glucocorticoid production and energy storage as fat.[43-45]

3α-Hydroxysteroid Dehydrogenases

The 3α-hydroxysteroid dehydrogenases (3αHSDs) are not familiar to most endocrinologists, but they are clinically important because of the discovery of the so-called backdoor pathway of steroidogenesis.[47] This remarkable pathway (Figure 13-6), first discovered as the mechanism by which the male marsupial fetal testis makes androgens,[47,48] plays a central role in human male sexual differentiation.[49] In this pathway 17OHP is converted to dihydrotestosterone without going through DHEA, androstenedione, or testosterone, and hence it provides a mechanism for 17OHP to contribute to the virilization of female fetuses with 21-hydroxylase deficiency.[22,50] There are four 3αHSD enzymes, sometimes termed types 1 through 4 but more properly termed AKR1C4-1[31]; the numbering of the 3αHSD nomenclature is reversed in the AKR1C nomenclature, which is confusing and most unfortunate. These enzymes are structurally very similar, are

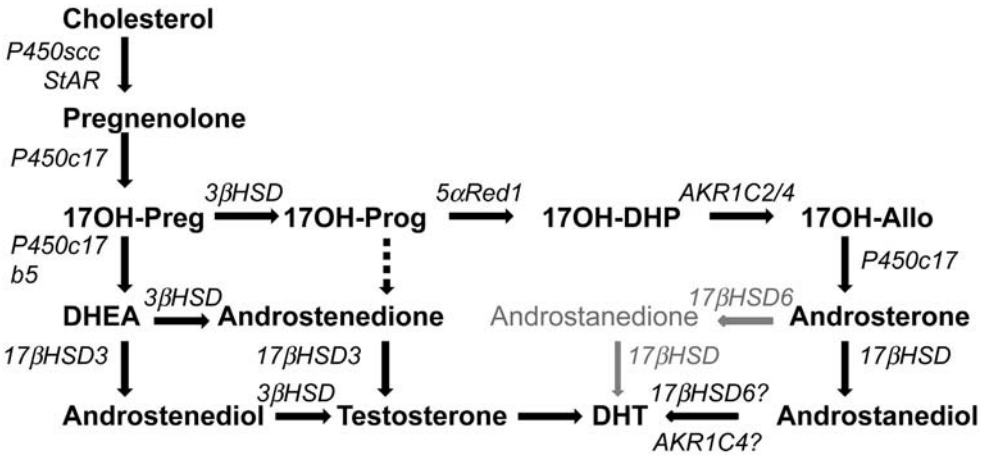


FIGURE 13-6 ■ Pathways to androgens in CAH. In the absence of P450c21 activity, the adrenal can produce androgens by three pathways. First, the pathway from cholesterol to DHEA remains intact in 21-hydroxylase deficiency, and increased production of DHEA will lead to some DHEA being converted to androstenedione and thence to testosterone. Second, although minimal amounts of 17OHP are converted to androstenedione in the normal adrenal, the huge amounts of 17OHP produced in CAH permit some 17OHP to be converted to androstenedione and then to testosterone. Third, the so-called backdoor pathway depends on the 5α and 3α reduction of 17OHP to 17OH-allopregnenolone. This steroid is readily converted to androstanediol, which can then be oxidized to DHT by a 3αHSD enzyme, AKR1C2. Mass spectrometric examinations of human urinary steroids indicate this pathway is a major contributor in CAH.

encoded by a gene cluster on chromosome 10p14-15, and catalyze a wide array of steroidal conversions and other reactions.[9] The backdoor pathway is characterized by both reductive and oxidative 3αHSD activities; the reductive activity can apparently be catalyzed by either AKR1C2 or AKR1C4.[49] The nature of the oxidative activity remains uncertain, but may be catalyzed by retinol dehydrogenase (RoDH).[9] AKR1C3, which converts androstenedione to testosterone in the adrenal, is also known as 17βHSD5. Further studies of the role of the backdoor pathway will be central to pediatric endocrinology.

Fetal Adrenal Steroidogenesis

Adrenocortical steroidogenesis begins early in embryonic life, around 7 weeks after fertilization. Steroidogenic enzymes are immunocytochemically detected principally in the fetal zone at 50 to 52 days postconception, and by 8 weeks postconception the adrenal contains cortisol and responds to ACTH in primary culture systems.[51] This cortisol synthesis is under the regulation of pituitary ACTH and involves transient expression of adrenal 3βHSD2; following the 9th week postconception, expression of 3βHSD2 and synthesis of cortisol wane; 3βHSD2 is barely detectable at 10 to 11 weeks and is absent at 14 weeks. At the same time, the fetal adrenal also produces 17βHSD5,[51] which can convert androstenedione to testosterone. Thus, the fetal adrenal makes cortisol at the same time during gestation that fetal testicular testosterone is virilizing the genitalia of the normal male fetus. This fetal adrenal cortisol apparently suppresses ACTH, which otherwise would drive adrenal testosterone synthesis via 17βHSD5.

Fetuses affected with genetic lesions in adrenal steroidogenesis can produce sufficient adrenal androgen to virilize a female fetus to a nearly male appearance, and this masculinization of the genitalia is complete by the 12th week of gestation. The definitive zone of the fetal adrenal produces steroid hormones according to the pathways in Figure 13-3. By contrast, the large fetal zone of the adrenal is relatively deficient in 3βHSD2 activity after 12 weeks. The fetal adrenal has relatively abundant 17,20 lyase activity of P450c17; low 3βHSD and high 17,20 lyase activity account for the abundant production of dehydroepiandrosterone (DHEA) and its sulfate (DHEAS) by the fetal adrenal, which are converted to estrogens by the placenta (Figure 13-7). The fetal adrenal also has considerable sulfotransferase activity but little steroid sulfatase activity, also favoring conversion of DHEA to DHEAS. The resulting DHEAS cannot be a substrate for adrenal 3βHSD2; instead, it is secreted, 16α-hydroxylated in the fetal liver, and then acted on by placental 3βHSD1, 17βHSD1, and P450aro to produce estriol, or the substrates can bypass the liver to yield estrone and estradiol. Placental estrogens inhibit adrenal 3βHSD activity, providing a feedback system to promote production of DHEAS. Fetal adrenal steroids account for 50% of the estrone and estradiol and 90% of the estriol in the maternal circulation.

Although the fetoplacental unit produces huge amounts of DHEA, DHEAS, and estriol, as well as other steroids, they do not appear to serve an essential role.[52] Successful pregnancy is wholly dependent on placental synthesis of progesterone, which suppresses uterine contractility and prevents spontaneous abortion; however, fetuses with genetic disorders of adrenal and gonadal steroidogenesis develop normally, reach term gestation, and undergo normal parturition and delivery. Mineralocorticoid production is only required postnatally, estrogens are not required, and androgens are only needed for male sexual differentiation. It appears that human fetal glucocorticoids are needed at about 8 to 12 weeks,[51] but it is not clear that they are needed thereafter; if they are, the small amount of maternal cortisol that escapes placental inactivation suffices. A single newborn has been described with profound glucocorticoid resistance who was homozygous for a frameshift mutation at codon

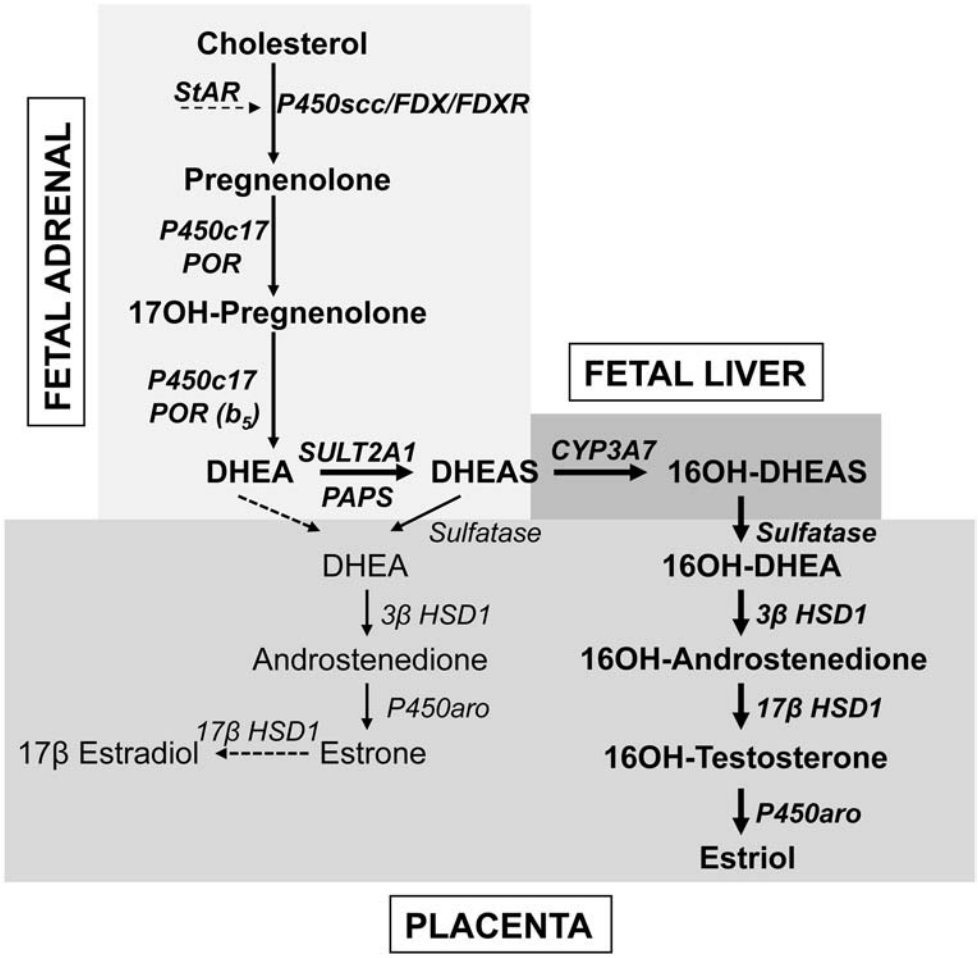


FIGURE 13-7 ■ Steroid synthesis by the fetoplacental system. The fetal adrenal has minimal 3βHSD activity, hence the pathway from cholesterol to DHEA predominates. Most DHEA is converted to DHEAS by the sulfotransferase SULT2A1 and is then 16α-hydroxylated by CYP3A7 in the fetal liver. The 16α-hydroxy DHEAS reaches the placenta, where the sequential action of steroid sulfatase, 3βHSD1, 17βHSD1, and aromatase (P450aro) yield estriol, the principal steroidal product of the placenta. Small amounts of DHEA reach the placenta without being 16α-hydroxylated, where 3βHSD1 and P450aro can convert it to estrone; small amounts of estrone may also be converted to estradiol. About 80% of placental estrogen is estriol, 15% is estrone, and only 5% is estradiol.

772 in the glucocorticoid-binding domain of the glucocorticoid receptor.[53] Although the infant had severe hypoglycemia and hypertension postnatally, pulmonary and other aspects of fetal development were normal, suggesting that glucocorticoid action is not required for normal human fetal development.

The regulation of steroidogenesis and growth of the fetal adrenal are not fully understood, but both are related to ACTH. ACTH effectively stimulates steroidogenesis by fetal adrenal cells in vitro, and excess ACTH is clearly involved in the adrenal growth and overproduction of androgens in fetuses affected with congenital adrenal hyperplasia. Experimental prenatal treatment of such fetuses by administering pharmacologic doses of dexamethasone to the mother at 6 to 10 weeks' gestation can significantly reduce fetal adrenal androgen production and thus reduce the virilization of female fetuses, indicating that the hypothalamic-pituitary-adrenal (HPA) axis functions very early in fetal life.[22] By contrast, anencephalic fetuses lacking pituitary ACTH have adrenals that contain a fairly normal complement of steroidogenic enzymes and retain their capacity for steroidogenesis. Thus, fetal adrenal steroidogenesis may be regulated by both ACTH-dependent and ACTH-independent mechanisms.

REGULATION OF STEROIDOGENESIS

The Hypothalamic-Pituitary-Adrenal Axis

Hypothalamus: CRF and AVP

The principal steroidal product of the human adrenal is cortisol, which is mainly secreted in response to adrenocorticotropic hormone (ACTH, corticotropin) produced in the pituitary; secretion of ACTH is stimulated primarily by corticotropin-releasing factor (CRF) from the hypothalamus. Hypothalamic CRF is a 41-amino-acid peptide synthesized mainly by neurons in the paraventricular nucleus. These same hypothalamic neurons also produce the decapeptide arginine vasopressin (AVP, also known as antidiuretic hormone or ADH).[54] Both CRF and AVP are proteolytically derived from larger precursors with the AVP precursor containing the sequence for neurophysin, which is the AVP-binding protein. CRF

and AVP travel through axons to the median eminence, which releases them into the pituitary portal circulation, although most AVP axons terminate in the posterior pituitary. AVP is co-secreted with CRF in response to stress, and both CRF and AVP stimulate the synthesis and release of ACTH, but they appear to do so by different mechanisms. CRF binds to a G-protein–coupled receptor on the membranes of pituitary corticotropes and activates adenylyl cyclase, increasing cAMP, which activates the protein kinase A (PKA) signaling pathway. PKA triggers ACTH secretion by concerted regulation of cellular potassium and calcium fluxes and enhances POMC gene transcription. AVP binds to its G-protein-coupled receptor and activates phospholipase C, which leads to the release of intracellular Ca++ and to the activation of protein kinase C (PKC). AVP seems to amplify the effects of CRF on ACTH secretion without affecting synthesis. However, CRF is the more important physiologic stimulator of ACTH release, although maximal doses of AVP can elicit a maximal ACTH response. When given together, CRF and AVP act synergistically, as would be expected from their independent mechanisms of action.

Pituitary: ACTH and POMC

Pituitary ACTH is a 39-amino-acid peptide derived from proopiomelanocortin (POMC), a 241-amino-acid protein.[55] POMC undergoes a series of proteolytic cleavages, yielding several biologically active peptides (Figure 13-8). The N-terminal glycopeptide (POMC 1-75) can stimulate steroidogenesis and may function as an adrenal mitogen. POMC 112-150 is ACTH 1-39, POMC 112-126 and POMC 191-207 constitute α- and β-MSH (melanocyte stimulating hormone), respectively, and POMC 210-241 is β-endorphin. POMC is also produced in small amounts by the brain, testis, liver, kidney, and placenta, but this extrapituitary POMC does not contribute significantly to circulating ACTH. Malignant tumors will commonly produce "ectopic ACTH" in adults and rarely in children; this ACTH derives from ectopic biosynthesis of the same POMC precursor. Only the first 20 to 24 amino acids of ACTH are needed for its full biologic activity, and synthetic ACTH 1-24 is widely used in diagnostic tests of adrenal function. However, these shorter forms of ACTH have a shorter half-life than does native ACTH 1-39. POMC gene transcription is stimulated by CRF and inhibited by glucocorticoids.[55]

Actions of ACTH

ACTH stimulates the G-protein–coupled melanocortin 2 receptor (MC2R), which is located almost exclusively in the adrenal cortex. Activation of MC2R triggers the production of cAMP, activating PKA that catalyzes the phosphorylation of many proteins involved in steroidogenesis, thereby modifying their activity. ACTH elicits both acute and long-term effects. ACTH stimulates the biosynthesis of LDL receptors and the uptake of LDL, which provides most of the cholesterol used for steroidogenesis, and stimulates transcription of the gene for HMG-CoA reductase, the rate-limiting step in cholesterol biosynthesis, but adrenal biosynthesis of cholesterol is quantitatively much less important than the uptake of LDL cholesterol.[10] Cholesterol is stored in steroidogenic tissues as cholesterol esters in lipid droplets. ACTH stimulates the activity of cholesterol esterase while inhibiting cholesterol ester synthetase, thus increasing the intracellular pool of free cholesterol, the substrate for P450scc. Finally, ACTH facilitates transport of cholesterol into mitochondria by stimulating the synthesis and phosphorylation of StAR, thus increasing the flow of free cholesterol into the mitochondria.[10] All of these actions are mediated by cAMP and occur within minutes, constituting the "acute" effect of ACTH on steroidogenesis.[14] The adrenal contains relatively modest amounts of steroid hormones; thus, release of preformed cortisol does not contribute significantly to the acute response to ACTH; acute responses occur by the rapid provision of large supplies of cholesterol to mitochondrial P450scc.[10,14]

The long-term "chronic" effects of ACTH are mediated directly at the level of the steroidogenic enzymes. ACTH via cAMP stimulates the accumulation of the

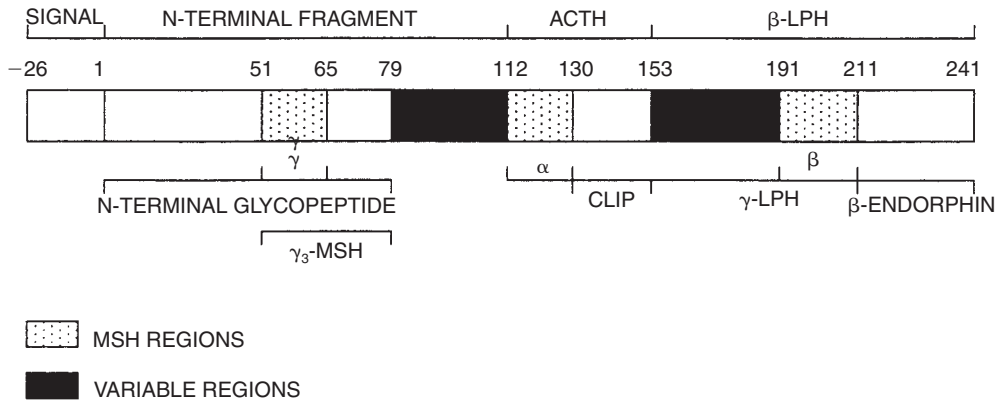


FIGURE 13-8 ■ Structure of human prepro-opiomelanocortin. The numbers refer to amino acid positions, with No. 1 assigned to the first amino acid of POMC after the 26-amino-acid signal peptide. The α-, β-, and γ-MSH regions, which characterize the three "constant" regions, are indicated by stippling; the "variable" regions are *solid.* The amino acid numbers shown refer to the N terminal amino acid of each cleavage site; because these amino acids are removed, the numbers do not correspond exactly with the amino acid numbers of the peptides as used in the text. ACTH, adrenocorticotropic hormone; CLIP, corticotropin-like intermediate lobe peptide; LPH, lipotropic hormone; MSH, melanocyte-stimulating hormone.

steroidogenic enzymes and their mRNAs by stimulating the transcription of their genes.[2,9] ACTH also increases adrenal blood flow, increasing the influx of oxygen and metabolic fuel and the delivery of newly secreted hormones to the circulation.[56] Thus, ACTH increases both the uptake of the cholesterol substrate and its conversion to steroidal products. The stimulation of this steroidogenesis occurs at each step in the pathway, not only at the rate-limiting step, P450scc.

The roles of ACTH and other peptides derived from POMC in stimulating growth of the adult adrenal remain uncertain. However, lack of pituitary POMC causes severe adrenal hypoplasia, and chronic ACTH excess causes adrenal hyperplasia. In the fetal adrenal ACTH stimulates the local production of insulin-like growth factor 2, basic fibroblast growth factor, and epidermal growth factor. These, and possibly other factors work together to mediate ACTH-induced growth of the fetal adrenal.[57]

Diurnal Rhythms of ACTH and Cortisol

Plasma concentrations of ACTH and cortisol tend to be high in the morning and low in the evening. Peak ACTH levels are usually seen at 4 to 6 a.m., and peak cortisol levels follow at about 8 a.m. Both ACTH and cortisol are released episodically in pulses every 30 to 120 minutes throughout the day, but the frequency and amplitude of these pulses are much greater in the morning. The basis of this diurnal rhythm is complex and incompletely understood. The hypothalamic content of CRF itself shows a diurnal rhythm with peak content at about 4 a.m. At least four factors appear to play a role in the rhythm of ACTH and cortisol: intrinsic rhythmicity of synthesis and secretion of CRF by the hypothalamus; light/dark cycles; feeding cycles; and inherent rhythmicity in the adrenal, possibly mediated by adrenal innervation. These factors are clearly interdependent and related. Dietary rhythms may play as large a role as light/dark cycles. Animal experiments show that altering the time of feeding can overcome the ACTH/cortisol periodicity established by a light/dark cycle. In normal human subjects, cortisol is released before lunch and supper, but not at these times in persons eating continuously during the day. Thus, glucocorticoids, which increase blood sugar, appear to be released at times of fasting and are inhibited by feeding.

As all parents know, infants do not have a diurnal rhythm of sleep or feeding. Infants acquire such behavioral rhythms in response to their environment long before they acquire a rhythm of ACTH and cortisol. The diurnal rhythms of ACTH and cortisol begin to be established at 6 to 12 months and often are not well established until after 3 years of age. Once the rhythm is well established in the older child or adult, it is changed only with difficulty. When people move to different parts of the world, their ACTH/cortisol rhythms generally take 15 to 20 days to adjust appropriately.

Physical stress (such as major surgery, severe trauma, blood loss, high fever, or serious illness) can increase the secretion of both ACTH and cortisol, but minor surgery and minor illnesses (such as upper respiratory infections) have little effect on ACTH and cortisol secretion.[58,59] Infection, fever, and pyrogens can stimulate the release of cytokines, such as IL-1 and IL-6, which stimulate secretion of CRH, and also stimulate IL-2 and TNF, which stimulate release of ACTH, providing further stimulus to cortisol secretion during inflammation.[60] Conversely, glucocorticoids inhibit cytokine production in the immune system, providing a negative feedback loop. Most psychoactive drugs, such as anticonvulsants, neurotransmitters, and antidepressants, do not affect the diurnal rhythm of ACTH and cortisol, although cyproheptadine (a serotonin antagonist) effectively suppresses ACTH release.

Adrenal: Glucocorticoid Feedback

The hypothalamic-pituitary-adrenal axis is a classic example of an endocrine feedback system. ACTH increases production of cortisol, and cortisol decreases production of ACTH. Cortisol and other glucocorticoids exert feedback inhibition of both CRF and ACTH (and AVP) principally through the glucocorticoid receptor. Like the acute and chronic phases of the action of ACTH on the adrenal, there are acute and chronic phases of the feedback inhibition of ACTH (and presumably CRF). The acute phase, which occurs within minutes, inhibits release of ACTH (and CRF) from secretory granules. With prolonged exposure, glucocorticoids inhibit ACTH synthesis by directly inhibiting the transcription of the gene for POMC (and AVP). Some evidence also suggests that glucocorticoids can directly inhibit steroidogenesis at the level of the adrenal fasciculata cell itself, but this appears to be a physiologically minor component of the regulation of cortisol secretion.

Mineralocorticoid Secretion: The Renin-Angiotensin System

Renin is a serine protease enzyme synthesized primarily by the juxtaglomerular cells of the kidney, but it is also produced in a variety of other tissues, including the glomerulosa cells of the adrenal cortex. The role of adrenally produced renin is not well established; it appears to maintain basal levels of P450c11AS, but it is not known if angiotensin II is involved in this action. Renin is synthesized as a precursor of 406 amino acids that is cleaved to prorenin (386 amino acids) and finally to the 340-amino acid protein found in plasma.[61] Decreased blood pressure, upright posture, sodium depletion, vasodilatory drugs, kallikrein, opiates, and β-adrenergic stimulation all promote release of renin. Renin enzymatically attacks angiotensinogen, the renin substrate, in the circulation. Angiotensinogen is a highly glycosylated protein and, therefore, has a highly variable molecular weight from 50,000 to 100,000 daltons. Renin proteolytically releases the aminoterminal 10 amino acids of angiotensinogen, referred to as angiotensin I. This decapeptide is biologically inactive until converting enzyme, an enzyme found primarily in the lungs and blood vessels, cleaves off its two carboxyterminal amino acids, to produce an octapeptide, termed angiotensin II. Angiotensin II binds to specific membrane receptors located in the zona glomerulosa of the adrenal cortex to stimulate aldosterone production. Angiotensin converting enzyme can be

inhibited by captopril and related agents; alternatively angiotensin II receptors may be blocked by pharmacologic agents such as irbesartan for the diagnosis and treatment of hyperreninemic hypertension.

Angiotensin II has two principal actions, both of which increase blood pressure. It directly stimulates arteriolar vasoconstriction within a few seconds and it stimulates synthesis and secretion of aldosterone within minutes. Increased plasma potassium is also a powerful and direct stimulator of aldosterone synthesis and release. Aldosterone, secreted by the glomerulosa cells of the adrenal cortex, has the greatest mineralocorticoid activity of all naturally occurring steroids. Aldosterone causes renal sodium retention and potassium loss, with a consequent increase in intravascular volume and blood pressure. Expansion of the blood volume provides the negative feedback signal for regulation of renin and aldosterone secretion. Angiotensin II functions through receptors that stimulate production of phosphatidylinositol, mobilize intracellular and extracellular Ca++, and activate PKC.[62] These intracellular second messengers then stimulate transcription of the P450scc gene by means independent of those employed by ACTH and cAMP. Potassium ion increases uptake of Ca++ with consequent hydrolysis of phosphoinositides to increase phosphatidylinositol. Thus, angiotensin II and potassium work at different levels of the same intracellular second messenger pathway, but these differ fundamentally from the action of ACTH.

Although the renin-angiotensin system is clearly the major regulator of mineralocorticoid secretion, ACTH and possibly other POMC-derived peptides such as γ_3-MSH can also promote secretion of aldosterone when used in high concentrations in animal systems, but the relevance of physiologic concentrations in human beings has not been established. Ammonium ion, hyponatremia, dopamine antagonists, and some other agents can also stimulate secretion of aldosterone, and atrial natriuretic factor is a potent physiologic inhibitor of aldosterone secretion.

Adrenal Androgen Secretion and the Regulation of Adrenarche

DHEA, DHEAS, and androstenedione, which are almost exclusively secreted by the adrenal zona reticularis, are generally referred to as adrenal androgens because they can be peripherally converted to testosterone. However, these steroids have little if any capacity to bind to and activate androgen receptors, hence they are only androgen precursors and not true androgens. The fetal adrenal secretes large amounts of DHEA and DHEAS, and these steroids are abundant in the newborn, but their concentrations fall rapidly as the fetal zone of the adrenal involutes following birth. After the first year of life, the adrenals of young children secrete very small amounts of DHEA, DHEAS, and androstenedione until the onset of adrenarche, usually around age 7 to 8 years, preceding the onset of puberty by about 2 years. Adrenarche is independent of puberty, the gonads, or gonadotropins, and the mechanism by which the onset of adrenarche is triggered remains unknown. The secretion of DHEA and DHEAS continues to increase during and after puberty and reaches maximal values in young adulthood, following which there is a slow, gradual decrease in the secretion of these steroids in the elderly ("adrenopause") (Figure 13-9).[63] Men have higher serum concentrations of DHEAS than women,[63] probably because men have a single copy of the X-linked steroid sulfatase gene.[64] Throughout much of adult life, adrenal secretion of DHEAS exceeds that of cortisol; in adult women, adrenal secretion of androgen precursors and androgens is equal to their secretion from the ovary. Despite the huge increases in the adrenal secretion of DHEA and DHEAS during adrenarche, circulating concentrations of ACTH and cortisol do not change with age. Thus, ACTH plays a permissive role in adrenarche but does not trigger it. Searches for hypothetical polypeptide hormones that might specifically stimulate the zona reticularis have been unsuccessful. Adrenarche is a unique phenomenon confined to few higher primates such as chimpanzees or orangutans, but the significance of adrenarche remains unknown.

Studies of adrenarche have focused on the roles of 3βHSD and P450c17. The abundance of 3βHSD in the zona reticularis appears to decrease with the onset of adrenarche, and the adrenal expression of cytochrome b_5, which fosters the 17,20 lyase activity of P450c17,[20,21] is almost exclusively confined to the zona reticularis; these factors strongly favor the production of DHEA.[65]

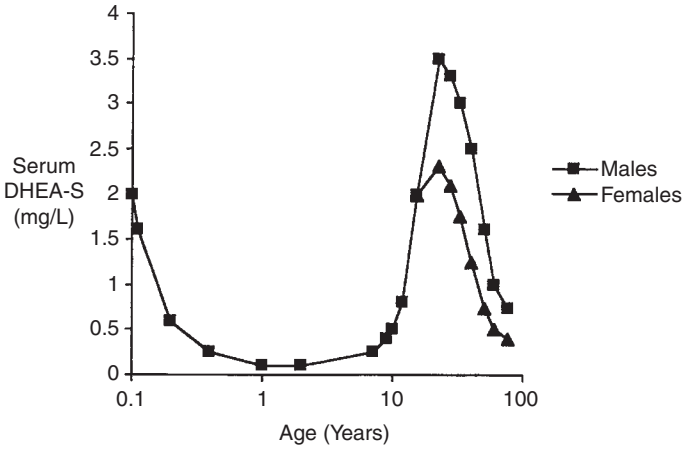


FIGURE 13-9 ■ Concentrations of DHEAS as a function of age. Note that the x-axis is on a log scale.

The phosphorylation of P450c17 also increases 17,20 lyase activity; the responsible kinase has recently been identified as p38α, but its role in adrenarche remains uncertain.[66] Premature and exaggerated adrenarche may be associated with insulin resistance, and girls with premature exaggerated adrenarche appear to be at much higher risk of developing the polycystic ovary syndrome as adults (characterized by hyperandrogenism, fewer ovulatory cycles, insulin resistance, and hypertriglyceridemia). Evidence suggests that infants born small for gestational age may be at increased risk for this syndrome. The suggestion that replacing the DHEA may improve memory and a sense of well-being in the elderly and in adrenal insufficiency remains controversial.[67] Thus, studies of physiology, biochemistry, and clinical correlates of adrenarche are pointing to premature adrenarche as an early sign of a metabolic disorder.

PLASMA STEROIDS AND THEIR DISPOSAL

Structure and Nomenclature

All steroid hormones are derivatives of pregnenolone (Figure 13-10). Pregnenolone and its derivatives that contain 21 carbon atoms are often termed C21 steroids. Each carbon atom is numbered, indicating the location at which the various steroidogenic reactions occur (e.g., 21-hydroxylation, 11-hydroxylation). The 17,20 lyase activity of P450c17 cleaves the bond between carbon atoms 17 and 20, yielding C19 steroids, which include all the androgens; P450aro converts C19 androgens to C18 estrogens. With the exception of estrogens, all steroid hormones have a single unsaturated carbon-carbon double bond. Steroids having this double bond between carbon atoms 4 and 5, including all the principal biologically active steroids, are termed Δ^4 steroids; their precursors having a double bond between carbon atoms 5 and 6 are termed Δ^5 steroids. The two isozymes of 3βHSD convert Δ^5 to Δ^4 steroids.

A rigorous, logically systematic, and unambiguous chemical terminology has been formulated to describe accurately the structure of all the steroid hormones and all their conceivable derivatives. However, this terminology is quite cumbersome (e.g., cortisol is 11β,17α, 21-trihydroxy-pregn-4-ene-3,20-dione, and dexamethasone is 9α-fluoro-11β,17α,21-trihydroxyprena-1,4-diene-3,20-dione). Therefore, we use only the standard "trivial names." Before the structures of the steroid hormones were determined in the 1930s, Reichstein, Kendall, and others identified them as spots on paper chromatograms and designated them A, B, C, and so forth. Unfortunately, some persist in using this outmoded terminology, so that corticosterone is sometimes termed *compound B*, cortisol *compound F*, and 11-deoxycortisol *compound S*. This archaic terminology obfuscates the precursor-product relationships of the steroids, confuses students, and should not be used.

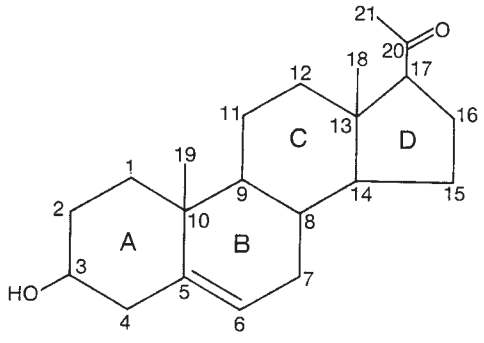


FIGURE 13-10 ■ Structure of pregnenolone. The carbon atoms are indicated by numbers and the rings are designated by letters according to standard convention. Pregnenolone is derived from cholesterol, which has a 6-carbon side chain attached to carbon #21. Pregnenolone is a Δ^5 compound, having a double bond between carbons #5 and 6; the action of 3β-hydroxysteroid dehydrogenase/isomerase moves this double bond from the B ring to carbons #4 and 5 in the A ring, forming Δ^4 compounds. All of the major biologically active steroid hormones are Δ^4 compounds.

Circulating Steroids

Although more than 50 different steroids have been isolated from adrenocortical tissue, the main pathways of adrenal steroidogenesis include only a dozen or so steroids, of which only a few are secreted in sizable quantities. The adult secretions of DHEAS and cortisol are each about 20 mg/24 hours, and the secretion of corticosterone, a weak glucocorticoid, is about 2 mg/24 hours. Although glucocorticoids, such as cortisol, and mineralocorticoids, such as aldosterone, are both needed for life and hence are of "equivalent" physiologic importance, diagrams such as Figure 13-3 fail to indicate that these steroids are not secreted in molar equivalents. The adult secretion rate of aldosterone is only about 0.1 mg/24 hour. This 100- to 1000-fold molar difference in the secretory rates of cortisol and aldosterone must be borne in mind when considering the effects of steroid-binding proteins in plasma and when conceptualizing the physiologic manifestations of incomplete defects in steroidogenesis due to single amino acid changes causing the partial loss of activity of a steroidogenic enzyme.

Most circulating steroids are bound to plasma proteins, including corticosteroid-binding globulin (CBG, also termed transcortin), albumin, and α_1 acid glycoprotein.[68,69] CBG has a very high affinity for cortisol but a relatively low binding capacity, albumin has a low affinity and high capacity, and α_1 acid glycoprotein is intermediate for both variables. The result is that about 90% of circulating cortisol is bound to CBG and a little more is bound to other proteins. These steroid-binding proteins are not transport proteins, as the biologically important steroids are water soluble in physiologically effective concentrations, and absence of CBG does not cause a detectable physiologic disorder. Instead, these plasma proteins act as a reservoir for steroids. This ensures that all peripheral tissues will be bathed in approximately equal concentrations of cortisol, and this greatly diminishes the physiologic effect of the great diurnal variation in cortisol secretion. Most synthetic glucocorticoids used in therapy do not bind significantly to CBG and bind poorly to albumin, partially accounting for their increased potencies, which are also associated with increased receptor-binding affinities. Aldosterone is not bound well by any plasma protein; hence, changes in plasma protein concentration do not affect plasma aldosterone concentrations but greatly influence plasma cortisol

concentrations. Estradiol and testosterone bind strongly to a different plasma protein termed sex steroid-binding globulin and also bind weakly to albumin.

Because steroids are hormones it is often thought that the concentration of "free" (i.e., unbound) circulating steroids determines biologic activity. However, the target tissues for many steroid hormones contain enzymes that modify those steroids. Thus, many actions of testosterone are actually due to dihydrotestosterone produced by local 5α-reductase; cortisol will have differential actions on various tissues due to the presence or absence of the two isozymes of 11βHSD, which can inactivate cortisol to cortisone or reactivate cortisone back to cortisol. Similar peripheral metabolism occurs via "extraglandular" 21-hydroxylase, P450aro, 3βHSD, and 17βHSD. Thus, circulating steroids are both classic hormones and precursors to locally acting autocrine or paracrine factors.

Steroid Catabolism

Only about 1% of circulating plasma cortisol and aldosterone are excreted unchanged in the urine; the liver metabolizes the remainder. A large number of hepatic metabolites of each steroid is produced; most contain additional hydroxyl groups and are linked to a sulfate or glucuronide moiety, rendering them more soluble and readily excretable by the kidney. A great deal is known about the various urinary metabolites of the circulating steroids because their measurement in pooled 24-hour urine samples has been an important means of studying adrenal steroids. The measurement of urinary steroid metabolites by modern mass spectrometric techniques has been a research tool but is beginning to be used by clinical reference laboratories, which will permit increased diagnostic capacities.

CLINICAL AND LABORATORY EVALUATION OF ADRENAL FUNCTION

Clinical Evaluation

Astute clinical evaluation can generally reveal the presence of primary adrenal deficiency or hypersecretion before performing laboratory tests. Thomas Addison described adrenal insufficiency in 1849, long before immunoassays became available. Virtually all patients with chronic adrenal insufficiency will have weakness, fatigue, anorexia, weight loss, hypotension, and hyperpigmentation. Patients with acute adrenal insufficiency may have hypotension, shock, weakness, apathy, confusion, anorexia, nausea, vomiting, dehydration, abdominal or flank pain, hyperthermia, or hypoglycemia. Deficient adrenal androgen secretion will compromise the acquisition of virilizing secondary sexual characteristics (pubic and axillary hair, acne, axillary odor) in female adolescents. Early signs of glucocorticoid excess include increased appetite, weight gain, and growth arrest without a concomitant delay in bone age. Chronic glucocorticoid excess in children results in typical cushingoid facies, but the "buffalo hump" and centripetal distribution of body fat that are characteristic of adult Cushing disease are seen only in long-standing undiagnosed disease. Mineralocorticoid excess is mainly characterized by hypertension, but patients receiving very low sodium diets (e.g., the newborn) will not be hypertensive, as mineralocorticoids increase blood pressure primarily by retaining sodium and thus increasing intravascular volume. Moderate hypersecretion of adrenal androgens is characterized by mild signs of virilization, whereas substantial hypersecretion of adrenal androgens is characterized by accelerated growth with a disproportionate increase in bone age, increased muscle mass, acne, hirsutism, deepening of the voice, and more profound degrees of virilism. A key feature of any physical examination of a virilized male is careful examination and measurement of the testes. Bilaterally enlarged testes suggest true (central) precocious puberty; unilateral testicular enlargement suggests testicular tumor; prepubertal testes in a virilized male indicate an extratesticular source of androgen, such as the adrenal.

Imaging studies are of limited utility in adrenal cortical disease. Computed tomography (CT) will only rarely detect pituitary tumors hypersecreting ACTH, and magnetic resonance imaging (MRI) will detect fewer than half of these, even with gadolinium enhancement. The small size, odd shape, and location near other structures also compromise the use of imaging techniques for the adrenals. Patients with Cushing disease or congenital adrenal hyperplasia will have modestly enlarged adrenals, but such enlargement is not detectable by imaging techniques with any useful degree of certainty. The gross enlargement of the adrenals in congenital lipoid adrenal hyperplasia, their hypoplasia in adrenal hypoplasia congenita or in the hereditary ACTH unresponsiveness syndrome, and many malignant tumors can be usefully diagnosed by imaging studies; however, many adrenal adenomas are too small to be detected. Thus, imaging studies may establish the presence of pituitary or adrenal tumors, but they can never establish their absence.

Laboratory Evaluation

The diagnostic evaluation of adrenal function is essentially chemical. The nonspecificity of many of the clinical signs described previously and the disappointing results with imaging studies remind us that any proper evaluation of hypothalamic-pituitary-adrenal function must rely on a series of carefully performed physiologic maneuvers associated with hormonal assays. The development of highly specific, exquisitely sensitive assays that can be done on small volumes of plasma now permit the direct examination of virtually every hormone involved in adrenal metabolism.

Plasma Concentrations of Cortisol and Other Steroids

Plasma cortisol is measured routinely by a variety of techniques including radioimmunoassay, immunoradiometric assay, and high-pressure liquid chromatography (HPLC). Other procedures, such as fluorimetric assays and competitive protein binding assays, are useful research tools but are not in general clinical use. It is of considerable importance to know what procedure one's laboratory is employing and

precisely what it is measuring. All immunoassays have some degree of cross-reactivity with other steroids. Most cortisol immunoassays will detect both cortisol and cortisone; by contrast, these are readily distinguished by HPLC. As the newborn's plasma contains mainly cortisone rather than cortisol during the first few days of life, a comparison of newborn data obtained by HPLC to published standards obtained by immunoassays may incorrectly suggests adrenal insufficiency. Tables 13-2 and 13-3 summarize the normal plasma concentrations for a variety of steroids. With the notable exception of dehydroepiandrosterone sulfate, most adrenal steroids exhibit a diurnal variation based on the diurnal rhythm of ACTH. Because the stress of illness or hospitalization can increase adrenal steroid secretion and because diurnal rhythms may not be well established in children younger than 3 years of age, it is best

TABLE 13-2 Mean Sex Steroid Concentration in Infants and Children

								T		DHT	
	PROG	17OHP	DHEA	DHEA-S	Δ^4-A	E_1	E_2	M	F	M	F
Cord blood	1100	62	21	6400	3.0	52	30	1.0	0.9	0.2	0.2
Prematures	11	8.1	28	11000	7.0			4.2	0.4	1.0	0.1
Term newborns		1.1	20	4400	5.2			6.9	1.4	0.9	0.3
Infants	1.0	1.0	3.8	820	0.7	<0.1	<0.1	6.6	<0.4	1.4	<0.1
Children 1-6 years			1.0	270	0.9	<0.1	<0.1	0.2		0.1	
6-8 years			3.1	540	0.9	<0.1	<0.1	0.2		0.1	
8-10 years			5.6	1400	0.9	<0.1	<0.1	0.2		0.1	
Males Pubertal stage I	0.6	1.3	5.6	950	0.9	0.0	0.0	0.2		<0.1	
II	0.6	1.6	10	2600	1.6	0.1	0.0	1.4		0.3	
III	0.8	2.0	14	3300	2.4	0.1	0.1	6.6		0.7	
IV	1.1	2.6	14	5400	2.8	0.1	0.1	13		1.2	
V	1.3	3.3	17	6300	3.5	0.1	0.1	19		1.6	
Adult	1.1	3.3	16	7300	4.0	0.1	0.1	22		1.7	
Females Pubertal stage I	0.6	1.0	5.6	1100	0.9	0.1	0.0		0.2		0.1
II	1.0	1.6	11	1900	2.3	0.1	0.1		0.7		0.3
III	1.3	2.3	14	2500	4.2	0.1	0.1		0.9		0.3
IV	9.2	2.9	15	3300	4.5	0.1	0.2		0.9		0.3
V	5.1	3.6	19	4100	6.0	0.2	0.4		1.0		0.3
Adult											
Follicular	1.0	1.5	16	4100	5.8	0.2	0.2		1.0		0.3
Luteal	24	5.4	16	4100	5.8	0.4	0.5		1.0		0.3

PROG, progesterone; 17OHP, 17-hydroxyprogesterone; DHEA, dehydroepiandrosterone; DHEA-S, DHEA sulfate; Δ^4-A, androstenedione; E_1, estrone; E_2, estradiol; T, testosterone; DHT, dihydrotestosterone; M, male; F, female.
All values are in nmol/L; to convert these values to ng/dL, multiply nmol/L by the following values: androstenedione, 28.6; DHEA, 28.8; DHT, 29; E_1, 27; E_2, 27.2; Prog, 31.5; 17OHP, 33.1. To convert DHEA-S to μg/dL, multiply by 0.0368.
Data adapted from Endocrine Sciences, Tarzana, California.

TABLE 13-3 Mean Glucocorticoid and Mineralocorticoid Concentrations

	Cortisol	DOC	Corticosterone	18OH Corticosterone	Aldosterone	Plasma Renin Activity
Cord blood	360	5.5	19		2.4	50
Prematures	180			5.5	2.8	222
Newborns	140		6.6	9.7	2.6	58
Infants	250	0.6	16	2.2	0.8	33
Children (8 a.m.)						
1-2 years	110-550			1.8	0.8	15
2-10 years	As adults	0.3		1.2	0.3→0.8*	8.3
10-15 years	As adults			0.7	0.1→0.6*	3.3
Adults (8 a.m.)	280-550	0.2	12	0.6	0.2→0.4*	2.8→4*
(4 p.m.)	140-280		3.8			

*Two values separated by an arrow indicate those in supine and upright posture.
All values in nmol/L except plasma renin activity (μg/L/s). To convert cortisol to μg/dL, multiply by 0.0363; to convert other values to ng/dL, multiply nmol/L by the following values: corticosterone, 34.7; 18OH corticosterone, 36.2; aldosterone, 36; DOC, 33.1.
DOC, deoxycorticosterone.

to obtain two or more samples for the measurement of any steroid.

As shown in Tables 13-2 and 13-3, data exist for the concentrations of a large number of steroid hormones throughout normal infancy, childhood, and adolescence. Not all endocrine laboratories perform all of these assays, and, depending on the assay procedures employed, various laboratories may have different "normal" values. Most central hospital and commercial laboratories are designed primarily to serve adult, rather than pediatric, patients. Thus, it is important to know whether the available assays will be sufficiently sensitive with small volumes of blood to be useful in measuring pediatric values. This is especially true for the measurement of sex steroids (and gonadotropins), which can exhibit pathologic elevations in children and still remain below the limit of detection of most "adult" assays.

Plasma Renin

Renin (which must not be confused with the digestive enzyme, rennin) is usually assayed by its enzymatic activity, although direct measurements of its concentration are becoming available. Plasma renin activity (PRA) is simply an immunoassay of the amount of angiotensin I generated per milliliter of serum per hour at 37° C. In normal serum, the concentration of both renin and angiotensinogen (the renin substrate) are limiting. Therefore, another test, plasma renin content (PRC), measures the amount of angiotensin I generated in 1 hour at 37° C in the presence of excess concentrations of angiotensinogen.

Plasma renin activity is sensitive to dietary sodium intake, posture, diuretic therapy, activity, and sex steroids. Because PRA values can vary widely with these variables, it is best to measure PRA twice, once in the morning after overnight supine posture and then again after maintenance of upright posture for 4 hours. A simultaneous 24-hour urine for total sodium excretion is generally needed to interpret PRA results. Decreased dietary and urinary sodium, decreased intravascular volume, diuretics, and estrogens will increase PRA. Sodium loading, hyperaldosteronemia, and increased intravascular volume decrease PRA.

The greatest use of renin measurements is in the evaluation of hypertension and in the management of CAH. However, several additional situations require assessment of the renin-angiotensin system. Children with simple virilizing adrenal hyperplasia who do not have clinical evidence of urinary salt wasting (hyponatremia, hyperkalemia, acidosis, hypotension, shock) may nevertheless have increased PRA, especially when dietary sodium is restricted. This was an early clinical sign that this form of 21-hydroxylase deficiency was simply a milder form of the more common, severe, salt-wasting form. Treatment of simple virilizing 21-hydroxylase deficiency with sufficient mineralocorticoid to suppress PRA into the normal range will reduce the child's requirement for glucocorticoids, thus maximizing final adult height. Children with CAH need to have their mineralocorticoid replacement therapy monitored routinely by measuring PRA.[22] Measurement of angiotensin II is also possible in some research laboratories, but most antibodies to angiotensin II strongly cross-react with angiotensin I. Thus, PRA remains the usual way of evaluating the renin-angiotensin-aldosterone system.

Urinary Steroid Excretion

The measurement of 24-hour urinary excretion of steroid metabolites is one of the oldest procedures for assessing adrenal function and still useful. Examination of the total 24-hour excretion of steroids eliminates the fluctuations seen in serum samples as a function of time of day, episodic bursts of ACTH and steroid secretion, and transient stress (such as a visit to the clinic or difficult venipuncture). Collection of a complete 24-hour urinary sample can be quite difficult in the infant or small child. Two consecutive 24-hour collections should be obtained, and each should be assayed for creatinine to monitor the completeness of the collection. Because of the diurnal and episodic nature of steroid secretion, one should never obtain 8- or 12-hour collections and attempt to infer the 24-hour excretory rate from such partial collections.

The analytic procedures for urinary steroid analysis typically rely on a chromatographic procedure for separating steroids followed by a colorimetric, immunologic, or other assay. Failure to employ a chromatographic separation step is the most common source of error. Such classic analyses of urinary steroids are now being replaced by gas chromatography followed by mass spectrometry (GC/MS). Advances in these techniques permit sensitive and specific assays of urinary steroids. However, each secreted steroid is metabolized to multiple forms before being excreted in urine, and this metabolism can vary with age and sex in pediatric populations, so that the analyses are complex and require specialized expertise that is not yet widely available.

Urinary 17-hydroxycorticosteroids (17OHCS), assayed by the colorimetric Porter-Silber reaction, measure 17,21-dihydroxy-20-ketosteroids by the generation of a colored compound after treatment with phenylhydrazine. The reaction is highly specific for the major urinary metabolites of cortisol and cortisone. It will also measure metabolites of 11-deoxycortisol, which will be increased in 11-hydroxylase deficiency or after treatment with metyrapone, a commonly used diagnostic agent (discussed later). Urinary 17OHCS secretion is increased in obesity, hyperthyroidism, and anorexia nervosa; it is decreased in starvation, hypothyroidism, renal failure, liver disease, and pregnancy. Drugs that induce hepatic enzymes, such as phenobarbital, can give low urinary 17OHCS values by stimulating hepatic metabolism of circulating steroids to excreted compounds not detected by the Porter-Silber reaction. Other drugs, including phenothiazines, spironolactone, hydroxyzine, and some antibiotics, can interfere with the colorimetric assay directly, giving falsely elevated values.

Measurement of 17OHCS should be replaced by measurement of urinary free cortisol, thus avoiding the nonspecificity and drug interference problems inherent in 17OHCS. In adults, this test is highly reliable in the diagnosis of Cushing syndrome. Free cortisol is extracted from the urine and measured by immunoassay or HPLC, providing the advantage of specificity. Excretion of urinary free cortisol and of total cortisol metabolites is

closely correlated with age, body surface area, and adiposity, but it is typically 11 ± 5 μg/m²/day.[70,71] Values vary substantially among different reference laboratories, reflecting variations in assay technologies, thus it is essential to utilize a laboratory with good data for normal children. It remains important to measure urinary creatinine to monitor the completeness of the collection.

Urinary 17-ketosteroids (17KS), assayed by the Zimmerman reaction, measure 17-ketosteroids by the generation of a colored compound after treatment with *meta*-dinitrobenzene and acid. The reaction principally measures metabolites of DHEA and DHEA sulfate and thus correlates with adrenal androgen production. Androstenedione will contribute significant 17KS and, if an alkali extraction is not used, estrone will also contribute. The principal androgens, testosterone and dihydrotestosterone, have hydroxyl rather than keto groups on carbon 17; hence, their metabolic products are not measured as 17KS. A wide variety of drugs, including penicillin, nalidixic acid, spironolactone, and phenothiazines, as well as nonspecific urinary chromogens, can spuriously increase values of 17KS. Measurement of urinary 17KS remains a useful, inexpensive screening test, and some clinicians prefer to follow 17KS to monitor therapy of CAH, but measurements of plasma steroids have now replaced the use of urinary 17KS in most centers.

Urinary 17-ketogenic steroids (17KGS) are occasionally confused with urinary 17-ketosteroids because of the similarity of the names; however, 17KGS are used to measure urinary metabolites of glucocorticoids, not sex steroids. Urinary 17KGS are assayed by oxidation of a variety of C-21 steroids to C-19 17-ketosteroids, which are then measured by the Zimmerman reaction as 17KS. All 17OHCS plus a number of other urinary steroids, including the 17KS, are measured, but the basal 17KS values are subtracted out. In addition to all the various problems of specificity and drug interference described earlier for 17OHCS and 17KS, a major disadvantage of 17KGS is that they will also detect pregnanetriol, the principal urinary metabolite of 17-hydroxyprogesterone. This is the steroid that shows the greatest elevations in congenital adrenal hyperplasia. Although some laboratories continue to perform measurements of 17KGS, this obsolete assay no longer has a place in modern pediatric practice.

Plasma ACTH and Other POMC Peptides

Accurate routine immunoassay of plasma ACTH is now available in most centers, but its measurement remains more difficult and variable than the assays for most other pituitary hormones. Handling of the samples must be done with care; samples must be drawn into a plastic syringe containing heparin or ethylenediamine tetraacetic acid (EDTA) and quickly transported in plastic tubes on ice, as ACTH adheres to glass and is quickly inactivated. Thus, elevated plasma ACTH concentrations can be highly informative, but most assays cannot detect low or low-normal values, and such values can be spurious if the samples are handled badly. In adults and older children who have well-established diurnal rhythms of ACTH, normal 8 a.m. values can rarely exceed 50 pg/mL, whereas 8 p.m. values are usually undetectable. Patients with Cushing disease often have normal morning values, but the diagnosis can be suggested by consistently elevated afternoon and evening values; patients with the ectopic ACTH syndrome can have values anywhere from 100 to 1000 pg/mL.

Secretory Rates

The secretory rates of cortisol and aldosterone (or other steroids) can be measured by administering a small dose of tritiated cortisol or aldosterone and measuring the specific activity of one or more known metabolites in a 24-hour urine collection. This procedure permitted measurement of certain steroids, such as aldosterone, before specific immunoassays became available. These procedures have also provided much information about the normal rate of production of various steroids. Based on this procedure most authorities have agreed that children and adults secrete about 6 to 8 mg of cortisol per square meter of body surface area per day.[72,73]

Dexamethasone Suppression Test

Administration of dexamethasone, a potent synthetic glucocorticoid, will suppress secretion of pituitary ACTH and of adrenal cortisol; the dexamethasone suppression test is a very useful procedure for distinguishing whether glucocorticoid excess is due primarily to pituitary disease or adrenal disease. As dexamethasone also suppresses adrenal androgen secretion, this test is useful for distinguishing between adrenal and gonadal sources of sex steroids. A complete, formal dexamethasone suppression test requires the measurement of basal values and those obtained in response to both low- and high-dose dexamethasone. This is described in the section on the evaluation of Cushing syndrome. Variations of this test are commonly used, notably the single 1 mg dose in adults or 0.3 mg/m² in children. This is a useful outpatient screening procedure for distinguishing Cushing syndrome from exogenous obesity. It can be useful for the same purpose in adolescents and older children, but it is otherwise of limited utility in pediatrics. An overnight high-dose dexamethasone suppression test is probably more reliable than the standard 2-day, high-dose test in differentiating adults with Cushing disease from those with the ectopic ACTH syndrome. However, the utility of this test in pediatric patients has not been established.

Stimulation Tests

Direct stimulation of the adrenal with ACTH is a rapid, safe, and easy way to evaluate adrenocortical function. The original ACTH test consisted of a 4- to 6-hour infusion of 0.5 units/kg of ACTH(1-39). This will maximally stimulate adrenal cortisol secretion, and thus effectively distinguishes primary adrenal insufficiency (Addison disease), in which the adrenal is incapable of responding, from secondary adrenal insufficiency due to hypopituitarism. In secondary adrenal insufficiency, some steroidogenic capacity is present, therefore some cortisol is produced in

TABLE 13-4 **Responses of Adrenal Steroids to a 60-Minute ACTH Test**

	Infants		Prepubertal		Pubertal	
	BASAL	STIMULATED	BASAL	STIMULATED	BASAL	STIMULATED
17OH-Pregnenolone	6.8		1.7	9.6	3.6	24
17OHP	0.8	5.8	1.5	5.8	1.8	4.8
DHEA	1.4		2.4	4.3	9.0	19
11-Deoxycortisol	2.3		1.8	5.8	1.7	4.9
Cortisol	280	830	360	830	280	690
DOC	0.6	2.4	0.2	1.7	0.2	1.7
Progesterone	1.1	3.2	1.1	4.0	1.9	4.8

All values are mean values in nmol/L; to convert these values to ng/dL, multiply nmol/L by the following values: 17OH-Pregnenolone, 33.3; 11-deoxycortisol, 34.6; DOC, 33.1 androstenedione, 28.6; DHEA, 28.8; DHT, 29; E_1, 27; E_2, 27.2; Prog, 31.5; 17OHP, 33.1. To convert cortisol to μg/dL, multiply by 0.363.

Data adapted from Endocrine Sciences, Tarzana, California.

response to the ACTH; thus, cortisol secretion is less than normal but greater than the negligible values seen in primary adrenal insufficiency.

The 4- to 6-hour intravenous ACTH test has been replaced in clinical practice by the 60-minute test, wherein a single bolus of ACTH(1-24) is administered intravenously and cortisol and possibly other steroids are measured at 0 and 60 minutes. Normal responses to a 60-minute test are shown in Table 13-4.[74] Synthetic ACTH(1-24) (cosyntropin) is preferred, as it has a more rapid action and shorter half-life than ACTH(1-39). The usual dose is 15 μg/kg in children up to 2 years of age, and 0.25 mg for children older than 2 years and adults. All of these doses are pharmacologic. A very-low-dose (1 μg) test may be useful in assessing adrenal recovery from glucocorticoid suppression. Newer data show that maximal steroidal responses can be achieved after only 30 minutes, but the best available standards are for a 60-minute test. One of the widest uses of intravenous ACTH tests in pediatrics is in diagnosing congenital adrenal hyperplasia (CAH). Stimulating the adrenal with ACTH increases steroidogenesis, resulting in an accumulation of steroids proximal to the disordered enzyme. For example, Figure 13-3 shows that impaired activity of P450c21 (21-hydroxylase) should lead to the accumulation of progesterone and 17-hydroxyprogesterone (17OHP). However, progesterone does not accumulate in appreciable quantities, because it, too, is converted to 17OHP. In routine practice, measuring the response of 17OHP to a 60-minute challenge with intravenous ACTH is the single most powerful and reliable means of diagnosing 21-hydroxylase deficiency; genetic testing can provide a useful confirmation.[22] Comparing the patient's basal to ACTH-stimulated values of 17OHP against those from large numbers of well-studied patients usually permits the discrimination of normal persons, heterozygotes, patients with nonclassic CAH, and patients with classic CAH, although there inevitably is some overlap between groups (Figure 13-11).[22] Measurement of testosterone or Δ^4 androstenedione in response to ACTH can distinguish normal persons from patients with classic CAH, but heterozygotes and patients with cryptic CAH have values overlapping both normals and classic CAH.

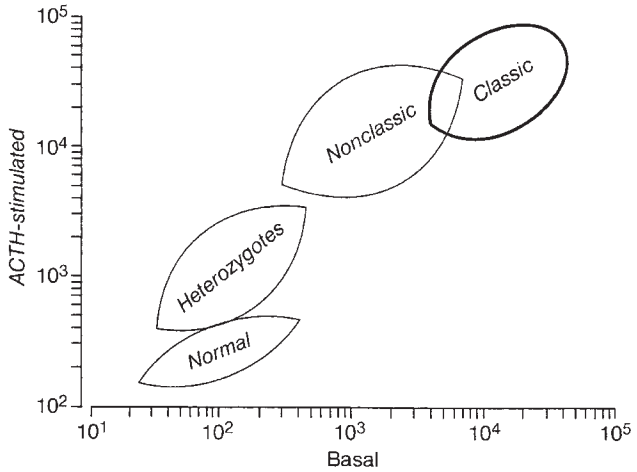


FIGURE 13-11 ■ 17OHP values (in ng/100 mL) before and after stimulation with ACTH in normals, patients with CAH, and heterozygotes.

Longer ACTH tests of up to 3 days have also been employed to evaluate adrenal function. It is important to remember that ACTH has both acute and chronic effects. Thus, short tests measure only the acute effects of ACTH—that is, the maximal stimulation of preexisting steroidogenic machinery. By contrast, a 3-day test will examine the more chronic effects of ACTH to stimulate increased capacity for steroidogenesis by increasing the synthesis of steroidogenic machinery. Few situations exist where a 3-day intramuscular ACTH test is indicated, although it is occasionally useful in diagnosing the rare syndromes of hereditary unresponsiveness to ACTH.[75]

Insulin-induced hypoglycemia is an effective but potentially risky test and hence is now rarely used. Insulin (0.1 U/kg/IV) is administered and blood is obtained at 0, 30, 45, and 60 minutes. The insulin-induced hypoglycemia will stimulate the release of "counter-regulatory" hormones that have actions to increase plasma glucose concentrations: ACTH and cortisol, growth hormone, epinephrine, and glucagon. Because of the inherent risk of convulsions as a result of hypoglycemia, an experienced physician must be in attendance (not merely

"available") throughout the course of the test. Blood glucose must fall to half of the initial value or to 45 mg/dL to achieve an adequate test, and it is wise to terminate the test after this level is reached. Most patients will experience hunger, irritability, diaphoresis, and tachycardia; when these are followed by drowsiness or sleep, blood sugar levels are likely below acceptable limits. If this occurs, a blood sample should be obtained and 2 mL/kg of 20% to 25% glucose given intravenously, to a maximum of 100 mL.

Metyrapone Test

Metyrapone blocks the action of P450c11β and, to a much lesser extent, P450scc. It is thus a chemical means of inducing a transient deficiency of 11-hydroxylase activity, which results in decreased cortisol secretion and subsequent increase in ACTH secretion. Metyrapone testing is done to assess the capacity of the pituitary to produce ACTH in response to a physiologic stimulus. This test is useful in evaluating the hypothalamic-pituitary axis in the presence of central nervous system lesions after neurosurgery or long-term suppression by glucocorticoid therapy.[76] Patients with a previous history of hypothalamic, pituitary, or adrenal disease or those who have been withdrawn from glucocorticoid therapy may be reevaluated with a metyrapone test. A normal response indicates recovery of the HPA axis and predicts that the patient will respond normally to the stress of surgery.

Metyrapone is generally given orally as 300 mg/m^2 every 4 hours for a total of six doses (24 hours). Unlike many other drugs, it is appropriate to continue to increase the dose in older or overweight patients, but the total dose should not exceed 3 g. Blood should be obtained for cortisol, 11-deoxycortisol and ACTH before and after the test, and a 24-hour urine collection should be obtained for 17OHCS before and during the test. In a normal response to metyrapone, cortisol decreases, ACTH increases, and 11-deoxycortisol (the substrate for P450c11β) increases greatly, to about 5 μg/dL. Metabolites of 11-deoxycortisol result in a doubling in urinary 17OHCS excretion. Adults and older children can be tested with the administration of a single oral dose of 30 mg/kg at midnight, given with food to reduce the gastrointestinal irritation. Blood samples are drawn at 8 a.m. the mornings before and after administering the drug.

GENETIC LESIONS IN STEROIDOGENESIS

Autosomal recessive genetic disorders disrupt each of the steps in the pathway shown in Figure 13-3. Most of these result in diminished synthesis of cortisol. In response to adrenal insufficiency, the pituitary synthesizes increased amounts of POMC and ACTH, which promotes increased steroidogenesis; ACTH and possibly other peptides derived from the amino terminal end of POMC also stimulate adrenal hypertrophy and hyperplasia. Thus, the term *congenital adrenal hyperplasia* refers to a group of diseases traditionally grouped together on the basis of the most prominent finding at autopsy.

In theory, the congenital adrenal hyperplasias are easy to understand. A genetic lesion in one of the steroidogenic enzymes interferes with normal steroidogenesis. The signs and symptoms of the disease derive from deficiency of the steroidal end product and the effects of accumulated steroidal precursors proximal to the blocked step. Thus, reference to the pathways shown in Figure 13-3 and knowledge of the biologic effects of each steroid should permit one to deduce the manifestations of the disease.

In practice, the congenital adrenal hyperplasias can be confusing, both clinically and scientifically. The key clinical, laboratory, and therapeutic features of each form of CAH are summarized in Table 13-5. Because each steroidogenic enzyme has multiple activities and many extra-adrenal tissues contain enzymes that have similar activities, the complete elimination of a specific adrenal enzyme may not result in the complete elimination of its steroidal products from the circulation. Furthermore, "partial deficiencies" in which some enzymatic activity remains are now recognized frequently, typically causing disease with later onset and milder clinical manifestations. The cloning of the genes for the steroidogenic enzymes has now permitted the direct study of these diseases, permitting an accurate understanding of their disordered physiology.

Congenital Lipoid Adrenal Hyperplasia

Lipoid CAH is the most severe genetic disorder of steroid hormone synthesis. This disorder is characterized by the absence of significant concentrations of all steroids, high basal ACTH and plasma renin activity, an absent steroidal response to long-term treatment with high doses of ACTH or hCG, and grossly enlarged adrenals laden with cholesterol and cholesterol esters.[77] These findings indicate a lesion in the first step in steroidogenesis—the conversion of cholesterol to pregnenolone. It was initially thought that the lesion was in an enzyme involved in this conversion, and, before the role of P450scc was understood, lipoid CAH was misnamed 20,22-desmolase deficiency. However, the gene for P450scc is normal in these patients, as are the mRNAs for adrenodoxin reductase and adrenodoxin.[77] Furthermore, placental steroidogenesis persists in lipoid CAH, permitting normal term gestation. The normal P450scc system plus the accumulation of cholesterol esters in the affected adrenal suggested that the lesion lay in an upstream factor involved in cholesterol transport into mitochondria. This factor was identified by cell biologic studies, cloned and named the steroidogenic regulatory protein (StAR)[13] and quickly found to be expressed in the adrenal and gonad, but not in the placenta, and then identified as the disordered step in lipoid CAH.[15,16]

Lipoid CAH is a StAR gene knockout experiment of nature, revealing the complex physiology of the StAR protein.[77] StAR promotes steroidogenesis by increasing the movement of cholesterol into mitochondria, but in the absence of StAR steroidogenic cells make steroids at about 14% of the StAR-induced level.[9,10,17] This observation led to the two-hit model of lipoid CAH[16] (Figure 13-12). The first hit is the loss of StAR itself,

TABLE 13-5 **Clinical and Laboratory Findings in the Congenital Adrenal Hyperplasias**

Enzyme Deficiency	Presentation	Laboratory Findings	Therapeutic Measures
Lipoid CAH (StAR or P450scc)	Salt-wasting crisis Male pseudohermaphroditism	Low/absent levels of all steroid hormones Decreased/absent response to ACTH Decreased/absent response to hCG in male pseudohermaphroditism ↑ ACTH and PRA	Glucocorticoid and mineralocorticoid replacement, salt supplementation Estrogen replacement at age > 12 years Gonadectomy of male pseudohermaphrodite and salt supplementation
3β-HSD	Salt-wasting crisis Male and female pseudohermaphroditism	↑ Δ^5 steroids before and after ACTH ↑ Δ^5/Δ^4 serum steroids Suppression of elevated adrenal steroids after glucocorticoid administration ↑ ACTH and PRA	Glucocorticoid and mineralocorticoid replacement Salt supplementation Surgical correction of genitalia Sex hormone replacement as necessary
P450c21	*Classic form:* Salt-wasting crisis Female pseudohermaphroditism Pre- and postnatal virilization *Nonclassic form:* Premature adrenarche, menstrual irregularity, hirsutism, acne, infertility	↑ 17OHP before and after ACTH ↑ Serum androgens and urine 17KS Suppression of elevated adrenal steroids after glucocorticoid Rx ↑ ACTH and PRA	Glucocorticoid and mineralocorticoid replacement Salt supplementation Surgical repair of female pseudohermaphroditism
P450c11β	Female pseudo-hermaphroditism Postnatal virilization in males and females	↑ 11-Deoxycortisol and DOC before and after ACTH ↑ Serum androgens and urine 17KS Suppression of elevated steroids after glucocorticoid administration ↑ ACTH and ↓ PRA Hypokalemia	Glucocorticoid administration Surgical repair of female pseudohermaphroditism
P450c11AS	Failure to thrive Weakness Salt loss	Hyponatremia, hyperkalemia ↑ Corticosterone ↓ Aldosterone ↑ PRA	Mineralocorticoid replacement Salt supplementation
P450c17	Male pseudohermaphroditism Sexual infantilism Hypertension	↑ DOC, 18-OHDOC, corticosterone, 18-hydroxycorticosterone Low 17α-hydroxylated steroids and poor response to ACTH Poor response to hCG in male pseudohermaphroditism Suppression of elevated adrenal steroids after glucocorticoid administration ↑ ACTH and ↓ PRA Hypokalemia	Glucocorticoid administration Surgical correction of genitalia and sex steroid replacement in male pseudohermaphroditism consistent with sex of rearing Estrogen replacement in female at >12 years Testosterone replacement if reared as male (rare)
POR	Male and female pseudohermaphroditism Antley-Bixler syndrome Infertility in adults	↑ ACTH, Prog, 17OHP ↓ DHEA, Andro, T Normal electrolytes	Glucocorticoid and sex steroid replacement Surgical correction of skeletal anomalies

ACTH, adrenocorticotropic hormone (corticotropin); DOC, deoxycorticosterone; hCG, human chorionic gonadotropin; PRA, plasma renin activity; 17OHP, 17-hydroxyprogesterone; 17KS, 17-ketosteroids; 18-OHDOC, 18-hydroxy deoxycorticosterone.

leading to a loss of most, but not all, steroidogenesis, leading to a compensatory rise in ACTH and LH. These hormones increase cellular cAMP, which increases biosynthesis of LDL receptors, their consequent uptake of LDL cholesterol, and de novo synthesis of cholesterol. In the absence of StAR, this increased intracellular cholesterol accumulates as in a storage disease, causing the second hit, which is the mitochondrial and cellular damage caused by the accumulated cholesterol, cholesterol esters, and their auto-oxidation products.[9,10,16]

The two-hit model explains the unusual clinical findings in lipoid CAH. In the fetal testis, which normally makes large amounts of testosterone in fetal life, the Leydig cells are destroyed early in gestation, eliminating testosterone biosynthesis; hence, an affected 46,XY fetus does not undergo normal virilization and is born with female external genitalia and a blind vaginal pouch. However, Wolffian duct derivatives are well developed, indicating the presence of some testosterone synthesis early in fetal life, as predicted by the two-hit model. The undamaged Sertoli cells produce Mullerian inhibitory hormone, so that the phenotypically female 46,XY fetus has no cervix, uterus, or fallopian tubes. The steroidogenically active fetal zone of the adrenal is similarly

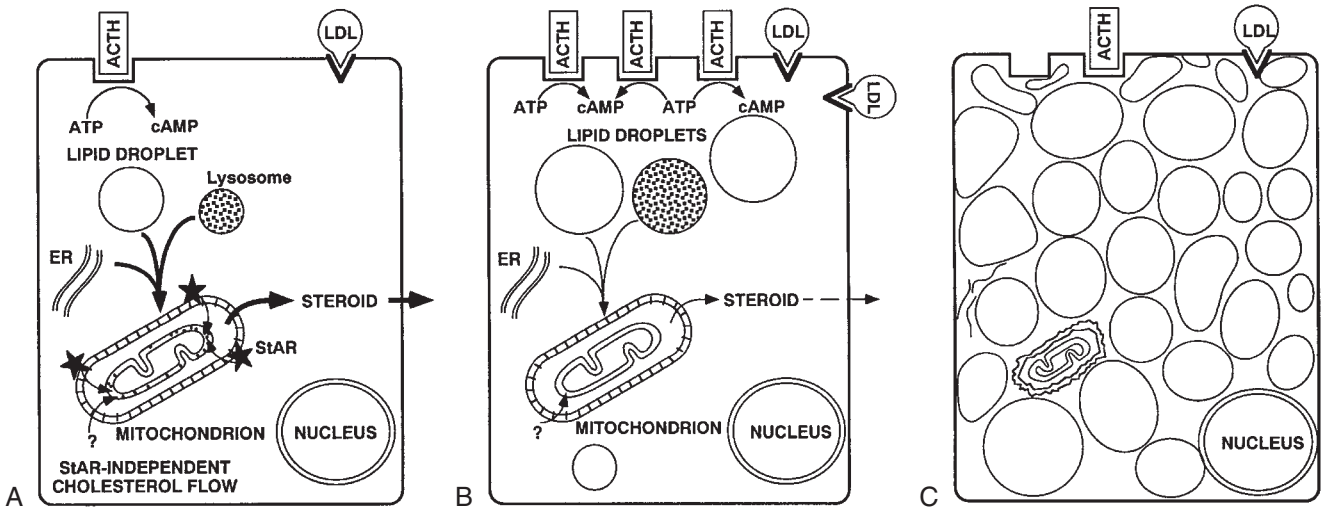


FIGURE 13-12 ■ Two-hit model of lipoid CAH. **A,** In a normal adrenal cell, cholesterol is primarily derived from LDL by receptor-mediated endocytosis and is processed in lysosomes before entering the cellular pool, but cholesterol can also be synthesized de novo from acetyl CoA. Cholesterol from both sources is stored as cholesterol esters in lipid droplets. Cholesterol reaches the mitochondria by poorly defined processes, then travels from the outer to inner mitochondrial membrane by both StAR-dependent and StAR-independent mechanisms. **B,** In early lipoid CAH, the absence of StAR reduces cholesterol flow and steroidogenesis, but some steroidogenesis persists via the StAR-independent pathway. The decreased secretion of cortisol leads to increased ACTH, which stimulates further cholesterol uptake and synthesis; this cholesterol accumulates in lipid droplets. **C,** Accumulating lipid droplets damage the cell, both through physical disruption of cytoarchitecture and by the chemical action of auto-oxidation products, eventually destroying all steroidogenic capacity. In the ovary, follicular cells remain unstimulated and undamaged until they are recruited at the beginning of each cycle. They can then produce small amounts of estradiol, as in panel **B,** leading to feminization and anovulatory cycles in affected females.

affected, eliminating most DHEA biosynthesis, and hence eliminating the fetoplacental production of estriol, so that midgestation maternal and fetal estriol levels are very low. The definitive zone of the fetal adrenal, which differentiates into the zonae glomerulosa and fasciculata, normally produces very little aldosterone, and as fetal salt and water metabolism are maintained by the placenta, stimulation of the glomerulosa by angiotensin II generally does not begin until birth. Consistent with this, many newborns with lipoid CAH do not have a salt-wasting crisis until after several weeks of life, when chronic stimulation then leads to cellular damage.[16,78]

The two-hit model also explains the spontaneous feminization of affected 46,XX females who are treated in infancy and reach adolescence.[9,10,16,79,80] The fetal ovary makes little or no steroids and contains no steroidogenic enzymes after the first trimester; consequently the ovary remains largely undamaged until it is stimulated by gonadotropins at the time of puberty, when it then produces some estrogen by StAR-independent steroidogenesis. Continued stimulation results in cholesterol accumulation and cellular damage, so that biosynthesis of progesterone in the latter part of the cycle is impaired. Because gonadotropin stimulation only recruits individual follicles and does not promote steroidogenesis in the whole ovary, most follicles remain undamaged and available for future cycles. Cyclicity is determined by the hypothalamic-pituitary axis and remains normal. With each new cycle, a new follicle is recruited and more estradiol is produced by StAR-independent steroidogenesis. Although net ovarian steroidogenesis is impaired, enough estrogen is produced (especially in the absence of androgens) to induce breast development, general feminization, monthly estrogen withdrawal, and cyclic vaginal bleeding.[16,79] However, progesterone synthesis in the latter half of the cycle is disturbed by the accumulating cholesterol esters so that the cycles are anovulatory. Measurements of estradiol, progesterone, and gonadotropins throughout the cycle in affected adult females with lipoid CAH confirm this model.[80] Similarly, examination of StAR-knockout mice confirms the two-hit model. Thus, examination of patients with lipoid CAH has elucidated the physiology of the StAR protein in each steroidogenic tissue.

Genetic analysis of patients with lipoid CAH has revealed numerous mutations in the StAR gene.[9,10] Lipoid CAH is common in Japan, and about 65% to 70% of affected Japanese alleles and virtually all affected Korean alleles carry the mutation Q258X. The carrier frequency for this mutation appears to be about 1 in 300 so that 1 in every 250,000 to 300,000 newborns in these countries is affected, for a total of about 500 patients in Japan and Korea. Other genetic clusters are found among Palestinian Arabs, most of whom carry the mutation R182L; in eastern Saudi Arabia, carrying R188C; and in Switzerland, carrying the mutation L260P. Deletion of only 10 carboxyterminal residues reduces StAR activity by half, and deletion of 28 carboxyterminal residues by the common Q258X mutation eliminates all activity. By contrast, deletion of the first 62 aminoterminal residues has no effect on StAR activity, even though this deletes the entire mitochondrial leader sequence and forces StAR to remain in the cytoplasm. Physical studies and partial proteolysis indicate that residues 63-193 of StAR (i.e., the domain that lacks most of the crucial residues identified by missense mutations) are protease-resistant and constitute a "pause-transfer" sequence, which permits

the bioactive loosely folded carboxyterminal molten globule domain to have increased interaction with the outer mitochondrial membrane.

The clinical findings in most patients with lipoid CAH are quite similar: an infant with normal-appearing female genitalia experiences failure to thrive and salt loss in the first weeks of life.[9,10,16,77] However, studies have revealed other clinical presentations, including apparent sudden infant death syndrome (SIDS) and late initial presentation of salt loss at about 1 year of age. An attenuated disease caused by mutations that retain about 20% to 25% of normal StAR activity has been described and is called "nonclassic lipoid CAH."[81] Those affected are usually children who first experience symptoms of adrenal insufficiency after several years, and the 46,XY patients have normal-appearing male external genitalia. Some patients have been diagnosed in adulthood and have been mistaken for having "familial glucocorticoid deficiency," a blanket term referring to disorders of ACTH action; most of these patients carry the R188C mutation in StAR.[82,83] Thus, the spectrum of clinical presentation of congenital lipoid adrenal hyperplasia is substantially broader than initially appreciated.

Treatment of lipoid CAH is straightforward if the diagnosis is made. Physiologic replacement with glucocorticoids, mineralocorticoids, and salt will permit survival to adulthood. The glucocorticoid requirement is less than in the virilizing adrenal hyperplasias because it is not necessary to oversuppress excess adrenal androgen production, so that growth in these patients should be normal. Severely affected 46,XY newborns have normal female external genitalia and may be advised to undergo orchiectomy later in life and be raised as females with sex hormone replacement therapy started at age of puberty. Affected 46,XX females typically have spontaneous pubertal feminization but anovulatory cycles and early secondary amenorrhea; they therefore also require sex hormone replacement therapy.

Disorders Resembling Lipoid CAH: P450scc Deficiency and SF1 Deficiency

Mutations in other genes can produce a clinical phenotype that is essentially indistinguishable from that caused by StAR mutations, but these disorders should not be called congenital lipoid adrenal hyperplasia. Beginning in 2001, several patients have been described with mutations in P450scc.[84] Their clinical and hormonal findings are indistinguishable from those with StAR mutations; however, to date no patient with a P450scc mutation has had the adrenal hyperplasia typically seen in lipoid CAH.[84,85] It would seem logical that elimination of all P450scc activity would be incompatible with term gestation, as the placenta, a fetal tissue, must produce progesterone in the second half of pregnancy to suppress maternal uterine contractions, thus preventing miscarriage. It is most likely that these few fetuses with P450scc mutations reached term gestation because of unusually protracted maintenance of the maternal corpus luteum of pregnancy, which normally involutes in the second trimester, but this has not been investigated directly. Nonclassic P450scc deficiency that is clinically and hormonally indistinguishable from nonclassic lipoid CAH has been reported in patients with P450scc mutations that retain 10% to 20% of wild-type activity.[86,87]

More than 50 patients have also been described carrying mutations in the gene for steroidogenic factor 1 (SF1), a transcription factor required for adrenal and gonadal, but not for placental, expression of genes for the steroidogenic enzymes.[88,89] There is broad phenotypic variability in SF1-deficient patients; some are 46,XY with a female phenotype and adrenal failure, thus resembling lipoid CAH, but in most cases the gonadal phenotype predominates and there is little if any impairment of adrenal steroidogenesis. SF1 mutations may be found in about 10% of 46,XY patients who have disordered sexual development. The Leydig cells may have lipid accumulation and progressive degeneration, similar to the findings in lipoid CAH.[89]

3β-Hydroxysteroid Dehydrogenase Deficiency

3βHSD deficiency is a rare cause of glucocorticoid and mineralocorticoid deficiency that is fatal if not diagnosed early in infancy. In its classic form, genetic females have clitoromegaly and mild virilization because the fetal adrenal overproduces large amounts of DHEA, a small portion of which is converted to testosterone by extra-adrenal 3βHSD1 and other enzymes. Genetic males also synthesize some androgens by peripheral conversion of adrenal and testicular DHEA, but the concentrations are insufficient for complete male genital development, so that these males have a small phallus and severe hypospadias.

There are two functional human genes for 3βHSD: the type 1 gene (*HSD3B1*) is expressed in the placenta and peripheral tissues, and the type 2 gene (*HSD3B2*) is expressed in the adrenals and gonads.[9] Genetic and endocrine studies of 3βHSD deficiency show that both the gonads and the adrenals are affected as a result of a single mutated 3βHSD2 gene that is expressed in both tissues. However, hepatic 3βHSD1 activity persists in the face of complete absence of adrenal and gonadal 3βHSD2 activity, thus complicating the diagnosis. Genetic studies have identified numerous mutations causing 3βHSD deficiency, all found in the type 2 gene.[90] Mutations have never been found in 3βHSD1, presumably because this would prevent placental biosynthesis of progesterone, resulting in a spontaneous first-trimester abortion.

The presence of peripheral 3βHSD activity complicates the hormonal diagnosis of this disease. One would expect that affected infants should have low concentrations of 17OHP, yet some newborns with 3βHSD deficiency have very high concentrations of serum 17OHP, approaching those seen in patients with classic 21-hydroxylase deficiency.[91] The high 17OHP concentrations are due to extra-adrenal 3βHSD1. The adrenal of a patient with 3βHSD2 deficiency will secrete very large amounts of three principal Δ^5 steroids, pregnenolone, 17-hydroxypregnenolone, and DHEA. Some of the secreted 17-hydroxypregnenolone is then converted to 17OHP by 3βHSD1. This 17OHP is not effectively picked up by the adrenal for subsequent conversion to cortisol because the circulating concentrations are

below the Michaelis constant (K_m) of P450c21 (1 μM 17OHP, or about 40,000 ng/dL). The ratio of the Δ^5 to the Δ^4 compounds remains high, consistent with the adrenal and gonadal deficiency of 3βHSD.[91] Thus, the principal diagnostic test in 3βHSD deficiency is intravenous administration of ACTH with measurement of the three Δ^5 compounds and the corresponding Δ^4 compounds. Unlike the case of 21-hydroxylase deficiency, where heterozygotes can be diagnosed by the response of 17OHP to ACTH, steroidal responses to ACTH cannot be used to identify carriers of 3βHSD deficiency.[92]

Mild or "partial" defects of adrenal 3βHSD activity have been reported on the basis of ratios of Δ^5 steroids to Δ^4 steroids following an ACTH test that exceed 2 or 3 standard deviations above the mean; these patients are typically young girls with premature adrenarche or young women with a history of premature adrenarche and complaints of hirsutism, virilism, and oligomenorrhea. However, these patients do not have 3βHSD deficiency as their 3βHSD2 genes are normal.[93] Patients with mild 3βHSD2 mutations have ratios of Δ^5 to Δ^4 steroids that exceed 8 standard deviations above the mean.[94] Thus ratios of Δ^5 to Δ^4 steroids are not reliable and cannot be used to diagnose 3βHSD deficiency; the diagnosis requires an ACTH test with a rise in Δ^5 steroids (typically a rise in 17OH-pregnenolone to $>$ 3000 ng/dL).[94] The basis of the mildly elevated ratios of Δ^5 to Δ^4 steroids in these hirsute individuals with normal 3βHSD genes is unknown. In adult women the hirsutism can be ameliorated and regular menses can be restored by suppressing ACTH with 0.25 mg of dexamethasone given orally each day, but such treatment is contraindicated in girls who have not yet reached their final adult height.

17α-Hydroxylase/17,20-Lyase Deficiency

P450c17 is the single enzyme that catalyzes both 17α-hydroxylase and 17,20-lyase activities.[9] 17-hydroxylase deficiency has been studied in detail at both clinical and genetic levels[95] and appears to be especially common in Brazil.[96] Deficient 17α-hydroxylase activity and deficient 17,20-lyase activity have been described as separate genetic diseases, but it is now clear that they represent different clinical manifestations of different lesions in the same gene.[21] Deficient 17α-hydroxylase activity results in decreased cortisol synthesis, overproduction of ACTH, and stimulation of the steps proximal to P450c17. These patients may have mild symptoms of glucocorticoid deficiency, but this is not life threatening, as the lack of P450c17 results in the overproduction of corticosterone, which also has glucocorticoid activity.[95] This is similar to the situation in rodents, whose adrenals lack P450c17 and consequently produce corticosterone as their glucocorticoid. Affected patients also typically overproduce DOC in the zona fasciculata, which causes sodium retention, hypertension, and hypokalemia and also suppresses plasma renin activity and aldosterone secretion from the zona glomerulosa, although the suppression of aldosterone is rather variable. When P450c17 deficiency is treated with glucocorticoids, DOC secretion is suppressed and plasma renin activity and aldosterone concentrations rise to normal.

The absence of 17α-hydroxylase and 17,20-lyase activities in complete P450c17 deficiency prevents the synthesis of adrenal and gonadal sex steroids. As a result, affected females are phenotypically normal but fail to undergo adrenarche and puberty and genetic males have absent or incomplete development of the external genitalia (male pseudohermaphroditism; 46,XY disorder of sexual development [DSD]). The classic presentation is that of a teenage female with sexual infantilism and hypertension. The diagnosis is made by finding low or absent 17-hydroxylated C-21 and C-19 plasma steroids, which respond poorly to stimulation with ACTH. Serum levels of DOC, corticosterone, and 18-OH-corticosterone are elevated, hyperresponsive to ACTH, and suppressible with glucocorticoids.

The *CYP17A1* gene encoding P450c17 is located on chromosome 10q24.3.[9] The molecular basis of 17α-hydroxylase deficiency has been determined in numerous patients by cloning and sequencing of the mutated gene identifying more than 50 distinct mutations. Four mutations appear recurrently: a duplication of four nucleotides causing a frameshift is found among descendants of Dutch Frieslanders, in-frame deletion of residues 487-489 is common in Asia, a deletion of phenylalanine at position 53 or 54, and the common W406R and R362C mutations, found among Brazilians of Spanish and Portuguese ancestry, respectively.[9,96] The genetic lesions identified include twelve mutations that cause frameshifts or premature translational termination; as expected, none of these mutants has any detectable 17α-hydroxylase or 17,20-lyase activity. Eleven missense and in-frame mutations have been found, most of which also eliminate all activity, whereas some others, such as P342T, reduce both activities by 80%.

Selective deficiency of the 17,20-lyase activity of P450c17 has been reported in some cases, which initially led to the incorrect conclusion that 17α-hydroxylase and 17,20-lyase are separate enzymes. One of the original patients was studied at the genetic level, showing two wholly inactivating mutations, which led to a corrected diagnosis of the patient as having complete 17α-hydroxylase deficiency.[21] Because both the 17α-hydroxylase and 17,20 lyase activities of P450c17 are catalyzed on the same active site, it was not clear that a syndrome of isolated 17,20 lyase deficiency could exist, until two patients with genital ambiguity, normal excretion of 17OHCS, and markedly reduced production of C_{19} steroids were studied at the molecular genetic level.[97] One patient was homozygous for the P450c17 mutation R347H, and the other was homozygous for R358Q; both mutations changed the distribution of surface charges in the redox-partner binding site of P450c17. When assayed in vitro, both mutants retained nearly normal 17α-hydroxylase activity but had no detectable 17,20 lyase activity, and enzymatic competition experiments showed that the substrate binding site remained normal. When an excess of P450 oxidoreductase and cytochrome b_5 was provided, some 17,20 lyase activity was restored, demonstrating that the loss in lyase activity was caused by impaired electron transfer. Several additional patients have been described with similar

mutations, and an active site mutation causing isolated 17,20 lyase deficiency has also been described.[21]

Computational modeling of P450c17 predicts the effects of all known mutations, including those with partial retention of both activities and those causing selective 17,20 lyase deficiency.[98] R347, R358, and several other arginine and lysine residues lie in the redox partner binding site; mutation of these residues cause varying degrees of selective loss of 17,20 lyase activity.[21,97,98]

P450 Oxidoreductase Deficiency

P450 oxidoreductase (POR) deficiency is a newly recognized form of CAH.[99,100] POR is the 2-flavin protein that transfers electrons from NADPH to all 50 microsomal forms of cytochrome P450, including P450c17, P450c21, and P450aro, as well as the drug-metabolizing P450 enzymes of the liver (see Fig 13-5).[12] Because POR participates in so many functions, its mutation might be expected to yield a severe phenotype, and POR-deficient mice die during fetal development. However, beginning in 1985, several patients were described with apparent combined deficiencies of P450c17 and P450c21, and it was suggested that a mutation in POR was responsible, but this was not proven until 2004.[99] A wide array of POR mutations has now been described, affecting various P450 enzymes to differing degrees, apparently explaining the great variability in the clinical and hormonal findings in POR deficiency.[99-103] The serum and urinary steroids indicate defects in both P450c17 and P450c21, and clinical findings vary from severely affected infants with ambiguous genitalia, cortisol deficiency, and the Antley-Bixler skeletal malformation syndrome (ABS) to mildly affected women who appear to have a form of polycystic ovary syndrome or mildly affected men with gonadal insufficiency. ABS is characterized by craniosynostosis, brachycephaly, radio-ulnar or radiohumeral synostosis, bowed femora, arachnodactyly, midface hypoplasia, proptosis, and choanal stenosis. When ABS is seen in association with abnormal steroids and ambiguous genitalia in either sex, the cause is an autosomal recessive mutation in POR[99,100,102]; by contrast, when ABS is seen without a lesion in steroidogenesis or genital development, the cause is an autosomal dominant, gain-of-function mutation in fibroblast growth factor receptor 2.[100] Thus, the term *Antley-Bixler syndrome* should be reserved for the phenotypic description of the skeletal malformations and should not be equated with POR deficiency, which may or may not be associated with ABS.[99,100]

Patients with POR deficiency will typically have normal electrolytes and mineralocorticoid function, nearly normal levels of cortisol that respond poorly to stimulation with ACTH, high concentrations of 17OHP that respond variably to ACTH, and low levels of C19 precursors to sex steroids. A remarkable feature of POR deficiency is that there is genital ambiguity in both sexes; females may be virilized and males may be underdeveloped, although there is considerable variation among individuals. As the 17,20 lyase activity of P450c17 is especially sensitive to perturbations in electron transport,[12,21,97] defects in fetal testicular steroidogenesis leading to incompletely developed external genitalia in 46,XY males is the predicted outcome. By contrast, the partial virilization seen in 46,XX genetic females appears to be due to two causes. First, placental aromatase (P450aro) requires POR. Pregnant women carrying a fetus with the POR mutation R457H (but not POR A287P) may experience virilization during pregnancy,[99-101] similar to that experienced by women carrying a fetus with P450aro deficiency.[39] The fetus normally disposes of large amounts of adrenal C19 steroids by excreting them through the placenta, which aromatizes them to the maternal estrogens of pregnancy (see Figure 13-7). A defect in this placental aromatase activity, either from mutation of POR or P450aro itself, will permit large amounts of fetal C19 steroids to enter and virilize the mother. This is evidenced by the low estriol values seen in women carrying a fetus with some POR mutations. Second, analysis of urinary steroids from patients with POR deficiency indicates that the alternative "backdoor pathway" of androgen production (see Figure 13-6) also contributes to the prenatal virilization of affected females.[101,104] The relative importance of these two distinct mechanisms for virilizing the fetus with POR deficiency remain unresolved. The mechanism linking defective POR activity to the ABS skeletal phenotype probably entails diminished activity of CYP26B1, the POR-dependent microsomal enzyme that degrades retinoic acid.[105] Studies of two families with CYP26B1 mutations and recreating such mutations in transgenic mice and zebrafish provides powerful evidence that retinoic acid must be degraded locally at embryonic sites that normally form skeletal joints and sutures; interference with this activity in POR deficiency appears to be the principal mechanism accounting for the skeletal phenotype.[105] Other mechanisms, including defective signaling by hedgehog proteins secondary to a POR-associated defect in cholesterol synthesis, may also play a role.[102]

Because the principal hepatic drug-metabolizing enzymes require POR, it is logical to expect impaired drug metabolism in POR-deficient patients. Although transgenic mice with liver-specific POR defects metabolize drugs poorly and accumulate hepatic lipids, similar problems have not yet been described in patients with POR deficiency. Although numerous studies of drug-metabolizing enzymes in vitro show major impairment by POR mutations,[106]only one study has found such an effect in a POR-deficient patient.[107] Much remains to be learned about POR deficiency.

The incidence of POR deficiency is unknown. Because the disorder is newly described, it may seem rare, but the rapid description of large numbers of patients and the potentially subtle clinical manifestations in individuals carrying mutations with partial activity suggest that POR deficiency may be fairly common. Two mutations are especially common: A287P, the predominant mutation in patients of European ancestry; and R457H, the predominant mutation in patients of Japanese ancestry. Because few patients have been studied in the newborn period, it has not been established whether newborn screening of 17OHP designed to detect 21-hydroxylase deficiency will also detect POR deficiency, although some reported POR patients were

initially misdiagnosed with 21-hydroxylase deficiency. Treatment of POR deficiency requires multidisciplinary management of craniosynostosis and other orthopedic problems, and DSD management including sex hormone replacement therapy starting at pubertal age in both sexes. Some patients may benefit from low-dose glucocorticoid replacement therapy, especially during severe illness; this should be determined individually by assessing the response of cortisol to ACTH.

Cytochrome b_5 Deficiency

Cytochrome b_5 is a small hemoprotein that acts as an allosteric factor to facilitate the interaction of P450c17 with POR, thus fostering 17,20 lyase activity.[20] Adrenal expression of b_5 is specific to the zona reticularis and coincides with the onset of adrenarche.[64] Cytochrome b_5 deficiency is a newly described form of androgen deficiency, apparently affecting both adrenal and testicular androgen synthesis. The first report of b_5 deficiency was a male pseudohermaphrodite with methemoglobinemia who was not evaluated hormonally.[108] Methemoglobinemia is an expected consequence of b_5 deficiency because the reduction of methemoglobin is the principal physiologic role of b_5, and the usual cause of methemoglobinemia is deficiency of cytochrome b_5 reductase. Two cases report male pseudohermaphroditism with elevated concentrations of methemoglobin, but not with clinical methemoglobinemia[109,110]; as the lesion only affects androgen synthesis, adrenal insufficiency is not part of this disorder, which is part of the syndrome of 17,20 lyase deficiency.[21]

21-Hydroxylase Deficiency

21-Hydroxylase deficiency, which is due to mutations in the *CYP21A2* gene encoding adrenal P450c21, is one of the most common inborn errors of metabolism and accounts for over 90% of all patients with CAH. Because of improved diagnosis and treatment in infancy, patients with severe 21-hydroxylase deficiency now routinely reach adulthood, so that the management of CAH concerns physicians dealing with all age groups.[22-24]

Pathophysiology

In severe 21-hydroxylase deficiency, there is an inability to convert progesterone to DOC, resulting in aldosterone deficiency that causes severe hyponatremia (Na+ often below 110 mEq/L), hyperkalemia (K+ often above 10 mEq/L), and acidosis (pH often below 7.1). The associated hypotension, shock and cardiovascular collapse may result in death in an untreated newborn. As the placenta and the mother's kidneys maintain the control of fluids and electrolytes in the fetus, this salt-losing crisis develops only after birth, usually during the second week of life. The inability to convert 17OHP to 11-deoxycortisol results in cortisol deficiency, which impairs postnatal carbohydrate metabolism and exacerbates cardiovascular collapse, because a permissive action of cortisol is required for full pressor action of catecholamines. High concentrations of cortisol in the adrenocortical capillary effluent that bathes the medulla are needed for the conversion of norepinephrine to epinephrine; hence, children with CAH have low epinephrine concentrations, which may exacerbate the hypoglycemia associated with cortisol deficiency.

Although the role of cortisol in fetal physiology is not well established,[51-53] cortisol deficiency is manifested prenatally. Low fetal cortisol stimulates ACTH secretion, which stimulates adrenal hyperplasia and transcription of the genes for all the steroidogenic enzymes, especially for P450scc, the rate-limiting enzyme in steroidogenesis. This increased transcription increases enzyme production and activity, with consequent accumulation of non-21-hydroxylated steroids. There are three pathways by which these steroids are diverted to androgens (see Figure 13-6). First, 17OH-pregnenolone may be converted to DHEA by the 17,20 lyase activity of P450c17; if not inactivated by sulfation to DHEAS, DHEA may be converted to androstenedione by 3βHSD2. Androstenedione may then be converted to testosterone in the fetal adrenal or in target tissues by 17βHSD5 (see Figure 13-3). Second, although 17OHP is not normally an effective substrate for the 17,20 lyase activity of P450c17, the very high levels of 17OHP characteristic of CAH will "force" some conversion to androstenedione by mass action. Third, there is an alternative or "backdoor pathway" to androgen, in which 17OHP is 5α and 3α reduced to 17OH-allopregnenolone, which is readily converted by P450c17 to androsterone and thence by 17βHSD and the oxidative action of 3αHSD to dihydrotestosterone, so that this most potent androgen is produced without DHEA, androstenedione, or testosterone as intermediates.[47] This pathway exists in the human fetus[49] and contributes substantially to the production of androgens in children with CAH.[50] The role of this pathway in virilizing the CAH fetus has not yet been established but is likely. Furthermore, as described previously (see "Fetal Adrenal Steroidogenesis"), fetal adrenal androgen synthesis is normally prevented during the time when the external genitalia may become virilized. The fetal adrenal transiently expresses 3βHSD2 at about 7 to 12 weeks, permitting cortisol synthesis, which in turn suppresses ACTH and hence suppresses fetal adrenal production of DHEA and other C-19 steroids.[51] In CAH, this transient production of cortisol is not possible, further driving the androgenization of female fetuses.

The fetal testes produce large amounts of testosterone in early to midgestation, which differentiates the pluripotent embryonic precursor structures into male external genitalia. In the male fetus with 21-hydroxylase deficiency, the additional testosterone produced in the adrenals has no phenotypic effect. By contrast, the fetal ovaries normally produce no sex steroids or other factors needed for differentiation of the female external genitalia. The testosterone inappropriately produced by the adrenals of the CAH female fetus causes varying degrees of external genital virilization, which can range from mild clitoromegaly with or without posterior fusion of the labioscrotal folds to complete labioscrotal fusion that includes a urethra traversing the enlarged clitoris (Figure 13-13). These infants have normal ovaries,

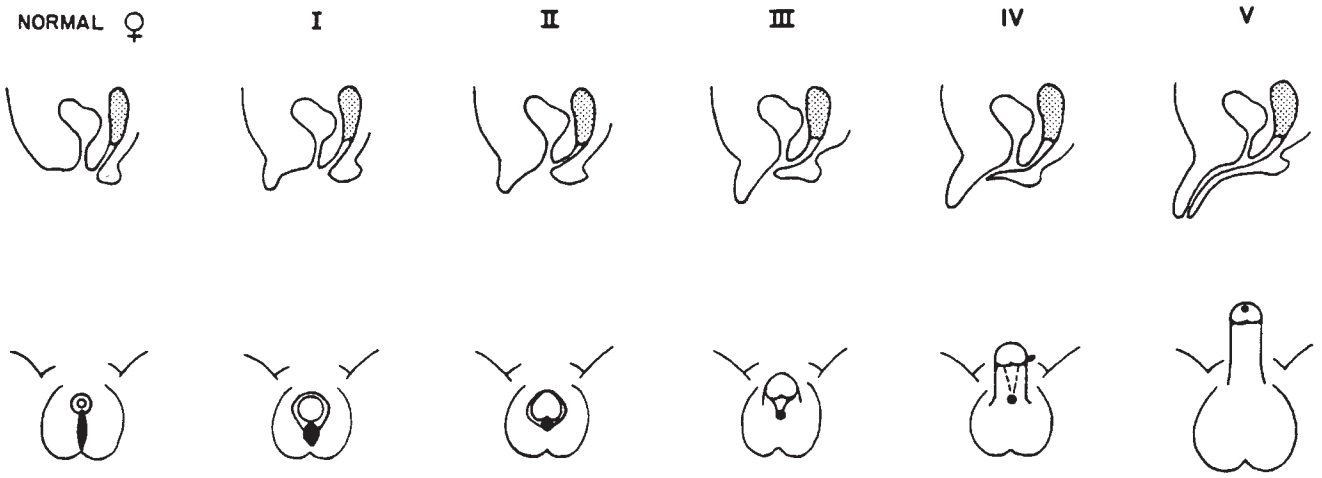


FIGURE 13-13 ■ Virilization of the external genitalia. A continuous spectrum is shown from normal female to normal male in both sagittal section (above) and perineal views (below), using the staging system of Prader. Disorders of external genitalia can occur either by the virilization of a normal female, as in congenital adrenal hyperplasia, or due to an error in testosterone synthesis in the male. In females with congenital adrenal hyperplasia due to 21-hydroxylase deficiency, the degree of virilization correlates poorly with the presence or absence of clinical signs of salt loss.

fallopian tubes, and a uterus, but they have "ambiguous" external genitalia or may be sufficiently virilized so that they appear to be male, resulting in errors of sex assignment at birth.

The diagnosis of 21-hydroxylase deficiency is suggested by genital ambiguity in females, a salt-losing episode in either sex, or rapid growth and virilization in males or females in infancy. Plasma 17OHP is markedly elevated (> 2000 ng/dL or 60 nmol/L and often exceeding 40,000 ng/dL or 1200 nmol/L) after 24 hours of age in an otherwise healthy full-term infant) and hyperresponsive to stimulation with ACTH (see Figure 13-11). It is important also to measure 11-deoxycortisol, DHEA, and androstenedione, both to distinguish among the forms of CAH and because adrenal or testicular tumors can also produce 17OHP. ACTH will also induce a substantial rise in serum 21-deoxycortisol in all forms of 21-hydroxylase deficiency, but not in normals, providing a useful adjunctive test when this steroid can be measured. High newborn 17OHP values that rise further after ACTH can also be seen in 3βHSD2 deficiency (because of the activity of hepatic 3βHSD1) and in P450c11 deficiency (because of end-product inhibition of P450c21).[91] 17OHP is normally high in cord blood but falls to normal newborn levels after 12 to 24 hours (Figure 13-14) so that assessment of 17OHP levels should not be made in the first 24 hours of life. Premature infants and term infants under severe stress (e.g., with cardiac or pulmonary disease) may have persistently elevated 17OHP concentrations with normal 21-hydroxylase. Newborn screening programs for CAH are now in place throughout the industrialized world, based on 17OHP measurements. The technologies employed and "cutoff" values used vary in different health care systems. When testing is done on full-term infants more than 24 hours after birth, the screening is reliable.[22] Endocrinologists and neonatologists must become familiar with local assays and the values found in the extremely premature,

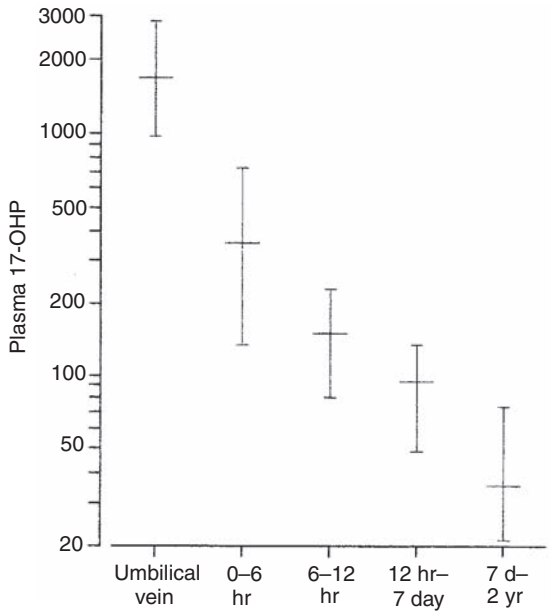


FIGURE 13-14 ■ Means and ranges of 17OHP in normal newborns (data are in ng/100 mL). Note that values can be very high and quite variable for the first 24 hours of life.

which may be read as false positives for CAH requiring repeat testing. Also, negative neonatal screening does not exclude milder 21-hydroxylase deficiency causing nonclassic CAH beyond the newborn period.

Clinical Forms of 21-Hydroxylase Deficiency

There is a broad spectrum of clinical manifestations of 21-hydroxylase deficiency, depending on the particular gene mutation. These different forms of 21-hydroxylase deficiency are not different diseases, as there is a continuous spectrum of manifestations, ranging from the severe

"salt-wasting" form to clinically inapparent forms that may be normal variants. Thus, the typical disease forms discussed in the following sections are mainly a clinical convenience.[22]

Salt-Wasting CAH. Salt-wasting CAH is caused by a nearly complete lack of P450c21 activity, effectively eliminating both glucocorticoid and mineralocorticoid synthesis. Females with this disorder are frequently diagnosed at birth because of virilization of the external genitalia; 21-hydroxylase deficiency is the most common form of 46,XX DSD. After appropriate resuscitation of the cardiovascular collapse, acidosis, and electrolyte disorders, if diagnosed late, the mineralocorticoids and glucocorticoids can be replaced orally and the ambiguous genitalia may be corrected with plastic surgical procedures. The steroidal replacement management is difficult because of the rapidly changing needs of a growing infant or child (see section on "Treatment," presented later in the chapter). Drug doses must be adjusted frequently, and there is also considerable individual variability in what constitutes "physiologic" replacement. As an underdosage of glucocorticoids can be life-threatening, especially during illness, most pediatricians have tended to err on the "safe side" and so these children usually receive inappropriately large doses of glucocorticoids. It is not possible to compensate for the growth lost during the first 2 years of life, when growth is fastest, so these children almost always end up shorter than predicted from their genetic potential. Adult females may have sexual dysfunction, marry with a low frequency, are more reluctant to form intimate relationships, and have decreased fertility. Prenatal androgens appear to affect behavior, but not sexual identity.[22] Males with this disorder are generally undiagnosed at birth and come to medical attention through the neonatal screening, during the salt-losing crisis that follows at day 5 to 15 of life, or die, invariably with an incorrect diagnosis. Adult males appear to have less steady heterosexual relationships and decreased fertility, especially with insufficient treatment, which promotes the occurrence of testicular adrenal rest tumors.[22]

Simple Virilizing CAH. Virilized females who have elevated concentrations of 17OHP but who do not suffer a salt-losing crisis have long been recognized as having the "simple virilizing" form of CAH. Unless detected by newborn screening, males with this disorder may escape diagnosis until age 3 to 7 years, when they come to medical attention because of early development of pubic, axillary, and facial hair; acne; and phallic growth. These signs may be mistaken for premature puberty, but the astute physician can readily differentiate such boys with sexual precocity from boys with true central precocious puberty, as the testes remain of prepubertal size in CAH, whereas gonadotropic stimulation in true precocious puberty results in pubertal-sized testes (≥ 4 mL). These children grow rapidly and are tall for age when diagnosed, but their epiphyseal maturation (bone age) advances disproportionately rapidly so that ultimate adult height is compromised. Untreated or poorly treated children with CAH may fail to undergo normal puberty, and boys may have small testes and azoospermia because of the feedback of the adrenally produced testosterone on pituitary gonadotropins. When treatment is begun at several years of age, suppression of adrenal testosterone secretion may remove tonic inhibition of the hypothalamus, occasionally resulting in true central precocious puberty, requiring treatment with a GnRH agonist. High concentrations of ACTH in poorly treated males may stimulate the enlargement of adrenal rests in the testes. These enlarged testes usually appear nodular by ultrasound, unlike the homogeneously enlarged testes in central precocious puberty. Because the adrenal normally produces 100 to 1000 times as much cortisol as aldosterone, mild defects (amino acid replacement mutations) in P450c21 are less likely to affect mineralocorticoid secretion than cortisol secretion. This is reflected physiologically by the increased plasma renin activity seen in these patients after moderate salt restriction.

Nonclassic CAH. Many people have very mild forms of 21-hydroxylase deficiency. These forms may be evidenced by mild to moderate hirsutism, virilism, acne, menstrual irregularities, and decreased fertility in adult women (so-called late-onset CAH), or there may be no phenotypic manifestations at all other than an increased response of plasma 17OHP to an intravenous ACTH test (so-called cryptic CAH).[22] Despite the minimal manifestations of this disorder, these individuals also have hormonal evidence of a mild impairment in mineralocorticoid secretion, evidenced by an unusually greater rise in PRA when dietary sodium is restricted.

There is some inconsistency in classifying patients into these three forms of CAH because each diagnostic category is not a separate disease but represents a typical picture in a continuous spectrum of disease, caused by a broad spectrum of genetic lesions. Furthermore, because some mutant P450c21 alleles are common in the general population, most patients are compound heterozygotes, carrying a different mutation on the allele inherited from each parent. Finally, factors other than the specific mutations found in P450c21 will influence the clinical phenotype, including the presence of extra-adrenal 21-hydroxylases, undiagnosed P450c21 promoter mutations, and variations in androgen metabolism and sensitivity. Thus, discordances between genotype and phenotype are to be expected.

Incidence of 21-Hydroxylase Deficiency

Perinatal screening for elevated concentrations of serum 17OHP in several countries has shown that the incidence of "classic" CAH (i.e., salt-wasting and simple virilizing CAH) is about 1 in 14,000, yielding a heterozygous carrier rate of 1 in 60. The screening of 1.9 million newborns in Texas yielded an overall incidence of 1 in 16,000, including incidences of 1 in 15,600 Caucasians, 1 in 14,500 Hispanics (primarily Mexican Americans of indigenous American ancestry), and 1 in 42,300 African Americans.[111] Because about 20% of the African-American gene pool is of European descent, the calculated incidence in individuals of wholly sub-Saharan African ancestry is about 1 in 250,000. Nonclassic CAH is clearly much

more common; its incidence varies substantially among ethnic groups, with the highest incidence among Ashkenazi Jews and Mediterranean peoples.[9,22,112] The high incidence, lack of mortality, and lack of decreased fertility in most individuals with nonclassic CAH suggest that this is a polymorphic variant and not a disease in the classic sense. Nevertheless, patients with nonclassic CAH may seek help for complaints of virilism, menstrual disorders, and fertility.[112]

Genetics of the 21-Hydroxylase Locus

21-Hydroxylase Genes. The functional 21-hydroxylase gene is formally termed *CYP21A2* and a nearby nonfunctional pseudogene is formally termed *CYP21A1P*. These genes, also termed P450c21B (functional gene) and P450c21A (pseudogene), are duplicated in tandem with the C4A and C4B genes encoding the fourth component of serum complement[9,23,24] (Figure 13-15). Although the P450c21A locus is transcribed, the resultant RNAs do not encode protein; only the P450c21B gene encodes 21-hydroxylase.[9] The P450c21 genes consist of 10 exons, are about 3.4 kb long, and differ in only 87 or 88 of these bases. This high degree of sequence similarity indicates that these two genes are evolving in tandem through intergenic exchange of DNA. The P450c21 genes of mice and cattle are also duplicated and linked to leukocyte antigen loci. However, whereas only the P450c21B gene functions in human beings, only the P450c21A gene functions in mice and both genes function in cattle.[9,23] Sequencing of the gene duplication boundaries show that the human locus duplicated after mammalian speciation, consistent with data that indicate that other mammals have single *CYP21* gene copies.[9,23]

HLA Linkage. The *CYP21* genes lie within the class III region of the human major histocompatibility complex (MHC) (see Figure 13-15). HLA typing has been used for prenatal diagnosis and to identify heterozygous family members but has been replaced by direct genetic analysis. Statistical associations (linkage disequilibrium) are well established between CAH and certain specific HLA types.[9,23,24] Salt-losing CAH is associated with HLA-B60 and HLA-40 in some populations, and HLA-Bw47 is strongly associated with salt-losing CAH. HLA-Bw51 is associated with simple virilizing CAH in some populations, and about 40% of haplotypes for nonclassic CAH carry HLA-B14. HLA-B14 is often associated with a duplication of the C4B gene, but all HLA-B alleles can be found linked to CAH. HLA-identical individuals

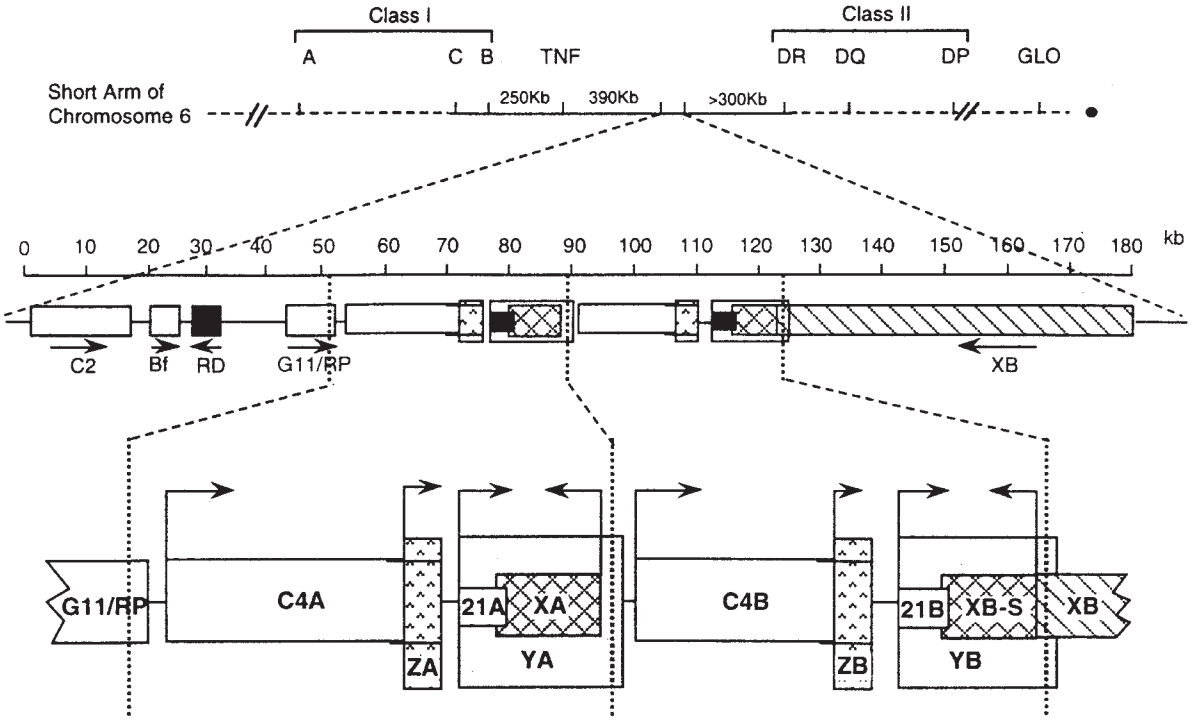


FIGURE 13-15 ■ Genetic map of the HLA locus containing the genes for P450c21. The top line shows the p21.1 region of chromosome 6, with the telomere to the left and the centromere to the right. Most HLA genes are found in the class I and class II regions; the class III region containing the P450c21 genes lies between these two. The second line shows the scale (in kilobases) for the diagram immediately below, showing (from left to right) the genes for complement factor C2, properdin factor Bf, and the RD and G11/RP genes of unknown function; arrows indicate transcriptional orientation. The bottom line shows the 21-hydroxylase locus on an expanded scale, including the C4A and C4B genes for the fourth component of complement, the inactive CYP21A gene (21A) and the active CYP21B gene (21B) that encodes P450c21. XA, YA, and YB are adrenal-specific transcripts that lack open reading frames. The XB gene encodes the extracellular matrix protein Tenascin-X; XB-S encodes a truncated adrenal-specific form of the Tenascin-X protein, whose function is unknown. ZA and ZB are adrenal-specific transcripts that arise within the C4 genes and have open reading frames, but it is not known if they are translated into protein; however, the promoter elements of these transcripts are essential components of the CYP21A and CYP21B promoters. The arrows indicate transcriptional orientation. The vertical dotted lines designate the boundaries of the genetic duplication event that led to the presence of A and B regions.

in a single family may have different clinical features of CAH despite HLA identity, possibly representing extra-adrenal 21-hydroxylation, de novo mutations, or multiple genetic crossover events.

Other Genes in the 21-Hydroxylase Locus. The tandemly duplicated C4A and C4B loci produce isoforms of complement component C4 that can be distinguished functionally and immunologically[9,23]; the C4B protein has greater hemolytic activity despite greater than 99% sequence identity with C4A. The C4A gene is always 22 kb long, but there are long (22 kb) and short (16 kb) forms of C4B due to a variation in one intron. The 3′ ends of the C4 genes are only 2466bp upstream from the transcriptional start sites of the P450c21 genes. Promoter sequences needed for the transcription of the human P450c21B gene lie within intron 35 of the C4B gene.[113] In addition to the P450c21 and C4 genes, there are several other genes within 100 kb of the P450c21 gene, including the genes for complement factor C2 and properdin factor Bf (see Figure 13-15). Lying just 3′ of the Bf gene on the opposite strand of DNA from P450c21 is the STK19 gene (also variously called RD, RP, and G11), which encodes a nuclear serine/threonine kinase.[110]

A pair of genes, termed XA and XB, is duplicated with the C4 and P450c21 genes.[9,23] These genes lie on the strand of DNA opposite from the C4 and P450c21 genes and overlap the 3′ end of P450c21. The last exon of XA and XB lies within the 3′ untranslated region of exon 10 in P450c21A and P450c21B, respectively. The XA gene was truncated during the duplication of the ancestral C4-P450c21-X genetic unit but is transcribed in the adrenal. The XB gene encodes a large extracellular matrix protein called Tenascin X that is expressed in most tissues, especially connective tissue.[115] The XB gene spans about 65kb of DNA and includes 43 exons encoding a 12kb mRNA. The XB gene also encodes a short truncated form of Tenascin-X having unknown function and arising from an intragenic promoter. Identification of a CAH patient with a "contiguous gene syndrome" comprising a deletion of both the P450c21B and XB genes demonstrated that Tenascin X deficiency results in Ehlers-Danlos syndrome (EDS).[116] Most forms of EDS are caused by autosomal dominant mutations in collagen genes; the recessive forms are caused by mutations in genes for collagen-modifying enzymes, including Tenascin-X, which is associated with and stabilizes collagen fibrils. Tenascin-X deficiency causes a clinically distinct, somewhat more severe, recessive form of EDS, either with or without associated 21-hydroxylase deficiency.[117]

P450c21 Gene Lesions Causing 21-Hydroxylase Deficiency

21-Hydroxylase deficiency can be caused by *CYP21A2* gene deletions, gene conversions, and apparent point mutations. The most common apparent point mutations are actually small gene conversion events,[9,23] so that gene conversions account for about 85% of the lesions in 21-hydroxylase deficiency. Each person has two *CYP21A2* alleles, one contributed by each parent. Most patients with 21-hydroxylase deficiency are compound heterozygotes, having different lesions on their two alleles. Because gene deletions and large conversions eliminate gene transcription, homozygosity for these lesions will cause salt-losing CAH. Some microconversions, such as those creating premature translational termination, are also associated with salt-losing CAH. Milder forms, such as simple virilizing and nonclassic CAH, are associated with amino acid replacements in the P450c21 protein caused by gene microconversion events. Patients with these forms of CAH are usually compound heterozygotes bearing a severely disordered allele and a mildly disordered allele so that the clinical manifestations are based on the nature of the mildly disordered allele.

Mapping of P450c21 Genes in Normals and in CAH. Although the P450c21B and P450c21A loci (*CYP21A2* and *CYP21A1P* genes) differ by only 87 or 88 nucleotides, they can be distinguished by restriction endonuclease digestion and Southern blotting. Two unusual and related features of the 21-hydroxylase locus complicate its analysis. First, the gene deletions in this locus are most unusual in that they extend 30 kb from one of several points in the middle of P450c21A to the precisely homologous point in P450c21B. Thus, the 15% of alleles that carry deletions do not yield a typical Southern blotting pattern with a band of a size different from that of one of the normal, unless one uses very rarely cutting enzymes and analyzes the resulting large DNA fragments by pulsed-field gel electrophoresis. The second unusual feature of this locus is that gene conversions are extremely common.[9,23]

Gene Conversions and Microconversions Causing CAH. If a segment of gene A replaces the corresponding segment of the related gene B, the structure of recipient gene B is said to be "converted" to that of donor gene A. The hallmark of gene conversion is that the number of closely related genes remains constant, whereas their diversity decreases. Two types of gene conversions commonly cause 21-hydroxylase deficiency: large gene conversions that can be mistaken for gene deletions and small microconversions that resemble point mutations. The relative frequency of large gene conversions versus gene deletions was formerly controversial, principally because initial studies used relatively small groups of patients from single locations or ethnic groups. A compilation of multiple studies shows that about 19% of mutant alleles have gene deletions, 8% have large gene conversions, 67% have microconversions, and 6% had other lesions[23] (Figure 13-16). Such numbers emphasize the more severely affected patients. About 75% of mutated P450c21B genes are grossly intact and appear to carry point mutations, but gene sequencing shows that most apparent point mutations are also found in the P450c21A pseudogene, indicating that they are actually representing micro gene conversion events (Table 13-6). Three changes in the pseudogene (8bp deletion, exon 3; T insertion, exon 7; Gly3 18Stop, exon 8) render its RNA product nonfunctional. Each change results in an altered reading frame or premature stop codon,

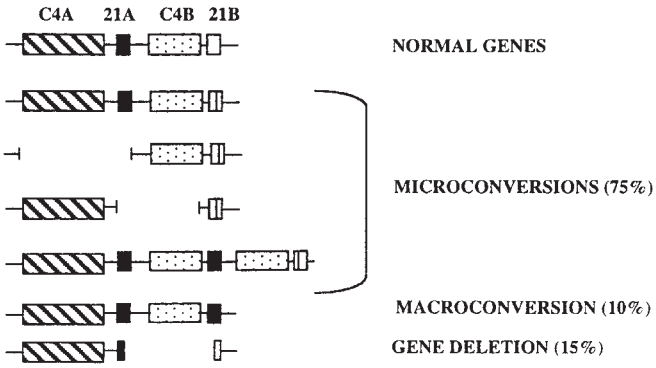


FIGURE 13-16 ■ Classes of genetic rearrangements causing 21-hydroxylase deficiency. Deletions or duplications of the C4A and C4B genes can occur with or without associated lesions in the P450c21B gene. Note that all "point mutations" in P450c21B are actually "microconversion." Many authors combine the "gene deletion" and "macroconversion" groups because these are difficult to distinguish by Southern blotting as both result in a loss of the P450c21B gene, but the genotypes are clearly distinct, as shown.

TABLE 13-6 **Microconversions in the *CYP21A2* Gene That Cause 21-Hydroxylase Deficiency**

Mutation	Location	Associated Phenotypes	Activity
Pro 30→Leu	Exon 1	NC/SV	30%-60%
A→G	Intron 2	SV/SW	minimal
8 bp deletion	Exon 3	SW	0
Ile 172→Asn Ile 236→Asp	Exon 4	SV	3%-7%
Val 237→Glu Met 239→Lys	Exon 6	SW	0
Val 281→Leu	Exon 7	NC	18% ± 9%
Gly 292→Ser	Exon 7	SW	
T insertion @ 306	Exon 7	SW	0
Gly 318→Stop	Exon 8	SW	0
Arg 339→His	Exon 8	NC	20%-50%
Arg 356→Trp	Exon 8	SV/SW	2%
Pro 453→Ser	Exon 10	NC	20%-50%
GG→C @ 484	Exon 10	SW	

eliminating all activity; all of these have been found in P450c21B alleles that cause severe salt-losing CAH. Three closely clustered base changes alter the normal amino acid sequence Ile-Val-Glu-Met at codons 236 to 239 in exon 6 to Asn-Glu-Glu-Lys in both P450c21A and in a small number of genes causing severe salt-losing CAH.

The most common lesion in classic CAH is an A→G change in the second intron, 13 bases upstream from the normal 3′ splice acceptor site of this intron; this is a microconversion found in over 25% of severely affected CAH alleles. This intronic mutation causes abnormal splicing of the encoded RNA, destroying activity. However, a small portion of this mRNA may be spliced normally in some patients so that the phenotypic presentation is variable; most such patients are salt losers, but some have non–salt-losing CAH. This intron 2 microconversion is often associated with the Ser/Thr polymorphism at codon 268; this is a true polymorphism, as S268T does not alter enzymatic activity. The microconversion R356W, which is found in about 10% of severely affected alleles, may retain slight activity and has been found in both salt-losing and simple virilizing CAH. A large number of rare mutations have been described in single individuals.

Mutations Causing Simple Virilizing and Nonclassic CAH. The microconversion I172N is the most common cause of simple virilizing CAH. When this residue is changed to Asn, Leu, Gln, or His and the resulting mutant P450c21 protein is assayed in vitro, it retains only 3% to 7% of normal 21-hydroxylase activity. The intron 2 microconversion is occasionally seen in simple virilizing CAH. The microconversion P30L is generally associated with classic CAH, but it is found in some patients with simple virilizing CAH.

The most common mutation causing nonclassic CAH is V281L, a microconversion linked to HLA-B14 and HLA-DR1 but also found in patients with other HLA types. The microconversion P30L is found in about 15% to 20% of nonclassic alleles, and the mutations R339H and P453S have been associated with nonclassic CAH; the P453S mutation is polymorphic in about 20% of P450c21A pseudogenes and hence also represents a microconversion event.

Prenatal Diagnosis of CAH

The prenatal diagnosis of 21-hydroxylase deficiency can be approached by measuring fetally produced 17OHP or Δ^4-androstenedione in amniotic fluid, but these steroids may not be elevated above the broad range of normal in non–salt-losing or nonclassic CAH. Second, HLA typing of fetal amniocytes can be informative if there is previous linkage analysis of an affected index case and parents. However, some HLA alleles are expressed weakly in cultured amniocytes, and the procedure is no longer widely used. Third, sequencing of the affected gene is now widely used, but the complex genetics of the CAH locus mean this procedure is reliable only when the genetic lesion in a previously affected sibling or the parents is known.

Diagnosis

In the absence of prenatal screening, genital ambiguity, neonatal screening or a salt-losing crisis will generally alert pediatricians to most cases of severe 21-hydroxylase deficiency. Salt-losing crises generally occur in the second week of life, and the child presents with vomiting, diarrhea, dehydration, hyperkalemia, and hyponatremia. Occasionally, such infants are thought to have viral syndromes or gastrointestinal obstructions; such a failure to make the diagnosis can result in the infant's death.

Similarly, boys with simple virilizing CAH may escape diagnosis until they are 3 to 7 years old, when they present with isosexual precocity, advanced bone age, and characteristically prepubertal testes. Teenage and adult females with nonclassic CAH may consult an internist, obstetrician, or dermatologist for virilism, hirsutism, menstrual irregularity, infertility, or acne.

The key diagnostic maneuver is to measure 17OHP and other steroids in response to intravenous synthetic ACTH. The usual doses are 15 μg/kg in children up to 2 years of age, and 0.25 mg in older children and adults. 17OHP and cortisol should be measured at 0 and 60 minutes. Individual patient responses must be compared to age- and sex-matched data from normal children. Normal responses are shown in Table 13-4 and Figure 13-11. Both basal and stimulated levels of 17OHP are markedly elevated in patients with salt-losing and simple virilizing forms of 21-hydroxylase deficiency. Basal levels are usually greater than 2000 ng/dL and increase to more than 5000 to 10,000 ng/dL after ACTH. Patients with the milder late-onset or cryptic forms typically have normal to mildly elevated basal levels but have supranormal responses to ACTH stimulation (i.e., 1500 to > 10,000 ng/dL). The cortisol response to ACTH is absent or subnormal in patients with the classic forms of CAH and is normal in patients with late-onset and cryptic forms. Basal plasma ACTH levels reflect the extent of 21-hydroxylase and cortisol deficiency (i.e., they are markedly elevated in severe forms and may be normal in patients with the milder forms who are not overtly adrenal insufficient).

Other ancillary tests are listed in Table 13-5. Urinary excretion of 17-ketosteroids will generally be elevated, but this test is no longer in general use and more useful for monitoring the efficacy of suppressive therapy than for initial diagnosis. When urinary steroids are measured, a complete 24-hour sample must be obtained, and a concomitant measurement of creatinine excretion is required to monitor the completeness of the collection. Less than 24 hour urine collections are not quantitatively accurate because of diurnal variations in steroid excretion. Measurements on spot urine samples may give some qualitative information when correlated to creatinine. Urine steroid profiling with mass-spectrometric methods (GC/MS or LC/MS-MS) typically shows elevated metabolites of 17OHP: pregnanetriol, 17-OH-pregnanolone, and pregnanetriolone.

Plasma renin activity and its response to salt restriction can be especially useful. Most patients with simple virilizing 21-hydroxylase deficiency have high plasma renin activity that increases further on sodium restriction, confirming that these patients are partially mineralocorticoid deficient and can maintain a normal serum sodium level only by hyperstimulation of the zona glomerulosa.

Treatment

The management of CAH remains difficult.[22] Overtreatment with glucocorticoids causes delayed growth, even when the degree of overtreatment is insufficient to produce signs and symptoms of Cushing syndrome. Undertreatment results in continued overproduction of adrenal androgens, which hastens epiphyseal maturation and closure, again compromising growth and other manifestations of androgen excess.

Doses of glucocorticoids should be based on the expected normal cortisol secretory rate. Several studies have established that the secretory rate of cortisol is about 6 to 8 mg/m^2 per day. However, effective suppression of ACTH and adrenal androgen production requires somewhat higher doses of about 10 to 15 mg/m^2 per day.[22] Newly diagnosed patients, especially newborns, require substantially higher initial dosages to suppress their hyperactive CRH-ACTH-adrenal axis. The glucocorticoid used is important. Most tables of glucocorticoid dose equivalencies are based on their equivalence in anti-inflammatory assays. However, the growth-suppressant equivalences of various glucocorticoids do not parallel their anti-inflammatory equivalencies: long-acting synthetic steroids such as dexamethasone have a disproportionately greater growth-suppressant effect and hence must be avoided in treating growing children and adolescents (Table 13-7). Most authorities favor the use of oral

TABLE 13-7 **Potency of Various Therapeutic Steroids (Set Relative to the Potency of Cortisol)**

Steroid	Anti-inflammatory Glucocorticoid Effect	Growth-Retarding Glucocorticoid Effect	Salt-Retaining Mineralocorticoid Effect	Plasma Half-Life (minutes)	Biologic Half-Life (hours)
Cortisol (hydrocortisone)	1.0	1.0	1.0	80-120	8
Cortisone acetate (oral)	0.8	0.8	0.8	80-120	8
Cortisone acetate (IM)	0.8	1.3	0.8		18
Prednisone	4	5	0.25	200	16-36
Prednisolone	4		0.25	120-300	16-36
Methylprednisolone	5	7.5	0.4		
Betamethasone	25		0	130-330	
Triamcinolone	5		0		
Dexamethasone	30	80	0	150-300	36-54
9α-Fludrocortisone	15		200		
DOC acetate	0		20		
Aldosterone	0.3		200-1000		

hydrocortisone or cortisone acetate in three divided daily doses in growing children. However, adults and older teenagers who already have fused their epiphyses may be managed with prednisone or dexamethasone.[22]

Mineralocorticoid therapy in CAH patients returns plasma volume to normal and eliminates the hypovolemic drive to ACTH secretion. Thus, mineralocorticoid therapy often permits the use of lower doses of glucocorticoids in patients with simple virilizing CAH, optimizing growth in children and diminishing unwanted weight gain in adults. Only one oral mineralocorticoid preparation, fludrocortisone (9α-fluorocortisol), is generally available. When the oral route is not available in severely ill patients, mineralocorticoid replacement is achieved through intravenous hydrocortisone plus sodium chloride. About 20 mg of hydrocortisone has a mineralocorticoid effect of about 0.1 mg of 9α-fluorocortisol (see Table 13-7). Mineralocorticoids are unique in pharmacology in that their doses are not based on body mass or surface area. In fact, newborns are quite insensitive to mineralocorticoids as reflected by their high serum aldosterone concentrations (see Figure 13-17) and often require larger doses than do adults (0.15 to 0.30 mg/day, depending on the sodium supplementation). In older children, the replacement dose of 9α-fluorocortisol is 0.05 to 0.15 mg daily. It must be emphasized that a mineralocorticoid is essentially useless unless adequate sodium is presented to the renal tubules. Thus, additional salt supplementation, usually 1 to 2 g NaCl/day in the newborn, is also needed. Some adult patients with severe salt-losing CAH can discontinue mineralocorticoid replacement and salt supplementation; this probably reflects the increased mineralocorticoid sensitivity in adults, their free access to salty foods, and the induction of hepatic extra-adrenal 21-hydroxylating enzymes.[27]

Long-term management requires careful clinical and laboratory monitoring. Measurements of growth should be made at 3- to 4-month intervals in children, along with an annual assessment of bone age. Each visit should be accompanied by measurement of blood pressure, plasma renin activity and serum Δ^4-androstenedione, DHEA, DHEA sulfate, and testosterone. Plasma 17OHP is generally measured but may be difficult to interpret because of its variation as a function of the timing of glucocorticoid doses, its diurnal variation and its hyperresponsiveness to stress (e.g., clinic visits).

Experimental Prenatal Treatment of CAH

Because the treatment of CAH involves administering a glucocorticoid to suppress the HPA axis, this approach has been proposed for treating the affected fetus by administering glucocorticoids to the mother. Female fetuses affected with CAH begin to become virilized at about 6 to 8 weeks' gestation, the same time at which the testes of normal male fetuses produce testosterone, causing fusion of the labioscrotal folds, enlargement of the genital tubercle into a phallus, and the formation of the phallic urethra. The adrenals of female fetuses with CAH may produce concentrations of testosterone that approach those of a normal male, resulting in varying degrees of masculinization of the external genitalia. If fetal adrenal steroidogenesis is suppressed in a female fetus with CAH, the virilization can theoretically be reduced or eliminated. Thus, some have advocated administering dexamethasone to the mother as soon as pregnancy is diagnosed; however, such treatment remains experimental and requires approval by an institutional review board (IRB) and informed parental consent.[22] This treatment should be done only when the parents are known to be heterozygotes by having already had an affected child. However, even in such pregnancies, only one in four fetuses will have CAH. Furthermore, as no prenatal treatment is needed for male fetuses affected with CAH, only one in eight pregnancies of heterozygous parents would harbor an affected female fetus that might potentially benefit from prenatal treatment. However, treatment must be started at about 6 weeks postconception (8 weeks of amenorrhea). Prenatal diagnostics cannot begin until 6 to 8 weeks into pregnancy when fetal sexing may be performed from mother's blood, allowing nontreatment or early cessation of dexamethasone treatment in male fetuses. In female fetuses, chorion biopsy at 12 to 13 weeks will allow the diagnosis of CAH. Thus, seven of eight pregnancies will be treated needlessly to treat one affected female fetus.[22]

The ethics of such prenatal treatment remain highly controversial.[22,118,119] The rationale is that dexamethasone, which is not metabolized by placental 11βHSD2, will cross the placenta, suppress fetal ACTH, and consequently suppress adrenal steroidogenesis. However, it is not known precisely when the fetal hypothalamus begins to produce CRH, when the fetal pituitary begins to produce ACTH, whether all fetal ACTH production is regulated by CRH, or whether these hormones are suppressible by dexamethasone in the early fetus. Although there is evidence that pharmacologic doses of glucocorticoids do not harm pregnant women, few data exist for the fetus. Pregnant women with diseases such as nephrotic syndrome and systemic lupus erythematosus are generally treated with prednisone, which does not reach the fetus because it is inactivated by placental 11βHSD. Treatment of a fetus with CAH requires the use of fluorinated steroids that escape metabolism by these enzymes, and few data are available about the long-term use of such agents throughout gestation.

The usual protocol employs dexamethasone doses of 20 μg/kg of maternal body weight (with a maximum dose of 1.5 mg/day). For a 60-kg woman, this is 1.2 mg, which is about six times physiologic replacement. However, the fetus normally develops in the presence of very low cortisol concentrations of only about 20 to 60 nmol/L (0.7 to 2 μg/dL),[120,121] which is only about 10% of the corresponding maternal level. Thus, the doses used in prenatal treatment appear to achieve effective concentrations of active glucocorticoid that may be up to 60 times physiologic for the fetus. The potential benefits of prenatal treatment are reduction or elimination of virilization of external genitalia and brain, reducing the risk of gender confusion and the need for surgery. The advocates of prenatal treatment report modest cushingoid features in the mother and no untoward effects in the offspring, including the 7 of 8 fetuses in whom the treatment is stopped once the diagnosis is made. However, animal

studies indicate prenatally administered dexamethasone increases risks of palatal clefting; impairs brain, kidney, and islet cell development; decreases birth weight; and increases risks of hypertension.[22,119,122] Human studies have been limited in methodology or size, but clinical studies in Sweden found that prenatally treated children without CAH had poorer working memory and poorer self-perception of scholastic competence and increased self-rated social anxiety[123]; there were also mild effects on sociability[124] and gender role behavior in boys,[125] leading the Swedish investigators to discontinue further research on this treatment.[126] Alternative approaches, such as a preimplantation genetic diagnosis, also carry some risks. Thus, the risks of prenatal treatment appear to outweigh the benefits.[119]

Experimental Postnatal Treatment of CAH

Growth is compromised in most children with CAH. The use of short-acting glucocorticoids and mineralocorticoid supplementation help, but adult heights are typically at least 1 SD below predicted heights (based on parental heights).[22] The loss of height in CAH is partially due to the effect of sex steroids on epiphyseal closure and partially due to glucocorticoid-induced resistance to the action of growth hormone; the most crucial times when height is lost are during the first 2 years of life and during the pubertal growth spurt. Consequently, several research studies have addressed optimizing the final height of children with CAH.[22]

Antiandrogens and Aromatase Inhibitors. Antiandrogens and aromatase inhibitors have been tried in the presence of low-dose mineralocorticoids and lower doses of glucocorticoids.[22] Because estrogen, not androgen, is the key hormone in promoting epiphyseal fusion, inhibiting the conversion of androgen to estrogen with an aromatase inhibitor (testolactone) promotes growth, whereas the antiandrogen (flutamide) ameliorates virilization. The principal benefit of this approach is that it permits the use of physiologic replacement doses of glucocorticoids (8 mg/m^2/day of hydrocortisone), rather than the usual supraphysiologic doses of 12 to 15 mg/m^2/day, thus further promoting normal growth. The drugs are expensive and not approved for this use, long-term safety and efficacy are not established, and antiandrogens are potentially hepatotoxic. Thus, like all experimental therapies, this approach should only be pursued in controlled, prospective, IRB-approved trials.[22]

Adrenalectomy. Adrenalectomy has been proposed in severe CAH. As the adrenal carrying severe P450c21 mutations (e.g., gene deletions) cannot produce aldosterone or cortisol, it has been argued that these affected glands do more harm than good and should be removed. The advent of laparoscopic adrenalectomy has made this suggestion feasible without undue surgical trauma to the patient. Among 18 adrenalectomized patients, five had adrenal crises when therapy was suboptimal and two became hypoglycemic during intercurrent illnesses.[22] These risks are similar to those faced by children with CAH who do not receive stress-dose steroids. Adrenalectomy also removes the adrenal medulla, but it does not appear to predispose to hypoglycemia because children with CAH are already epinephrine deficient. Thus, although adrenalectomy is an extreme measure, it may be appropriate in selected cases.[22]

Growth Hormone and GnRH Agonist Therapy. Growth hormone and GnRH agonist therapy have been proposed in children near the age of puberty, as pharmacologic GH therapy may partially overcome the effects of higher doses of glucocorticoids, and GnRH agonists will delay the progression of puberty, permitting more time to grow. Small preliminary studies with these agents are promising, but both agents are expensive and neither is approved for this use; controlled prospective trials are needed.[22]

Lesions in Isozymes of P450c11

11β-Hydroxylase Deficiency

There are two distinct forms of 11-hydroxylase.[9] P450c11β, encoded by the *CYP11B1* gene, mediates the 11β-hydroxylation of 11-deoxycortisol to cortisol and that of DOC to corticosterone in the zonae fasciculata and glomerulosa. P450c11AS, or aldosterone synthase, encoded by the *CYP11B2* gene, is found only in the zona glomerulosa and mediates 11β-hydroxylation, 18-hydroxylation, and 18-oxidation; thus, it is the sole enzyme required to convert DOC to aldosterone. P450c11β is found in both the glomerulosa and fasciculata, and mediates 11β-hydroxylation and some 18-hydroxylation, but it has no 18-methyl oxidase activity. Deficient P450c11β activity causes about 5% of CAH in persons of European ancestry but is more common in both Moslem and Jewish Middle Eastern populations.[127,128] Severe deficiency of P450c11β decreases the secretion of cortisol, causing CAH and virilization of affected females. The defect in the pathway to cortisol results in accumulation of 11-deoxycortisol and the defect in the 17-deoxy pathway in the synthesis of corticosterone in the fasciculata may lead to overproduction of DOC. Because DOC is a mineralocorticoid, these patients can retain sodium. Although DOC is less potent than aldosterone, it is secreted at high levels in 11β-hydroxylase deficiency, so that salt is retained and the serum sodium remains normal. Overproduction of DOC frequently leads to hypertension; as a result, 11β-hydroxylase deficiency is often referred to as "the hypertensive form of CAH" when detected in older children. However, newborns often manifest mild, transient salt loss as a result of the normal newborn resistance to mineralocorticoids (Figure 13-17); this may lead to incorrect diagnosis and treatment. Thus, there may be a poor correlation between DOC concentrations, serum potassium, and blood pressure or between the degree of virilization in affected females and the electrolyte and cardiovascular manifestations. Newborns may also have elevated concentrations of 17OHP, presumably as a "backup" phenomenon of high concentrations of 11-deoxycortisol inhibiting P450c21, so that P450c11β deficiency may be detected in newborn screening for P450c21 deficiency.[111] The diagnosis is established by demonstrating elevated basal concentrations of DOC and

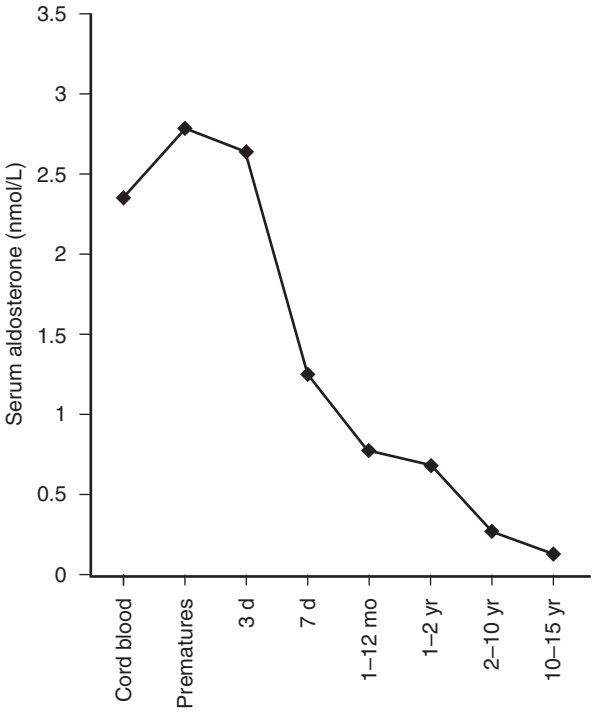


FIGURE 13-17 ■ Concentrations of aldosterone as a function of age.

11-deoxycortisol which hyperrespond to ACTH; a normal or suppressed plasma renin activity is also a hallmark of this disease.

The *CYP11B1* mutations causing 11β-hydroxylase deficiency have been reviewed; common mutations include R448H, which is common among Moroccan Jews, and Q356X and G379V found in Tunisian Arabs.[128] A milder, nonclassic form of 11β-hydroxylase deficiency, analogous to nonclassic 21-hydroxylase deficiency has been reported in otherwise asymptomatic women with hirsutism, virilism, and menstrual irregularities. However, true nonclassic 11β-hydroxylase deficiency is rare; only two of five hyperandrogenemic women who had 11-deoxycortisol values more than three times higher than the 95th percentile in response to stimulation with ACTH had mutations of P450c11β, all of which retained 15% to 37% of normal activity.[129] Repeated ACTH testing in two of the three women who lacked mutations showed much lower (but still elevated) 11-deoxycortisol values. Thus, just as in the case of nonclassic 3βHSD deficiency, an abnormal steroid response to ACTH is not sufficient to diagnose a genetic lesion.

Corticosterone Methyl Oxidase Deficiencies

P450c11AS, encoded by the CYP11B2 gene, has 93% amino acid sequence identity with P450c11β and is expressed exclusively in the zona glomerulosa, where it catalyzes 11β-hydroxylase, 18-hydroxylase, and 18 methyl oxidase activities. Disorders of P450c11AS cause the so-called corticosterone methyl oxidase (CMO) deficiencies, wherein aldosterone biosynthesis is impaired while the zona fasciculata continues to produce corticosterone and DOC. The absence of aldosterone biosynthesis will generally result in a salt-wasting crisis in infancy, at which time the normal secretory rate of DOC is insufficient to meet the newborn's mineralocorticoid requirements (similarly to the newborn with P450c11β deficiency). Thus, these infants typically present with hyponatremia, hyperkalemia, and metabolic acidosis, but the salt-wasting syndrome is typically less severe than in patients with 21-hydroxylase deficiency or lipoid CAH because of the persistent secretion of DOC. These patients may recover spontaneously and grow to adulthood without therapy. This probably reflects the increasing sensitivity to mineralocorticoid action with advancing age in childhood, as reflected by the usual, age-related decrease in serum aldosterone (see Figure 13-17). Consistent with this, plasma renin activity is markedly elevated in affected children but may be normal in affected adults.

CMOI deficiency results from a complete loss of P450c11AS activity so that no 18-hydroxylase or 18 methyl oxidase activity persists, eliminating the biosynthesis of 18OH-corticosterone and aldosterone, while preserving the biosynthesis of corticosterone by P450c11β. Thus, the diagnosis for CMOI deficiency is usually based on an increased ratio of corticosterone to 18OH-corticosterone. A frameshift mutation, a premature stop codon, and the missense mutation R384P are known to cause this disorder.[9]

CMOII deficiency results from amino acid replacement mutations in P450c11AS that selectively delete the 18 methyl oxidase activity while preserving the 18-hydroxylase activity. The diagnosis of CMOII deficiency requires an increased 18OH-corticosterone and very low aldosterone concentration. CMOII deficiency is common in Sephardic Jews of Iranian origin, where all affected individuals appear to be homozygous for two different mutations, R181W and V385A.[130] Family members who were homozygous for only one of these mutations were clinically unaffected; both mutations are required to cause disease.

The distinction between CMOI and CMOII is not precise, and these disorders should be regarded as different degrees of severity on a continuous clinical spectrum just as the various forms of 21-hydroxylase deficiency are part of a broad clinical spectrum. The close linkage and genetic similarity of the *CYP11B1* and *CYP11B2* genes is reminiscent of the P450c21A and P450c21B genes. However, gene conversion is rare, apparently due to the higher recombinational frequency in the HLA region carrying the P450c21 genes.

Glucocorticoid-Suppressible Hyperaldosteronism

Although *CYP11B* gene conversions are rare, an unusual gene duplication causes glucocorticoid-suppressible hyperaldosteronism. A homologous recombination event creates a third CYP11B gene that fuses the 5′ flanking DNA of the CYP11B1 gene for P450c11β onto the CYP11B2 gene for P450c11AS, thus placing the regulation of P450c11AS under the control of ACTH rather than the renin-angiotensin system, so that these patients make P450c11AS in response to physiology that should stimulate P450c11β.[131] The excess P450c11AS causes hyperaldosteronism and hypertension; this is then

suppressible by glucocorticoid suppression of ACTH, which normally suppresses P450c11β, hence the name "glucocorticoid-remediable hypertension"; this disorder appears to account for about 2% of hypertension.[132]

Lesions in Isozymes of 11β-Hydroxysteroid Dehydrogenase

Cortisone and prednisone are inactive prohormones that must be reduced to cortisol or prednisolone in order to bind to and activate the glucocorticoid receptor. The interconversion of these keto- and hydroxysteroids is catalyzed by the two isozymes of 11β-hydroxysteroid dehydrogenase, 11βHSD1 (encoded by *HSD11B1*) and 11βHSD2 (encoded by *HSD11B2*). Both enzymes are reversible in vitro, hence both can act as either an oxidase or reductase, depending on the availability of cofactors, but under physiologic situations 11βHSD1 generally acts to activate cortisone to cortisol whereas 11βHSD2 reverses this activation.[9,11,45] 11βHSD1 is primarily expressed in liver and fat, and 11βHSD2 is expressed in mineralocorticoid-responsive tissues where it inactivates cortisol, permitting low concentrations of aldosterone to activate mineralocorticoid receptors (which also bind cortisol); 11βHSD2 is inactive against aldosterone, DOC, and fluorocortisol. Interest in these enzymes extends far beyond their deficiency states, as they play central roles in metabolism[43-45]; this has stimulated interest in using inhibitors of 11βHSD1 to treat the metabolic syndrome, but such agents are not yet clinically available.[133]

Lesions in 11βHSD1: (Apparent) Cortisone Reductase Deficiency

Defective 11βHSD1 activity, diagnosed by reduced ratios of urinary metabolites of cortisol to those of cortisone, impairs cortisol feedback at the hypothalamic-pituitary axis, increasing the secretion of ACTH and consequently increasing adrenal C19 steroid secretion resulting in hyperandrogenism, sexual precocity, and polycystic ovaries. These patients may have heterozygous mutations (K187N, R137C) in the *HSD11B1* gene encoding 11βHSD1; because 11βHSD1 normally functions as a dimer, the presence of some mutant protein can exert dominant negative effects.[134] Alternatively, a genetic lesion may be found in the *H6PDH* gene,[135] which encodes hexose-6-phosphate dehydrogenase (H6PDH), the enzyme that generates the NADPH used by 11βHSD1 in the lumen of the endoplasmic reticulum. Although it was initially thought that mutations in both 11βHSD1 and H6PDH interacted to cause this disease,[136] mutations of H6PDH in patients appear to manifest with a more severe phenotype and are sufficient to cause this disorder.[134,135,137,138]

Lesions In 11βHSD2: Apparent Mineralocorticoid Excess (AME)

Patients with AME have hypervolemic hypertension, salt retention, and hypokalemic alkalosis—the classic picture of hyperaldosteronism—but with suppressed plasma renin activity and without measurable serum mineralocorticoids due to recessive mutations of 11βHSD2.[42] About 30 different mutations in 11βHSD2 have been described in about 60 patients with AME, and heterozygous carriers may have an increased risk of hypertension.[139] Typical features of children with AME include failure to thrive, delayed puberty, polydipsia, polyuria, muscle weakness, and hypertension. The hypertension is severe, often causing end-organ damage at an early age. Diagnosis is made from the high ratio of urinary metabolites of cortisol to cortisone. Treatment includes antagonism of the mineralocorticoid receptor with spironolactone, correction of the hypokalemia, low-salt diets, and diuretics, but it is only partially successful, and 10% of patients die from cerebrovascular accidents.[140]

ADRENAL INSUFFICIENCY

Many conditions cause adrenal insufficiency, including CAH, hypopituitarism with ACTH deficiency, and primary adrenal disorders. Primary adrenal insufficiency is commonly termed Addison disease, but this is a vague term that encompasses many disorders. Up to World War II, most patients with "Addison disease" had tuberculosis of the adrenal, but over 80% of contemporary adult patients have autoimmune adrenalitis, therefore, the term *Addison disease* is now widely used to indicate an autoimmune or idiopathic cause.

The spectrum of adrenal disorders presenting in infants, children, and adolescents differs from that presenting in adulthood (Box 13-1). CAH and autoimmune adrenal disease represent the largest proportions of cases, but some of the inherited developmental and metabolic causes of adrenal failure are also fairly common.[141,142] Diagnosing some of these disorders is important for assessing potential associated features, initiating long-term management, and instituting genetic counseling.[143] Adrenal disorders are typically divided into chronic and acute causes, but many acute presentations reflect an undiagnosed underlying chronic or developmental process (Box 13-2). Acute presentations may be triggered by intercurrent illness, trauma, or surgery, with poor fluid and sodium intake.

Acute Primary Adrenal Insufficiency

Acute adrenal crisis occurs most commonly in the child with undiagnosed chronic adrenal insufficiency who is subjected to an additional severe stress such as major illness, trauma, or surgery. The major presenting symptoms and signs include abdominal pain, fever, hypoglycemia with seizures, weakness, apathy, nausea, vomiting, anorexia, hyponatremia, hypochloremia, acidemia, hyperkalemia, hypotension, shock, cardiovascular collapse, and death. Treatment consists of fluid and electrolyte resuscitation, ample doses of glucocorticoids, chronic glucocorticoid and mineralocorticoid replacement, and treatment of the precipitating illness.

Massive adrenal hemorrhage with shock due to blood loss can occur in large infants who have had a traumatic delivery. A flank mass is usually palpable and can be distinguished from renal vein thrombosis by microscopic rather than gross hematuria; the diagnosis is then confirmed by

BOX 13-1 Causes of Adrenal Insufficiency

Primary Adrenal Insufficiency

- Congenital adrenal hyperplasia
  - Autoimmune disorders
  - Autoimmune adrenalitis
- Autoimmune polyglandular syndromes
- Adrenal hypoplasia congenita
  - X-linked adrenal hypoplasia
  - Other (*SF1*, IMAGe syndrome)
- ACTH resistance syndromes
  - Familial glucocorticoid deficiencies, types 1 and 2
  - Triple A (Allgrove) syndrome
- Metabolic disorders
  - Adrenoleukodystrophy
  - Peroxisome biogenesis disorders (e.g., Zellweger)
  - Cholesterol metabolism (Smith-Lemli-Opitz, Wolman)
  - Mitochondrial (Kearn-Sayers, mitochondrial deletions)
- Infectious disorders
  - Sepsis
  - Tuberculosis
  - Fungal infections
  - Viral
- Infiltrative/destructive causes
  - Hemorrhage
  - Amyloidosis, sarcoidosis, metastases
- Drugs inhibiting steroid biosynthesis

Secondary Adrenal Insufficiency

- Hypothalamic tumors, radiation, or surgery
- Hypopituitarism
- Isolated ACTH insufficiency
- Defects in POMC synthesis and processing
- Withdrawal from glucocorticoid therapy

BOX 13-2 Signs and Symptoms of Adrenal Insufficiency

Features Shared by Acute and Chronic Insufficiency

- Anorexia
- Apathy and confusion
- Dehydration
- Fatigue
- Hyperkalemia
- Hypoglycemia
- Hyponatremia
- Hypovolemia and tachycardia
- Nausea and vomiting
- Postural hypotension
- Prolonged neonatal jaundice
- Salt craving
- Weakness

Features of Acute Insufficiency (Adrenal Crisis)

- Abdominal pain
- Fever

Features of Chronic Insufficiency (Addison Disease)

- Decreased pubic and axillary hair
- Diarrhea
- Hyperpigmentation
- Low-voltage electrocardiogram
- Small heart on x-ray
- Weight loss

computed tomography or ultrasonography. Massive adrenal hemorrhage is more commonly associated with meningococcemia (Waterhouse-Friderichsen syndrome). Meningitis is often, but not always, present. The characteristic petechial rash of meningococcemia can progress rapidly to large ecchymoses; the blood pressure drops and respirations become labored, frequently leading rapidly to coma and death. Immediate intervention with intravenous fluids, antibiotics, and glucocorticoids is not always successful. A similar adrenal crisis may also occur rarely with septicemia from *Streptococcus*, *Pneumococcus*, *Pseudomonas*, diphtheria, and methicillin-sensitive and resistant isolates of *Staphylococcus aureus*.[144] Adrenal hemorrhage has also been reported with the antiphospholipid syndrome and in patients on anticoagulant therapy.

Chronic Primary Adrenal Insufficiency

Autoimmune Disorders

Autoimmune adrenalitis is most commonly seen in adults 25 to 45 years old, about 60% to 70% of whom are women, with an adult prevalence of about 1 in 25,000.[145] Autoimmune destruction of other endocrine tissues is frequently associated with autoimmune adrenalitis. Chronic adrenal insufficiency is suggested by poor weight gain or weight loss, weakness, fatigue, anorexia, hypotension, hyponatremia, hypochloremia, hyperkalemia, frequent illnesses, nausea, and vague gastrointestinal complaints (see Box 13-2), reflecting chronic deficiency of both glucocorticoids and mineralocorticoids. Early in the course of autoimmune adrenalitis one may see signs of glucocorticoid deficiency (weakness, fatigue, weight loss, hypoglycemia, anorexia) without signs of mineralocorticoid deficiency (hyponatremia, hyperkalemia, acidosis, tachycardia, hypotension, low voltage on EKG, small heart on chest x-ray) or evidence of mineralocorticoid deficiency without glucocorticoid deficiency. Thus, an initial clinical presentation that spares one category of adrenal steroids does not mean it will be spared in the long run. The symptoms listed in Box 13-2 can be seen in chronic adrenal insufficiency that is either primary or secondary. In primary chronic adrenal insufficiency, the low concentrations of plasma cortisol stimulate the hypersecretion of ACTH and other POMC peptides, including the various forms of melanocyte-stimulating hormone (MSH); consequently, chronic primary adrenal insufficiency is also characterized by

hyperpigmentation of the skin and mucous membranes, whereas secondary adrenal insufficiency is not. Such hyperpigmentation is most prominent in skin exposed to sun and in flexor surfaces such as knees, elbows, and knuckles. The diagnosis is suggested by the previously listed signs and symptoms, verified by a low morning cortisol level with a high ACTH, and confirmed by a minimal response of cortisol to a 60-minute intravenous ACTH test. Hyponatremia, hyperkalemia, low aldosterone, and elevated PRA suggest a disturbance in mineralocorticoid production. Associated findings may include the appearance of a small heart on chest x-ray, anemia, azotemia, eosinophilia, lymphocytosis, and hypoglycemia. Treatment of chronic primary adrenal insufficiency consists of physiologic glucocorticoid and mineralocorticoid replacement therapy.

Autoimmune adrenalitis is strongly associated with specific HLA haplotypes and with polymorphisms in the gene for cytotoxic T lymphocyte–associated antigen 4 (CLTA 4), which may be broadly involved in susceptibility to autoimmune disease.[146,147] The diagnosis of autoimmune chronic adrenal insufficiency is based largely on finding circulating antibodies directed against adrenal cells or adrenal cellular contents. In many cases the adrenal antigens are steroidogenic cytochrome P450 enzymes, especially P450scc, P450c17, and P450c21.[145] It is not clear how these enzymes reach immune cells to elicit an antibody response, but autopsy studies show infiltration of the adrenal cortex.[141] Thus, it is likely that cell-mediated immunity is responsible for destruction of adrenocortical cells, resulting in a secondary discharge of cellular contents (including P450 enzymes) into the circulation, with the subsequent development of secondary "marker" antibodies against these P450s. About half of adult patients with lymphocytic adrenalitis will also have autoimmune disease of another endocrine tissue with high titers of antibodies directed against specific contents of the affected tissue. This finding has led to the definition of specific autoimmune polyendocrine syndromes (APS), some of which are more prevalent in childhood.

Type 1 Autoimmune Polyendocrine Syndrome. Type 1 autoimmune polyendocrine syndrome (APS1), also known as autoimmune polyendocrinopathy-candidiasis-ectodermal dysplasia (APECED), is characterized by chronic mucocutaneous candidiasis, autoimmune Addison disease, and hypoparathyroidism. At least two of these features must be present to make the diagnosis, and their age of onset can be highly variable. In general, chronic mucocutaneous candidiasis appears in early childhood and affects the mouth and nails. Acquired hypoparathyroidism can present with clinical hypocalcemia during middle or late childhood, although in some cases hypocalcemia may be masked by untreated adrenal insufficiency. The adrenal disorder usually presents in childhood or adolescence; autoimmune adrenal disease may be a presenting feature in about 5% of cases.[148] Additional autoimmune features of this condition include alopecia and vitiligo; gastritis, chronic diarrhea, and malabsorption with or without pernicious anemia; hypergonadotropic hypogonadism, especially in women; and less commonly hepatitis, thyroiditis, interstitial nephritis, myositis, dental enamel hypoplasia, acquired asplenia, and type 1 diabetes mellitus. Keratoconjunctivitis is an important associated feature that requires careful monitoring and treatment to prevent blindness. Oral or esophageal squamous cell carcinoma occurs in 10% of individuals as adults.[148] APS1 is rare in most populations but is common among people of Finnish (1:15,000), Sardinian, and Iranian Jewish (1:9,000) ancestry.[141] APS1 is caused by recessively inherited mutations in a 58-kilodalton transcription factor called AIRE ("autoimmune regulator").[149,150] More than 50 different mutations in the *AIRE* gene have been described, although the homozygous or compound heterozygous R257X change is especially common in the Finnish population. The *AIRE* gene is widely expressed in developing tissues of the immune system. The specific mechanisms by which these mutations result in the pleiotropic findings of APS1 are not yet clear, although deletion of AIRE in mice results in ectopic expression of peripheral tissue antigens in thymic medullary epithelial cells, resulting in the development of an autoimmune disorder similar to APS1/APECED.[151]

Type 2 Autoimmune Polyendocrine Syndrome. Type 2 autoimmune polyendocrine syndrome (APS2), also known as Schmidt syndrome, refers to the relatively common association of autoimmune adrenalitis with thyroiditis or type 1 diabetes.[145] APS2 is more common in females (3:1 ratio), is HLA-linked, and is generally seen in young or middle-aged adults but can present at almost any age. Primary (hypergonadotropic) ovarian failure is seen in up to one quarter of postpubertal females with APS2, but primary testicular failure is rare.[141] Pernicious anemia, hepatitis, vitiligo, and alopecia may also be seen, but the hypoparathyroidism and mucocutaneous candidiasis typical of APS1 are not seen in APS2. APS2 is associated with the same HLA markers as idiopathic autoimmune adrenalitis, which may simply be a form of APS2.

Adrenal Hypoplasia Congenita

Adrenal hypoplasia congenita, also known as congenital adrenal hypoplasia, is a disorder of adrenal development resulting in primary adrenal insufficiency. This condition can occur with several different inheritance patterns and with a variety of associated or syndromic features.

X-linked Adrenal Hypoplasia Congenita. X-linked adrenal hypoplasia congenita (AHC) is caused by mutations of the *NR0B1* gene encoding DAX1 on chromosome Xp21. In this most common form of primary adrenal hypoplasia, the definitive zone of the fetal adrenal does not develop, and the fetal zone is vacuolated and cytomegalic. About half of boys with AHC present with salt loss and glucocorticoid insufficiency in early infancy; the rest present more insidiously with chronic adrenal insufficiency throughout childhood.[142] Hypogonadotropic hypogonadism and incomplete pubertal development are associated features, although early puberty with subsequent pubertal arrest has been reported in rare cases. An underlying defect in spermatogenesis may also be present.

DAX1 is a nuclear transcription factor involved in adrenal and testicular development, as well as being expressed in pituitary gonadotropes. About two thirds of boys with AHC have point mutations,[142] the other one third have *NR0B1* gene deletions either in isolation or as part of a contiguous gene deletion syndrome involving a telomeric X-linked mental retardation locus (*IL1RAPL1*) or centromeric genes for glycerol kinase deficiency (*GKD*) and sometimes ornithine transcarbamylase (*OTC*) and Duchenne muscular dystrophy (*DMD*). An adult-onset form of AHC due to point mutations has also been described.[152]

Boys with AHC respond well to glucocorticoid and mineralocorticoid replacement therapy. Testosterone treatment to induce secondary sexual characteristics is needed in adolescence; spontaneous fertility is rare and attempts to induce spermatogenesis with gonadotropins are rarely successful. Female carriers are unaffected, but half of their sons will be affected. Close monitoring and genetic counseling can help to prevent life-threatening adrenal crises in other family members or future pregnancies. Thus, a family history of adrenal failure, unexplained death, or pubertal abnormalities in the male relatives of a boy with adrenal insufficiency should suggest AHC; indeed, a substantial proportion of boys with sporadic adrenal hypoplasia have DAX1 mutations.[142]

Autosomal Forms of Adrenal Hypoplasia. In addition to X-linked AHC, autosomal recessive forms of adrenal hypoplasia have been reported and are yielding to genetic analysis. Mutations in steroidogenic factor-1 (SF1, *NR5A1*), which were discussed earlier as a disorder of steroidogenesis that resembles lipoid CAH, are sometimes classified as a form of adrenal hypoplasia. However, SF1 mutations affect the gonad more severely than the adrenal and have not been reported in phenotypic males with adrenal hypoplasia.[142] Primary adrenal failure has also been associated with Pena-Shokeir syndrome type 1, pseudotrisomy 13, Meckel syndrome, and Pallister-Hall syndrome (*GLI3*), and with defects in *WNT3*.[4]

IMAGe Syndrome. Primary adrenal hypoplasia and neonatal adrenal crisis are also part of the IMAGe syndrome (*I*ntrauterine growth retardation, *M*etaphyseal dysplasia, *A*drenal hypoplasia, *Ge*nitourinary anomalies).[153] A large, multigenerational family with multiple, well-characterized individuals[154] has now been studied by linkage analysis, identifying a dominant missense mutation in the *CDKN1C* gene as the cause of this disorder.[155] In addition, four other unrelated patients had four different missense mutations in *CDKN1C*.[155] The *CDKN1C* gene encodes the Wilms tumor supressor p57KIP2, which inhibits several cyclin-dependent kinases. This gene lies in the imprinted region of chromosome 11p15.5, so that only the maternal allele is expressed. Different mutations in this same gene cause Beckwith-Wiedemann syndrome.[156] The Beckwith mutations inhibit cell cycle, whereas the IMAGe mutations do not, and, when expressed in *Drosophila*, the Beckwick mutations have no effect on eye development, but the IMAGe mutations cause restricted growth.[155] Thus, mutations in the same gene cause both the IMAGe adrenal hypoplasia syndrome and the Beckwith-Wiedemann adrenal hyperplasia syndromes.

ACTH Resistance Syndromes

Hereditary unresponsiveness to ACTH, also termed familial glucocorticoid deficiency (FGD), can present as an acute adrenal crisis precipitated by an intercurrent illness in an infant or with the signs and symptoms of chronic adrenal insufficiency in childhood. Several different recessively inherited causes of FGD have been identified. Unlike individuals with autoimmune adrenalitis, adrenal hypoplasia, or other forms of destruction of adrenal tissue, patients with hereditary unresponsiveness to ACTH typically continue to produce mineralocorticoids, because production of aldosterone by the adrenal zona glomerulosa is regulated principally by the renin-angiotensin system. Thus, the presenting picture consists of failure to thrive, lethargy, pallor, hyperpigmentation, and hypoglycemia, often associated with seizures. Rare cases may also entail electrolyte abnormalities or increased plasma renin activity, leading to misdiagnosis as a different form of adrenal insufficiency.

MC2R Mutations: Familial Glucocorticoid Deficiency Type 1 (FGD1). The ACTH receptor is a 7-transmembrane, G-protein–coupled member of the family of melanocortin receptors termed MC2R.[157] *MC2R* mutations appear to be the most common cause of ACTH resistance, with several dozen reported cases, but the statistical distribution of these cases is unclear, as many different clinical criteria have been applied.[82] These patients present with glucocorticoid deficiency and hyperpigmentation, often around 2 years of age; hypoglycemia is common and ACTH levels are grossly elevated, accounting for the hyperpigmentation. Tall stature and increased head circumference has been reported in several cases. Treatment with replacement doses of glucocorticoids typically prevents adrenal crises but may not suppress elevated ACTH levels completely; nevertheless, the use of supraphysiologic doses of steroids to suppress ACTH should be avoided.

MRAP Mutations: Familial Glucocorticoid Deficiency Type2 (FGD2). The melanocortin 2 receptor accessory protein, MRAP, is a small transmembrane protein that forms unusual antiparallel dimers and serves two functions: it facilitates trafficking of MC2R from the endoplasmic reticulum to the cell membrane, and it interacts with MC2R on the cell surface to facilitate receptor action.[158] Both MRAP and the related MRAP2 protein serve similar functions with other members of the MCR family, but MRAP2 mutations have not yet been reported. MRAP mutations apparently are the second-most common cause of FGD, but MRAP mutations are clinically indistinguishable from MC2R mutations.[159]

Other Forms of Familial Glucocorticoid Deficiency. Mild mutations in StAR that cause nonclassic lipoid CAH[81] may be mistaken for familial glucocorticoid deficiency.[82] Linkage analysis in families with apparent FGD has identified mutations in *NNT*, the gene encoding

nicotinamide nucleotide transhydrogenase, as another cause of this disease.[160] The NNT protein is located on the inner mitochondrial membrane, where it participates in the generation of NADPH, which is necessary for the action of P450scc to convert cholesterol to pregnenolone and for responses to oxidative stress. In the Irish Traveller population, the *MCM4* gene encoding minichromosome maintenance complex component 4 has also been found to be associated with FGD; patients with MCM4 mutations also presented with growth failure, increased chromosomal breakage, and natural killer cell deficiency.[161]

Triple A (Allgrove) Syndrome. Triple A (Allgrove) syndrome consists of (1) ACTH-resistant *a*drenal (glucocorticoid) deficiency (80% of individuals), (2) *a*chalasia of the cardia (85%), and (3) *a*lacrima (90%). The alacrima is the earliest and most consistent symptom, and the achalasia and adrenal insufficiency develop over the first two decades, although the achalasia is usually noted first. Mineralocorticoid insufficiency is reported in about 15% of cases, and up to 60% of patients develop progressive neurologic symptoms such as intellectual impairment, sensorineural deafness, peripheral and cranial neuropathies, optic atrophy, Parkinsonism, and autonomic dysfunction.[75] About 80% of affected patients have autosomal recessive mutations in *AAAS*, which encodes a WD-repeat protein termed ALADIN[162,163]; the basis of the disease in patients lacking AAAS mutations is not yet known. ALADIN co-localizes to the cytoplasmic side of the nuclear pore, where it interacts with and participates in the nuclear translocation of ferritin heavy chain protein, FTH1, thus rendering cells progressively susceptible to oxidative damage.[164] Clinical findings can be quite variable even within the same family. Adrenal insufficiency is rarely the presenting feature. Thus, a detailed family history of achalasia, alacrima, or neurologic disorders is important when evaluating a patient with primary adrenal failure.

Metabolic Disorders

Metabolic disorders can also cause chronic primary adrenal insufficiency, including adrenoleukodystrophy (Schilder disease), peroxisome biosynthesis disorders (e.g., Zellweger syndrome spectrum), disorders of cholesterol synthesis and metabolism (e.g., Wolman disease, cholesterol ester storage disease, Smith-Lemli-Opitz syndrome), and mitochondrial disorders (e.g., Kearns-Sayre syndrome).

Adrenoleukodystrophy (ALD). This peroxisomal disease is the most common metabolic cause of adrenal failure. Most cases are caused by mutations in the peroxisomal membrane protein ALDP encoded by the *ABCD1* gene on chromosome Xq28[165,166]; ALDP belongs to the superfamily of ATP-binding cassette transporters. There are many clinical forms of ALD, including the childhood cerebral form (CCALD) with cerebral demyelination, the adolescent cerebral form, the adult cerebral form, adrenomyeloneuropathy with axonopathy of the pyramidal and somatosensory tracts and peripheral neuropathy, the olivo-ponto-cerebellar form, and a form presenting only with Addison disease.[167] CCALD is the most common phenotype. The prevalence of this condition is generally reported to be between 1:20,000 to 100,000, although the overall frequency may be as high as 1:17,000.[168] A rare autosomal recessive form of this condition also exists, which usually presents in infancy (discussed later).

ALDP imports activated acyl-CoA derivatives of very long chain fatty acids (VLCFA) into peroxisomes where they are shortened by β-oxidation.[169,170] Consequently, ALD is characterized by high ratios of C26 to C22 VLCFA in plasma and tissues, permitting the diagnosis of individual patients and affected fetuses.[171] Carriers can usually be detected by VLCFA screening, although genetic analysis may be necessary in some cases. Symptoms of X-linked CCALD commonly develop in mid-childhood; adrenomyeloneuropathy presents in adulthood.[172] The same ALDP mutation can cause both adrenoleukodystrophy and adrenomyeloneuropathy, hence it is likely that other genetic loci are also involved.[173] The earliest findings in CCALD are associated with the central nervous system leukodystrophy and include behavioral changes, poor school performance, dysarthria, and poor memory progressing to severe dementia. Symptoms of adrenal insufficiency usually appear after symptoms of white matter disease, but adrenal insufficiency may be the initial finding in up to 20% of children or young adults.[168,174,175] By contrast, adrenomyeloneuropathy typically begins with adrenal insufficiency in childhood and adolescence, and signs of neurologic disease follow 10 to 15 years later. About 1% to 3% of female carriers of X-linked ALD may develop neurologic symptoms or adrenal dysfunction. Because screening is accurate and the diagnosis has long-term implications, VLCFA should be analyzed in all boys presenting with adrenal failure where the diagnosis is not clear.

Dietary therapy with so-called Lorenzo's oil (a 4:1 mixture of glyceryl-trioleate and glyceryl-trierucate) improves VLCFA levels, but it has been ineffective for the treatment of established cerebral disease, although a role in preventing the onset of cerebral disease has been evaluated.[168,173] Other therapeutic options include hematopoietic stem cell transplantation,[168,170] which is most successful for early cerebral disease with a reported 5- to 8-year survival rate of 56%.[176] Lovastatin treatment decreases plasma C24:0 and C26:0 but is no longer recommended because it has no effect on C26:0 in cells or on VLCFAs.[177]

Peroxisome Biogenesis Disorders. Peroxisome biogenesis disorders (PBDs) are a group of autosomal recessive conditions caused by mutations in PEX proteins. Zellweger syndrome, neonatal adrenoleukodystrophy, and infantile Refsum disease form the "Zellweger spectrum" and are clinically distinct from rhizomelic chondrodysplasia punctata due to PEX7 gene mutations.[178,179] These disorders are characterized by developmental delay, hypotonia, neurosensory deafness, optic atrophy, and dysmorphic facial development. Patients often develop seizures, especially those with neonatal adrenoleukodystrophy. The diagnosis can be confirmed by the presence of C26:1, increased

C26:0, and an increased ratio of C26:C22 and C24:C22 VLCFA. Most children with the severe forms of Zellweger syndrome and neonatal adrenoleukodystrophy do not survive past 2 years, although other variants of the Zellweger spectrum are associated with longer survival.[180]

Wolman Disease. Most cellular cholesterol derives from the uptake of circulating lipoproteins that contain cholesterol esters; these esters are cleaved to free cholesterol by lysosomal acid lipase (cholesterol esterase), encoded by the *LIPA* gene on chromosome 10q23.31.[10] Mutations of *LIPA* cause Wolman disease (primary xanthomatosis) and its milder variant, cholesterol ester storage disease.[181-186] In Wolman disease, cholesteryl esters and triglycerides accumulate in the liver, spleen, lymph nodes, and other tissues. In the adrenal, there is insufficient free cholesterol available for steroidogenesis, causing adrenal insufficiency. The disease is less severe than congenital lipoid adrenal hyperplasia with respect to steroidogenesis, and patients may survive for several months after birth. However, the disease affects all cells, not just steroidogenic cells, as all cells must store and utilize cholesterol; hence, the disorder is relentless and fatal. Vomiting, steatorrhea, failure to thrive, hepatosplenomegaly, jaundice, and anemia are the usual presenting findings, sometimes beginning in the first week of life, leading to developmental delay and malabsorptive malnutrition. Characteristic bilateral subcapsular adrenal calcification that outlines the adrenals may be seen on pre- or postnatal ultrasound scan. The diagnosis is established by bone marrow aspiration yielding foam cells containing large lysosomal vacuoles engorged with cholesterol esters, and it is confirmed by finding absent cholesterol esterase activity in fibroblasts, leukocytes, bone marrow cells, or cultured amniocytes (for prenatal diagnosis). Treatment by transplanting bone marrow or other hematopoietic cells appears to ameliorate the course of the disease in about half of cases, but the mechanism of this effect is unclear.[187,188] Wolman disease is rare, causing about 3% of cases of primary adrenal insufficiency in one large series.[141] Cholesterol ester storage disease appears to be a milder defect in the same enzyme, generally presenting in childhood or adolescence.[186] Hypercholesterolemia is typical, and accumulated neutral fats and cholesterol esters in the arteries predispose to atherosclerosis. Massive hepatomegaly and hepatic fibrosis may lead to esophageal varices.

Smith-Lemli-Opitz Syndrome. Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive defect in cholesterol biosynthesis resulting from abnormalities in the sterol Δ-7-reductase gene, *DHCR*7.[189] The incidence of SLOS is estimated from 1:80,000 to 1:13,000. More than 100 mutations have been described, but two predominate: the c.964G>C mutation is mostly found in North America and Western Europe, whereas the W151X mutation is frequently found in Central and Eastern Europe, suggesting founder effects for these two mutations.[190] The clinical features of SLOS include microcephaly, developmental delay, a typical facial appearance, proximal thumbs and syndactyly of the second and third toes, cardiac abnormalities, and underdeveloped genitalia in males. Adrenal insufficiency is present in some cases, especially during times of stress or when LDL-derived cholesterol sources are inadequate (e.g., dietary insufficiency/bile salt depletion).[191] The clinical spectrum of SLOS is extremely broad, likely reflecting differences in residual intrauterine cholesterol biosynthesis or differences in the transplacental supply of cholesterol supporting fetal development.[190] Biochemical analysis of sterol Δ-7-reductase activity, coupled with genetic analysis, can confirm the diagnosis. Alternatively, LC-MS/MS is a good method for sterol detection that can be used for diagnostic confirmation of SLOS. This approach may be adopted for newborn screening for SLOS via dried blood samples in the future. Life expectancy varies with disease severity. Dietary cholesterol supplementation, ranging from 20 to 300 mg/kg/day, has become an established therapeutic intervention. Beneficial effects include providing cholesterol to tissues outside the CNS and down-regulating HMG-CoA reductase, probably suppressing 7-DHC synthesis. Although this may result in amelioration of the extra-CNS phenotype, a beneficial effect on brain is unlikely, as plasma cholesterol does not cross the blood-brain barrier. Adding simvastatin to cholesterol supplementation is of uncertain benefit.[190]

Mitochondrial Disorders. Mitochondrial disorders can be associated with primary adrenal dysfunction.[192] Typical clinical features include lactic acidosis, cataracts, sensorineural deafness, and myopathy/ophthalmoplegia. Adrenal failure is rare in childhood, but subclinical adrenocortical insufficiency may be found in adults with multisystem disease, and is a poor prognostic factor. The Kearns-Sayre syndrome results from large-scale deletions of mitochondrial DNA and can be associated with additional endocrinopathies such as hypothyroidism, hypogonadism, diabetes, growth failure, and hypoparathyroidism.

Other metabolic disorders have also been reported to cause adrenal insufficiency, including Niemann-Pick disease type B, a lysosomal lipid storage disease due to mutations in the sphingomyelin phosphodiesterase 1 (SMPD1) gene manifesting with hepatosplenomegaly and pulmonary disease.

Other Causes

Chronic adrenal insufficiency may result from other causes. Hemorrhage and infections, discussed earlier as causes of acute primary adrenal insufficiency, may spare some adrenal tissue, leaving severely compromised, rather than totally absent, adrenal function. The result, as with autoimmune adrenalitis, is a chronic disorder with insidious onset of the broad range of nonspecific findings described earlier. Tuberculosis, fungal infections (e.g., histoplasmosis, coccidioidomycosis), viral infections (e.g., HIV, CMV), metastases, amyloidosis, and sarcoidosis may cause a similar clinical picture. Drugs such as aminoglutethimide, etomidate, suramin, and ketoconazole can also inhibit cortisol biosynthesis; these drugs can cause adrenal insufficiency and must be used with caution.

Secondary Adrenal Insufficiency

ACTH is required for adrenal cellular growth and for transcription of genes for steroidogenic factors, hence any impairment of ACTH synthesis or release can cause secondary adrenal insufficiency. Examples include hypothalamic defects, hypopituitarism, disorders of POMC synthesis and processing, and suppression of the hypothalamic-pituitary axis following exogenous steroid treatment.

Most forms of secondary adrenal insufficiency affect glucocorticoid and androgen synthesis rather than mineralocorticoid release, as angiotensin II is the primary drive to the zona glomerulosa. However, the clinical and biochemical assessment may be complicated as glucocorticoids are necessary for renal free water clearance and concomitant vasopressin (AVP, ADH) insufficiency may be present. In fact, treatment of secondary adrenal insufficiency can unmask a previously inapparent deficiency of antidiuretic hormone and thus precipitate diabetes insipidus, so close attention must be given to fluid and electrolyte balance when steroid replacement is introduced. Conversely, the hypothyroidism resulting from TSH deficiency will result in slowed metabolism of the small amount of cortisol produced and, therefore, protects the patient from the symptoms of adrenal insufficiency. Treatment of hypothyroidism with thyroxine will accelerate metabolism of these small amounts of cortisol, thus unmasking adrenal insufficiency due to ACTH deficiency and, on occasion, can precipitate an acute adrenal crisis. Therefore, careful evaluation of the pituitary-adrenal axis is required in hypopituitarism with secondary hypothyroidism. Many clinicians will choose to "cover" a patient with small doses of glucocorticoids (one fourth to one half of physiologic replacement) during initial treatment of such secondary hypothyroidism. Finally, it is important to appreciate that combined deficiency of growth hormone and ACTH will strongly predispose the patient to hypoglycemia, as both hormones act to raise plasma glucose. This effect is especially important in infancy and early childhood, when children are vulnerable to hypoglycemia during periods of prolonged fasting.

Hypothalamic Causes

Hypothalamic causes of ACTH insufficiency include tumors and radiotherapy. Tumors, such as craniopharyngioma, are associated with ACTH deficiency in about 25% of patients.[193,194] The frequency may be higher in tumors such as germinoma and astrocytoma. Adrenal insufficiency is rarely the presenting complaint but may contribute to the clinical picture. After surgery or radiation therapy, the great majority of patients with hypothalamic tumors will have ACTH deficiency as part of their surgical or radiation-induced hypothalamic-pituitary damage. Therefore, all such patients should receive glucocorticoid coverage during treatment, irrespective of the status of the HPA axis at the time the tumor is identified. Because treatment of secondary adrenal insufficiency can precipitate diabetes insipidus, close attention to water balance is essential. ACTH insufficiency can also result following whole-brain irradiation for brain tumors and other central malignancies. This may involve both hypothalamic and pituitary mechanisms. The frequency of ACTH insufficiency in such cases is much lower than following the treatment of hypothalamic tumors, but may manifest some years after treatment.[195]

ACTH Deficiency

ACTH may be deficient as part of a multiple pituitary hormone deficiency (MPHD, panhypopituitarism) or as isolated ACTH insufficiency. MPHD can result from pituitary surgery or radiotherapy, from an infiltrative process (e.g., Langerhans cell histiocytosis), or from a poorly understood form of hypothalamic dysfunction. In most cases, growth hormone secretion is lost first, followed in order by gonadotropins, TSH, and ACTH, so ongoing vigilance and assessment of these patients is required for many years. MPHD can also result from disorders of hypothalamic-pituitary development involving transcription factors such as HESX1, LHX4, and SOX3. Associated features such as optic nerve hypoplasia (HESX1) and cerebellar abnormalities (LHX4) may help to focus the diagnosis. Impaired ACTH secretion has also been described in individuals with PROP1 mutations, one of the most frequent genetic causes of MPHD. This finding can occur many years after presentation with GH, TSH, and gonadotropin deficiency, highlighting the importance of long-term follow-up of patients with pituitary disorders.[196,197]

Patients with MPHD often have a relatively mild form of adrenal insufficiency. Mineralocorticoid secretion is normal, whereas cortisol secretion is reduced but not absent. However, adrenal reserve is severely compromised by the chronic understimulation of steroidogenic enzyme biosynthesis. Because some cortisol synthesis continues, the diagnosis may not be apparent unless a CRF or metyrapone test of pituitary ACTH production capacity and an intravenous ACTH test of adrenal reserve are performed. This can be especially true when TSH deficiency is a component of hypopituitarism, as outlined earlier; treatment of secondary hypothyroidism with thyroxine will accelerate metabolism of these small amounts of cortisol, thus unmasking adrenal insufficiency due to ACTH deficiency, and, on occasion, can precipitate an acute adrenal crisis.

Isolated ACTH insufficiency is a rare condition that can be caused by recessively inherited mutations in the *TPIT* gene. *TPIT* encodes a T-box factor (TBX19) that regulates transcription of the POMC promoter in corticotropes.[198] These patients usually present with severe, early-onset ACTH insufficiency.[199,200] Hypoglycemia and prolonged jaundice are frequently present, and neonatal death may result.[201] As this defect is confined to POMC synthesis in the corticotropes, the additional features of generalized POMC deficiency are not present. TPIT mutations are not found in approximately half of patients with isolated ACTH insufficiency, suggesting that other causes are yet to be found.[202] Hypocortisolemia due to isolated ACTH insufficiency has also been described with hippocampal (memory) defects and hair abnormalities (alopecia) as part of the "triple H syndrome," a possible autoimmune association.[203]

Disorders of POMC

Defects in POMC synthesis and processing can also cause abnormal ACTH production and action. In patients with POMC mutations, plasma ACTH and cortisol concentrations are usually extremely low, but depending on the genetic defect, plasma ACTH concentrations may also be elevated reflecting the production of an immunoreactive but bioinactive protein.[204] Recessively inherited mutations or deletion of the POMC gene will affect multiple POMC peptides, including MSH and β-endorphin. Thus, red hair, pale skin, and obesity are associated features of this form of secondary adrenal insufficiency.[205] These clinical signs may be more subtle in individuals with naturally dark hair and pigmented skin, or red hair may darken in adulthood.[206] Mutations in prohormone convertase 1 (PC1, *PCSK1*), which is required for the processing of POMC to ACTH, cause abnormal cleavage and processing of several hormone systems, including the generation of bioactive ACTH from POMC.[207] Patients with this rare recessive disorder can have hypocortisolemia together with abnormal glucose metabolism, obesity, hypogonadotropic hypogonadism, and persistent malabsorptive diarrhea.[207,208]

Long-Term Steroid Therapy

Long-term glucocorticoid therapy can suppress POMC gene transcription and the synthesis and storage of ACTH. Furthermore, long-term therapy apparently decreases the synthesis and storage of CRF and diminishes the abundance of receptors for CRF in the pituitary. Therefore, recovery of the hypothalamic-pituitary axis from long-term glucocorticoid therapy entails recovery of multiple components in a sequential cascade and, hence, often requires considerable time (see the section on "Glucocorticoid Therapy and Withdrawal," presented later in the chapter). Patients successfully withdrawn from glucocorticoid therapy or successfully treated for Cushing disease may exhibit a fairly rapid normalization of plasma cortisol values while continuing to have diminished adrenal reserve for over 12 months. Inhaled steroids, nasal sprays, and even steroid eye drops can cause suppression of the adrenal axis, so vigilance may be needed following their withdrawal or at time of additional stress (e.g., surgery, intercurrent illness).[209-211] Although treatment with cortisone and prednisone during pregnancy will result in minimal suppression of the fetal adrenal, because of the protective effects of placental 11βHSD, dexamethasone treatment in pregnancy can affect fetal adrenal steroidogenesis.

ADRENAL EXCESS

Cushing Syndrome

The term *Cushing syndrome* describes any form of glucocorticoid excess; *Cushing disease* designates hypercortisolism due to pituitary overproduction of ACTH. The related disorder caused by ACTH of nonpituitary origin is termed the *ectopic ACTH syndrome*. The term *Cushing syndrome* is sometimes used to refer specifically to hypersecretion of cortisol from adrenal tumors, but this is ambiguous and should be avoided. Other causes of Cushing syndrome include adrenal adenoma, adrenal carcinoma, and multinodular adrenal hyperplasia. All of these are distinct from *iatrogenic Cushing syndrome*, which is the similar clinical constellation resulting from administration of supraphysiologic quantities of ACTH or glucocorticoids.

Although generally described in great detail and illustrated with striking photographs in endocrine texts, Cushing disease is fairly rare in adults.[212] Furthermore, about 25% of patients referred to large centers for Cushing disease are children, thus it is becoming increasingly clear that pediatric Cushing disease is more common than previously recognized. Many patients first seen as adults actually experience the onset of symptoms in childhood or adolescence. Harvey Cushing's original patient was a young woman of only 23 years whose history and clinical features indicated long-standing disease. Hence, many patients with Cushing syndrome can be detected in the pediatric age group. In adults and children over 7 years of age, the most common cause of Cushing syndrome is true Cushing disease (adrenal hyperplasia due to hypersecretion of pituitary ACTH).[213] Boys are more frequently affected than girls in the prepubertal period, although the sex ratios are equal during adolescence and women have a higher incidence of Cushing disease in adulthood.[214] In infants and children under 7 years, adrenal tumors predominate. Among 60 infants under 1 year of age with Cushing syndrome, 48 had adrenal tumors[215] (Table 13-8).

Clinical Findings

The physical features of Cushing syndrome are familiar to virtually all physicians. Central obesity, "moon facies," hirsutism, and facial flushing are seen in over 80% of adults with Cushing syndrome. Striae, hypertension, muscular weakness, back pain, "buffalo hump" fat distribution, psychological disturbances, acne, and easy bruising are also commonly described (35% to 80%). However, these

TABLE 13-8 **Etiology of Cushing Syndrome in Infancy**

	Males	Females
Adrenal tumors (n = 48)		
—Carcinoma	5	20
—Adenoma	4	16
—Not defined	2	1
Ectopic ACTH syndrome	1	1
Nodular adrenal hyperplasia	1	4
Undefined adrenal hyperplasia	2	2
ACTH-producing tumor	1	0
TOTAL	16	44

Data from Miller, W. L., Townsend, J. J., Grumbach, M. M., & Kaplan, S. L. (1979). An infant with Cushing's disease due to an adrenocorticotropin-producing pituitary adenoma. J Clin Endocrinol Metab, 48, *1017–1025.*

are the signs and features of advanced Cushing disease. When annual photographs of such patients are available, it is often apparent that these features can take 5 years or longer to develop. Thus, the classic cushingoid appearance will usually not be the initial picture seen in the child with Cushing syndrome. The earliest, most reliable indicators of hypercortisolism in children are weight gain and growth arrest[216] (Table 13-9). Cumulative data from three large studies of pediatric Cushing disease identified weight gain at presentation in 91/97 (94%) cases and growth failure in 82/95 (86%) cases.[216-218] Thus, any overweight child who stops growing should be evaluated for Cushing syndrome. Glucocorticoids suppress growth by increasing hypothalamic secretion of somatostatin, suppressing growth hormone secretion and IGF-1 production, and by acting directly on the epiphyses to inhibit sulfation of cartilage, inhibit mineralization, and inhibit cell proliferation. By contrast, children with simple dietary obesity often grow more rapidly and are tall for their age (presumably due to chronic secondary hyperinsulinism). The obesity of Cushing disease in children is initially generalized rather than centripetal, and a "buffalo hump" is evidence of long-standing disease. Psychological disturbances, especially compulsive overachieving behavior, are seen in about 40% of children and adolescents with Cushing disease [216] and are distinctly different from the depression typically seen in adults.[219] Emotional lability has been described in approximately 30% of cases.[217] An underappreciated aspect of pediatric Cushing disease is the substantial degree of bone loss and undermineralization in these patients.[216,220,221] It is likely that Cushing disease is generally regarded as a disease of young adults because the diagnosis was missed, rather than absent, during adolescence. Rarely, Cushing syndrome caused by adrenal carcinoma or the ectopic ACTH syndrome can produce a rapid fulminant course.

TABLE 13-9 **Findings in 39 Children with Cushing Disease**

Sign/Symptom	Number of Patients	%
Weight gain	36/39	92
Growth failure	31/37	84
Osteopenia	14/19	74
Fatigue	26/39	67
Hypertension	22/35	63
Delayed or arrested puberty	21/35	60
Plethora	18/39	46
Acne	18/39	46
Hirsutism	18/39	46
Compulsive behavior	17/39	44
Striae	14/39	36
Bruising	11/39	28
Buffalo hump	11/39	28
Headache	10/39	26
Delayed bone age	2/23	13
Nocturia	3/39	8

Data from Devoe, D. J., Miller, W. L., Conte, F. A., et al. (1997). Long-term outcome in children and adolescents after transsphenoidal surgery for Cushing's disease. J Clin Endocrinol Metab, 82, *3196–3202.*

Cushing Disease

Among adults, over 90% of patients with Cushing disease have identifiable pituitary microadenomas.[219,222] These tumors are generally 2 to 10 mm in diameter, are not encapsulated, have ill-defined boundaries, and are frequently detectable with a contrast-enhanced pituitary MRI. These tumors are often identifiable only by minor differences in their appearance and texture from surrounding tissue, thus the frequency of surgical cure is correlated with the technical skill of the surgeon.

Among children and adolescents, about 80% to 85% of those with Cushing disease have surgically identifiable microadenomas.[216,223,224] Although removal of the tumor usually appears curative, 20% of such "cured" patients suffer relapse and again manifest Cushing disease within about 5 years so that the net cure rate is 65% to 75%.[216-218,225,225] Transsphenoidal surgery offers the best initial approach for rapid and complete cure of most patients, but alternative approaches may be necessary in younger children if sphenoid sinus aeration has not yet occurred. Control of hypercortisolemia is important in the perioperative period. Careful monitoring for recovery of the HPA axis is necessary over several months, as stress responses may be diminished despite normal basal cortisol secretion. Short-term consequences of transsphenoidal surgery include transient diabetes insipidus and cerebrospinal fluid rhinorrhea.[217,218,227] Persistent panhypopituitarism is rare, but the effects of hypercortisolism on growth hormone secretion may remain for 1 to 2 years after treatment, and growth hormone deficiency can occur even in those children who have not received irradiation.[217,218,227] Final height may be reduced by 1.5 to 2 SD by the long-term hypercortisolism.[216,228] Treatment with growth hormone may ameliorate this growth loss in patients with GH insufficiency.[229,230]

The high cure rate of transsphenoidal microadenomectomy in Cushing disease indicates that the majority of such patients have primary disease of the pituitary itself, rather than secondary hyperpituitarism resulting from hyperstimulation of the pituitary by CRF or other agents. Careful follow-up studies of these patients confirm this.[216,224,225] In most postoperative patients, the circadian rhythms of ACTH and cortisol return to normal, ACTH and cortisol respond appropriately to hypoglycemia, cortisol is easily suppressed by low doses of dexamethasone, and the other hypothalamic-pituitary systems return to normal.

However, some patients with Cushing disease have no identifiable microadenoma, and some "cured" patients relapse. This suggests that this smaller population of patients may have a primary hypothalamic disorder, or that some of the pituitary tissue responsible for ACTH-hypersecretion was not excised. Effective treatment of Cushing disease with cyproheptidine, a serotonin antagonist, has been reported in adults, further suggesting a hypothalamic disturbance. Thus, present clinical

investigation suggests that Cushing disease is usually caused by a primary pituitary adenoma, but that sometimes it is caused by hypothalamic dysfunction. Microsurgery can be curative in the former, but not the latter. Unfortunately, no diagnostic maneuver is available to distinguish the two possibilities; thus, transsphenoidal exploration remains the preferred initial therapeutic approach to the patient with Cushing disease.

The management of nonresponsive or relapsed Cushing disease is challenging. Repeat transsphenoidal surgery is typically the first approach, especially if there is evidence of a distinct lesion or lateral hypersecretion of ACTH identified by petrosal sinus sampling. Second-line approaches include hypophysectomy, gamma-knife irradiation, cyproheptadine, cabergoline, adrenalectomy, and drugs that inhibit adrenal function. All have significant disadvantages, especially in children. Hypophysectomy also eliminates pituitary secretion of growth hormone, TSH, and gonadotropins, causing growth failure, hypothyroidism, and failure to progress in puberty, respectively. Although hypothyroidism is easily treated with oral thyroxine replacement, growth hormone deficiency requires very expensive replacement therapy. Sex steroid replacement can be used to achieve secondary sexual characteristics at the age of puberty; however, gonadotropin replacement or pulsatile gonadotropin-releasing hormone therapy will be needed to achieve fertility. Pituitary irradiation has been touted to avoid many of these problems and is effective in treating Cushing disease, but growth-hormone deficiency occurs in most cases and additional endocrinopathies can occur with time.[231] The interval from radiotherapy to cure can be more than 1 year, during which time therapeutic blockage of hypercortisolemia is necessary to prevent the ongoing effects of Cushing disease on growth, weight, and bone mineralization. Furthermore, large doses of radiation increase the risk of cerebral arteritis, leukoencephalopathy, leukemia, glial neoplasms, and bone tumors involving the skull; stereotactic radiotherapy may reduce these potential effects, but few data exist yet in children. Cyproheptadine has met with virtually no success in pediatric Cushing disease, in part due to the unacceptable side effects (weight gain, irritability, hallucinations) often seen with the needed doses.

Laparoscopic adrenalectomy is the preferred approach in our centers when two transsphenoidal procedures fail. In addition to the obvious effects of eliminating normal production of glucocorticoids and mineralocorticoids, removal of the adrenal eliminates the physiologic feedback inhibition of the pituitary. In some adults, this results in the development of pituitary macroadenomas, producing large quantities of ACTH. These can expand and impinge on the optic nerves and can produce sufficient POMC to yield enough MSH to produce profound darkening of the skin (Nelson syndrome), but this is rarely seen in children. There is relatively little pediatric experience with ketoconazole and other drugs that inhibit steroidogenesis, but these may provide a useful form of therapy for selected patients or for controlling hypercortisolemia in the short term.[232] Metyrapone is not useful for long-term therapy; ortho, para-DDD (mitotane), an adrenolytic agent, may be used to effect a "chemical adrenalectomy," but its side effects of nausea, anorexia, and vomiting are severe. Etomidate may be useful in the acute setting for severe or life-threatening Cushing disease prior to surgery.[233,234]

Other Causes of Cushing Syndrome

Ectopic ACTH Syndrome. The ectopic ACTH syndrome is commonly seen in adults with oat cell carcinoma of the lung, carcinoid tumors, pancreatic islet cell carcinoma, and thymoma. Ectopically produced POMC and ACTH are derived from the same gene that produces pituitary POMC, but they are not sensitive to glucocorticoid feedback in the malignant cells. This phenomenon permits distinction between pituitary and ectopic ACTH by suppressibility of the former by high doses of dexamethasone. Although the ectopic ACTH syndrome is rare in children, it has been described in infants younger than 1 year of age. Associated tumors have included neuroblastoma, pheochromocytoma, islet cell carcinoma of the pancreas, and neuroendocrine tumors of the thymus.[235] The ectopic ACTH syndrome is typically associated with ACTH concentrations 10 times higher than those seen in Cushing disease. However, both adults and children with this disorder may show little or no clinical evidence of hypercortisolism, probably due to the typically rapid onset of the disease and to the general catabolism associated with malignancy. Unlike patients with Cushing disease, adults and children with the ectopic ACTH syndrome frequently have hypokalemic alkalosis, presumably because the extremely high levels of ACTH stimulate the production of DOC by the adrenal fasciculata and may also stimulate the adrenal glomerulosa in the absence of hyperreninemia.

Adrenal Tumors. Adrenal tumors, especially adrenal carcinomas, are the more typical cause of Cushing syndrome in infants and small children (see Table 13-8). These tend to occur with much greater frequency in girls; the reason for this is unknown. Adrenal adenomas almost always secrete cortisol with minimal secretion of mineralocorticoids or sex steroids. By contrast, adrenal carcinomas tend to secrete both cortisol and androgens and are often associated with progressive virilization.[236,237] Adrenal adenoma or carcinoma may be associated with congenital bodily asymmetry (hemihypertrophy), sometimes as part of the Beckwith-Wiedemann syndrome, or with germline mutations or loss-of-heterozygosity of the tumor suppressor gene p53, sometimes as part of the Li-Fraumeni syndrome.[238] CT and MRI are useful in the diagnosis of adrenal tumors, and steroid analysis can be informative at the time of presentation and for monitoring for potential relapse. The treatment for both adenoma and carcinoma is surgical, and complete resection is needed for cure. In some cases, the histologic differentiation of adrenal adenomas and carcinomas is difficult, but a worse prognosis is associated with increased tumor size, capsular or vascular invasion, retroperitoneal lymph nodes, metastases, or failure to normalize hormonal values postoperatively.[237,239-241] Although a few patients with residual or metastatic disease have done well with

adjunctive therapy with ortho, para-DDD (mitotane), or other chemotherapeutic strategies, the general prognosis remains poor. To improve outcome for such rare diseases, therapeutic strategy now follows international protocols, which are linked to registries for controlled trials to monitor short and- long-term outcome.[241]

ACTH-Independent Multinodular Adrenal Hyperplasias. ACTH-independent multinodular adrenal hyperplasias comprise benign adrenal hyperplasia such as ACTH-independent macronodular adrenocortical hyperplasia (also known as massive macronodular adrenocortical disease) and micronodular hyperplasias, mostly primary pigmented nodular adrenocortical disease (PPNAD). Macronodular disorders are histologically associated with nodules larger than 1 cm and micronodular disorders encompass nodules that are smaller than 1 cm. In childhood, bilateral macroadenomatous hyperplasia is mostly seen with McCune-Albright syndrome due to somatic mutations in the gene encoding guanine nucleotide–binding protein, alpha-stimulating polypeptide GNAS1. Patients typically present with the triad of polyostotic fibrous dysplasia, café-au-lait skin spots with irregular margins and gonadotropin-independent sexual precocity. However, as the disorder is caused by somatic cell mutations, rather than germline mutations, its manifestations are clinically heterogeneous and may include other endocrine disorders such as Cushing syndrome, thyrotoxicosis, hyperparathyroidism, pituitary gigantism, and hyperprolactinemia. Cushing syndrome is rare in McCune-Albright syndrome and usually presents before 6 months of age. Most cases of macronodular adrenocortical hyperplasia are sporadic, isolated, and occur in middle age, although some cases are associated with MEN 1.

The group of micronodular benign adrenal hyperplasias comprises PPNAD and isolated micronodular adrenocortical disease. PPNAD is a rare entity characterized by the secretion of both cortisol and adrenal androgens.[242,243] It is seen in infants, children, and young adults, with females affected more frequently. It is usually seen as part of the "Carney complex," which is a form of multiple endocrine neoplasia (MEN), consisting of pigmented lentigines and blue nevi on the face, lips, and conjunctiva, and a variety of tumors including schwannomas and atrial myxomas, and occasionally GH-secreting pituitary adenomas, Leydig cell tumors, calcifying Sertoli cell tumors (which may secrete estrogens), and medullary carcinoma of the thyroid.[243,244] Typical features of Cushing syndrome are often seen in the pediatric population.[245] The adrenals are not truly hyperplastic but consist of discrete pigmented nodules surrounded by atrophic tissue, which permits their identification by MRI or CT. Because the hypercortisolism is resistant to suppression with high doses of dexamethasone and because both glucocorticoids and sex steroids are produced, this entity is clinically difficult to distinguish from the ectopic ACTH syndrome, but plasma ACTH assays are usually diagnostic. Complete adrenalectomy is usually indicated, although some successes have been reported with subtotal resections.

Carney complex, like other MEN disorders, is typically autosomal dominant. Loss of heterozygosity and mutations in a regulatory subunit of protein kinase (PRKAR1A) on chromosome 17q22-24 have been found in 73% of patients with Carney complex,[246-250] or as sporadic germline or somatic events in isolated adrenal tumors (isolated PPNAD).[251] In general, patients with Carney complex harboring PRKAR1A mutations manifest at a younger age and have more myxomas, schwannomas, thyroid tumors, and gonadal tumors than patients without PRKAR1A mutations. Patients with isolated PPNAD typically carry the PRKAR1A mutations c.709-7del6 or c.1A>G / p.M1V, which is important for genetic counseling and screening.[250]

Patients with micronodular benign adrenal hyperplasia without Carney complex and PRKAR1A mutations may harbor mutations in other cAMP signaling proteins. Mutations in the gene encoding phosphodiesterase 11A4 (PDE11A) have been reported in individuals with isolated micronodular adrenocortical disease and PPNAD and mutations in phosphodiesterase 8B (PDE8B) in isolated micronodular adrenocortical disease.[250,252] Both PDE11A and PDE8B catalyze the hydrolysis of cAMP and cGMP and are expressed in several endocrine tissues including the adrenals. Thus, it is becoming clear that abnormalities in signaling pathways play an important role in adrenal hyperplasia and tumorigenesis.[253]

Differential Diagnosis

Cushing syndrome in children is usually suggested by weight gain, growth arrest, mood change, and change in facial appearance (plethora, acne, hirsutism). The diagnosis in children may be subtle and difficult when it is sought at a relatively early point in the natural history of the disease. For initial screening, three laboratory investigations are recommended: (1) diurnal ACTH and cortisol profiles from blood (the latter may be performed on saliva); (2) 24-hour urinary free cortisol measurements, and (3) the overnight 1 mg dexamethasone suppression test.[243] Absolute elevations above the "upper limits of normal" for concentrations of plasma ACTH and cortisol are often absent. Rather than finding morning concentrations of cortisol $>$ 20 μg/dL or of ACTH $>$ 50 pg/mL, it is more typical to find mild, often equivocal elevations in the afternoon and evening values. This loss of the diurnal rhythm, evidenced by continued secretion of ACTH and cortisol throughout the afternoon, evening, and nighttime, is usually the earliest reliable laboratory index of Cushing disease. A single plasma cortisol measurement obtained at midnight from an indwelling venous catheter while the patient remains asleep should be less than 2 μg/dL in normal individuals and more than 2 μg/dL in Cushing disease.[254] By contrast, the values for ACTH and cortisol are typically extremely high in the ectopic ACTH syndrome, whereas cortisol is elevated but ACTH is suppressed in adrenal tumors and in multinodular adrenal hyperplasia (Table 13-10). In all forms of Cushing syndrome, monitoring of the 24-hour urinary free cortisol (UFC) assists in deciding whether further investigations are warranted. A normal 24-hour UFC value is generally $<$ 70 μg/m^2/day (with radioimmunoassays). Several repeat collections may be necessary, and it is important to use normal ranges adjusted for size

TABLE 13-10 **Diagnostic Values in Various Causes of Cushing Syndrome**

Test		Normal Values	Adrenal Carcinoma	Adrenal Adenoma	Nodular Adrenal Hyperplasia	Cushing Disease	Ectopic ACTH Syndrome
Plasma cortisol concentration	AM	>14	↑	↑	↑	ı	↑↑
	PM	<8	↑	↑	↑	↑	↑↑
Plasma ACTH concentration	AM	<100	↓	↓	↓	↑	↑↑
	PM	<50	↓	↓	↓	↑	↑↑
Low-dose dex suppression	Cortisol	<3	No Δ	No Δ	No Δ	*	No Δ
	ACTH	<30	No Δ	No Δ	No Δ	*	No Δ
	17OHCS	<2	No Δ	No Δ	No Δ	*	No Δ
High-dose dex suppression	Cortisol	↓↓	No Δ	No Δ	†	↓	No Δ
	ACTH	↓↓	No Δ	No Δ	†	↓	No Δ
	17OHCS	↓↓	No Δ	No Δ	†	↓	No Δ
IV ACTH test	Cortisol	>20	No Δ	± ↑	± ↑	↑	No Δ
Metyrapone test	Cortisol	↓	± ↓	No Δ	± ↓	↓	± ↓
11 Deoxycortisol		↑	± ↑	No Δ	± ↑	↑	± ↑
	ACTH	↑	No Δ	No Δ	± ↑	↑	No Δ
	17OHCS	↑	No Δ	No Δ	±	↑	No Δ
24-Hour urinary excretion (basal)	17OHCS		↑↑	↑	↑	↑	↑
	17KS		↑↑	± ↑	↑	↑	↑
Plasma concentration DHEA or DHEA-S			↑↑	↓	± ↑	↑	↑

*Incomplete response (i.e., ±.)
†Usually no Δ.
Dex, dexamethasone
AM typically refers to 8 a.m.; PM to 4 p.m.
Cortisol concentration in μg/dL
ACTH concentration in pg/mL.
17OHCS in mg/24 hours.

as well as age, as children with simple obesity have higher cortisol secretion rates. Another baseline test is the low-dose (1 mg) dexamethasone overnight suppression test with a cutoff for cortisol < 1.8 μg/dL (50 nmol/L). If both the 24-hour UFC and the low-dose dexamethasone overnight suppression test are normal, the diagnosis of Cushing syndrome is usually excluded. Exceptions are patients with intermittent or periodic cortisol hypersecretion who need longer follow-up and repeat testing for diagnosis of Cushing syndrome.

Once the diagnosis Cushing syndrome is established, further investigations may be needed to find the origin of disease. Low- and high-dose dexamethasone suppression tests can be useful when done with care. To achieve reliable results in pediatric patients, children should be hospitalized, preferably on a pediatric clinical research ward. Two days of baseline (control) data should be obtained. Low-dose dexamethasone (20 μg/kg/day, up to a maximum of 2 mg) should be given, divided into equal doses given every 6 hours for 2 days followed by high-dose dexamethasone (80 μg/kg/day) given in the same fashion. Eight a.m. and 8 p.m. (or midnight) values for ACTH and cortisol and 24-hour urine collections for 17OHS, 17KS, free cortisol, and creatinine (to monitor the completeness of the collection) should be obtained on each of the 6 days of the test. Measurements of either urinary free cortisol or 17OHCS are probably equally reliable if the laboratory has established good pediatric standards. Because of variations due to episodic secretion of ACTH, the 8 a.m. and 8 p.m. blood values should be drawn in triplicate at 8:00, 8:15, and 8:30. In patients with exogenous obesity or other non-Cushing disorders, cortisol, ACTH, and urinary steroids will be suppressed readily by low-dose dexamethasone. Plasma cortisol should be less than 5 μg/dL, ACTH less than 20 pg/mL, and 24-hour urinary 17OHS less than 1 mg/g of creatinine. Patients with adrenal adenoma, adrenal carcinoma, or the ectopic ACTH syndrome will have values relatively insensitive to both low- and high-dose dexamethasone, although some patients with multinodular adrenal hyperplasia may respond to high-dose suppression and a paradoxical rise in cortisol following dexamethasone has been reported with the Carney complex.[255,256] Patients with Cushing disease classically respond with a suppression of ACTH, cortisol, and urinary steroids during the high-dose treatment but not during the low-dose treatment. However, some children, especially those early in the course of their illness, may exhibit partial suppression in response to low-dose dexamethasone. Thus, if the low dose that is given exceeds 20 μg/kg/day or if the assays used are insufficiently sensitive to distinguish partial from complete suppression, false-negative tests may result.

Petrosal sinus sampling is widely used in adults with Cushing disease, to distinguish pituitary Cushing disease from the ectopic ACTH syndrome. The smaller vascular bed in children increases the risk of this procedure, but inferior petrosal venous sampling has been used with some success in adolescents in an attempt to localize pituitary adenomas prior to surgery.[257] Such approaches should only be undertaken in specialist centers; jugular

venous sampling may provide an alternative approach, although extensive data in the pediatric population are not available.[258] In general, the diagnosis of Cushing disease is considerably more difficult to establish in children than in adults. In addition to laboratory tests, imaging studies may help in establishing the exact diagnosis of Cushing syndrome. An adrenal CT scan or MRI may visualize an adrenal cortex tumor or a macro-/micronodular adrenal hyperplasia, whereas adrenal ultrasound often fails and therefore should not be trusted as a diagnostic tool for adrenal disorders. MRI is also the preferred method for visualizing the hypothalamus and the anterior pituitary for lesions.

Virilizing and Feminizing Adrenal Tumors

Most virilizing adrenal tumors are adrenal carcinomas producing a mixed array of androgens and glucocorticoids; virilizing and feminizing adrenal adenomas are quite rare. Virilizing tumors in boys have a presentation similar to that of simple virilizing congenital adrenal hyperplasia. There will be phallic enlargement, erections, pubic and axillary hair, acne, increased muscle mass, deepening of the voice, acne, and scrotal thinning, but the testicular size will be prepubertal. Elevated concentrations of testosterone in young boys alter behavior, with increased irritability, rambunctiousness, hyperactivity, and rough play, but without evidence of libido. Diagnosis is based on hyperandrogenemia that is non-suppressible by glucocorticoids. The treatment is surgical; all such tumors should be handled as if they are malignant, with care exerted not to cut the capsule and seed cells onto the peritoneum. The pathologic distinction between adrenal adenoma and carcinoma is difficult, especially in pediatric patients.

Feminizing adrenal tumors are extremely rare in either sex. P450aro, the enzyme aromatizing androgenic precursors to estrogens, is not normally found in the adrenals but is found in peripheral tissues such as fat and is also found in some adrenal carcinomas. It is not known whether most feminizing adrenal tumors exhibit ectopic adrenal production of this enzyme, whether some other enzyme mediates aromatization in the tumor, or whether these are truly androgen-producing, virilizing tumors occurring in a setting where there is unusually effective peripheral aromatization of adrenal androgens. Feminizing adrenal (or extra-adrenal) tumors can be distinguished from true (central) precocious puberty in girls by the absence of increased circulating concentrations of gonadotropins and by a prepubertal response of luteinizing hormone to an intravenous challenge of gonadotropin releasing hormone (LRF, GnRH). In boys, such tumors will cause gynecomastia, which will resemble the benign gynecomastia that often accompanies puberty. However, as with virilizing adrenal tumors, testicular size and the gonadotropin response to LRF testing will be prepubertal. The diagnosis of a feminizing tumor in a pubertal boy can be extremely difficult, but it is usually suggested by an arrest in pubertal progression and can be proved by the persistence of circulating plasma estrogens after the administration of testosterone.

Other Disorders

Primary Hyperaldosteronism: Conn Syndrome

Conn syndrome, characterized by hypertension, polyuria, hypokalemic alkalosis, and low plasma renin activity due to an aldosterone-producing adrenal adenoma, is well described in adults but is rare in children. The diagnostic task is to differentiate primary aldosteronism from physiologic secondary hyperaldosteronism occurring in response to another physiologic disturbance. Any loss of sodium, retention of potassium, or decrease in blood volume will result in hyperreninemic secondary hyperaldosteronism. Renal tubular acidosis, treatment with diuretics, salt-wasting nephritis, or hypovolemia due to nephrosis, ascites, or blood loss are typical settings for physiologic secondary hyperaldosteronism. Primary aldosteronism is characterized by hypertension and hypokalemic alkalosis. The cause is a small adrenal adenoma, usually confined to one adrenal. Laparoscopic surgery permits both adrenals to be explored surgically because adrenal vein catheterization is not possible in children and is difficult in adults. Aldosterone is normally produced in glomerulosa cells in response to intravascular volume depletion via the renin-angiotensin system or high plasma potassium. In primary aldosteronism the adrenal constitutively produces aldosterone in the absence of angiotensin II and hyperkalemia. Somatic mutations in the gene encoding the K+ channel KCNJ5 (also known as Kir3.4) have been found in tumor tissue of some adult patients with aldosterone producing adenomas and have also been found as germ line mutations in four unrelated families with severe hypertension, aldosteronism, and adrenal hyperplasia.[259-262] These KCNJ5 mutations affect the ion selection filter of the K+ channel, causing an increase in Na+ influx and permanent depolarization of the cell membrane.[259] Membrane depolarization then activates voltage-gated Ca2+ channels, which increases intracellular Ca2+ thus providing the normal signal for aldosterone production and glomerulosa cell proliferation. Somatic mutations of KCNJ5 are found in 34% of aldosterone producing adenomas; such mutations are more prevalent in females and at a younger age, and they manifest with higher preoperative aldosterone levels.[263] By contrast, germ line KCNJ5 mutations appear to be rare and are more likely to be associated with bilateral adrenal hyperplasia than with aldosterone producing adenomas.

Familial Glucocorticoid Resistance

Familial glucocorticoid resistance is a rare disorder caused by mutations in the α-isoform of the glucocorticoid receptor. Decreased glucocorticoid action results in grossly increased ACTH secretion, which, in addition to stimulating the production of cortisol, also stimulates the production of other adrenal steroids. Thus, these patients may present with fatigue, hypertension, and hypokalemic alkalosis, suggesting a mineralocorticoid excess syndrome, and they also have symptoms of hyperandrogenism.[264,265] By contrast, they typically lack cushingoid features with biochemical hypercortisolism.

Circadian rhythmicity of the HPA axis is maintained, and resistance to dexamethasone suppression is observed. Patients have been described who are homozygous for missense mutations,[266] heterozygous for a gene deletion,[267] or homozygous for a wholly null mutation.[53] Heterozygous point mutations with incomplete dominant negative activity or multiple effects on GRα action have also been described.[268] Point mutations may interfere with GRα-dependent transcriptional regulation through altered DNA binding, impaired ligand binding, delayed nuclear localization, abnormal nuclear aggregation, and disrupted interaction with coactivators, depending on the position of the mutation.[264] Thus, familial glucocorticoid resistance is usually a syndrome of partial resistance to the action of glucocorticoids. Treatment consists of supraphysiologic doses of dexamethasone that will elicit a physiologic response with the poorly functioning receptor, usually starting with 0.25 to 0.5 mg/day at bedtime and titrating to the suppression of morning ACTH and thus androgens, blood pressure, and serum potassium levels.[265] The dose of dexamethasone may be reduced to a minimum according to individual needs. Hypertension may require additional treatment with aldosterone antagonists.

Pseudohypoaldosteronism

Pseudohypoaldosteronism (PHA) is a rare salt-wasting disorder of infancy characterized by hyponatremia, hyperkalemia, and increased plasma renin activity in the face of elevated aldosterone concentrations reflecting aldosterone resistance.[269] The more common, more severe, systemic, autosomal recessive form of PHA ("pseudohypoaldosteronism type 2") is caused by inactivating mutations in any of the three subunits (α, β, γ) of the amiloride-sensitive sodium channel, ENaC (encoded by the *SCNN1A*, *B*, and *G* genes).[270] This condition is often associated with lower respiratory tract disease consisting of chest congestion, cough, and wheezing (but not pulmonary infections) as ENaC mutations increase the volume of pulmonary fluid.[271] This disease persists into adulthood, requiring vigorous salt-replacement therapy throughout life. Gain-of-function mutations due to carboxyterminal truncation of β-ENaC cause Liddle syndrome, an autosomal dominant form of salt-retaining hypertension.[270]

Autosomal dominant renal type 1 pseudohypoaldosteronism ("PHA type 1") is caused by inactivating mutations in the mineralocorticoid receptor (encoded by the *NR3C2* gene).[272] More than 50 different mutations have been found in this receptor, which interfere with mineralocorticoid binding and gene transcription.[273] This disease is milder than the recessive forms of PHA caused by ENaC mutations and remits with age, but it requires sodium replacement therapy in infancy and childhood. Rarely, point mutations in the mineralocorticoid receptor have been found in association with an autosomal dominant form of severe hypertension, which begins in adolescence and worsens in pregnancy.[274] In these cases, alterations in the structure of the ligand-binding domain of the mineralocorticoid receptor result in mild constitutive activation and permit binding and activation of the receptor by progesterone.

An acquired, transient form of PHA is often seen in infants with obstructive uropathy, especially shortly following surgical relief of the obstruction or with urinary tract infections. The lesion is renal tubular,[275] so that mineralocorticoid treatment is generally ineffective; salt replacement generally suffices while the renal lesion resolves.

GLUCOCORTICOID THERAPY AND WITHDRAWAL

Since their introduction into clinical medicine in the early 1950s, glucocorticoids have been used to treat virtually every known disease. At present their rational use falls into two broad categories: replacement in adrenal insufficiency and pharmacotherapeutic use. The latter category is largely related to the anti-inflammatory properties of glucocorticoids but also includes their actions to lyse leukemic leukocytes, lower plasma calcium concentrations, and reduce increased intracranial pressure. Virtually all of these actions are mediated through glucocorticoid receptors, which are found in most cells. Because there appears to be only one major type of glucocorticoid receptor, clearly all glucocorticoids will affect all tissues containing such receptors. Thus, with the exception of the distinction between glucocorticoids and mineralocorticoids, tissue-specific, disease-specific, or response-specific analogs of naturally occurring glucocorticoids cannot be produced. The only differences among the various glucocorticoid preparations are their ratio of glucocorticoid to mineralocorticoid activity, their capacity to bind to various binding proteins, their molar potency, and their biologic half-life. Dexamethasone is commonly used to reduce increased intracranial pressure and brain edema. Neurosurgical experience indicates that the optimal doses are 10 to 100 times those that would thoroughly saturate all available receptors, suggesting that this action of dexamethasone may not be mediated through the glucocorticoid receptor.

Glucocorticoids are so termed because of their major actions to increase plasma concentrations of glucose. This occurs by their induction of the transcription of the genes encoding the enzymes of the Embden-Meyerhof glycolytic pathway and other hepatic enzymes that divert amino acids, such as alanine, to the production of glucose. Thus, the coordinated action to increase the transcription of these genes can result in increased plasma concentrations of glucose, obesity, and muscle wasting. The other features of Cushing syndrome are similarly attributable to the increased transcriptional activity of specific glucocorticoid-sensitive genes.

In the normal population, there is a wide interindividual variation in glucocorticoid sensitivity, which is at least in part explained by genetic variants of the glucocorticoid receptor (GR) gene.[265] Several single nucleotide polymorphisms have been associated with changes in glucocorticoid sensitivity. A3669G (rs6198) is associated with glucocorticoid insensitivity and a more active immune system with an increased risk for autoimmune disease; it is present in about 35% of the normal population. ER22/23EK polymorphism (rs6189 and rs6190)

present in about 7% of the population is associated with mild glucocorticoid resistance and a healthier metabolic profile. By contrast, polymorphisms N363S (rs1695) and Bcl1 (rs41423247), present in about 8% and 45% of people, respectively, are associated with mild glucocorticoid hypersensitivity yielding a less fortunate metabolic profile (more body fat, less lean mass, increased cholesterol and insulin resistance, etc.).[265] Some of the variability in glucocorticoid sensitivity is explained by a large number of GR subtypes arising from alternative processing of the GR gene. Various GR isoforms may be generated through alternative splicing and alternate sites of translational initiation. These isoforms may then also be subjected to tissue-specific posttranslational modifications such as phosphorylation, ubiquitination, sumoylation, and acetylation. These processes allow cells to generate a broad range of glucocorticoid responsiveness to specifically regulate glucocorticoid-dependent genes.[276] Thus, individual glucocorticoid sensitivity plays a role in modulating the risk for diseases (cardiovascular, autoimmune, metabolic) and may also affect responsiveness to glucocorticoid treatment.

Replacement Therapy

Glucocorticoid replacement therapy is complicated by undesirable side effects with even minor degrees of overtreatment or undertreatment. Overtreatment can cause the signs and symptoms of Cushing syndrome; and even minimal overtreatment can impair the growth of children. Undertreatment will cause the signs and symptoms of adrenal insufficiency (see Box 13-2) only if the extent of undertreatment (dose and duration) is considerable. However, undertreatment may impair the individual's capacity to respond to stress. Glucocorticoid replacement therapy is most commonly employed in congenital adrenal hyperplasia due to 21-hydroxylase deficiency; however, in this setting, undertreatment will lead to overproduction of adrenal androgens, which will hasten epiphyseal maturation and closure, thus compromising ultimate adult height. Therefore, when formulating a program of adrenal replacement therapy for the growing child, it is crucial to mimic the normal endogenous production of glucocorticoids.

To optimize pediatric glucocorticoid replacement therapy, astute physicians have gauged their therapy to resemble the endogenous secretory rate of cortisol. Several studies indicate that the cortisol secretory rate is 6 to 8 mg/m^2/day in children and adults,[72,73] but data are not available for infants and children under 5 years. The range of normal values varies considerably, indicating that therapy must be tailored and individualized for each patient to achieve optimal results. The management of this delicate balance between overtreatment and undertreatment of the child requiring replacement therapy is thus confounded by considerable variation in the "normal" cortisol secretory rate among different children of the same size and the probability that most conventional guidelines err on the side of overtreatment. However, several additional factors must be considered in tailoring a specific child's glucocorticoid replacement regimen.

The specific form of adrenal insufficiency being treated significantly influences therapy. When treating autoimmune adrenalitis or any other form of "Addison disease," it is prudent to err slightly on the side of undertreatment. This will eliminate the possibility of glucocorticoid-induced iatrogenic growth retardation and will permit the pituitary to continue to produce normal to slightly elevated concentrations of ACTH. This ACTH will continue to stimulate the remaining functional adrenal steroidogenic machinery and also provide a fairly convenient means of monitoring the effects of therapy. By contrast, when treating severe, virilizing congenital adrenal hyperplasia, the adrenal should be suppressed more completely, as essentially all adrenal steroidogenesis will result in the production of unwanted androgens, with their consequent virilization and rate of advancement of bony maturation that is more rapid than the rate of advancement of height. However, overtreatment will also compromise growth.

The presence or absence of associated mineralocorticoid deficiency is an important variable. Children with mild degrees of mineralocorticoid insufficiency, such as those with "simple virilizing" congenital adrenal hyperplasia, may continue to have mildly elevated ACTH values, suggesting insufficient glucocorticoid replacement in association with elevated PRA. In some children, the ACTH is elevated in response to chronic, compromised hypovolemia, attempting to stimulate the adrenal to produce more mineralocorticoid. In these children, who do not manifest overt signs and symptoms of mineralocorticoid insufficiency, treatment with mineralocorticoid replacement may permit one to decrease the amount of glucocorticoid replacement needed to suppress plasma ACTH and urinary 17KS. This reduction in glucocorticoid therapy reduces the likelihood that adult height will be compromised.

The specific formulation of glucocorticoid used is also of great importance. Extremely potent, long-acting glucocorticoids, such as dexamethasone or prednisone, may be used in the treatment of adults but are rarely appropriate for replacement therapy in children. As children are continually growing and changing their weights and body surface areas, it is necessary to adjust their dose frequently. Small, incremental changes are more easily done with relatively weaker glucocorticoids. The use of short-acting steroids permits the patient to have physiologically low nighttime glucocorticoid activity, facilitating rest, growth, and pituitary stimulation. The efficacy of attempting to mimic the physiologic diurnal variation in steroid hormone secretion remains controversial. As ACTH and cortisol concentrations are high in the morning and low in the evening, it is intellectually and logically appealing to attempt to duplicate this circadian rhythm in replacement therapy. However, the results do not clearly indicate that better growth is achieved by giving relatively larger doses in the morning and lower doses at night. This probably reflects the fact that ACTH and cortisol secretion are episodic throughout the day and that this well-established circadian variation is not smooth. The pattern of high in the morning and low in the evening is only an averaged result. Furthermore, the adrenal releases cortisol episodically throughout the day in response to various physiologic demands

(hypoglycemia, exercise, stress, etc.); thus, under normal circumstances the plasma concentrations are high when the clearance and disposal rates are also high. A planned program of replacement therapy cannot possibly anticipate these day-to-day variations.

Finally, *dosage equivalents among various glucocorticoids can be misleading*. Virtually all handbooks of therapy publish tables of equivalency for the most commonly used pharmaceutical preparations of glucocorticoids. A similar set of equivalencies is shown in Table 13-7. Because most preparations of glucocorticoids are intended for pharmacotherapeutic use rather than replacement therapy, and because the most common indication for pharmacologic doses of glucocorticoids is for their anti-inflammatory properties, virtually all tables of glucocorticoid equivalencies are based on anti-inflammatory, immunosuppressive equivalencies. However, the differences in the plasma half-life and ability to bind to plasma proteins result in different biologic equivalencies when one assesses, for instance, anti-inflammatory versus growth-suppressant equivalencies. For example, dexamethasone is widely reported as being about 30 times more potent than cortisol when its anti-inflammatory capacities are measured, but the growth suppressant activity of dexamethasone is about 80 times that of cortisol (Table 13-7). Thus, all of the variables discussed earlier explain why there is little unanimity in recommendations for designing a glucocorticoid replacement regimen. However, an understanding of these variables will permit appropriate monitoring of the patient and encourage the physician to vary the treatment according to the responses and needs of the individual child.

Commonly Used Glucocorticoid Preparations

Numerous chemical derivatives and variants of the naturally occurring steroids are commercially available in a huge array of dosage forms, vehicles, and concentrations. Choosing the appropriate product can be simplified by considering only the most widely used steroids listed in Table 13-7.

There are four relevant considerations in the choice of which drug to use. First, the glucocorticoid potency of the various drugs is generally calculated and described according to the anti-inflammatory potency, but this may not be the appropriate consideration in many cases. Second, the growth-suppressant effect of a glucocorticoid preparation may be significantly different from its anti-inflammatory effect. This is due to differences in half-life, metabolism and protein binding, and receptor affinity (potency). Third, the mineralocorticoid activity of various glucocorticoid preparations varies widely. Both glucocorticoid and mineralocorticoid hormones can bind to both glucocorticoid (type 1) and mineralocorticoid (type 2) receptors, and most authorities now regard these as two different types of glucocorticoid receptors. Mineralocorticoid activity is intimately related to the activity of 11βHSD2, which metabolizes glucocorticoids but not mineralocorticoids to forms that cannot bind the receptor. Thus, the relative mineralocorticoid potency of various steroids is determined by both their affinity for the type 2 receptor and their resistance to the activity of 11βHSD2. An understanding that some commonly used glucocorticoids, such as cortisol, cortisone, prednisolone, and prednisone, have significant mineralocorticoid activity is especially important when large doses of glucocorticoids are used as "stress doses" in a patient on replacement therapy. Such stress doses of the glucocorticoid preparation may provide sufficient mineralocorticoid activity to meet physiologic needs; therefore, mineralocorticoid supplementation is not needed. Fourth, the plasma half-life and biologic half-life of the various preparations may be discordant and will vary widely. This is mainly related to binding to plasma proteins, hepatic metabolism, and hepatic activation. For example, cortisone and prednisone are biologically inactive (and even have mild steroid antagonist actions) until they are metabolized by hepatic 11βHSD1 to their active forms, cortisol and prednisolone. Thus, the relative glucocorticoid potency of these preparations will also be affected by hepatic function. Cortisone and prednisone are cleared more rapidly in patients receiving drugs such as phenobarbital or phenytoin, which induce hepatic enzymes, and are cleared more slowly in patients with liver failure.

In addition to these chemical considerations in the choice of glucocorticoid, the route of administration is important. Glucocorticoids are available for oral, intramuscular, intravenous, intrathecal, intra-articular, inhalant, and topical use; topical preparations include those designed for use on skin, mucous membranes, and conjunctiva. Each preparation is designed to deliver the maximal concentration of steroid to the desired tissue while delivering less steroid systemically. However, all such preparations are absorbed to varying extents, so that the widely used inhalant preparations used to treat asthma can, in sufficient doses, cause growth retardation and other signs of Cushing syndrome. In general, and in contradistinction to many other drugs, orally administered steroids are absorbed rapidly, but incompletely, whereas intramuscularly administered steroids are absorbed slowly, but completely. Thus, if the secretory rate of cortisol is 8 mg/m^2 of body surface area, the intramuscular or intravenous replacement dose of cortisol (hydrocortisone) would be 8 mg/m^2. The efficiency of absorption of glucocorticoids can vary considerably depending on diet, gastric acidity, bowel transit time, and other individual factors. This emphasizes that the dosage equivalents listed in Table 13-7 and similar tables are only approximations, and doses must be tailored to the clinical response.

ACTH can also be used for glucocorticoid therapy by its action to stimulate endogenous adrenal steroidogenesis. Although intravenous and intramuscular ACTH are extremely useful in diagnostic tests of adrenal function, the use of ACTH as a therapeutic agent is no longer favored, principally because it will stimulate synthesis of mineralocorticoids and adrenal androgens as well as glucocorticoids. Furthermore, the need to administer ACTH parenterally further diminishes its usefulness. Intramuscular ACTH 1-39 in a gel form is recommended for and is the treatment of choice for infantile spasms and possibly also for other forms of epilepsy in infants resistant to conventional anticonvulsants.

Whether this action is mediated by ACTH itself, by other peptides in the biologic preparation, by ACTH-induced adrenal steroids, or by ACTH-responsive synthesis of novel "neurosteroids"[277] in the brain has not been determined. When pharmacologic doses of ACTH are used therapeutically, as in infantile spasms, the patient should be given a low-sodium diet to ameliorate steroid hypertension. Although greatly elevated concentrations of ACTH, as in the ectopic ACTH syndrome, will cause pituitary suppression, treatment with daily injections of ACTH results in less hypothalamic-pituitary suppression than does treatment with equivalent doses of oral glucocorticoids, presumably because the effect on the adrenal is transient. Also, adrenal suppression obviously does not occur in ACTH therapy. Because the effects of ACTH on adrenal steroidogenesis are highly variable, it is even more difficult to determine dosage equivalencies for ACTH and oral steroid preparations than it is among the various steroids, as discussed previously. A very rough guide from studies in adults is that 40 units of ACTH(1-39) gel is approximately equivalent to 100 mg of cortisol.

Pharmacologic Therapy

Pharmacologic doses of glucocorticoids are used in a variety of clinical situations including immune suppression in organ transplantation, tumor chemotherapy, treatment of "autoimmune" collagen vascular and nephrotic syndromes, regional enteritis, and ulcerative colitis. Asthma, pseudotumor cerebri, dermatitis, certain infections, neuritis, and certain anemias are also often treated with glucocorticoids. The choice of glucocorticoid preparation to be used is guided by the pharmacologic parameters described previously and in Table 13-7 and by custom (e.g., the use of betamethasone rather than dexamethasone to induce fetal lung maturation in impending premature deliveries). There is substantial variation in the relative glucocorticoid and mineralocorticoid activities of each steroid, depending on the assay used,[278] hence Table 13-7 is an integration of multiple studies and can only be taken as a rough guide.

Pharmacologic doses of glucocorticoids administered for more than 1 or 2 weeks will cause the signs and symptoms of iatrogenic Cushing syndrome. These are similar to the glucocorticoid-induced findings in Cushing disease, but they may be more severe because of the high doses involved (Table 13-11). Iatrogenic Cushing syndrome is also not associated with adrenal androgen effects, and mineralocorticoid effects are rare. Alternate-day therapy can decrease the toxicity of pharmacologic glucocorticoid therapy, especially suppression of the HPA axis and suppression of growth. The basic premise of alternate-day therapy is that the disease state can be suppressed with intermittent therapy, whereas there is significant recovery of the HPA axis during the "off" day. Thus, alternate-day therapy requires the use of a relatively short-acting glucocorticoid administered only once in the morning of each therapeutic day to ensure that the "off" day is truly "off." Long-acting glucocorticoids, such as dexamethasone, should not be employed for alternate-day therapy; results are best with oral prednisone or methylprednisolone.

TABLE 13-11 **Complications of High-Dose Glucocorticoid Therapy**

Short-Term Therapy	Long-Term Therapy
Gastritis	Gastric ulcers
Growth arrest	Short stature
↑ Appetite	Weight gain
Hypercalciuria	Osteoporosis, fractures
Glycosuria	Slipped epiphyses
Immune suppression	Ischemic bone necrosis
Masked symptoms of infection, especially fever and inflammation	Poor wound healing
	Catabolism
	Cataracts
Toxic psychoses	Bruising (capillary fragility)
	Adrenal/pituitary suppression
	Toxic psychosis

Withdrawal of Glucocorticoid Therapy

Withdrawal of glucocorticoid therapy can be difficult and can lead to symptoms of glucocorticoid insufficiency. When glucocorticoid therapy has been used for only 1 week or 10 days, therapy can be discontinued abruptly, even if high doses have been used.[279] Although only one or two doses of glucocorticoid are needed to suppress the HPA axis, this axis recovers very rapidly from short-term suppression. When therapy has persisted for 2 weeks or longer, recovery of HPA function is slower, and tapered doses of glucocorticoids are indicated. Acute discontinuation of therapy in such patients will lead to symptoms of glucocorticoid insufficiency, the so-called steroid withdrawal syndrome. This symptom complex does not include salt loss, as adrenal glomerulosa function, regulated principally by the renin-angiotensin system, remains normal. However, blood pressure can fall abruptly, as glucocorticoids are required for the action of catecholamines in maintaining vascular tone. The most prominent symptoms of the steroid withdrawal syndrome include malaise, anorexia, headache, lethargy, nausea, and fever. In reducing pharmacologic doses of glucocorticoids, it might appear logical to reduce the dosage precipitously to "physiologic" replacement doses. However, this is rarely successful and occasionally disastrous. Even when given "physiologic" replacement, patients who have been receiving pharmacologic doses of glucocorticoids will experience steroid withdrawal. Long-term pharmacologic glucocorticoid therapy inhibits synthesis of glucocorticoid receptors, so that physiologic concentrations of glucocorticoids will elicit subphysiologic cellular responses, resulting in the steroid withdrawal syndrome. Thus, it is necessary to taper gradually from the outset. The duration of glucocorticoid therapy is a critical consideration in designing a glucocorticoid withdrawal program. Therapy for a couple of months

will completely suppress the HPA axis but will not cause adrenal atrophy. Therapy that continues for years may result in almost total atrophy of the adrenal fasciculata/reticularis and hence may require a withdrawal regimen that takes months.

Procedures for tapering steroids are empirical. Their success is determined by the length and mode of therapy and by individual patient responses. Patients who have been on alternate-day therapy can be withdrawn more easily than those receiving daily therapy, especially daily therapy with a long-acting glucocorticoid such as dexamethasone. In patients on long-standing therapy, a 25% reduction in the previous level of therapy is generally recommended weekly. A patient with a body surface area of 1 m^2 will have a secretory rate of cortisol of about 9 mg/day. If the patient has been on daily therapy equivalent to 100 mg of cortisone for many months, a tapering protocol over 8 to 10 weeks may be needed. A protocol of 75% of the previous week's dose would thus be 75 mg/day for the first week, 56 mg/day for the second, then 42, 31.5, 24, 18, 13.5, 10, 7.5, 5.5 mg/day, then off treatment. A more practical regimen based on the sizes of tablets available would be 75, 50, 37.5, 25, 17.5, 12.5, 10, 7.5, and 5 mg/day. Most patients can be tapered more rapidly, but all patients need to be followed closely. When withdrawal is done with steroids other than cortisone or cortisol, measurement of morning cortisol values can be a useful adjunct. Morning cortisol values of 10 μg/dL or more indicate that the dose can be reduced safely.

Even after the successful discontinuation of therapy, the HPA axis is not wholly normal. Just as in the patient successfully treated for Cushing disease, the HPA axis may be incapable of responding to severe stress for 6 to 12 months after successful withdrawal from long-term, high-dose glucocorticoid therapy. Thus, evaluation of the hypothalamus and pituitary by a CRF or metyrapone test, and evaluation of adrenal responsiveness to pituitary stimulation with an intravenous low-dose ACTH test, should be done at the conclusion of a withdrawal program and 6 months thereafter. The results of these tests will indicate if there is a need for "steroid coverage" in acute surgical stress or illness.

Stress Doses of Glucocorticoids

The cortisol secretory rate increases significantly during physiologic stress such as trauma, major surgery, or severe illness. Patients receiving glucocorticoid replacement therapy or those recently withdrawn from pharmacologic therapy need coverage with "stress doses" of steroids in such situations. However, the specific indications for this coverage and the appropriate dosage are controversial and difficult to establish; most practitioners prefer to err on the "safe" side of steroid overdosage. This is the safest tactic in the short term, but it can have a significant effect on growth over a period of years.

It is generally said that doses 3 to 10 times physiologic replacement are needed for "the stress of surgery." The stress accompanying a surgical procedure can vary greatly. Modern techniques of anesthesiology, better anesthetic, analgesic, and muscle-relaxing drugs, and increased awareness of the particular needs of children in managing intraoperative fluids and electrolytes have greatly reduced the stress of surgery. In the past, a significant portion of such stress had to do with pain and hypovolemia, but when managed appropriately on contemporary pediatric services, minor surgical procedures elicit minimal changes in cortisol levels.[58]. Similarly, part of the stress of acute illness is fever and fluid loss, factors now familiar to all pediatricians. Although it remains appropriate and necessary to give about three times physiologic requirements during such periods of stress, it is probably not necessary to give much higher doses. Similarly, it is not necessary to triple a child's physiologic replacement regimen during simple colds, upper respiratory infection, otitis media, or after immunizations.

The preparation of the hypoadrenal patient on replacement therapy for surgery requires coordination with the anesthesia team. The best approach is to place a stress dose of 25 mg hydrocortisone per m^2 in the intravenous fluid, so that the glucocorticoid is delivered continuously during surgery, rather than being given a single bolus at the beginning of the procedure. It appears that the greatest stress is at the time of anesthesia reversal, rather than at the time of anesthesia induction.[57] Regular therapy at two to three times physiologic requirements can then be reinstituted on the day after the surgical procedure.

Mineralocorticoid Replacement

Replacement therapy with mineralocorticoids is indicated in salt-losing congenital adrenal hyperplasia and in syndromes of adrenal insufficiency that affect the zona glomerulosa. Only one mineralocorticoid, 9α-fluorocortisol (Florinef), is currently available. There is no parenteral mineralocorticoid preparation so that hydrocortisone plus salt must be used.

Because of increasing mineralocorticoid sensitivity with age (see Figure 13-17), mineralocorticoid doses are similar in children and adults. Newborns are quite insensitive to mineralocorticoids and typically require substantially larger doses than adults. The adult replacement dose of 9α-fluorocortisol is usually 0.05 to 0.1 mg daily, but newborns with CAH may require up to 0.4 mg. Sodium must be available to the nephrons for mineralocorticoids to promote reabsorption of sodium, thus, the newborn with salt-losing congenital adrenal hyperplasia must be treated with both mineralocorticoids and sodium chloride. Similarly, mineralocorticoids will cause hypertension only by retaining sodium.

Cortisol has significant mineralocorticoid activity: approximately 20 mg of cortisol or cortisone intravenously has a mineralocorticoid action equivalent to 0.1 mg of 9α-fluorocortisol. Thus, when cortisol or cortisone is given in stress doses, each provides adequate mineralocorticoid activity, and mineralocorticoid replacement can be interrupted. This is frequently seen when patients with salt-losing congenital adrenal hyperplasia undergo surgery: the stress doses of intramuscular cortisone acetate and the intravenous saline solutions administered during and after surgery suffice for the patient's mineralocorticoid requirements. Additional 9α-fluorocortisol

is not needed until the supraphysiologic stress doses of cortisol are decreased. Because 9α-fluorocortisol can be administered only orally, and because this may not be possible in the postoperative period, the appropriate drug for glucocorticoid replacement is cortisol or cortisone, both of which have mineralocorticoid activity, rather than synthetic steroids such as prednisone or dexamethasone, which have little mineralocorticoid activity.

CONCLUDING REMARKS

Because the adrenal cortex is principally concerned with steroid synthesis, most of its disorders reflect genetic lesions in adrenal development and steroidogenesis. Overproduction and underproduction of steroids having varied and complex physiologic actions lead to complex phenotypes and clinical presentations. These primary, genetic disorders typically present themselves in infancy and childhood. By contrast, secondary disorders, such as Cushing disease (usually a disorder of the pituitary) and Addison disease (usually a disorder of cellular immunity) may be seen at any age. Thus, the pediatric endocrinologist must have a detailed understanding of the cell biology, genetics, and biochemistry of steroid hormone biosynthesis. Improvements in the speed, efficiency, accuracy, and economy of DNA sequencing now permit direct genetic diagnosis of many genetic diseases. However, it is unlikely that measurements of steroid hormones will become obsolete in the foreseeable future, and an understanding of steroid physiology will always be needed to comprehend clinical presentations, formulate differential diagnoses, choose genes for study, and monitor therapy. Thus, genetics will continue to enhance clinical physiology but will not replace it.

REFERENCES

1. Miller, W. L. (2013). A brief history of adrenal research: Steroidogenesis—the soul of the adrenal. *Mol Cell Endocrinol*, *371*, 5–14.
2. Miller, W. L. (1988). Molecular biology of steroid hormone synthesis. *Endocr Rev*, *9*, 295–318.
3. Evans, R. M. (1988). The steroid and thyroid hormone receptor superfamily. *Science*, *240*, 889–895.
4. Else, T., & Hammer, G. D. (2005). Genetic analysis of adrenal absence: agenesis and aplasia. *Trends Endocrinol Metab*, *16*, 458–468.
5. Mesiano, S., & Jaffe, R. B. (1997). Developmental and functional biology of the primate fetal adrenal cortex. *Endocr Rev*, *18*, 378–403.
6. Hammer, G. D., Parker, K. L., & Schimmer, B. P. (2005). Transcriptional regulation of adrenocortical development. *Endocrinology*, *146*, 1018–1024.
7. Kim, A. C., Barlaskar, F. M., Heaton, J. H., et al. (2009). In search of adrenocortical stem and progenitor cells. *Endocr Rev*, *30*, 241–263.
8. Axelrod, J., & Reisine, T. D. (1984). Stress hormones: their interaction and regulation. *Science*, *224*, 452–459.
9. Miller, W. L., & Auchus, R. J. (2011). The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. *Endocr Rev*, *32*, 81–151.
10. Miller, W. L., & Bose, H. S. (2011). Early steps in steroidogenesis: intracellular cholesterol trafficking. *J Lipid Res*, *52*, 2111–2135.
11. Agarwal, A. K., & Auchus, R. J. (2005). Cellular redox state regulates hydroxysteroid dehydrogenase activity and intracellular hormone potency. *Endocrinology*, *146*, 2531–2538.
12. Miller, W. L. (2005). Regulation of steroidogenesis by electron transfer. *Endocrinology*, *146*, 2544–2550.
13. Stocco, D. M., & Clark, B. J. (1996). Regulation of the acute production of steroids in steroidogenic cells. *Endocr Rev*, *17*, 221–244.
14. Miller, W. L. (2007). StAR search—what we know about how the steroidogenic acute regulatory protein mediates mitochondrial cholesterol import. *Mol Endocrinol*, *21*, 589–601.
15. Lin, D., Sugawara, T., Strauss, J. F., 3rd, et al. (1995). Role of steroidogenic acute regulatory protein in adrenal and gonadal steroidogenesis. *Science*, *267*, 1828–1831.
16. Bose, H. S., Sugawara, T., Strauss, J. F., 3rd, & Miller, W. L. (1996). The pathophysiology and genetics of congenital lipoid adrenal hyperplasia. *N Engl J Med*, *335*, 1870–1878.
17. Bose, H. S., Lingappa, V. R., & Miller, W. L. (2002). Rapid regulation of steroidogenesis by mitochondrial protein import. *Nature*, *417*, 87–91.
18. Papadopoulos, V., & Miller, W. L. (2012). Role of mitochondria in steroidogenesis. *Best Pract Res Clin Endocrinol Metab*, *26*, 771–790.
19. Simard, J., Ricketts, M. L., Gingras, S., et al. (2005). Molecular biology of the 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase gene family. *Endocr Rev*, *26*, 525–582.
20. Auchus, R. J., Lee, T. C., & Miller, W. L. (1998). Cytochrome b5 augments the 17,20-lyase activity of human P450c17 without direct electron transfer. *J Biol Chem*, *273*, 3158–3165.
21. Miller, W. L. (2012). The syndrome of 17,20 lyase deficiency. *J Clin Endocrinol Metab*, *97*, 59–67.
22. Speiser, P. W., Azziz, R., Baskin, L. S., et al. (2010). Congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab, *95*, 4133–4160.
23. Morel, Y., & Miller, W. L. (1991). Clinical and molecular genetics of congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Adv Hum Genet*, *20*, 1–68.
24. Speiser, P. W., & White, P. C. (2003). Congenital adrenal hyperplasia. *N Engl J Med*, *349*, 776–788.
25. Mellon, S. H., & Miller, W. L. (1989). Extraadrenal steroid 21-hydroxylation is not mediated by P450c21. *J Clin Invest*, *84*, 1497–1502.
26. Yamazaki, H., & Shimada, T. (1997). Progesterone and testosterone hydroxylation by cytochromes P450 2C19, 2C9, and 3A4 in human liver microsomes. *Arch Biochem Biophys*, *346*, 161–169.
27. Gomes, L. G., Huang, N., Agrawal, V., et al. (2009). Extraadrenal 21-hydroxylation by CYP2C19 and CYP3A4: effect on 21-hydroxylase deficiency. *J Clin Endocrinol Metab*, *94*, 89–95.
28. White, P. C., Curnow, K. M., & Pascoe, L. (1994). Disorders of steroid 11β–hydroxylase isozymes. *Endocr Rev*, *15*, 421–438.
29. Fardella, C. E., & Miller, W. L. (1996). Molecular biology of mineralocorticoid metabolism. *Annu Rev Nutr*, *16*, 443–470.
30. Labrie, F., Luu-The V., Lin, S. X., et al. (1997). The key role of 17β-hydroxysteroid dehydrogenases in sex steroid biology. *Steroids*, *62*, 148–158.
31. Penning, T. M. (1997). Molecular endocrinology of hydroxysteroid dehydrogenases. *Endocr Rev*, *18*, 281–305.
32. Moghrabi, N., & Andersson, S. (1998). 17β-hydroxysteroid dehydrogenases: physiological roles in health and disease. *Trends Endocrinol Metab*, *9*, 265–270.
33. Penning, T. M., Burczynski, M. E., Jez, J. M., et al. (2000). Human 3α-hydroxysteroid dehydrogenase isoforms (AKR1C1-AKR1C4) of the aldo-keto reductase superfamily: functional plasticity and tissue distribution reveals roles in the inactivation and formation of male and female sex hormones. *Biochem J*, *351*, 67–77.
34. Nakamura, Y., Hornsby, P. J., Casson, P., et al. (2009). Type 5 17β-hydroxysteroid dehydrogenase (AKR1C3) contributes to testosterone production in the adrenal reticularis. *J Clin Endocrinol Metab*, *94*, 2192–2198.
35. Falany, C. N. (1997). Enzymology of human cytosolic sulfotransferases. *FASEB J*, *11*, 206–216.
36. Strott, C. A. (2002). Sulfonation and molecular action. *Endocr Rev*, *23*, 703–732.
37. Noordam, C., Dhir, V., McNelis, J.C., et al. (2009) Inactivating PAPSS2 mutations in a patient with premature pubarche. *N Engl J Med 360*, 2310-2318.
38. Simpson, E. R., Mahendroo, M. S., Means, G. D., et al. (1994). Aromatase cytochrome P450, the enzyme responsible for estrogen biosynthesis. *Endocr Rev*, *15*, 342–355.

39. Grumbach, M. M., & Auchus, R. J. (1999). Estrogen: consequences and implications of human mutations in synthesis and action. *J Clin Endocrinol Metab*, *84*, 4677–4694.
40. Thigpen, A. E., Silver, R. I., Guileyardo, J. M., et al. (1993). Tissue distribution and ontogeny of steroid 5α-reductase isozyme expression. *J Clin Invest*, *92*, 903–910.
41. Wilson, J. D. (1999). The role of androgens in male gender role behavior. *Endocr Rev*, *20*, 726–737.
42. White, P. C., Mune, T., & Agarwal, A. K. (1997). 11β-hydroxysteroid dehydrogenase and the syndrome of apparent mineralocorticoid excess. *Endocr Rev*, *18*, 135–156.
43. Walker, E. A., & Stewart, P. M. (2003). 11β-hydroxysteroid dehydrogenase: unexpected connections. *Trends Endocrinol Metab*, *14*, 334–339.
44. Seckl, J. R., Morton, N. M., Chapman, K. E., & Walker, B. R. (2004). Glucocorticoids and 11β-hydroxysteroid dehydrogenase in adipose tissue. *Recent Prog Horm Res*, *59*, 359–393.
45. Tomlinson, J. W., Walker, E. A., Bujalska, I. J., et al. (2004). 11β-hydroxysteroid dehydrogenase type 1: a tissue-specific regulator of glucocorticoid response. *Endocr Rev*, *25*, 831–866.
46. Hewitt, K. N., Walker, E. A., & Stewart, P. M. (2005). Hexose-6-phosphate dehydrogenase and redox control of 11β-hydroxysteroid dehydrogenase type 1 activity. *Endocrinology*, *146*, 2539–2543.
47. Auchus, R. J. (2004). The backdoor pathway to dihydrotestosterone. *Trends Endocrinol Metab*, *15*, 432–438.
48. Wilson, J. D., Auchus, R. J., Leihy, M. W., et al. (2003). 5α-androstane-3α,17β-diol is formed in tammar wallaby pouch young testes by a pathway involving 5α-pregnane-3α,17α-diol-20-one as a key intermediate. *Endocrinology*, *144*, 575–580.
49. Fluck, C. E., Meyer-Boni, M., Pandey, A. V., et al. (2011). Why boys will be boys: two pathways of fetal testicular androgen biosynthesis are needed for male sexual differentiation. *Am J Hum Genet*, *89*, 201–218.
50. Kamrath, C., Hochberg, Z., Hartmann, M. F., et al. (2012). Increased activation of the alternative "backdoor" pathway in patients with 21-hydroxylase deficiency: evidence from urinary steroid hormone analysis. *J Clin Endocrinol Metab*, *97*, E367–E375.
51. Goto, M., Piper Hanley, K., Marcos, J., et al. (2006). In humans, early cortisol biosynthesis provides a mechanism to safeguard female sexual development. *J Clin Invest*, *116*, 953–960.
52. Miller, W. L. (1998). Steroid hormone biosynthesis and actions in the materno-feto-placental unit. *Clin Perinatol*, *25*, 799–817.
53. McMahon, S. K., Pretorius, C. J., Ungerer, J. P. J., et al. (2010). Neonatal complete generalized glucocorticoid resistance and growth hormone deficiency caused by a novel homozygous mutation in helix 12 of the ligand binding domain of the glucocorticoid receptor gene (NR3C1). *J Clin Endocrinol Metab*, *95*, 297–302.
54. Sawchenko, P. E., Swanson, L. W., & Vale, W. W. (1984). Co-expression of corticotropin-releasing factor and vasopressin immunoreactivity in parvocellular neurosecretory neurons of the adrenalectomized rat. *Proc Natl Acad Sci U S A*, *81*, 1883–1887.
55. Lundblad, J. R., & Roberts, J. L. (1988). Regulation of proopiomelanocortin gene expression in pituitary. *Endocr Rev*, *9*, 135–158.
56. Ehrhart-Bornstein, M., Hinson, J. P., Bornstein, S. R., et al. (1998). Intraadrenal interactions in the regulation of adrenocortical steroidogenesis. *Endocr Rev*, *19*, 101–143.
57. Mesiano, S., & Jaffe, R. B. (1997). Role of growth factors in the developmental regulation of the human fetal adrenal cortex. *Steroids*, *62*, 62–72.
58. Udelsman, R., Norton, J. A., Jelenich, S. E., et al. (1987). Responses of the hypothalamic-pituitary-adrenal and renin-angiotensin axes and the sympathetic system during controlled surgical and anesthetic stress. *J Clin Endocrinol Metab*, *64*, 986–994.
59. Taylor, L.K., Auchus, R.J., Baskin, L.S., and Miller, W.L. (2013) Cortisol response to operative stress with anesthesia in healthy children. *J Clin Endocrinol Metab 98*: 3687-3693.
60. Chrousos, G. P. (1995). The hypothalamic-pituitary-adrenal axis and immune-mediated inflammation. *N Engl J Med*, *332*, 1351–1362.
61. Hardman, J. A., Hort, Y. J., Catanzaro, D. F., et al. (1984). Primary structure of the human renin gene. *DNA*, *3*, 457–468.
62. Barrett, P. Q., Bollag, W. B., Isales, C. M., et al. (1989). Role of calcium in angiotensin II-mediated aldosterone secretion. *Endocr Rev*, *10*, 496–518.
63. Orentreich, N., Brind, J. L., Rizer, R. L., & Vogelman, J. H. (1984). Age changes and sex differences in serum dehydroepiandrosterone sulfate concentrations throughout adulthood. *J Clin Endocrinol Metab*, *59*, 551–555.
64. Miller, W. L. (2009). Androgen synthesis in adrenarche. *Rev Endocr Metab Disord*, *10*, 3–17.
65. Hui, X. G., Akahira, J., Suzuki, T., et al. (2009). Development of the human adrenal zona reticularis: morphometric and immunohistochemical studies from birth to adolescence. *J Endocrinol*, *203*, 241–252.
66. Tee, M.K., Miller, W.L. (2013) Phosphorylation of human cytochrome P450c17 by p38α selectively increases 17,20 lyase activity and androgen synthesis. *J Biol Chem 288*: 22903-23913.
67. Alkatib, A. A., Cosma, M., Elamin, M. B., et al. (2009). A systematic review and meta-analysis of randomized placebo-controlled trials of DHEA treatment effects on quality of life in women with adrenal insufficiency. *J Clin Endocrinol Metab*, *94*, 3676–3681.
68. Hammond, G. L. (1990). Molecular properties of corticosteroid binding globulin and the sex-steroid binding proteins. *Endocr Rev*, *11*, 65–79.
69. Rosner, W. (1990). The functions of corticosteroid-binding globulin and sex hormone-binding globulin: recent advances. *Endocr Rev*, *11*, 80–91.
70. Dimitriou, T., Maser-Gluth, C., & Remer, T. (2003). Adrenocortical activity in healthy children is associated with fat mass. *Am J Clin Nutr*, *77*, 731–736.
71. Remer, T., Maser-Gluth, C., Boye, K. R., et al. (2006). Exaggerated adrenarche and altered cortisol metabolism in Type 1 diabetic children. *Steroids*, *71*, 591–598.
72. Kerrigan, J. R., Veldhuis, J. D., Leyo, S. A., et al. (1993). Estimation of daily cortisol production and clearance rates in normal pubertal males by deconvolution analysis. *J Clin Endocrinol Metab*, *76*, 1505–1510.
73. Linder, B. L., Esteban, N. V., Yergey, A. L., et al. (1990). Cortisol production rate in childhood and adolescence. *J Pediatr*, *117*, 892–896.
74. Lashansky, G., Saenger, P., Fishman, K., et al. (1991). Normative data for adrenal steroidogenesis in a healthy pediatric population: age- and sex-related changes after adrenocorticotropin stimulation. *J Clin Endocrinol Metab*, *73*, 674–686.
75. Clark, A. J., & Weber, A. (1998). Adrenocorticotropin insensitivity syndromes. *Endocr Rev*, *19*, 828–843.
76. Avgerinos, P. C., Yanovski, J. A., Oldfield, E. H., et al. (1994). The metyrapone and dexamethasone suppression tests for the differential diagnosis of the adrenocorticotropin-dependent Cushing syndrome: a comparison. *Ann Intern Med*, *121*, 318–327.
77. Miller, W. L. (1997). Congenital lipoid adrenal hyperplasia: the human gene knockout for the steroidogenic acute regulatory protein. *J Mol Endocrinol*, *19*, 227–240.
78. Chen, X., Baker, B. Y., Abduljabbar, M. A., & Miller, W. L. (2005). A genetic isolate of congenital lipoid adrenal hyperplasia with atypical clinical findings. *J Clin Endocrinol Metab*, *90*, 835–840.
79. Bose, H. S., Pescovitz, O. H., & Miller, W. L. (1997). Spontaneous feminization in a 46,XX female patient with congenital lipoid adrenal hyperplasia due to a homozygous frameshift mutation in the steroidogenic acute regulatory protein. *J Clin Endocrinol Metab*, *82*, 1511–1515.
80. Fujieda, K., Tajima, T., Nakae, J., et al. (1997). Spontaneous puberty in 46,XX subjects with congenital lipoid adrenal hyperplasia: ovarian steroidogenesis is spared to some extent despite inactivating mutations in the steroidogenic acute regulatory protein (StAR) gene. *J Clin Invest*, *99*, 1265–1271.
81. Baker, B. Y., Lin, L., Kim, C. J., et al. (2006). Nonclassic congenital lipoid adrenal hyperplasia: a new disorder of the steroidogenic acute regulatory protein with very late presentation and normal male genitalia. *J Clin Endocrinol Metab*, *91*, 4781–4785.
82. Metherell, L. A., Naville, D., Halaby, G., et al. (2009). Nonclassic lipoid congenital adrenal hyperplasia masquerading as familial glucocorticoid deficiency. *J Clin Endocrinol Metab*, *94*, 3865–3871.
83. Sahakitrungruang, T., Soccio, R. E., Lang-Muritano, M., et al. (2010). Clinical, genetic, and functional characterization of four patients carrying partial loss-of-function mutations in the steroidogenic acute regulatory protein (StAR). *J Clin Endocrinol Metab*, *95*, 3352–3359.
84. Tajima, T., Fujieda, K., Kouda, N., et al. (2001). Heterozygous mutation in the cholesterol side chain cleavage enzyme (P450scc) gene in a patient with 46,XY sex reversal and adrenal insufficiency. *J Clin Endocrinol Metab*, *86*, 3820–3825.

85. Tee, M. K., Abramsohn, M., Loewenthal, N., et al. (2013). Varied clinical presentations with mutations in CYP11A1 encoding the cholesterol side chain cleavage enzyme, P450scc. *J Clin Endocrinol Metab*, *98*, 713–720.
86. Rubtsov, P., Karmanov, M., Sverdlova, P., et al. (2009). A novel homozygous mutation in CYP11A1 gene is associated with late-onset adrenal insufficiency and hypospadias in a 46,XY patient. *J Clin Endocrinol Metab*, *94*, 936–939.
87. Sahakitrungruang, T., Tee, M. K., Blackett, P. R., & Miller, W. L. (2011). Partial defect in the cholesterol side-chain cleavage enzyme P450scc (CYP11A1) resembling nonclassic congenital lipoid adrenal hyperplasia. *J Clin Endocrinol Metab*, *96*, 792–798.
88. Achermann, J. C., Ito, M., Hindmarsh, P. C., & Jameson, J. L. (1999). A mutation in the gene encoding steroidogenic factor-1 causes XY sex reversal and adrenal failure in humans. *Nat Genet*, *22*, 125–126.
89. Camats, N., Pandey, A. V., Fernandez-Cancio, M., et al. (2012). Ten novel mutations in the NR5A1 gene cause disordered sex development in 46,XY and ovarian insufficiency in 46,XX individuals. *J Clin Endocrinol Metab*, *97*, E1294–E1306.
90. Moisan, A. M., Ricketts, M. L., Tardy, V., et al. (1999). New insight into the molecular basis of 3β-hydroxysteroid dehydrogenase deficiency: identification of eight mutations in the HSD3B2 gene eleven patients from seven new families and comparison of the functional properties of twenty-five mutant enzymes. *J Clin Endocrinol Metab*, *84*, 4410–4425.
91. Cara, J. F., Moshang, T., Jr., Bongiovanni, A. M., & Marx, B. S. (1985). Elevated 17-hydroxyprogesterone and testosterone in a newborn with 3β-hydroxysteroid dehydrogenase deficiency. *N Engl J Med*, *313*, 618–621.
92. Pang, S., Carbunaru, G., Haider, A., et al. (2003). Carriers for type II 3β-hydroxysteroid dehydrogenase (HSD3B2) deficiency can only be identified by HSD3B2 genotype study and not by hormone test. *Clin Endocrinol* (Oxf), *58*, 323–331.
93. Pang, S. (1998). The molecular and clinical spectrum of 3β-hydroxysteroid dehydrogenase deficiency disorder. *Trends Endocrinol Metab*, *9*, 82–86.
94. Mermejo, L. M., Elias, L. L., Marui, S., et al. (2005). Refining hormonal diagnosis of type II 3β-hydroxysteroid dehydrogenase deficiency in patients with premature pubarche and hirsutism based on HSD3B2 genotyping. *J Clin Endocrinol Metab*, *90*, 1287–1293.
95. Auchus, R. J. (2001). The genetics, pathophysiology, and management of human deficiencies of P450c17. *Endocrinol Metab Clin North Am*, *30*, 101–119.
96. Costa-Santos, M., Kater, C. E., & Auchus, R. J. (2004). Two prevalent CYP17 mutations and genotype-phenotype correlations in 24 Brazilian patients with 17-hydroxylase deficiency. *J Clin Endocrinol Metab*, *89*, 49–60.
97. Geller, D. H., Auchus, R. J., Mendonca, B. B., & Miller, W. L. (1997). The genetic and functional basis of isolated 17,20-lyase deficiency. *Nat Genet*, *17*, 201–205.
98. Auchus, R. J., & Miller, W. L. (1999). Molecular modeling of human P450c17 (17α-hydroxylase/17,20-lyase): insights into reaction mechanisms and effects of mutations. *Mol Endocrinol*, *13*, 1169–1182.
99. Fluck, C. E., Tajima, T., Pandey, A. V., et al. (2004). Mutant P450 oxidoreductase causes disordered steroidogenesis with and without Antley-Bixler syndrome. *Nat Genet*, *36*, 228–230.
100. Huang, N., Pandey, A. V., Agrawal, V., et al. (2005). Diversity and function of mutations in P450 oxidoreductase in patients with Antley-Bixler syndrome and disordered steroidogenesis. *Am J Hum Genet*, *76*, 729–749.
101. Fukami, M., Horikawa, R., Nagai, T., et al. (2005). Cytochrome P450 oxidoreductase gene mutations and Antley-Bixler syndrome with abnormal genitalia and/or impaired steroidogenesis: molecular and clinical studies in 10 patients. *J Clin Endocrinol Metab*, *90*, 414–426.
102. Scott, R. R., & Miller, W. L. (2008). Genetic and clinical features of P450 oxidoreductase deficiency. *Horm Res*, *69*, 266–275.
103. Krone, N., Reisch, N., Idkowiak, J., et al. (2012). Genotype-phenotype analysis in congenital adrenal hyperplasia due to P450 oxidoreductase deficiency. *J Clin Endocrinol Metab*, *97*, E257–E267.
104. Shackleton, C., Marcos, J., Arlt, W., & Hauffa, B. P. (2004). Prenatal diagnosis of P450 oxidoreductase deficiency (ORD): a disorder causing low pregnancy estriol, maternal and fetal virilization, and the Antley-Bixler syndrome phenotype. *Am J Med Genet A*, *129A*, 105–112.
105. Laue, K., Pogoda, H. M., Daniel, P. B., et al. (2011). Craniosynostosis and multiple skeletal anomalies in humans and zebrafish result from a defect in the localized degradation of retinoic acid. *Am J Hum Genet*, *89*, 595–606.
106. Miller, W. L., Agrawal, V., Sandee, D., et al. (2011). Consequences of POR mutations and polymorphisms. *Mol Cell Endocrinol*, *336*, 174–179.
107. Tomalik-Scharte, D., Maiter, D., Kirchheiner, J., et al. (2010). Impaired hepatic drug and steroid metabolism in congenital adrenal hyperplasia due to P450 oxidoreductase deficiency. *Eur J Endocrinol*, *163*, 919–924.
108. Giordano, S. J., Kaftory, A., Steggles, A. W. (1994). A splicing mutation in the cytochrome b5 gene from a patient with congenital methemoglobinemia and pseudohermaphrodism. *Hum Genet*, *93*, 568–570.
109. Kok, R. C., Timmerman, M. A., Wolffenbuttel, K. P., et al. (2010). Isolated 17,20-lyase deficiency due to the cytochrome b5 mutation W27X. *J Clin Endocrinol Metab*, *95*, 994–999.
110. Idkowiak, J., Randell, T., Dhir, V., et al. (2012). A missense mutation in the human cytochrome b5 gene causes 46,XY disorder of sex development due to true isolated 17,20 lyase deficiency. *J Clin Endocrinol Metab*, *97*, E465–E475.
111. Therrell, B. L., Jr., Berenbaum, S. A., Manter-Kapanke, V., et al. (1998). Results of screening 1.9 million Texas newborns for 21–hydroxylase-deficient congenital adrenal hyperplasia. *Pediatrics*, *101*, 583–590.
112. Witchel, S. F. (2012). Nonclassic congenital adrenal hyperplasia. *Curr Opin Endocrinol Diabetes Obes*, *19*, 151–158.
113. Wijesuriya, S. D., Zhang, G., Dardis, A., & Miller, W. L. (1999). Transcriptional regulatory elements of the human gene for cytochrome P450c21 (steroid 21-hydroxylase) lie within intron 35 of the linked C4B gene. *J Biol Chem*, *274*, 38097–38106.
114. Gomez-Escobar, N., Chou, C. F., Lin, W. W., et al. (1998). The G11 gene located in the major histocompatibility complex encodes a novel nuclear serine/threonine protein kinase . *J Biol Chem*, *273*, 30954–30960.
115. Bristow, J., Tee, M. K., Gitelman, S. E., et al. (1993). Tenascin-X: a novel extracellular matrix protein encoded by the human XB gene overlapping P450c21B. *J Cell Biol*, *122*, 265–278.
116. Burch, G. H., Gong, Y., Liu, W., et al. (1997). Tenascin-X deficiency is associated with Ehlers-Danlos syndrome. *Nat Genet*, *17*, 104–108.
117. Schalkwijk, J., Zweers, M. C., Steijlen, P. M., et al. (2001). A recessive form of the Ehlers-Danlos syndrome caused by tenascin-X deficiency. *N Engl J Med*, *345*, 1167–1175.
118. Miller, W. L. (1999). Dexamethasone treatment of congenital adrenal hyperplasia in utero: an experimental therapy of unproven safety. *J Urol*, *162*, 537–540.
119. Miller, W. L., & Witchel, S. F. (2013). Prenatal treatment of congenital adrenal hyperplasia: Risks outweigh benefits. *Am J Obstet Gynecol*, *208*, 354–359.
120. Partsch, C. J., Sippell, W. G., MacKenzie, I. Z., Aynsley-Green, A. (1991). The steroid hormonal milieu of the undisturbed human fetus and mother at 16-20 weeks gestation. *J Clin Endocrinol Metab*, *73*, 969–974.
121. Kari, M. A., Raivio, K. O., Stenman, U. H., & Voutilainen, R. (1996). Serum cortisol, dehydroepiandrosterone sulfate, and steroid-binding globulins in preterm neonates: effect of gestational age and dexamethasone therapy. *Pediatr Res*, *40*, 319–324.
122. Witchel, S. F., & Miller, W. L. (2012). Prenatal treatment of congenital adrenal hyperplasia-not standard of care. *J Genet Couns*, *21*, 615–624.
123. Hirvikoski, T., Nordenstrom, A., Lindholm, T., et al. (2007). Cognitive functions in children at risk for congenital adrenal hyperplasia treated prenatally with dexamethasone. *J Clin Endocrinol Metab*, *92*, 542–548.
124. Hirvikoski, T., Nordenstrom, A., Lindholm, T., et al. (2008). Long-term follow-up of prenatally treated children at risk for congenital adrenal hyperplasia: does dexamethasone cause behavioural problems? *Eur J Endocrinol*, *159*, 309–316.
125. Hirvikoski, T., Lindholm, T., Lajic, S., & Nordenstrom, A. (2011). Gender role behaviour in prenatally dexamethasone-treated

children at risk for congenital adrenal hyperplasia: a pilot study. *Acta Paediatr*, *100*, e112–e119.
126. Hirvikoski, T., Nordenstrom, A., Wedell, A., et al. (2012). Prenatal dexamethasone treatment of children at risk for congenital adrenal hyperplasia: the Swedish experience and standpoint. *J Clin Endocrinol Metab*, *97*, 1881–1883.
127. Chabraoui, L., Abid, F., Menassa, R., et al. (2010). Three novel CYP11B1 mutations in congenital adrenal hyperplasia due to steroid 11β-hydroxylase deficiency in a Moroccan population. *Horm Res Paediatr*, *74*, 182–189.
128. Parajes, S., Loidi, L., Reisch, N., et al. (2010). Functional consequences of seven novel mutations in the CYP11B1 gene: four mutations associated with nonclassic and three mutations causing classic 11β-hydroxylase deficiency. *J Clin Endocrinol Metab*, *95*, 779–788.
129. Joehrer, K., Geley, S., Strasser-Wozak, E. M., et al. (1997). CYP11B1 mutations causing non-classic adrenal hyperplasia due to 11β-hydroxylase deficiency. *Hum Mol Genet*, *6*, 1829–1834.
130. Pascoe, L., Curnow, K. M., Slutsker, L., et al. (1992). Mutations in the human CYP11B2 (aldosterone synthase) gene causing corticosterone methyloxidase II deficiency. *Proc Natl Acad Sci U S A*, *89*, 4996–5000.
131. Dluhy, R. G., & Lifton, R. P. (1999). Glucocorticoid-remediable aldosteronism. *J Clin Endocrinol Metab*, *84*, 4341–4344.
132. Fardella, C. E., Mosso, L., Gomez-Sanchez, C., et al. (2000). Primary hyperaldosteronism in essential hypertensives: prevalence, biochemical profile, and molecular biology. *J Clin Endocrinol Metab*, *85*, 1863–1867.
133. Furst-Recktenwald, S., Dorr, H. G., Quinkler, M., et al. (2012). Is there sufficient evidence to consider the use of 11β-hydroxysteroid dehydrogenase type 1 inhibition in children? *Clin Endocrinol* (Oxf), *77*, 159–168.
134. Lawson, A. J., Walker, E. A., Lavery, G. G., et al. (2011). Cortisone-reductase deficiency associated with heterozygous mutations in 11β-hydroxysteroid dehydrogenase type 1. Proc Natl Acad Sci U S A, 108, 4111–4116.
135. Lavery, G. G., Walker, E. A., Tiganescu, A., et al. (2008). Steroid biomarkers and genetic studies reveal inactivating mutations in hexose-6-phosphate dehydrogenase in patients with cortisone reductase deficiency. *J Clin Endocrinol Metab*, *93*, 3827–3832.
136. Draper, N., Walker, E. A., Bujalska, I. J., et al. (2003). Mutations in the genes encoding 11β-hydroxysteroid dehydrogenase type 1 and hexose-6-phosphate dehydrogenase interact to cause cortisone reductase deficiency. *Nat Genet*, *34*, 434–439.
137. San Millan, J. L., Botella-Carretero, J. I., Alvarez-Blasco, F., et al. (2005). A study of the hexose-6-phosphate dehydrogenase gene R453Q and 11β-hydroxysteroid dehydrogenase type 1 gene 83557insA polymorphisms in the polycystic ovary syndrome. *J Clin Endocrinol Metab*, *90*, 4157–4162.
138. White, P. C. (2005). Genotypes at 11β-hydroxysteroid dehydrogenase type 11B1 and hexose-6-phosphate dehydrogenase loci are not risk factors for apparent cortisone reductase deficiency in a large population-based sample. *J Clin Endocrinol Metab*, *90*, 5880–5883.
139. Quinkler, M., & Stewart, P. M. (2003). Hypertension and the cortisol-cortisone shuttle. *J Clin Endocrinol Metab*, *88*, 2384–2392.
140. Palermo, M., Quinkler, M., & Stewart, P. M. (2004). Apparent mineralocorticoid excess syndrome: an overview. *Arq Bras Endocrinol Metabol*, *48*, 687–696.
141. Perry, R., Kecha, O., Paquette, J., et al. (2005). Primary adrenal insufficiency in children: twenty years experience at the Sainte-Justine Hospital, Montreal. *J Clin Endocrinol Metab*, *90*, 3243–3250.
142. Lin, L., Gu, W. X., Ozisik, G., et al. (2006). Analysis of DAX1 (NR0B1) and steroidogenic factor-1 (NR5A1) in children and adults with primary adrenal failure: ten years' experience. *J Clin Endocrinol Metab*, *91*, 3048–3054.
143. Lin, L., & Achermann, J. C. (2004). Inherited adrenal hypoplasia: not just for kids! *Clin Endocrinol* (Oxf), *60*, 529–537.
144. Adem, P. V., Montgomery, C. P., Husain, A. N., et al. (2005). Staphylococcus aureus sepsis and the Waterhouse-Friderichsen syndrome in children. *N Engl J Med*, *353*, 1245–1251.
145. Betterle, C., Dal Pra, C., Mantero, F., & Zanchetta, R. (2002). Autoimmune adrenal insufficiency and autoimmune polyendocrine syndromes: autoantibodies, autoantigens, and their applicability in diagnosis and disease prediction. *Endocr Rev*, *23*, 327–364.
146. Ueda, H., Howson, J. M., Esposito, L., et al. (2003). Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. *Nature*, *423*, 506–511.
147. Ghaderi, M., Gambelunghe, G., Tortoioli, C., et al. (2006). MHC2TA single nucleotide polymorphism and genetic risk for autoimmune adrenal insufficiency. *J Clin Endocrinol Metab*, *91*, 4107–4111.
148. Perheentupa, J. (2006). Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. *J Clin Endocrinol Metab*, *91*, 2843–2850.
149. Finnish-German, A. C. (1997). An autoimmune disease, APECED, caused by mutations in a novel gene featuring two PHD-type zinc-finger domains. *Nat Genet*, *17*, 399–403.
150. Nagamine, K., Peterson, P., Scott, H. S., et al. (1997). Positional cloning of the APECED gene. *Nat Genet*, *17*, 393–398.
151. Villasenor, J., Benoist, C., & Mathis, D. (2005). AIRE and APECED: molecular insights into an autoimmune disease. *Immunol Rev*, *204*, 156–164.
152. Tabarin, A., Achermann, J. C., Recan, D., et al. (2000). A novel mutation in DAX1 causes delayed-onset adrenal insufficiency and incomplete hypogonadotropic hypogonadism. *J Clin Invest*, *105*, 321–328.
153. Vilain, E., Le Merrer, M., Lecointre, C., et al. (1999). IMAGe, a new clinical association of intrauterine growth retardation, metaphyseal dysplasia, adrenal hypoplasia congenita, and genital anomalies. *J Clin Endocrinol Metab*, *84*, 4335–4340.
154. Bergada, I., Del Rey, G., Lapunzina, P., et al. (2005). Familial occurrence of the IMAGe association: additional clinical variants and a proposed mode of inheritance. *J Clin Endocrinol Metab*, *90*, 3186–3190.
155. Arboleda, V. A., Lee, H., Parnaik, R., et al. (2012). Mutations in the PCNA-binding domain of CDKN1C cause IMAGe syndrome. *Nat Genet*, *44*, 788–792.
156. Hatada, I., Ohashi, H., Fukushima, Y., et al. (1996). An imprinted gene p57KIP2 is mutated in Beckwith-Wiedemann syndrome. *Nat Genet*, *14*, 171-173.
157. Clark, A. J. L., Metherell, L. A., Cheetham, M. E., & Huebner, A. (2005). Inherited ACTH insensitivity illuminates the mechanisms of ACTH action. *Trends Endocrinol Metab*, *16*, 451–457.
158. Webb, T. R., Clark, A. J. L. (2010). The melanocortin 2 receptor accessory proteins. *Mol Endocrinol*, *24*, 475–484.
159. Metherell, L. A., Chapple, J. P., Cooray, S., et al. (2005). Mutations in MRAP, encoding a new interacting partner of the ACTH receptor, cause familial glucocorticoid deficiency type 2. *Nat Genet*, *37*, 166–170.
160. Meimaridou, E., Kowalczyk, J., Guasti, L., et al. (2012). Mutations in NNT encoding nicotinamide nucleotide transhydrogenase cause familial glucocorticoid deficiency. *Nat Genet*, *44*, 740–742.
161. Hughes, C. R., Guasti, L., Meimaridou, E., et al. (2012). MCM4 mutation causes adrenal failure, short stature, and natural killer cell deficiency in humans. *J Clin Invest*, *122*, 814–820.
162. Tullio-Pelet, A., Salomon, R., Hadj-Rabia, S., et al. (2000). Mutant WD-repeat protein in triple-A syndrome. *Nat Genet*, *26*, 332–325.
163. Handschug, K., Sperling, S., Yoon, S. J., et al. (2001). Triple A syndrome is caused by mutations in AAAS, a new WD-repeat protein gene. *Hum Mol Genet*, *10*, 283–290.
164. Storr, H. L., Kind, B., Parfitt, D. A., et al. (2009). Deficiency of ferritin heavy-chain nuclear import in triple a syndrome implies nuclear oxidative damage as the primary disease mechanism. *Mol Endocrinol*, *23*, 2086–2094.
165. Ligtenberg, M. J., Kemp, S., Sarde, C. O., et al. (1995). Spectrum of mutations in the gene encoding the adrenoleukodystrophy protein. *Am J Hum Genet*, *56*, 44–50.
166. Watkins, P. A., Gould, S. J., Smith, M. A., et al. (1995). Altered expression of ALDP in X-linked adrenoleukodystrophy. *Am J Hum Genet*, *57*, 292–301.
167. Shimozawa, N. (2011). Molecular and clinical findings and diagnostic flowchart of peroxisomal diseases. *Brain Dev*, *33*, 770–776.
168. Moser, H. W., Raymond, G. V., & Dubey, P. (2005). Adrenoleukodystrophy: new approaches to a neurodegenerative disease. *JAMA*, *294*, 3131–3134.

169. McGuinness, M. C., Lu, J. F., Zhang, H. P., et al. (2003). Role of ALDP (ABCD1) and mitochondria in X-linked adrenoleukodystrophy. *Mol Cell Biol*, *23*, 744–753.
170. Kemp, S., & Wanders, R. J. (2007). X-linked adrenoleukodystrophy: very long-chain fatty acid metabolism, ABC half-transporters and the complicated route to treatment. *Mol Genet Metab*, *90*, 268–276.
171. Watkins, P. A., Naidu, S., & Moser, H. W. (1987). Adrenoleukodystrophy: biochemical procedures in diagnosis, prevention and treatment. *J Inherit Metab Dis*, *10*(Suppl. 1), 46–53.
172. Moser, H. W., Moser, A. E., Singh, I., & O'Neill, B. P. (1984). Adrenoleukodystrophy: survey of 303 cases: biochemistry, diagnosis, and therapy. *Ann Neurol*, *16*, 628–641.
173. Moser, H. W. (1995). Adrenoleukodystrophy. *Curr Opin Neurol*, *8*, 221–226.
174. Laureti, S., Casucci, G., Santeusanio, F., et al. (1996). X-linked adrenoleukodystrophy is a frequent cause of idiopathic Addison's disease in young adult male patients. *J Clin Endocrinol Metab*, *81*, 470–474.
175. Sadeghi-Nejad, A., & Senior, B. (1990). Adrenomyeloneuropathy presenting as Addison's disease in childhood. *N Engl J Med*, *322*, 13–16.
176. Peters, C., Charnas, L. R., Tan, Y., et al. (2004). Cerebral X-linked adrenoleukodystrophy: the international hematopoietic cell transplantation experience from 1982 to 1999. *Blood*, *104*, 881–888.
177. Engelen, M., Ofman, R., Dijkgraaf, M. G., et al. (2010). Lovastatin in X-linked adrenoleukodystrophy. *N Engl J Med*, *362*, 276–277.
178. Steinberg, S., Chen, L., Wei, L., et al. (2004). The PEX Gene Screen: molecular diagnosis of peroxisome biogenesis disorders in the Zellweger syndrome spectrum. *Mol Genet Metab*, *83*, 252–263.
179. Wanders, R. J. A., & Waterham, H. R. (2005). Peroxisomal disorders I: biochemistry and genetics of peroxisome biogenesis disorders. *Clin Genet*, *67*, 107–133.
180. Mandel, H., & Korman, S. H. (2003). Phenotypic variability (heterogeneity) of peroxisomal disorders. *Adv Exp Med Biol*, *544*, 9–30.
181. Klima, H., Ullrich, K., Aslanidis, C., et al. (1993). A splice junction mutation causes deletion of a 72-base exon from the mRNA for lysosomal acid lipase in a patient with cholesteryl ester storage disease. *J Clin Invest*, *92*, 2713–2718.
182. Anderson, R. A., Byrum, R. S., Coates, P. M., & Sando, G. N. (1994). Mutations at the lysosomal acid cholesteryl ester hydrolase gene locus in Wolman disease. *Proc Natl Acad Sci U S A*, *91*, 2718–2722.
183. Pagani, F., Pariyarath, R., Garcia, R., et al. (1998). New lysosomal acid lipase gene mutants explain the phenotype of Wolman disease and cholesteryl ester storage disease. *J Lipid Res*, *39*, 1382–1388.
184. Lohse, P., Maas, S., Sewell, A. C., et al. (1999). Molecular defects underlying Wolman disease appear to be more heterogeneous than those resulting in cholesteryl ester storage disease. *J Lipid Res*, *40*, 221–228.
185. Zschenker, O., Jung, N., Rethmeier, J., et al. (2001). Characterization of lysosomal acid lipase mutations in the signal peptide and mature polypeptide region causing Wolman disease. *J Lipid Res*, *42*, 1033–1040.
186. Pisciotta, L., Fresa, R., Bellocchio, A., et al. (2009). Cholesteryl ester storage disease (CESD) due to novel mutations in the LIPA gene. *Mol Genet Metab*, *97*, 143–148.
187. Gramatges, M. M., Dvorak, C. C., Regula, D. P., et al. (2009). Pathological evidence of Wolman's disease following hematopoietic stem cell transplantation despite correction of lysosomal acid lipase activity. *Bone Marrow Transplant*, *44*, 449–450.
188. Tolar, J., Petryk, A., Khan, K., et al. (2009). Long-term metabolic, endocrine, and neuropsychological outcome of hematopoietic cell transplantation for Wolman disease. *Bone Marrow Transplant*, *43*, 21–27.
189. Correa-Cerro, L. S., & Porter, F. D. (2005). 3β-hydroxysterol Δ7-reductase and the Smith-Lemli-Opitz syndrome. *Mol Genet Metab*, *84*, 112–126.
190. DeBarber, A. E., Eroglu, Y., Merkens, L. S., et al. (2012). Smith-Lemli-Opitz syndrome. *Expert Rev Mol Med*, *13*, e24.
191. Andersson, H. C., Frentz, J., Martinez, J. E., et al. (1999). Adrenal insufficiency in Smith-Lemli-Opitz syndrome. *Am J Med Genet*, *82*, 382–384.
192. Nicolino, M., Ferlin, T., Forest, M., et al. (1997). Identification of a large-scale mitochondrial deoxyribonucleic acid deletion in endocrinopathies and deafness: report of two unrelated cases with diabetes mellitus and adrenal insufficiency, respectively. *J Clin Endocrinol Metab*, *82*, 3063–3067.
193. DeVile, C. J., Grant, D. B., Hayward, R. D., & Stanhope, R. (1996). Growth and endocrine sequelae of craniopharyngioma. *Arch Dis Child*, *75*, 108–114.
194. Karavitaki, N., Brufani, C., Warner, J. T., et al. (2005). Craniopharyngiomas in children and adults: systematic analysis of 121 cases with long-term follow-up. *Clin Endocrinol* (Oxf), *62*, 397–409.
195. Rose, S. R., Danish, R. K., Kearney, N. S., et al. (2005). ACTH deficiency in childhood cancer survivors. *Pediatr Blood Cancer*, *45*, 808–813.
196. Mendonca, B. B., Osorio, M. G., Latronico, A. C., et al. (1999). Longitudinal hormonal and pituitary imaging changes in two females with combined pituitary hormone deficiency due to deletion of A301,G302 in the PROP1 gene. *J Clin Endocrinol Metab*, *84*, 942–945.
197. Pernasetti, F., Toledo, S. P., Vasilyev, V. V., et al. (2000). Impaired adrenocorticotropin-adrenal axis in combined pituitary hormone deficiency caused by a two-base pair deletion (301-302delAG) in the prophet of Pit-1 gene . *J Clin Endocrinol Metab*, *85*, 390–397.
198. Lamolet, B., Pulichino, A. M., Lamonerie, T., et al. (2001). A pituitary cell-restricted T box factor, Tpit, activates POMC transcription in cooperation with Pitx homeoproteins. *Cell*, *104*, 849–859.
199. Metherell, L. A., Savage, M. O., Dattani, M., et al. (2004). TPIT mutations are associated with early-onset, but not late-onset isolated ACTH deficiency. *Eur J Endocrinol*, *151*, 463–465.
200. Pulichino, A. M., Vallette-Kasic, S., Couture, C., et al. (2003). Human and mouse TPIT gene mutations cause early onset pituitary ACTH deficiency. *Genes Dev*, *17*, 711–716.
201. Vallette-Kasic, S., Brue, T., Pulichino, A. M., et al. (2005). Congenital isolated adrenocorticotropin deficiency: an underestimated cause of neonatal death, explained by TPIT gene mutations. *J Clin Endocrinol Metab*, *90*, 1323–1331.
202. Couture, C., Saveanu, A., Barlier, A., et al. (2012). Phenotypic homogeneity and genotypic variability in a large series of congenital isolated ACTH-deficiency patients with TPIT gene mutations. *J Clin Endocrinol Metab*, *97*, E486–E495.
203. Farooqi, I. S., Jones, M. K., Evans, M., et al. (2000). Triple H syndrome: a novel autoimmune endocrinopathy characterized by dysfunction of the hippocampus, hair follicle, and hypothalamic-pituitary adrenal axis. *J Clin Endocrinol Metab*, *85*, 2644–2648.
204. Samuels, M. E., Gallo-Payet, N., Pinard, S., et al. (2012). Isolated glucocorticoid deficiency caused by immunoreactive but biologically inactive ACTH. *Horm Res Paed*, *78*(Suppl. 1), Abst PL 4–5.
205. Krude, H., Biebermann, H., Luck, W., et al. (1998). Severe early-onset obesity, adrenal insufficiency and red hair pigmentation caused by POMC mutations in humans. *Nat Genet*, *19*, 155–157.
206. Krude, H., Biebermann, H., Schnabel, D., et al. (2003). Obesity due to proopiomelanocortin deficiency: three new cases and treatment trials with thyroid hormone and ACTH4-10. *J Clin Endocrinol Metab*, *88*, 4633–4640.
207. Jackson, R. S., Creemers, J. W., Ohagi, S., et al. (1997). Obesity and impaired prohormone processing associated with mutations in the human prohormone convertase 1 gene. *Nat Genet*, *16*, 303–306.
208. Jackson, R. S., Creemers, J. W., Farooqi, I. S., et al. (2003). Small-intestinal dysfunction accompanies the complex endocrinopathy of human proprotein convertase 1 deficiency. *J Clin Invest*, *112*, 1550–1560.
209. Kannisto, S., Korppi, M., Remes, K., & Voutilainen, R. (2000). Adrenal suppression, evaluated by a low dose adrenocorticotropin test, and growth in asthmatic children treated with inhaled steroids. *J Clin Endocrinol Metab*, *85*, 652–657.
210. Todd, G. R., Acerini, C. L., Ross-Russell, R., et al. (2002). Survey of adrenal crisis associated with inhaled corticosteroids in the United Kingdom. *Arch Dis Child*, *87*, 457–461.
211. Paton, J., Jardine, E., McNeill, E., et al. (2006). Adrenal responses to low dose synthetic ACTH (Synacthen) in children receiving high dose inhaled fluticasone. *Arch Dis Child*, *91*, 808–813.

212. Carpenter, P. C. (1988). Diagnostic evaluation of Cushing's syndrome. *Endocrinol Metab Clin North Am*, *17*, 445–472.
213. McArthur, R. G., Cloutier, M. D., Hayles, A. B., & Sprague, R. G. (1972). Cushing's disease in children: findings in 13 cases. *Mayo Clin Proc*, *47*, 318–326.
214. Storr, H. L., Isidori, A. M., Monson, J. P., et al. (2004). Prepubertal Cushing's disease is more common in males, but there is no increase in severity at diagnosis. J Clin Endocrinol Metab, *89*, 3818–3820.
215. Miller, W. L., Townsend, J. J., Grumbach, M. M., & Kaplan, S. L. (1979). An infant with Cushing's disease due to an adrenocorticotropin-producing pituitary adenoma. *J Clin Endocrinol Metab*, *48*, 1017–1025.
216. Devoe, D. J., Miller, W. L., Conte, F. A., et al. (1997). Long-term outcome in children and adolescents after transsphenoidal surgery for Cushing's disease. *J Clin Endocrinol Metab*, *82*, 3196–3202.
217. Joshi, S. M., Hewitt, R. J., Storr, H. L., et al. (2005). Cushing's disease in children and adolescents: 20 years of experience in a single neurosurgical center. *Neurosurgery*, *57*, 281–285.
218. Kanter, A. S., Diallo, A. O., Jane, J. A., Jr., et al. (2005). Single-center experience with pediatric Cushing's disease. *J Neurosurg*, *103*, 413–420.
219. Tyrrell, J. B., Brooks, R. M., Fitzgerald, P. A., et al. (1978). Cushing's disease: selective trans-sphenoidal resection of pituitary microadenomas. *N Engl J Med*, *298*, 753–758.
220. Hermus, A. R., Smals, A. G., Swinkels, L. M., et al. (1995). Bone mineral density and bone turnover before and after surgical cure of Cushing's syndrome. *J Clin Endocrinol Metab*, *80*, 2859–2865.
221. Leong, G. M., Abad, V., Charmandari, E., et al. (2007). Effects of child- and adolescent-onset endogenous Cushing syndrome on bone mass, body composition, and growth: a 7-year prospective study into young adulthood. *J Bone Miner Res*, *22*, 110–118.
222. Boggan, J. E., Tyrrell, J. B., & Wilson, C. B. (1983). Transsphenoidal microsurgical management of Cushing's disease: report of 100 cases. *J Neurosurg*, *59*, 195–200.
223. Styne, D. M., Grumbach, M. M., Kaplan, S. L., et al. (1984). Treatment of Cushing's disease in childhood and adolescence by transsphenoidal microadenomectomy. *N Engl J Med*, *310*, 889–893.
224. Magiakou, M. A., Mastorakos, G., Oldfield, E. H., et al. (1994). Cushing's syndrome in children and adolescents: presentation, diagnosis, and therapy. *N Engl J Med*, *331*, 629–636.
225. Leinung, M. C., Kane, L. A., Scheithauer, B. W., et al. (1995). Long term follow-up of transsphenoidal surgery for the treatment of Cushing's disease in childhood. *J Clin Endocrinol Metab*, *80*, 2475–2479.
226. Storr, H. L., Afshar, F., Matson, M., et al. (2005). Factors influencing cure by transsphenoidal selective adenomectomy in paediatric Cushing's disease. *Eur J Endocrinol*, *152*, 825–833.
227. Massoud, A. F., Powell, M., Williams, R. A., et al. (1997). Transsphenoidal surgery for pituitary tumours. *Arch Dis Child*, *76*, 398–404.
228. Magiakou, M. A., Mastorakos, G., & Chrousos, G. P. (1994). Final stature in patients with endogenous Cushing's syndrome. *J Clin Endocrinol Metab*, *79*, 1082–1085.
229. Lebrethon, M. C., Grossman, A. B., Afshar, F., et al. (2000). Linear growth and final height after treatment for Cushing's disease in childhood. *J Clin Endocrinol Metab*, *85*, 3262–3265.
230. Davies, J. H., Storr, H. L., Davies, K., et al. (2005). Final adult height and body mass index after cure of paediatric Cushing's disease. *Clin Endocrinol* (Oxf), *62*, 466–472.
231. Storr, H. L., Plowman, P. N., Carroll, P. V., et al. (2003). Clinical and endocrine responses to pituitary radiotherapy in pediatric Cushing's disease: an effective second-line treatment. *J Clin Endocrinol Metab*, *88*, 34–37.
232. Nieman, L. K. (2002). Medical therapy of Cushing's disease. *Pituitary*, *5*, 77–82.
233. Schulte, H. M., Benker, G., Reinwein, D., et al. (1990). Infusion of low dose etomidate: correction of hypercortisolemia in patients with Cushing's syndrome and dose-response relationship in normal subjects. *J Clin Endocrinol Metab*, *70*, 1426–1430.
234. Greening, J. E., Brain, C. E., Perry, L. A., et al. (2005). Efficient short-term control of hypercortisolaemia by low-dose etomidate in severe paediatric Cushing's disease. *Horm Res*, *64*, 140–143.
235. Neary, N. M., Lopez-Chavez, A., Abel, B. S., et al. (2012). Neuroendocrine ACTH-producing tumor of the thymus: experience with 12 patients over 25 years. *J Clin Endocrinol Metab*, *97*, 2223–2230.
236. Perry, R. R., Nieman, L. K., Cutler, G. B., Jr., et al. (1989). Primary adrenal causes of Cushing's syndrome: diagnosis and surgical management. *Ann Surg*, *210*, 59–68.
237. Michalkiewicz, E., Sandrini, R., Figueiredo, B., et al. (2004). Clinical and outcome characteristics of children with adrenocortical tumors: a report from the International Pediatric Adrenocortical Tumor Registry. *J Clin Oncol*, *22*, 838–845.
238. Ribeiro, R. C., Sandrini, F., Figueiredo, B., et al. (2001). An inherited p53 mutation that contributes in a tissue-specific manner to pediatric adrenal cortical carcinoma. *Proc Natl Acad Sci U S A*, *98*, 9330–9335.
239. Wolthers, O. D., Cameron, F. J., Scheimberg, I., et al. (1999). Androgen secreting adrenocortical tumours. *Arch Dis Child*, *80*, 46–50.
240. Wieneke, J. A., Thompson, L. D., & Heffess, C. S. (2003). Adrenal cortical neoplasms in the pediatric population: a clinicopathologic and immunophenotypic analysis of 83 patients. *Am J Surg Pathol*, *27*, 867–881.
241. Ribeiro, R. C., Pinto, E. M., Zambetti, G. P., & Rodriguez-Galindo, C. (2012). The International Pediatric Adrenocortical Tumor Registry initiative: contributions to clinical, biological, and treatment advances in pediatric adrenocortical tumors. *Mol Cell Endocrinol*, *351*, 37–43.
242. Young, W. F., Jr., Carney, J. A., Musa, B. U., et al. (1989). Familial Cushing's syndrome due to primary pigmented nodular adrenocortical disease. Reinvestigation 50 years later. *N Engl J Med*, *321*, 1659–1664.
243. Stratakis, C. A. (2008). Cushing syndrome caused by adrenocortical tumors and hyperplasias (corticotropin-independent Cushing syndrome). *Endocr Dev*, *13*, 117–132.
244. Rothenbuhler, A., & Stratakis, C. A. (2010). Clinical and molecular genetics of Carney complex. *Best Pract Res Clin Endocrinol Metab*, *24*, 389–399.
245. Storr, H. L., Mitchell, H., Swords, F. M., et al. (2004). Clinical features, diagnosis, treatment and molecular studies in paediatric Cushing's syndrome due to primary nodular adrenocortical hyperplasia. *Clin Endocrinol* (Oxf), *61*, 553–559.
246. Kirschner, L. S., Carney, J. A., Pack, S. D., et al. (2000). Mutations of the gene encoding the protein kinase A type 1-(regulatory subunit in patients with the Carney complex. *Nat Genet*, *26*, 89–92.
247. Kirschner, L. S., Sandrini, F., Monbo, J., et al. (2000). Genetic heterogeneity and spectrum of mutations of the PRKAR1A gene in patients with the Carney complex. *Hum Mol Genet*, *9*, 3037–3046.
248. Bossis, I., & Stratakis, C. A. (2004). PRKAR1A: normal and abnormal functions. *Endocrinology*, *145*, 5452–5458.
249. Bertherat, J., Horvath, A., Groussin, L., et al. (2009). Mutations in regulatory subunit type 1A of cyclic adenosine 5'-monophosphate-dependent protein kinase (PRKAR1A): phenotype analysis in 353 patients and 80 different genotypes. *J Clin Endocrinol Metab*, *94*, 2085–2091.
250. Almeida, M. Q., & Stratakis, C. A. (2010). Carney complex and other conditions associated with micronodular adrenal hyperplasias. *Best Pract Res Clin Endocrinol Metab*, *24*, 907–914.
251. Bourdeau, I., Matyakhina, L., Stergiopoulos, S. G., et al. (2006). 17q22-24 chromosomal losses and alterations of protein kinase a subunit expression and activity in adrenocorticotropin-independent macronodular adrenal hyperplasia. *J Clin Endocrinol Metab*, *91*, 3626–3632.
252. Horvath, A., Boikos, S., Giatzakis, C., et al. (2006). A genome-wide scan identifies mutations in the gene encoding phosphodiesterase 11A4 (PDE11A) in individuals with adrenocortical hyperplasia. *Nat Genet*, *38*, 794–800.
253. Kirk, J. M., Brain, C. E., Carson, D. J., et al. (1999). Cushing's syndrome caused by nodular adrenal hyperplasia in children with McCune-Albright syndrome. *J Pediatr*, *134*, 789–792.
254. Newell-Price, J., Trainer, P., Besser, M., & Grossman, A. (1998). The diagnosis and differential diagnosis of Cushing's syndrome and pseudo-Cushing's states. *Endocr Rev*, *19*, 647–672.
255. Stratakis, C. A., Kirschner, L. S., & Carney, J. A. (2001). Clinical and molecular features of the Carney complex: diagnostic criteria and recommendations for patient evaluation. *J Clin Endocrinol Metab*, *86*, 4041–4046.

256. Stratakis, C. A., Sarlis, N., Kirschner, L. S., et al. (1999). Paradoxical response to dexamethasone in the diagnosis of primary pigmented nodular adrenocortical disease. *Ann Intern Med*, *131*, 585–591.
257. Lienhardt, A., Grossman, A. B., Dacie, J. E., et al. (2001). Relative contributions of inferior petrosal sinus sampling and pituitary imaging in the investigation of children and adolescents with ACTH-dependent Cushing's syndrome. *J Clin Endocrinol Metab*, *86*, 5711–5714.
258. Ilias, I., Chang, R., Pacak, K., et al. (2004). Jugular venous sampling: an alternative to petrosal sinus sampling for the diagnostic evaluation of adrenocorticotropic hormone-dependent Cushing's syndrome. *J Clin Endocrinol Metab*, *89*, 3795–3800.
259. Choi, M., Scholl, U. I., Yue, P. et al. (2011). K+ channel mutations in adrenal aldosterone-producing adenomas and hereditary hypertension. *Science*, *331*, 768–772.
260. Charmandari, E., Sertedaki, A., Kino, T., et al. (2012). A novel point mutation in the KCNJ5 gene causing primary hyperaldosteronism and early-onset autosomal dominant hypertension. *J Clin Endocrinol Metab*, *97*, E1532–E1539.
261. Mulatero, P., Tauber, P., Zennaro, M. C., et al. (2012). KCNJ5 mutations in European families with nonglucocorticoid remediable familial hyperaldosteronism. *Hypertension*, *59*, 235–240.
262. Scholl, U. I., Nelson-Williams, C., Yue, P., et al. (2012). Hypertension with or without adrenal hyperplasia due to different inherited mutations in the potassium channel KCNJ5. *Proc Natl Acad Sci U S A*, *109*, 2533–2538.
263. Boulkroun, S, Beuschlein, F., Rossi, G. P., et al. (2012). Prevalence, clinical, and molecular correlates of KCNJ5 mutations in primary aldosteronism. Hypertension, 59, 592–598.
264. Charmandari, E., Kino, T., & Chrousos, G. P. (2004). Familial/sporadic glucocorticoid resistance: clinical phenotype and molecular mechanisms. *Ann N Y Acad Sci*, *1024*, 168–181.
265. van Rossum, E. F. C., & van den Akker, E. L. T. (2011). Glucocorticoid resistance. *Endocr Dev*, *20*, 127–136.
266. Hurley, D. M., Accili, D., Stratakis, C. A., et al. (1991). Point mutation causing a single amino acid substitution in the hormone binding domain of the glucocorticoid receptor in familial glucocorticoid resistance. *J Clin Invest*, *87*, 680–686.
267. Karl, M., Lamberts, S. W., Detera-Wadleigh, S. D., et al. (1993). Familial glucocorticoid resistance caused by a splice site deletion in the human glucocorticoid receptor gene. J Clin Endocrinol Metab, *76*, 683–689.
268. Kino, T., Stauber, R. H., Resau, J. H., et al. (2001). Pathologic human GR mutant has a transdominant negative effect on the wild-type GR by inhibiting its translocation into the nucleus: importance of the ligand-binding domain for intracellular GR trafficking. *J Clin Endocrinol Metab*, *86*, 5600–5608.
269. Zennaro, M. C., Hubert, E. L., & Fernandes-Rosa, F. L. (2012). Aldosterone resistance: structural and functional considerations and new perspectives. *Mol Cell Endocrinol*, *350*, 206–215.
270. Chang, S. S., Grunder, S., Hanukoglu, A., et al. (1996). Mutations in subunits of the epithelial sodium channel cause salt wasting with hyperkalaemic acidosis, pseudohypoaldosteronism type 1. *Nat Genet*, *12*, 248–253.
271. Kerem, E., Bistritzer, T., Hanukoglu, A., et al. (1999). Pulmonary epithelial sodium-channel dysfunction and excess airway liquid in pseudohypoaldosteronism. *N Engl J Med*, *341*, 156–162.
272. Geller, D. S., Rodriguez-Soriano, J., Vallo Boado, A., et al. (1998). Mutations in the mineralocorticoid receptor gene cause autosomal dominant pseudohypoaldosteronism type I. *Nat Genet*, *19*, 279–281.
273. Sartorato, P., Lapeyraque, A. L., Armanini, D., et al. (2003). Different inactivating mutations of the mineralocorticoid receptor in fourteen families affected by type I pseudohypoaldosteronism. *J Clin Endocrinol Metab*, *88*, 2508–2517.
274. Geller, D. S., Farhi, A., Pinkerton, N., et al. (2000). Activating mineralocorticoid receptor mutation in hypertension exacerbated by pregnancy. *Science*, *289*, 119–123.
275. Chandar, J., Abitbol, C., Zilleruelo, G., et al. (1996). Renal tubular abnormalities in infants with hydronephrosis. *J Urol*, *155*, 660–663.
276. Oakley, R. H., & Cidlowski, J. A. (2011). Cellular processing of the glucocorticoid receptor gene and protein: new mechanisms for generating tissue-specific actions of glucocorticoids. *J Biol Chem*, *286*, 3177–3184.
277. Mellon, S. H., & Griffin, L. D. (2002). Neurosteroids: biochemistry and clinical significance. *Trends Endocrinol Metab*, *13*, 35–43.
278. Grossmann, C., Scholz, T., Rochel, M., et al. (2004). Transactivation via the human glucocorticoid and mineralocorticoid receptor by therapeutically used steroids in CV-1 cells: a comparison of their glucocorticoid and mineralocorticoid properties. *Eur J Endocrinol*, *151*, 397–406.
279. Streck, W. F., & Lockwood, D. H. (1979). Pituitary adrenal recovery following short-term suppression with corticosteroids. *Am J Med*, *66*, 910–914.

QUESTIONS

1. A newborn 46,XX baby was born after a normal term pregnancy and left the nursery with the mother at 2 days of life after having been checked and declared healthy by a pediatrician. The newborn screening reveals a 17OH progesterone of 1986 ng/dl (60 nmol/L). On recall at 2 weeks of age, the screening test reveals a similar value. The child is now referred to a pediatric endocrine center. What could be the cause of this elevated 17OH progesterone?
   a. Defect in HSD3B2
   b. Defect in P450 oxidoreductase
   c. Defect in CYP11B1
   d. Defect in either b or c
   e. Defect in a, b, or c

Answer: e

2. An 8-year-old boy is referred for precocious puberty and overgrowth. At 5 years of age he had two small soft tissue tumors of the skin removed by the dermatologist and has a remarkable amount of freckles characterizing his face. Otherwise he is a healthy, smart second grader. Physical exam reveals a height of +2 SD and weight of +2 SD; pubertal stage Tanner 3-4 with pubic hair 3-4, penis length 7 cm (90th percentile), and testes R 4 ml, L 6 ml with palpable tumor (confirmed by ultrasound). Bone age is 12 years. What is the likely diagnosis?
   a. Central precocious puberty
   b. Late-onset CAH with TART
   c. Leydig cell tumor
   d. Carney complex
   e. Leopard syndrome

Answer: d

3. The following genes have been associated with familial glucocorticoid deficiency (FGD), also termed *hereditary unresponsiveness to ACTH*, *except* which one?
   a. MRAP
   b. NNT
   c. MC2R
   d. PRKAR1A
   e. StAR

Answer: d

4. A 9-year-old obese girl is referred for possible Cushing syndrome. Which of the following clinical findings is strongest to support this hypothesis and prompt further workup?
   a. Early morning serum cortisol 23.6 ug/dl (= 650 nmol/L)
   b. Exaggerated weight gain in the past 2 to 3 years
   c. Growth arrest
   d. Visceral adiposity
   e. Acne and pubic hair Tanner 3

Answer: c

5. Which of the following genetic defects of steroid biosynthesis may cause both 46,XY DSD and 46,XX DSD at birth?
   a. HSD3B2
   b. POR
   c. HSD17B3
   d. Both b and c
   e. Both a and b

Answer: e

6. The following statements are true for the alternative steroid "backdoor" pathway to dihydrotestosterone, *except* which one?
   a. Was first described in the tammar wallaby
   b. Contributes to androgen production in 21-hydroxylase deficiency
   c. Genetic defects in this pathway cause 46,XY DSD and adrenal insufficiency
   d. Shares the involvement of the CYP17 gene/enzyme with the classic pathway for its functionality
   e. Is involved in the virilization of a 46,XX fetus with P450 oxidoreductase deficiency during pregnancy

Answer: c

CHAPTER 14

Pheochromocytoma and Multiple Endocrine Neoplasia Syndromes

Steven G. Waguespack, MD • Anita K. Ying, MD

CHAPTER OUTLINE

INTRODUCTION

Endocrine neoplasms comprise a variety of benign and malignant tumors that arise from the endocrine glands or neuroendocrine tissues. Although most childhood endocrine neoplasms are sporadic, without an identifiable germline mutation, others are hereditary and are secondary to mutations in one of many known tumor-predisposing genes. Catecholamine-producing tumors and medullary thyroid carcinoma (MTC) are major examples of tumors that, when diagnosed during childhood, typically occur within the context of a broader tumor-predisposition syndrome such as von Hippel-Lindau (VHL) disease and the multiple endocrine neoplasia (MEN) type 2 syndromes, respectively. Advances in genetic testing and research have led to the discovery of new hereditary endocrine neoplasia syndromes and tumor-predisposing genes as well as a better understanding of underlying pathophysiology in these disorders. Knowledge regarding genotype-phenotype relationships has evolved, as has clinical practice regarding the age of genetic testing, presymptomatic screening for endocrine tumors, and the timing of therapeutic intervention. Given the rapidly changing field, it has become imperative for pediatric patients with an endocrine tumor to be evaluated in programs with known multidisciplinary expertise for such problems. In addition, formal genetic counseling and results of genetic testing should be fully incorporated into treatment planning and long-term follow-up. This chapter reviews the pathophysiology, diagnosis, and management of pediatric neuroendocrine tumors and the most common genetic syndromes associated with their diagnosis.

GENETIC COUNSELING AND TESTING

A diagnosis of an endocrine tumor in a child should always raise concern for an underlying hereditary condition, which can subsequently have medical, reproductive, psychological, or social consequences for the patient and family. Genetic counseling is a process of communication that promotes understanding, decision making, and coping related to the impact of genetic disease.[1] It should be incorporated into all stages of care, both at diagnosis and

during long-term follow-up, because patients' counseling and information needs change over time and also because genetic testing and management recommendations are likely to evolve. Genetic testing, best exemplified in MEN2,[2] is a multistep process that begins with an affected patient. MEN2 is one of few hereditary cancer syndromes for which predictive genetic testing is clearly indicated during childhood because an intervention (i.e., early thyroidectomy) can prevent future morbidity and possible mortality due to incurable metastatic MTC. In other disorders, such as MEN1 and VHL, genetic testing and early presymptomatic screening of an asymptomatic child may lead to an earlier diagnosis of disease but not disease that can be prevented by a prophylactic intervention. In all cases, despite the anticipated medical benefits afforded from an early diagnosis, genetic testing in children also has the potential for psychosocial harm: alteration of the child's self-image and of the parents' perception of the child, modification of the patient's outlook on life, worry about the potential for genetic discrimination, early "medicalization" of an otherwise healthy child, changes in family relationships, and concerns regarding future reproductive issues. Online resources for genetic counseling and testing include the National Society of Genetic Counselors (www.nsgc.org), the National Cancer Institute "Cancer Genetics" website (www.cancer.gov/cancertopics/genetics), and "Gene Tests" (www.genetests.org), a publicly funded project that provides current and authoritative information on genetic disease and testing.

PHEOCHROMOCYTOMA AND PARAGANGLIOMA

Pheochromocytomas (PHEO) and paragangliomas (PGL) are uncommon neuroendocrine tumors that arise from neural crest–derived cells. PHEO (Figure 14-1) is the term used for a catecholamine-producing paraganglioma that occurs in the adrenal medulla, whereas PGL (Figure 14-2) refers to extra-adrenal tumors that arise from both sympathetic and parasympathetic paraganglia located outside the cerebrospinal axis.[3-5] The term *PHEO* is often used interchangeably with PGL, but it is best to maintain a distinction between these two neoplasms due to underlying differences in genetics, clinical presentation, and malignant potential (Table 14-1).

PHEO/PGL represent < 7% of tumors that arise from the sympathetic nervous system and have an estimated incidence of 0.3 cases/million/year or less.[6,7] Up to

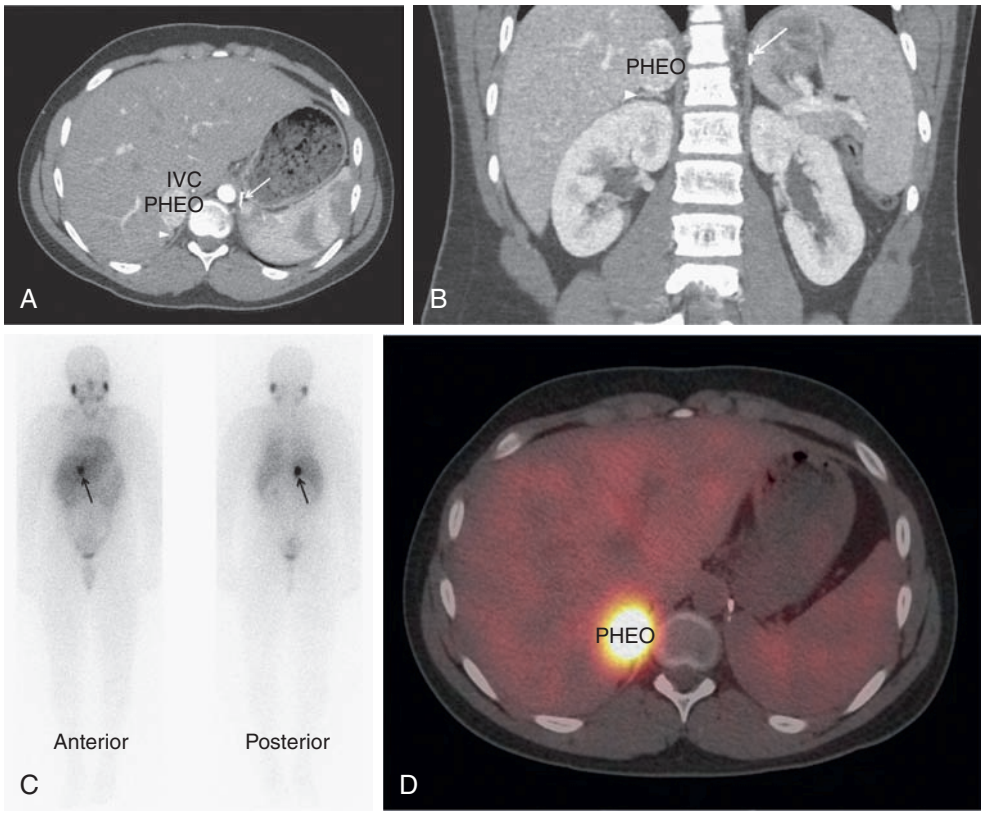


FIGURE 14-1 ■ Pheochromocytoma. A normotensive 14-year-old male with von Hippel-Lindau disease and a history of a left pheochromocytoma and abdominal paraganglioma diagnosed at the age of 7 was found to have elevated norepinephrine and normetanephrine levels after screening with a 24-hour urine collection. Axial CT postcontrast (**A**) and coronal reconstruction (**B**) identified a vascular neoplasm (PHEO) arising from the superior right adrenal gland. IVC, inferior vena cava; arrowhead, normal adrenal gland; arrow, surgical clips from previous adrenalectomy. MIBG scan confirmed the functional nature of the tumor and ruled out other sites of disease. Planar (**C**) and fused SPECT/CT axial images (**D**) in the same patient, 24 hours after the administration of ^{123}I MIBG. *(This image can be viewed in full color online at ExpertConsult.)*

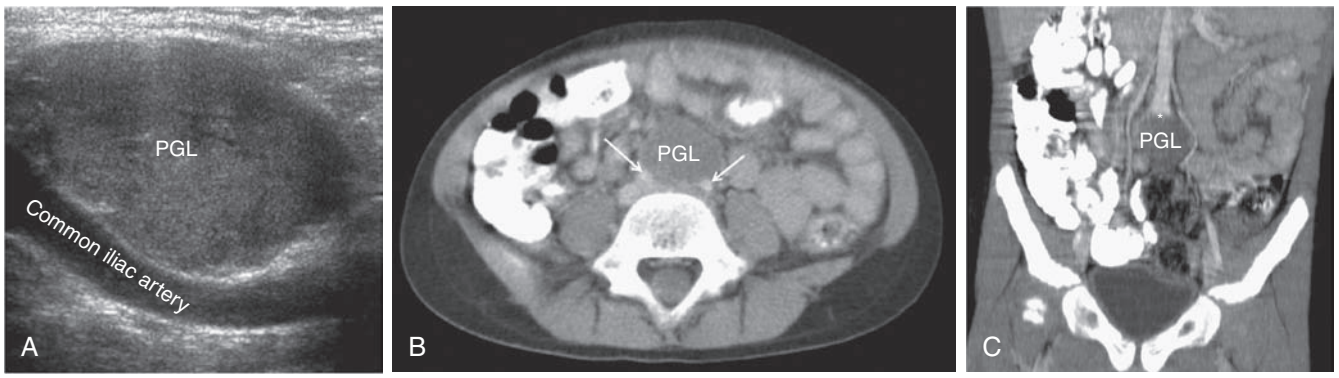


FIGURE 14-2 ■ Paraganglioma. A 6-year-old female with a malignant paraganglioma (PGL) and an *SDHB* mutation presented with severe hypertension during a well-child examination. **A,** Abdominal ultrasound (sagittal view) revealed a homogeneous, hypervascular 3.3 cm mass at the level of the aortic bifurcation. **B,** Axial CT postcontrast confirmed a low attenuation tumor (arrows refer to common iliac arteries); **C,** Coronal reconstruction detailed the location of the neoplasm at the aortic bifurcation (∗) in the organ of Zuckerkandl.

TABLE 14-1 **Major Disorders and Genes Associated with Pheochromocytoma and Paraganglioma**

	Gene (Chromosome)	Type of Tumor	Clinical Phenotype[a]	Earliest Age of DX[b]	Screening Guidelines[c]
Multiple endocrine neoplasia type 2A (MEN2A)	*RET* (10q11.2)	PHEO/rare PGL	Adrenergic Malignancy very rare Background of adrenal medullary hyperplasia	5-8[d141,153] 12[e168,455]	Start by age 8 years in codon 634 mutations; by age 20 years in lower risk codons
Multiple endocrine neoplasia type 2B (MEN2B)	*RET* (10q11.2)	PHEO	Adrenergic Malignancy rare Background of adrenal medullary hyperplasia	12[350]	Start by age 8 years
Neurofibromatosis type 1 (NF1)	*NF1* (17q11.2)	PHEO/PGL Rare composite PHEO[f]	Adrenergic Malignancy uncommon PHEO usually in adults (mean age 42[427])	7[456]	Screen any NF1 patient with HTN or other signs/symptoms of catecholamine excess or an incidental adrenal or para-aortic mass Consider testing in young women of child-bearing age who plan to become pregnant and in any patient prior to an elective surgical procedure
Familial paraganglioma syndrome 1 (PGL1)	SDHD (11q23)	PGL/PHEO	Primarily nonfunctional malignancy rare	5[9]	Start by age 10 years
Familial paraganglioma syndrome 3 (PGL3)	*SDHC* (1q21)	Primarily head and neck PGL	Primarily nonfunctional malignancy rare	13[457]	Start by age 10 years
Familial paraganglioma syndrome 4 (PGL4)	*SDHB* (1p36.1-p35)	PGL (primarily abdominal)/PHEO	Noradrenergic Malignancy common	3[458]	Start by age 5 years
von Hippel-Lindau disease (VHL)	*VHL* (3p25.3)	PHEO/PGL	Noradrenergic Malignancy uncommon	2[393]	Start by age 5 years

[a]**Noradrenergic** tumors almost exclusively secrete norepinephrine and normetanephrine whereas **adrenergic** tumors secrete epinephrine and metanephrine, in addition to norepinephrine and normetanephrine.
[b]Earliest age of diagnosis of PHEO/PGL and reference(s).
[c]In the opinion of the authors and based on review of the literature, age at which annual screening for PHEO/PGL should be initiated for patients with a known gene mutation.
[d]Ages of earliest PHEO onset reported in consensus guidelines via personal communication.
[e]Age of earliest PHEO onset published in the medical literature.
[f]A composite PHEO is a mixed tumor comprised of PHEO and neuroblastoma, ganglioneuroma, or ganglioneuroblastoma.
DX, diagnosis; HTN, hypertension; PGL, paraganglioma; PHEO; pheochromocytoma.
Adapted from Waguespack, S. G., Rich, T., Grubbs, E., et al. (2010). A current review of the etiology, diagnosis, and treatment of pediatric pheochromocytoma and paraganglioma. J Clin Endocrinol Metab, 95, *2023–2037.*

20% of PHEO/PGL are identified during childhood at an average age of 11 years; there is a slight predominance in boys, particularly when diagnosed under the age of 10.[7-15] Less than 2% of children diagnosed with hypertension will harbor a catecholamine-producing neoplasm.[16,17]

All functional PHEO/PGL produce and metabolize catecholamines and contain chromaffin tissue, which refers to the brown-black color resulting from the oxidation of catecholamines after staining with chromium salts. PGL occur in all locations where paraganglia are found (from the skull base to the pelvis) and are either functional (sympathetic) or nonfunctional (parasympathetic) neoplasms, depending on the site of origin and underlying pathophysiology[18-20] (see Table 14-1). PGL arising in the head and neck region are almost exclusively nonfunctional, whereas most intra-abdominal PGL (most commonly occurring within the organ of Zuckerkandl) (see Figure 14-2) are secretory tumors. The majority of these tumors diagnosed during childhood are PHEO, which synthesize and secrete catecholamines (dopamine, norepinephrine, or epinephrine) and their metabolites (including 3-methoxytyramine, normetanephrine, and metanephrine, respectively)[18,21,22] (Figure 14-3). Multicentric tumors are more common in childhood presentations of PHEO/PGL.[8,10,23]

Biosynthesis and Actions of Catecholamines

Dopamine, norepinephrine, and epinephrine (collectively known as "catecholamines") are chemical neurotransmitters and hormones that play important roles in the regulation of numerous physiologic processes and the development of neurologic, psychiatric, endocrine, and cardiovascular diseases.[24-27] The catecholamines are composed of a catechol (1,2-dihydroxybenzene) moiety and a side-chain amine group. They are synthesized from the amino acid tyrosine, which is converted to 3,4-dihydroxyphenylalanine (dopa) by the enzyme tyrosine hydroxylase, the rate-limiting step in catecholamine biosynthesis (see Figure 14-3). Subsequent enzymatic decarboxylation and hydroxylation of dopa yields dopamine and norepinephrine, respectively, and norepinephrine is subsequently converted to epinephrine via the cytosolic enzyme phenylethanolamine N-methyltransferase (PNMT).

The catecholamines are synthesized and stored in granules within the adrenal medulla, where they are released via exostosis into the systemic circulation in response to stressful stimuli. Dopamine and norepinephrine are also produced by postganglionic neurons in the sympathetic nervous system. Epinephrine is made only in the adrenal medulla, where it represents the predominant catecholamine (~80%) because PNMT expression is dependent on and regulated by high local concentrations of glucocorticoids (as occurs only in the adrenal medulla, surrounded by the cortisol-synthesizing cortex with a distinct concentration gradient toward the adrenal medulla).[24,28,29] The effects of catecholamines are terminated via rapid reuptake into nerve terminals by the norepinephrine transporter and via metabolism by two major enzymes: monoamine oxidase (MAO) and catechol-*O*-methyltransferase (COMT)[18,24,27] (see Figure 14-3).

The complex actions of norepinephrine and epinephrine are mediated by the G protein–coupled α- and β-adrenergic receptors, whereas dopamine binds to a different class of G protein–coupled dopamine receptors (five distinct receptors that are divided into two families: D1-like and D2-like)[24-26] (Table 14-2). The initial classification of adrenergic receptors was based on epinephrine's ability to both excite (α-receptor) and inhibit (β–receptor) smooth muscle. Specific agonists and antagonists characterize the adrenergic receptor subtype (α_1, α_2, β_1, β_2, and β_3) and can be used as therapeutic agents. The D2 receptor is the primary dopamine receptor that is targeted for drug therapy.

Clinical Presentation

The clinical presentation of pediatric PHEO/PGL is highly variable. Children with these tumors can come to attention due to symptomatic catecholamine hypersecretion, symptoms due to tumor mass effect (e.g., pain), an incidental radiographic finding, or because of screening for one of the associated hereditary tumor syndromes[7,30,31] (see Table 14-1). PHEO/PGL may also arise in the setting of cyanotic congenital heart disease.[32,33] Given their neuroendocrine origin, PHEO/PGL can very rarely co-secrete other hormones that result in a clinical syndrome of ectopic hormone excess, such as gigantism (growth hormone-releasing hormone), Cushing syndrome (corticotropin-releasing hormone or adrenocorticotropic hormone), hypercalcemia (parathyroid hormone–related peptide), the syndrome of inappropriate antidiuretic hormone secretion, or secretory diarrhea (vasoactive intestinal peptide).[18,34]

The clinical presentation of a functional PHEO/PGL depends on differences in catecholamine secretion and release as well as individual patient sensitivities to catecholamines.[35] Signs and symptoms of catecholamine excess include hypertension, which is typically sustained in the majority of pediatric cases; severe headaches; paroxysmal episodes with the classic triad of headaches, palpitations, and diaphoresis (less common in children); orthostatic hypotension and syncope; pallor; tremor; or anxiety.* PHEO/PGL in children can also cause nonspecific signs and symptoms such as blurred vision; abdominal pain, diarrhea, and other gastrointestinal symptoms; weight loss; hyperglycemia; polyuria and polydipsia; low-grade fever; and behavioral problems/decline in school performance.† Bladder PGL can present with hematuria and paroxysmal symptoms during micturition.[34,39]

Complications of catecholamine excess can include hypertensive crisis, cardiomyopathy (*takotsubo* cardiomyopathy), arrhythmias, pancreatitis, severe constipation and intestinal pseudo-obstruction, stroke, seizures, and

*See references 3, 8, 12, 14, 16, 18, 19, 30, and 36.
†See references 8, 12, 14, 16, 18, 19, and 36-38.

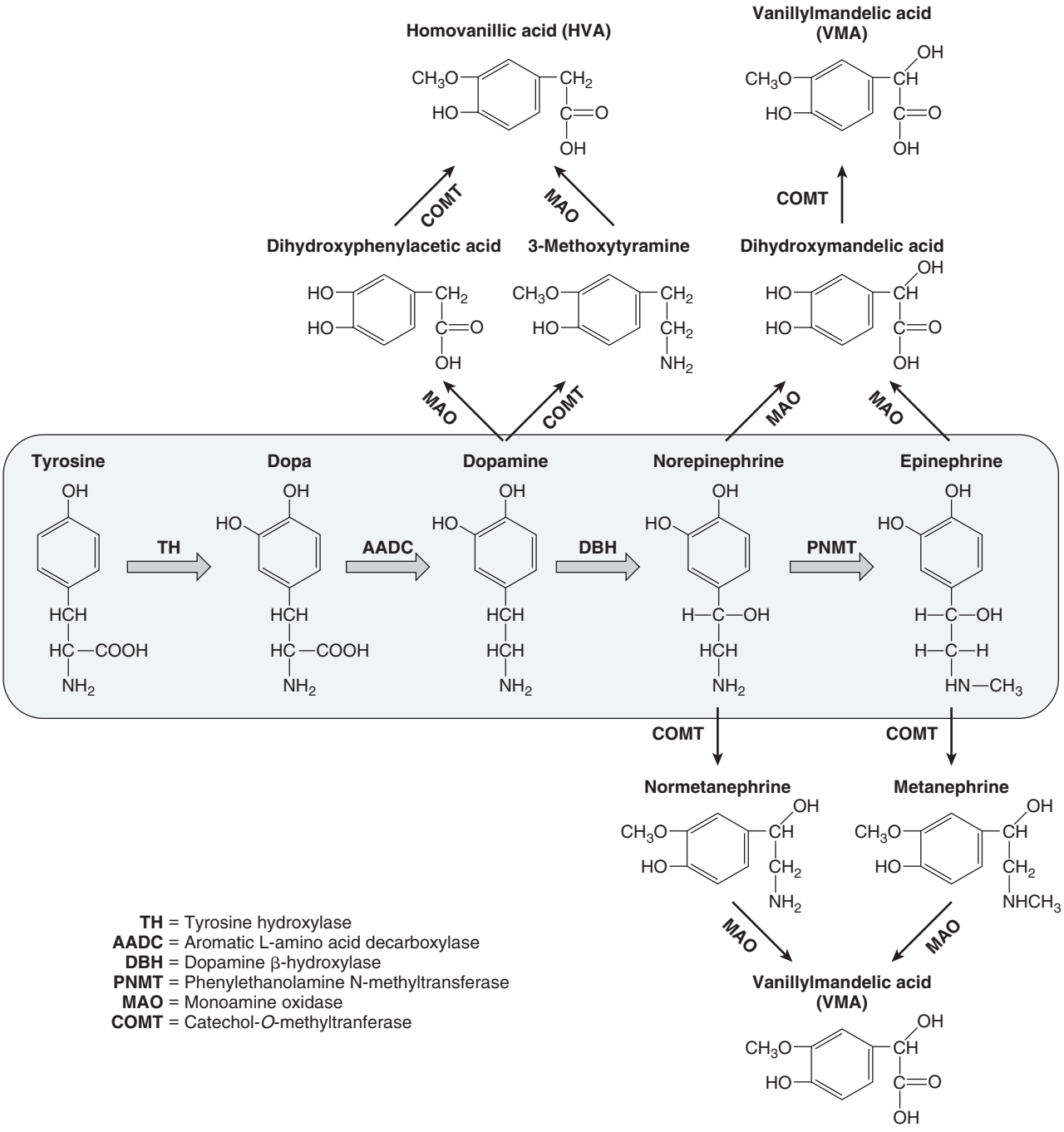


FIGURE 14-3 ■ Catecholamine synthesis and metabolism. The catecholamines are synthesized from the amino acid tyrosine, which is converted to 3,4-dihydroxyphenylalanine (Dopa) by the enzyme tyrosine hydroxylase (TH), the rate-limiting step in catecholamine biosynthesis. Subsequent enzymatic decarboxylation (aromatic L-amino acid decarboxylase; AADC) and hydroxylation (dopamine β-hydroxylase; DBH) yields dopamine and norepinephrine, respectively, and norepinephrine is subsequently converted to epinephrine via the cytosolic enzyme phenylethanolamine N-methyltransferase (PNMT). The catecholamines are metabolized by two major enzymes: monoamine oxidase (MAO) and catechol-*O*-methyltransferase (COMT).

even multisystem crisis and death.[19,34,36,38,40-42] Symptoms of parasympathetic PGL include hearing loss, pulsatile tinnitus, neck mass and other symptoms of mass effect such as voice hoarseness, pharyngeal fullness, and dysphagia.[43]

Compared with sporadic disease, PHEO/PGL identified during the course of prospective presymptomatic screening within the context of a familial disorder are smaller and less symptomatic (frequently asymptomatic) tumors.[44-46] Although this type of clinical presentation is becoming more commonplace, there is currently no consensus as to how to approach such patients with small asymptomatic tumors, particularly in clinical settings where the risk for malignancy is low.

TABLE 14-2 **Catecholamine Receptor Classification, Function, and Pharmacology**

Receptor Type	Primary Pharmacologic Agonist(s)	Primary Pharmacologic Antagonist(s)	Major Biologic Effects
α_1	Phenylephrine, midodrine	Doxazosin, prazosin, terazosin, etc.	Vasoconstriction; promotes cardiac growth and structure
α_2	Clonidine, methyldopa, tizanidine	Yohimbine	Inhibition of norepinephrine release; vasoconstriction; inhibits adrenal catecholamine release and modulates CNS dopamine neurotransmission
β_1	Dobutamine	Atenolol, bisoprolol, esmolol, metoprolol	Increases heart rate and contractility
β_2	Albuterol, levalbuterol, salmeterol, terbutaline, etc.	Propranolol (prototypic β_2 antagonist that is also a β_1 antagonist) and others	Smooth muscle relaxation (arteriolar and venous dilation; relaxation of tracheobronchial muscles)
β_3	Investigational	Investigational	Metabolic effects in adipose tissue and skeletal muscle
D1-like (D_1 and D_5)	L-DOPA	No primary agents	Vasodilation; increased renin secretion; promotes norepinephrine and epinephrine release; various CNS functions
D2-like (D_2, D_3, and D_4)	Bromocriptine, cabergoline, pramipexole, ropinirole	Aripiprazole, chlorpromazine, haloperidol, metoclopramide, prochlorperazine, etc.	Decreases prolactin secretion; decreases renin secretion; inhibits norepinephrine and epinephrine release; various CNS functions

Data from Westfall, T. C, & Westfall, D. P. (2011). Neurotransmission: the autonomic and somatic motor nervous systems. In L. L. Brunton (Ed.), *Goodman & Gilman's the pharmacological basis of therapeutics* (12th ed.) (pp. 171–218). New York: McGraw-Hill.; Westfall, T. C., & Westfall, D. P. (2011). Catecholamines and sympathomimetic drugs. In L. L. Brunton (Ed.), *Goodman & Gilman's the pharmacological basis of therapeutics* (12th ed.) (pp. 277–333). New York: McGraw-Hill.; Sanders-Bush, E., & Hazelwood, L. (2011). 5-Hydroxytryptamine (serotonin) and dopamine. In L. L. Brunton (Ed.), *Goodman & Gilman's the pharmacological basis of therapeutics* (12th ed.) (pp. 335–361). New York: McGraw-Hill.

Evaluation

Biochemical Diagnosis

The diagnosis of PHEO/PGL has been simplified by advances in the assays used to detect and quantify levels of catecholamines and their metabolites in blood and urine. The measurement of fractionated plasma or urine metanephrines (metanephrine and normetanephrine) is the most sensitive test (approaching 100% sensitivity) for the diagnosis of a sympathetic chromaffin tumor and should be the primary diagnostic test in the initial evaluation of suspected PHEO/PGL[3,18,47-54] (Figure 14-4).

The high sensitivity of metanephrine testing is based on the fact that there is continuous intratumoral metabolism of catecholamines, a process that occurs independently of catecholamine release, which can occur intermittently or at low rates.[50] An elevation of metanephrines greater than fourfold above the reference range is associated with almost 100% probability of the presence of a catecholamine-secreting tumor.[55] Any drugs known to interfere with these assays (e.g., acetaminophen, tricyclic antidepressants, phenoxybenzamine, and decongestants, among others[3]) should be discontinued prior to testing. Dietary restrictions need not be routinely employed but should be considered if the assay utilized measures only deconjugated normetanephrines or if a dopamine-secreting tumor is suspected.[56] The measurement of the catecholamine metabolites vanillylmandelic acid (VMA) and homovanillic acid (HVA) is no longer recommended for the evaluation of PHEO/PGL. However, testing for HVA and VMA in spot urine samples remains a critical component of the evaluation of neuroblastoma, where these analytes have a high sensitivity and specificity for tumor detection.[57]

In patients with mildly elevated metanephrine levels in whom a false-positive test result is suspected, consideration should be given to measuring these analytes in the supine position, 30 minutes after an indwelling needle or catheter is inserted into the vein.[3,53] Clonidine suppression and glucagon stimulation tests[58-60] have been a component of the diagnostic algorithm in adults but are rarely required and, in the case of the glucagon stimulation test, have been largely abandoned due to insufficient diagnostic sensitivity.[61] Furthermore, these tests have not been validated in the diagnosis of childhood PHEO/PGL and are not currently recommended.

Catecholamine-producing tumors can be subclassified as being either noradrenergic or adrenergic based uon their pattern of catecholamine release.[62,63] Noradrenergic PHEO/PGL secrete norepinephrine and normetanephrine, as seen in VHL disease and in tumors associated with the familial PGL syndromes.[15,62,64-66] Adrenergic tumors secrete both epinephrine and norepinephrine and their metabolites, and these tumors are more commonly PHEO that arise sporadically or within the clinical context of MEN2 or NF1.[50,62,64,66] This differential secretion of catecholamines is due to decreased expression of PNMT in noradrenergic tumors.[64]

Dopamine-secreting tumors are rare and are typically extra-adrenal *SDHx*-mediated paragangliomas.[67-69]

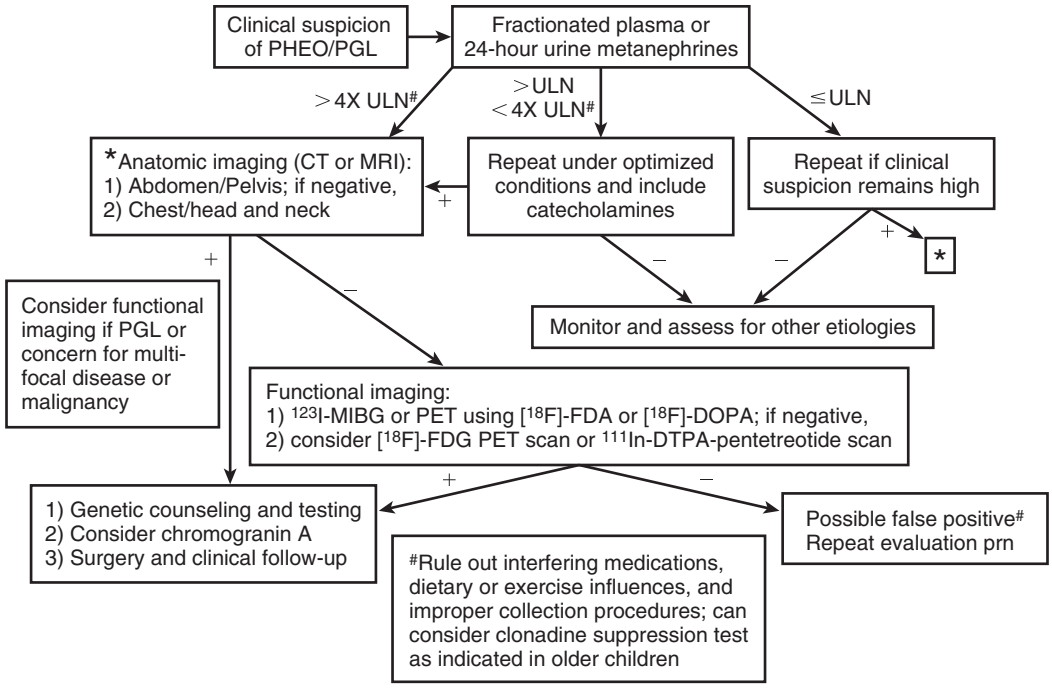


FIGURE 14-4 ■ **Diagnosis of pediatric pheochromocytoma/paraganglioma.** (Adapted from Waguespack, S. G., Rich, T., Grubbs, E., et al. (2010). A current review of the etiology, diagnosis, and treatment of pediatric pheochromocytoma and paraganglioma. *J Clin Endocrinol Metab, 95*, 2023–2037.)

The measurement of plasma methoxytyramine (see Figure 14-3) may help to identify such a tumor, particularly in the context of an *SDHx* mutation,[70] but this test is not widely available. A dopamine-secreting tumor should be considered in normotensive patients identified to have a mass that appears consistent with a PHEO/PGL, in which case dopamine and its metabolites, homovanillic acid and methoxytyramine (if available), should also be measured.[68,70-72] For prospective screening in patients with an *SDHx* mutation, total catecholamines should also be checked in addition to metanephrines due to this possibly.

Chromogranin A is a major secretory protein present in the soluble matrix of chromaffin granules that serves as an effective tumor marker that may correlate with PHEO/PGL size and malignant potential.[15,47,73-75] Chromogranin A also appears to be a useful marker in the biochemically silent, *SDHB*-related paraganglioma,[20,69] making it a potentially useful test in the screening of asymptomatic *SDHx* mutation carriers.

Radiographic Studies

Once a diagnosis of catecholamine excess is established from biochemical testing, radiographic studies should be undertaken to identify the location of the tumor(s)[19,53] (see Figures 14-1, 14-2, and 14-4). These tests include both anatomic imaging using computed tomography (CT) or magnetic resonance imaging (MRI) and functional nuclear scintigraphy obtained concomitantly with single-photon emission computed tomography (SPECT)/CT, chiefly using radiolabeled meta-iodobenzylguanidine (MIBG).[76] Initial radiographic studies should include anatomic cross-sectional imaging of the abdomen and pelvis, followed by imaging of the neck and chest if the initial studies are unrevealing[18,50] (see Figure 14-4). CT and MRI have similar diagnostic sensitivities, and so the imaging test of choice is best determined by local practices and patient preference.[53,77] Abdominal ultrasound may also be considered in young children, if local expertise permits (see Figure 14-2). Catecholamine-producing tumors are highly vascular (and hence enhancing) neoplasms that commonly contain necrotic, cystic, or hemorrhagic areas; on MRI, they may exhibit a classic hyperintense appearance on T2-weighted images.[18,78]

MIBG, a synthetic compound that bears structural similarity to norepinephrine (except it also has a guanidine side chain that resists metabolism), accumulates preferentially in adrenergic tissues, namely due to reuptake via the norepinephrine transporter system.[79,80] MIBG utilized for diagnostic purposes is radiolabeled with either ^{123}I or ^{131}I, although ^{123}I-MIBG is the agent of choice because of its superior imaging properties and substantially lower radiation dose.[76] ^{123}I-MIBG scanning is a sensitive and specific test (94% and 92%, respectively[76]) that can confirm the catecholamine-producing nature of a tumor, localize tumors not seen with cross sectional imaging, and potentially identify other sites of disease, although its use is more limited in malignant disease.[50,51,81-84] Whether or not MIBG scintigraphy is required in all cases where tumor is located by CT or MRI remains an area of debate,[53] but it should be considered in known syndromic disease where there is a higher risk of multifocal disease. Prior to ^{123}I-MIBG scanning, care should be taken to ensure that the patient is not taking medications (decongestants, calcium channel blockers, or labetalol) that are known to decrease MIBG uptake, and potassium iodide should be administered to

block thyroid uptake of radioactive iodine.[80,85] Because of the limitations of MIBG testing, other nuclear imaging modalities have been studied: somatostatin receptor scintigraphy using ^{111}In-DTPA-pentetreotide (octreotide), [^{18}F] fluoro-dihydroxyphenylalanine (^{18}F-DOPA) positron emission tomography (PET), [^{18}F]-fluorodopamine (^{18}F-FDA) PET, or [^{18}F] fluorodeoxyglucose (FDG) PET.[50,77,86-88] Although some of these functional studies, particularly ^{18}F-DOPA and ^{18}F-FDA PET, are likely to be superior to scanning with MIBG,[89-91] not all centers can perform these studies. [^{18}F]FDG PET appears to be superior in the evaluation and workup of malignant PHEO/PGL, particularly in *SDHB* mutation carriers.[91-94]

Genetic Issues

The majority of apparently-sporadic PHEO/PGL that present in children and young adults are due to an identifiable germline mutation in one of several tumor-predisposing genes[9,15] (see Table 14-1). The most commonly associated syndrome is VHL followed by the familial paraganglioma syndromes (PGL1-4) and MEN2 (see separate sections presented later in the chapter). In all cases, synchronous or metachronous tumors can occur in both adrenal glands as well as extra-adrenal sites, underscoring the need for lifelong follow-up testing and appropriate genetic counseling and testing. Table 14-1 lists the major hereditary syndromes associated with PHEO/PGL in children. Knowledge regarding the genetic causes of catecholamine-producing tumors is rapidly expanding and genes associated with the development of PHEO/PGL include egl nine homolog 1 [*C. elegans*] (*EGLN1* also known as *PHD2*)[95]; kinesin family member 1B (*KIF1B*)[96]; transmembrane protein 127 (*TMEM127*)[97]; succinate dehydrogenase complex, subunit A (SDHA)[98]; MYC associated factor X (*MAX*)[99]; and succinate dehydrogenase complex assembly factor 2 (*SDHAF2*), the cause of PGL2.[100] Most recently, gain-of-function germline and somatic mutations in HIF-1 alpha-like factor (*HIF2A*; also known as *EPAS1*) have been associated with the development of PGL in individuals with congenital polycythemia.[101,102]

Family history, clinical presentation of the patient, and differences in the biochemical phenotype help to prioritize genetic testing.[31,103] PHEO/PGL that arise in the context of VHL or the familial paraganglioma syndromes occur at younger ages and are typically noradrenergic tumors, producing almost exclusively norepinephrine and normetanephrine.[15,66] Adrenergic tumors (which secrete epinephrine and metanephrine, in addition to norepinephrine and normetanephrine), are seen in MEN2, NF1, and sporadic cases. In general, *VHL* is the major gene of interest in children with PHEO and *SDHB* is the suspected gene in patients with PGL or malignant disease.[9,15] Evaluation for *RET* proto-oncogene germline mutations is recommended only in the rare case of a child with an apparently sporadic PHEO who exhibits an adrenergic phenotype, because medullary thyroid carcinoma usually presents before PHEO in most individuals with MEN2.[2,31] NF1 is usually clinically diagnosed and therefore testing for mutations in the *NF1* gene in the context of an apparently sporadic tumor will be of very low yield and therefore is not recommended.

Management

Surgical Therapy

Surgical resection is the mainstay in the treatment of PHEO/PGL. A preoperative biopsy is not indicated and potentially dangerous.[104] The procedure of choice for most PHEO is laparoscopic adrenalectomy, either using transperitoneal or retroperitoneal approaches.[4,105-111] Laparotomy should be contemplated in patients with large PHEO or a concern for underlying malignancy based on the clinical presentation, genetic background, or radiographic appearance of the tumor.[4] In the setting of bilateral PHEO or known hereditary PHEO, a cortical-sparing procedure should be performed to minimize the risk of the lifelong glucocorticoid and mineralocorticoid replacement and the attendant risks of primary adrenal insufficiency.[4,107,112-114] Because it is extremely difficult to preserve a vascularized portion of adrenal cortex sufficient to prevent corticosteroid dependence without also leaving some residual adrenal medulla, there is a risk for recurrent PHEO in the remnant; limited data suggest that recurrence rates in this setting are between 10% and 38%.[10,30,112,115] The surgical approach for removal of a PGL depends on the location of the tumor but in selected cases of abdominal disease can also be performed laparoscopically.[19,108] Head and neck PGL can be expectantly monitored or treated with surgery or radiation.[4]

It is important that the anesthesiologist have experience with the intraoperative management of PHEO/PGL, because dysrhythmias can occur and blood pressures can be quite labile.[116,117] Both intravenous antihypertensive medications (esmolol, labetalol, nitroprusside, phentolamine, etc.) and vasopressors (e.g., phenylephrine and norepinephrine) should be readily available for intraoperative use. The greatest risk for hypertension occurs during anesthesia induction and manipulation of the tumor, whereas hypotension is most likely to occur after ligation of the adrenal vein, when the abrupt decline in catecholamine concentrations leads to vasodilation.[4] Postoperatively, the patient should be monitored for the two major complications of hypotension and hypoglycemia.[3,4,116,117] Hypertension may persist for days to weeks following surgery. In patients who have had a cortical-sparing adrenalectomy in the context of bilateral PHEO resection, stress glucocorticoids should be provided and a high-dose cosyntropin stimulation test obtained prior to hospital discharge to determine the need for adrenal steroid replacement.

Medical Preparation for Surgery

Once the diagnosis of a PHEO/functional PGL has been confirmed, medical therapy to normalize the blood pressure and mitigate the signs and symptoms of catecholamine excess should be initiated (Table 14-3). If surgery

TABLE 14-3 **Preoperative Medical Management of Pheochromocytoma/Sympathetic Paraganglioma**

Drug Class	Drug	Mechanism of Action	Initial Pediatric Dose
α-adrenergic receptor blockers	Doxazosin	α_1-antagonist	0.5-1 mg daily
	Phenoxybenzamine	α_1- and α_2-antagonist	0.2-0.5 mg/kg/day divided BID (max 10 mg BID)
	Prazosin	α_1-antagonist	0.05-0.1 mg/kg/day divided TID (max 1 mg TID)
β-adrenergic receptor blockers	Atenolol	β_1- antagonist	0.5-1 mg/kg/dose daily (max 50 mg daily)
	Metoprolol	β_1- antagonist	1-2 mg/kg/day divided BID (max 50 mg BID)
	Propranolol	β_1- and β_2-antagonist	0.5-1 mg/kg/day divided BID (max 40 mg BID)
Calcium channel blockers	Nifedipine (sustained release)	Calcium channel blocker	0.25-0.5 mg/kg/day daily or BID (max 60 mg total daily dose)
Inhibitors of catecholamine synthesis	Metyrosine	Tyrosine hydroxylase inhibitor	125-250 mg divided BID-TID

is planned, medical treatment should be taken for at least 1 to 2 weeks prior to surgery. This is done to minimize the potential complications that may arise from acute catecholamine surges during the induction of anesthesia and manual manipulation of the tumor.[11,19,116,118] No universal algorithm exists for the medical management of a PHEO/PGL prior to surgery. Nevertheless, blockade of α-adrenergic receptors is usually the therapy of choice and effective α-receptor blockade improves symptoms, lowers blood pressure, and expands the vascular bed and blood volume. The primary agent used in children is the nonselective α-blocker phenoxybenzamine.[7,18,19,116] Side effects of phenoxybenzamine can include nasal congestion, symptomatic orthostasis, and tachycardia. Due to its long half-life, it may also increase the risk of postoperative hypotension.[3,11,51,60] Selective α_1-blockers such as prazosin and doxazosin and calcium channel blockers such as nifedipine can also be utilized.[11,60,119-121] Although labetalol and carvedilol, drugs with both α- and β-antagonist activity, are attractive options for preoperative blockade, they are not universally recommended for primary medical treatment because of their lower α-adrenergic receptor blockade relative to their β-antagonist activity.[121] Metyrosine is a competitive inhibitor of tyrosine hydroxylase, the rate-limiting step of catecholamine biosynthesis (see Figure 14-3), and it can also be utilized as part of the preoperative preparative regimen.[121,122] However, not all centers use metyrosine routinely due to its potential significant side effects (sedation, diarrhea, and extrapyramidal manifestations) and unclear benefit in most cases.[4,18,31] Symptomatic postural hypotension may be seen at the beginning of medical therapy, particularly with large biochemically active tumors, so it is imperative to start at low doses and increase the dose or frequency every few days until the blood pressure is normal for age and height and the patient is minimally orthostatic. Phenoxybenzamine is only supplied as a single dose (10-mg capsule), so it will need to be compounded by the pharmacy to allow for the administration of the lower doses needed in younger children. Phenoxybenzamine is also an expensive drug, which makes the selective α_1-blockers a more attractive option in many cases.

Once alpha blockade has been established, a β-blocking agent (see Table 14-3) is typically added to control reflex tachycardia.[121] A β-blocker should not be used as a single agent because of the possibility of worsening symptoms and hypertension due to unopposed catecholamine effects at α-adrenergic receptors.[3] A few days prior to surgery, oral salt loading (either via increased dietary intake or with sodium chloride tablets) is recommended to expand the blood volume in order to mitigate postoperative hypotension. Some centers also routinely admit patients for intravenous fluids prior to PHEO/PGL resection,[121] and this should be considered for very symptomatic children with large tumors.

Prognosis and Follow-up

The prognosis of children diagnosed with PHEO/PGL is excellent with 5- and 10-year survival rates of 98% and a 20-year survival rate of 84%.[15] Based on data from a British tumor registry, the incidence of malignant disease in children is estimated to be 0.02 per million per year.[7] Approximately 12% of pediatric PHEO/PGL are malignant,[8] although some referral centers report a malignancy rate in tumors diagnosed during childhood as high as 65%.[14,15,30] The high rate of malignancy from some studies may in part reflect a referral bias. On the other hand, the latency period between diagnosis and confirmation of metastatic disease is 9 years on average[15]; thus, the true rate of malignancy may be higher than previously recognized because it can only be identified during long-term systematic follow-up of patients diagnosed with PHEO/PGL during childhood. Children with metastatic disease typically demonstrate a more indolent clinical course with average overall survival $>$ 6 years after the diagnosis of metastatic disease.[15]

There is no single histologic feature or immunohistochemical profile that is independently able to predict metastatic potential in a resected PHEO/PGL, but features noted more frequently in malignant tumors include extra-adrenal location, confluent tumor necrosis, absence of hyaline globules, coarse nodularity of the primary tumor, high proliferative index, and size greater than 5 cm, among others.[22,123-125] Malignancy is therefore only established by the identification of distant metastases in a site where paraganglia are not normally located (primarily bones but also lymph nodes, liver, or lungs).[15,22,123] The risk of

malignant disease is greater for extra-adrenal sympathetic PGL than for PHEO or nonsecretory head and neck PGL, and overall prognosis is worse for these patients.[15,125] The highest risk for malignancy and death is in SDHB-related sympathetic PGL, which represents 50% or more of malignant tumors.[15,65,69,126-130]

Because PHEO/PGL can have unpredictable behavior and because children are at risk for the development of metachronous primary tumors, delayed metastasis from previously treated neoplasms, and local recurrence (in the case of cortical-sparing procedures), long-term follow-up with biochemical screening and intermittent imaging studies is required, particularly for children with a PGL or a known *SDHB* mutation.[3,7,10,15,19,22,30,112,115] For asymptomatic children with an identified genetic mutation predisposing them to the development of a PHEO/PGL, annual biochemical screening is advised, with the age of initial screening determined by the specific gene mutation (see Table 14-1). Furthermore, occasional cross-sectional imaging, typically MRI because of the lack of radiation exposure, is recommended periodically for follow-up of patients at high risk of recurrence or malignant disease (e.g., abdominal paragangliomas) or at risk for developing a PHEO/PGL that may not be identified on biochemical testing alone (e.g., familial paraganglioma syndromes), although the optimal screening strategy has yet to be determined.[131]

MEDULLARY THYROID CARCINOMA

MTC is a malignant neuroendocrine tumor that arises from the neural crest–derived, calcitonin-producing parafollicular C cells of the thyroid gland.[132,133] It comprises only a small minority of thyroid malignancies diagnosed in patients under the age of 21 years, although it is the most common thyroid malignancy diagnosed at less than age 5 years.[134] The overall age-adjusted incidence of MTC during childhood is < 0.5 cases per million per year, with a fairly equal female:male ratio, unlike the differentiated thyroid carcinomas, which are more frequent in girls than boys.[134-136]

When diagnosed during childhood, MTC primarily results from a dominantly inherited or de novo activating mutation in the multiple endocrine neoplasia (MEN) type 2A or type 2B or familial MTC (FMTC). FMTC is more commonly being recognized as a phenotypic variant of MEN2A with decreased penetrance or delayed onset of the other neoplastic manifestations.[141,142] *RET* is a member of the cadherin superfamily and encodes a receptor tyrosine kinase that has an extracellular binding domain and an intracellular tyrosine kinase (Figure 14-5). The endogenous ligands (which activate RET via a high-affinity ligand-binding coreceptor, GFRα) are members of the glial cell–derived neurotrophic factor (GDNF) family, which

Exon	Codon/Mutations	MTC	+PHEO	+PHPT	+CLA	+HSPR	MEN 2B Phenotype	ATA Risk	Age of Testing[a]
10	C609R/G/F/S/Y	○	○	○		○		B	5y
	C611R/G/F/S/W/Y	○	○	○		○		B	5y
	C618R/G/F/S/Y	○	○	○		○		B	5y
	C620R/G/F/S/W/Y	○	○			○		B	5y
11	C630R/F/S/Y	○		○				B	5y
	C634R/G/F/S/W/Y	○	○	○	○			C	3-5y
13	E768D	○	○					A	5-10y
	L790F	○	○					A	5-10y
14	V804L/M	○	○	○	○			A	5-10y[b]
15	A883F	○	○				○	D	<6 mo[c]
	S891A	○	○	○				A	5-10y
16	M918T	○	○				○	D	<6 mo

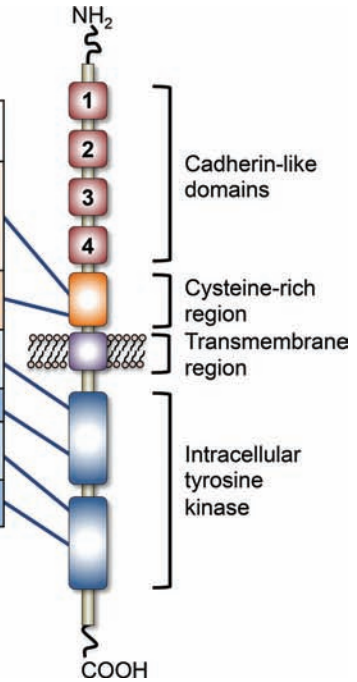


FIGURE 14-5 ■ The RET receptor and commonly mutated codons and associated phenotypes in the MEN2 syndromes, including the recent ATA risk stratification. [a]Age of testing refers to the age at which clinical testing with thyroid ultrasound and basal calcitonin levels would be appropriate. It is not meant to indicate the age of testing for the presence of a *RET* mutation, which may be done earlier after appropriate genetic counseling, or the absolute age for early thyroidectomy. [b]Although a case of very aggressive MTC in a child diagnosed at age 6 years has been reported, an older age of disease onset is generally observed with this *RET* mutation. [c]Only rare cases of MEN2B secondary to the A8883F mutation have been published, and the MTC phenotype of this mutation remains largely unknown. MTC, medullary thyroid carcinoma; PHEO, pheochromocytoma; PHPT, primary hyperparathyroidism; CLA, cutaneous lichen amyloidosis; HSCR, Hirschsprung disease. (*This image can be viewed in full color online at ExpertConsult.*) (From Waguespack, S. G., Rich, T. A., Perrier, N. D., et al. (2011). Management of medullary thyroid carcinoma and MEN2 syndromes in childhood. *Nat Rev Endocrinol, 7*, 596–607.)

are involved in the regulation of neural tissue development.[143] The RET protein therefore plays a crucial role in the development of neural crest–derived cells, the urogenital system, and the central and peripheral nervous systems, notably the enteric nervous system.[144,145]

Although sporadic nonheritable tumors account for up to 75% of adult cases of MTC,[141] such tumors are rare in children. In contrast to sporadic MTC, hereditary MTC is typically multifocal, bilateral, and located in the middle to upper regions of the thyroid lobes (Figure 14-6), an area where C cells are the most highly concentrated.[133,146,147] Microscopic examination and calcitonin staining of the thyroid will often identify C-cell hyperplasia, which is the initial stage in an oncologic cascade that leads to the development of microscopic noninvasive MTC and ultimately lymph node and distant metastatic disease due to frankly invasive carcinoma (Figure 14-6).[133,148] Patients with hereditary MTC have an age-related progression of malignant disease, with lymph node and distant metastases typically occurring years after the onset of C-cell hyperplasia.[2,148] The cervical and mediastinal lymph node basins are the most common sites of metastatic disease,

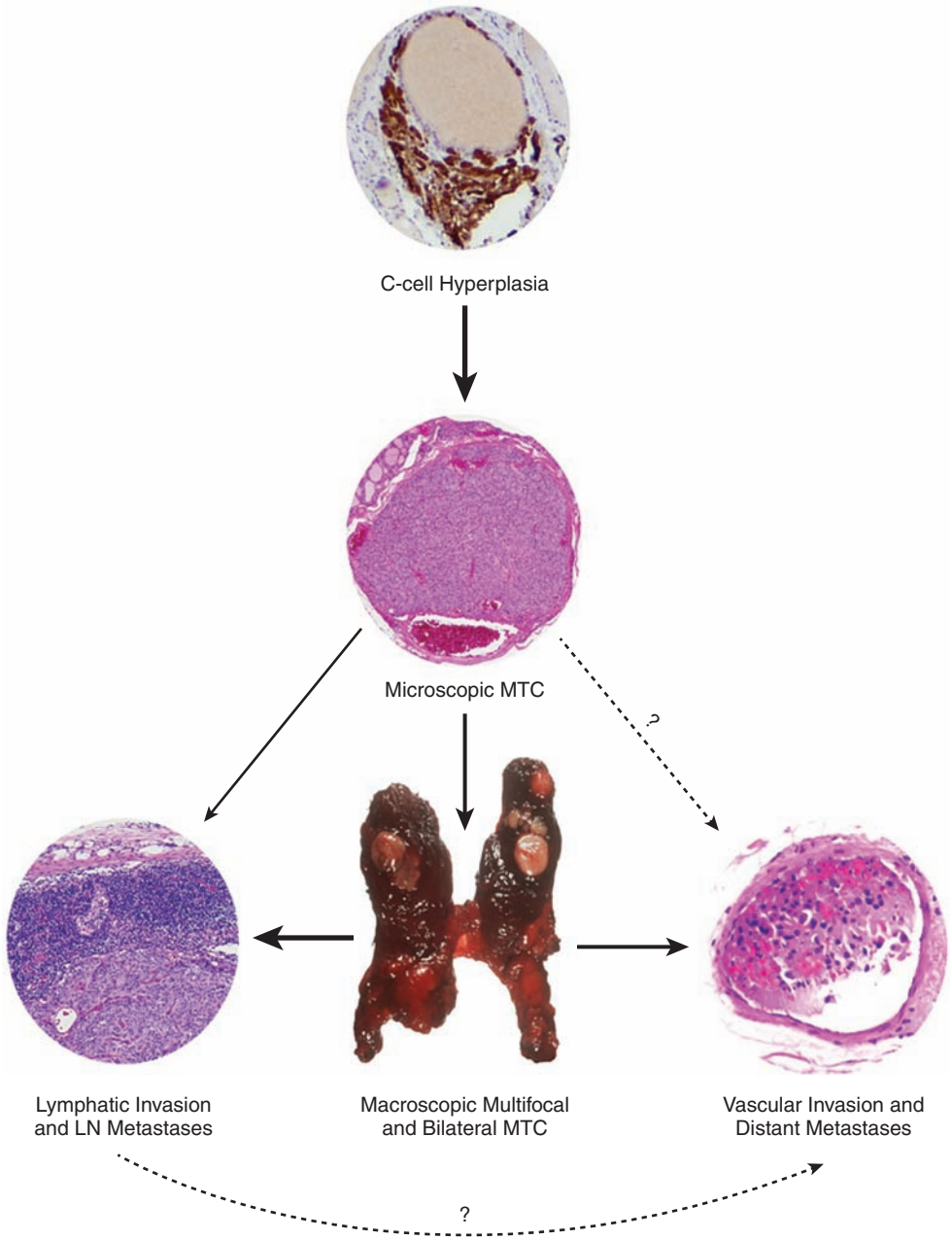


FIGURE 14-6 ■ The development and progression of hereditary MTC in childhood. C-cell hyperplasia is the initial stage in an oncologic cascade that ultimately leads to the development of microscopic noninvasive MTC and ultimately lymph node and distant metastatic disease due to frankly invasive carcinoma. The weight of the arrows denotes the hypothetical probability of the event occurring in the typical pediatric patient with a *RET* codon 634 mutation. LN, lymph node; MTC, medullary thyroid carcinoma. *(This image can be viewed in full color online at ExpertConsult.)* (From Waguespack, S. G., Rich, T. A., Perrier, N. D., et al. (2011). Management of medullary thyroid carcinoma and MEN2 syndromes in childhood. *Nat Rev Endocrinol, 7*, 596–607.)

whereas distant sites for MTC spread typically include the lungs, liver, and bone or bone marrow. Positive lymph node status and higher stage at diagnosis predict lower disease-free survival and higher mortality.[136,149-151]

Clinical Presentation

Children with sporadic MTC present similar to other thyroid malignancies, typically with a palpable thyroid nodule or cervical lymphadenopathy. They do not have the characteristic facial or other features found in MEN2B as described later. On the other hand, children with hereditary disease due to a *RET* mutation rarely present with overt clinical disease, outside of the newly identified MEN2A kindred or MTC associated with MEN2B, a diagnosis that remains uniformly delayed.[152] Therefore, in the 21st century, the predominant clinical presentation of MTC during childhood is one of the presymptomatic identification of a positive *RET* mutation and the ultimate identification of microscopic MTC after early thyroidectomy.

Evaluation and Management

Several guidelines and review papers are available to assist the clinician in the specific evaluation and management of the child with suspected or proven MTC.[2,141,153-156] As with all pediatric thyroid malignancies, surgery is the cornerstone of therapy. Given the potential higher complication rates in children than adults,[157] children should be operated on by a surgeon skilled in thyroid surgery and with "high volume" experience for this procedure.[158] Apart from the decision of when to intervene, the thyroid surgeon must also consider the patient's *RET* genotype and clinical data and incorporate this knowledge into the decision-making process to determine the optimal surgical approach.[2] Meticulous and safe removal of all thyroid tissue, including the posterior capsule, is the goal of the prophylactic thyroidectomy.[154] Routine central compartment (level VI) neck dissection[159] is not performed in the setting of a purely prophylactic thyroidectomy because lymph node metastases are quite rare in that setting.[141,148-150,154,160,161] However, if the operation is for a clinically evident hereditary tumor or for sporadic MTC, thyroidectomy and a concomitant central neck dissection should be performed. Dissection of the lateral cervical lymph node compartments (levels IIA to V) is generally performed only in cases where there is clinical evidence of lateral neck involvement.

Fortunately, life-threatening MTC rarely occurs during childhood and typically only in the clinical context of MEN2B. MTC is not sensitive to standard cytotoxic chemotherapy, which historically has incorporated the agent dacarbazine.[142] Targeted molecular therapies that inhibit RET and other receptor tyrosine kinases known to be involved in angiogenesis have shown great promise in the treatment of metastatic MTC.[162-164] The drug vandetanib became the first such agent approved by the U.S. Food and Drug Administration (FDA) for the treatment of adults with MTC, and preliminary results from a phase I/II trial in children with MTC have been encouraging.[165]

Prognosis

The prognosis of MTC diagnosed during childhood is generally excellent, with 5-year and 15-year survival rates of 95% and 86%, respectively.[134] Mean survival after diagnosis is 28.3 years and the presence of distant disease at diagnosis, compared with locoregional disease, portends a worse prognosis.[134,166,167] As the tumor stage increases, the risk of both locoregional and distant metastatic disease rises,[136,166] although lymph node metastases may still occur when the tumor is < 1 cm in size.[168-171] Many children who present with clinical MTC already have metastatic disease at diagnosis. Consequently, the majority of cases of childhood MTC that are not diagnosed before lymph node metastases occur represent incurable, albeit indolent, cancers. The aggressiveness of the clinical course can be predicted by the presence of certain *RET* mutations (see MEN2 section), the child's clinical presentation, and the use of calcitonin and carcinoembryonic antigen (CEA) as tumor markers. The loss of calcitonin expression, a CEA level out of proportion to calcitonin, and a rapid CEA or calcitonin doubling time are all harbingers of an aggressive disease course.[141,151,172,173]

HEREDITARY ENDOCRINE NEOPLASIA SYNDROMES

Carney Complex

The Carney complex (CNC) is a genetically and clinically heterogeneous disorder with autosomal dominant inheritance first described in 1985.[174] It is characterized by spotty skin pigmentation affecting the lips, conjunctiva, and other mucosal surfaces (Figure 14-7); myxomas of the breast, heart, and skin; endocrine tumors or overactivity, classically primary pigmented nodular adrenocortical disease (PPNAD)[175] and growth hormone/prolactin hypersecretion; and psammomatous melanotic schwannoma, among other clinical manifestations.[174,176,177] Two or more distinct genetic loci are associated with CNC: the gene *PRKAR1A*[178,179] on chromosome 17q23-q24 and an unknown gene on chromosome 2p16.[180] *PRKAR1A* encodes for the RI-α subunit of protein kinase A, the major mediator of intracellular cAMP signaling. Most *PRKAR1A* mutations are inactivating point mutations, and about 30% of CNC patients have de novo disease.[176,181] In patients with CNC, the overall *PRKAR1A* mutation detection rate is 62%.[181]

The major endocrine phenotype of the CNC is PPNAD, occurring either alone or with other CNC manifestations, in 60% of cases.[181] PPNAD is a rare form of ACTH-independent hypercortisolism pathologically associated with multiple small (< 1 cm) black or brown nodules (containing lipofuscin) in an otherwise atrophic cortex[175,182,183] (see Figure 14-7). The median age of onset is 34 years (with about 20% of cases occurring during childhood), and there is a female predilection after puberty.[181] The hypercortisolism is typically insidious in

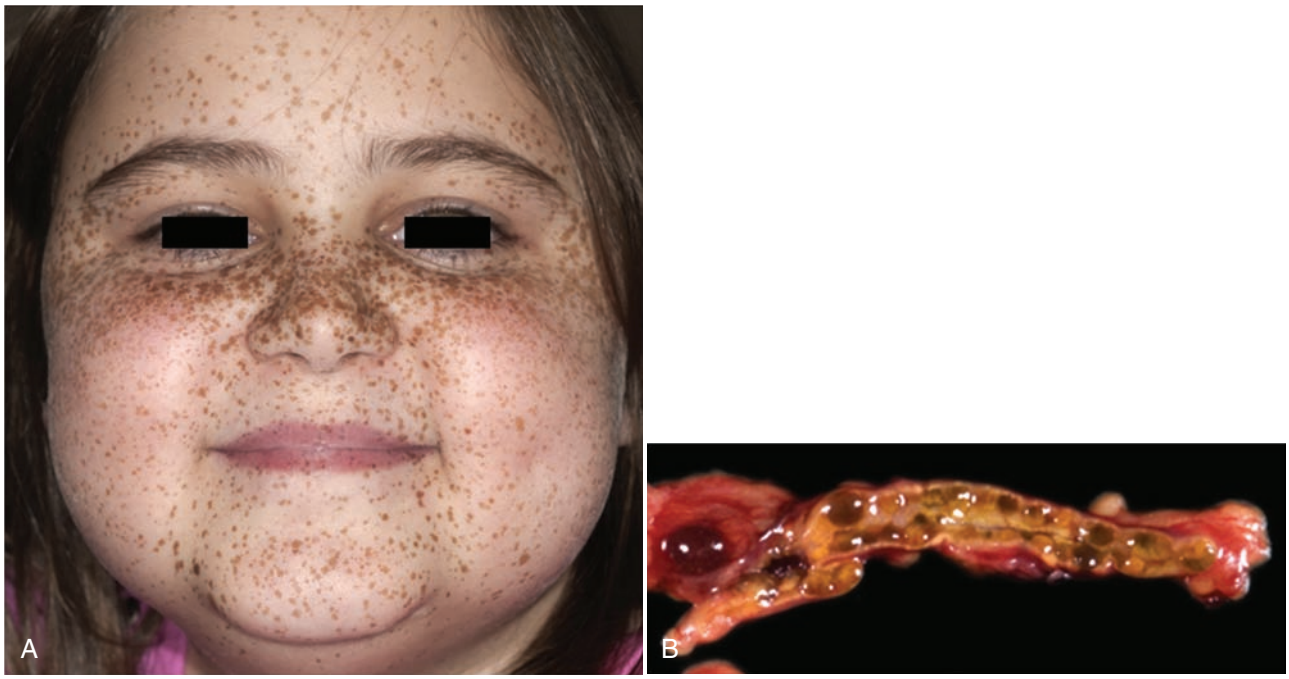


FIGURE 14-7 ■ Carney complex and primary pigmented nodular adrenocortical disease (PPNAD). **A,** A 10-year-old female with ACTH-independent Cushing syndrome and the Carney complex demonstrates facial rounding, plethora, and the classic distribution of facial lentigines. **B,** Gross pathologic specimen of the right adrenal gland in another patient with PPNAD and the Carney complex. *(This image can be viewed in full color online at ExpertConsult.)*

its onset, and a classic finding in PPNAD-related Cushing syndrome is the paradoxical increase in glucocorticoid excretion during high-dose dexamethasone administration for the Liddle test.[184] Adrenal imaging is of limited value for the confirmation of PPNAD, but it can suggest diffuse micronodular disease on high-resolution studies.[182,183,185] The treatment of symptomatic PPNAD is bilateral adrenalectomy.

Growth-hormone hypersecretion (causing gigantism or acromegaly, depending on the age of onset) is the primary pituitary phenotype of the CNC, occurring in 10% to 12% of CNC patients.[174,181,186] Pathologically, this may be associated with an adenoma or hyperplasia.[186,187] Concomitant hyperprolactinemia is prevalent and is similarly caused by underlying hyperplasia or frank adenomas in rare cases.[187,188] Subtle abnormalities in growth hormone and prolactin secretion may be identified in up to 75% of patients with CNC.[186] Thyroid neoplasia occurs in 25% of CNC patients and differentiated thyroid carcinoma (both papillary and follicular carcinomas) have been identified in 2.5% of cases.[181] Males with CNC are at risk for testicular tumors, primarily bilateral large-cell calcifying Sertoli cell tumors (LCCSCT), clinically identified in 33% to 41% of patients.[181,186] Similar to the Peutz-Jeghers syndrome (discussed later), gynecomastia or palpable mass(es) may be a clinical presentation of LCCSCT, which can also be malignant in rare cases.[186,189]

Once CNC is diagnosed clinically or an asymptomatic child is found to harbor a germline *PRKAR1A* mutation, prospective monitoring for the development of the tumors and endocrine disorders characteristic of the syndrome can be undertaken,[176,186] although optimal screening strategies remain largely unknown.

Familial Isolated Pituitary Adenomas

Hereditary pituitary adenomas can occur within families outside of the context of MEN1 and other autosomal dominant, heritable endocrine neoplasia syndromes. The term *familial isolated pituitary adenomas (FIPA)* was first proposed in 2006.[190] Pituitary tumors occurring within FIPA families clinically present similar to sporadic pituitary adenomas and all hormonal subtypes are represented. The disorder was ultimately identified in a subset of FIPA kindreds to be caused by germline mutations in the aryl hydrocarbon receptor-interacting protein (*AIP*) gene.[191,192]

Mutations in *AIP* represent about 15% of FIPA cases (50% of kindreds with familial somatotropinomas), and more than 50 AIP mutations have been described to date.[192,193] Penetrance is incomplete and is estimated to be around 30%.[193] Median age of diagnosis is 23 years (range 8 to 74 years) and half of cases present during childhood or adolescence.[194] In patients < age 18 years presenting with an apparently-sporadic pituitary macroadenoma, 20% may harbor a germline *AIP* mutation.[195] FIPA individuals with *AIP* mutations have tumors that are larger (overwhelmingly macroadenomas) and diagnosed at a much younger age compared with *AIP*-negative patients.[192,194] The majority of *AIP*-mutated adenomas are somatotroph or mammosomatotroph adenomas, which typically present with gigantism in males.[192,194] These AIP-mutated growth-hormone secreting tumors are more recalcitrant to the typical therapies prescribed for gigantism/acromegaly.[194]

Given the identification of *AIP*-related FIPA, there are no consensus guidelines as to the timing of genetic testing

for *AIP* mutations in sporadic pituitary adenoma cases or at-risk members of an affected kindred. Recommendations for prospective clinical screening in asymptomatic *AIP*-mutation carriers are also not well established. However, some authors have proposed an approach to care of such patients that includes an annual hormonal and auxologic evaluation.[193,196]

Familial Paraganglioma Syndromes

The familial paraganglioma syndromes, also referred to as the familial paraganglioma and pheochromocytoma syndromes (FPPS),[131] are characterized primarily by the development of parasympathetic and sympathetic PGL and PHEO.[197] Four autosomal dominantly inherited FPPS syndromes (PGL1-4) have been described (see Table 14-1) and are due to germline mutations in discrete genes encoding either the subunits of the succinate dehydrogenase (SDH) enzyme gene (*SDHB*, *SDHC*, and *SDHB*) or a protein necessary for the flavination of SDHA: PGL1(*SDHD*),[198] PGL2(*SDHAF2*),[100] PGL3 (*SDHC*),[199] and PGL4 (*SDHB*).[200] The *SDHx* genes constitute the subunits for complex II of the mitochondrial respiratory chain that, when mutated, lead to stabilization of hypoxia inducible factor 1 with a subsequent state of pseudohypoxia.[131] The clinical phenotype differs among the four syndromes and remains poorly characterized in some cases, such as PGL2. As an example, mutations in *SDHC* primarily cause benign parasympathetic head and neck PGL (also referred to as "glomus tumors" or "chemodectomas"), whereas mutations in *SDHB* are associated more with abdominal PGL and a high risk of malignancy (see Table 14-1, Figure 14-2). In all cases, penetrance of the clinical phenotype is less than 100% and increases with age.[127,197,201,202] Mutations in *SDHD* (and possibly also *SDHAF2*) demonstrate parent-of-origin effects, with disease generally occurring only when the mutation is inherited from the father.[197,203] *SDHx*-related tumors are associated with gastrointestinal stromal tumors (GIST), a finding that has also been referred to as the Carney-Stratakis syndrome or dyad.[204,205] Other non-paraganglial malignancies described (chiefly in patients with *SDHB* mutations) include renal cell carcinoma and papillary thyroid carcinoma.[201,206,207] A patient with an *SDHD* mutation was identified to have a growth hormone-secreting pituitary macroadenoma, suggesting a possible role of SDH defects and pituitary tumorigenesis.[208]

Hyperparathyroidism-Jaw Tumor Syndrome

The hyperparathyroidism-jaw tumor (HPT-JT) syndrome is a heritable disorder primarily characterized by parathyroid neoplasia and primary hyperparathyroidism associated with ossifying fibromas of the maxilla or mandible. First reported in 2002, *CDC73* (formerly known as *HRPT2*) is the only known gene in which mutations cause HPT-JT, and *CDC73* mutations are identified in about 60% of HPT-JT syndrome kindreds.[209] Primary hyperparathyroidism due to a single benign parathyroid adenoma is the most common clinical manifestation, but parathyroid carcinomas are quite prevalent in the syndrome, representing up to 15% of HPT cases.[210] The youngest age of diagnosis of HPT has been seven years.[211] Ossifying fibromas, also known as cemento-ossifying fibromas, can be quite aggressive and occur in 30% to 40% of individuals with HPT-JT syndrome.[210] They are chiefly treated with surgical extirpation. Other clinical manifestations of the HPT-JT syndrome include renal lesions (cysts, hamartomas, and nephroblastoma) and uterine tumors.[212,213] Prospective clinical monitoring of individuals with a *CDC73* mutation includes periodic biochemistries, dental imaging, and ultrasonography of the kidney and uterus.[210]

Multiple Endocrine Neoplasia 1 (MEN1)

MEN1 is an autosomal dominantly inherited tumor syndrome characterized by the occurrence of glandular hyperplasia and benign or malignant neoplasia in two or more specific endocrine glands, chiefly the parathyroids, pituitary, and neuroendocrine cells of the duodenum and pancreas[153,214-217] (Table 14-4). The tumors can be hormonally active or inactive and multifocality is common, except for pituitary tumors. Some patients also develop adrenocortical adenomas, carcinoid tumors, benign tumors of the skin (angiofibromas, collagenomas, lipomas), central nervous system (CNS) tumors (meningiomas and ependymomas), or uterine leiomyomas.[216,217]

The hereditary nature of MEN1 was first recognized by Wermer in 1954.[218] Genetic linkage analysis among families with a clinical diagnosis of MEN1 initially localized the inherited abnormality to chromosome 11q13,[219,220] which ultimately led to the identification in 1997 of germline mutations in several families with MEN1.[221,222] The MEN1 gene is a tumor suppressor gene that encodes a nuclear protein (menin), which in turn plays a role in transcriptional regulation, genome stability, cell division, and proliferation.[215,223,224] Advances in genetic testing have made it possible to establish a specific molecular diagnosis and screen unaffected relatives. The challenge for pediatric physicians, other caregivers, and parents is determining when to perform appropriate clinical surveillance and intervention for patients known to harbor an MEN1 gene mutation.

Epidemiology and Pathogenesis

The prevalence of MEN1 is estimated at 1 to 10 per 100,000 individuals.[225,226] The "two-hit" mechanism of disease, first described by Knudson in hereditary retinoblastoma,[227] is illustrated in Figure 14-8. Patients with MEN1 carry one wild-type and one inactive mutant allele of the *MEN1* gene (a germline mutation) in all cells. This in itself is insufficient to induce tumor formation. Subsequently, a somatic mutation (the "second hit") in a single cell deletes the only normally functioning *MEN1* gene, leads to a loss of heterozygosity at the *MEN1* locus in tumor DNA, and attenuates the ordinary constraints on cell growth by menin. Thus, tumor formation is initiated from a single clone of cells.

Most patients with MEN1 inherit the mutant allele from an affected parent, but about 10% of individuals

TABLE 14-4 **Characteristic Tumors and Associated Abnormalities in MEN1**

Organ System	Tumors	Estimated Penetrance[a]	Earliest Age of Onset (References)	Product Hypersecreted
Parathyroid	Hyperplasia/adenoma	90%-100%	8y[226]	Parathyroid hormone (PTH)
Anterior pituitary		15%-50%		
	Prolactinoma	20%	5y[†129]	Prolactin (PRL)
	Somatotropinoma	10%	9y[230]	Growth hormone (GH)
	Corticotropinoma	<5%	12y[459]	Adrenocorticotropic hormone (ACTH)
	Nonfunctioning	< 5%	12y[460]	
Enteropancreatic neuroendocrine		30%-80%		
	Insulinoma	10%	8y[461]	Insulin
	Gastrinoma	40%	10y[230]	Gastrin
	Glucagonoma	< 1%		Glucagon
	VIP producing	< 1%		Vasoactive intestinal polypeptide
	Nonfunctioning and PPoma	40%-80%	12y[460]	Pancreatic polypeptide
Stomach	Type II gastric carcinoid	10%	31y[230]	None
Thymus	Carcinoid	2%	16y[254]	ACTH, GH-releasing hormone, or none
Bronchus	Carcinoid	2%	15y[230]	None
Adrenal	Adrenocortical adenoma	40%	25[230]	Cortisol, aldosterone, or none
	Pheochromocytoma	< 1%		Metanephrines/ catecholamines
Skin	Angiofibromas	85%		
	Collagenomas	70%		
	Lipomas	30%		
CNS	Meningiomas	8%		
	Ependymomas	1%		
Uterus	Leiomyomas	Rare		

*Percentage of patients that eventually develop these clinical manifestations.[153,216,217,273]
†This patient had a mammosomatotroph tumor, co-secreting prolactin, and growth hormone.

with MEN1 represent de novo mutations.[222,224,228] More than 90% of tumors from MEN1 patients have loss of heterozygosity due to a subchromosomal rearrangement or deletion of the entire chromosome; other mechanisms for the second hit include point mutations, small deletions or insertions within the *MEN1* gene.[214,215,224] There have been more than 1100 germline mutations of the *MEN1* gene characterized thus far, and these mutations occur via multiple mechanisms and are distributed throughout the *MEN1* gene.[214,215,222,224,228] Testing by direct DNA sequencing identifies most MEN1 mutations, but approximately 5% to 10% of people with MEN1 do not have a mutation in the coding region or splicing sites of *MEN1*.[217,224] Such patients may represent a phenocopy (see the section on MEN4), or they may harbor a mutation in untranslated regions or introns or a large gene deletion, which requires other technologies such as multiplex ligation-dependent probe amplification (MLPA) to detect.[229]

Clinical Presentation and Management

The clinical presentation of MEN1 is highly variable, even among members of the same kindred, and will depend on the location and functionality of the underlying tumor(s). Not surprisingly, functional tumors typically present 5 to 10 years earlier than nonsecretory neoplasms.[230] There is almost complete penetrance of the phenotype, so MEN1 clinical and biochemical manifestations will generally develop in 80% and > 98% of patients, respectively, by the fifth decade of life.[153,228,230] An age-related progression of the various endocrine tumors exists in MEN1.[228,230] Penetrance of the phenotype in children with MEN1 is estimated to be < 1% before age 5 years, 7% by age 10 years, 28% by age 15, and 52% by age 20 years[228] (Figure 14-9). Prospective screening of asymptomatic *MEN1* mutation carriers leads to an earlier age of diagnosis,[228,230-235] and thus penetrance rates are likely to increase as clinical practice evolves. In contrast to MEN2, as described in the subsequent section, there are no clear genotype-phenotype correlations in MEN1, so a physician cannot rely on the specific mutation or family history to predict the age of onset, severity, or type of an MEN1 manifestation.[215,228,230]

In the absence of treatment, MEN1-associated endocrine tumors are associated with higher mortality (50% probability of death by the age of 50 years), and the cause of death in 50% to 70% of patients with MEN1 is usually

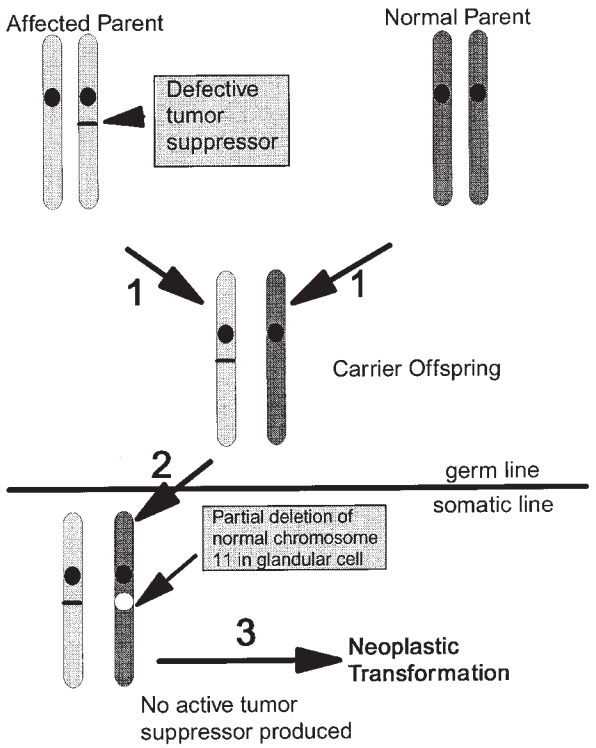


FIGURE 14-8 ■ "Two-hit hypothesis" of tumorigenesis in MEN1.[1] An affected parent passes a mutated *MEN1* gene to an offspring, who inherits a normal gene from the other parent.[2] Loss of heterozygosity in a somatic cell (a "second hit" that typically occurs via a subchromosomal rearrangement or deletion of the entire chromosome) deletes the remaining normal *MEN1* gene.[3] The absence of menin tumor suppressor activity in a cell leads to tumor formation.

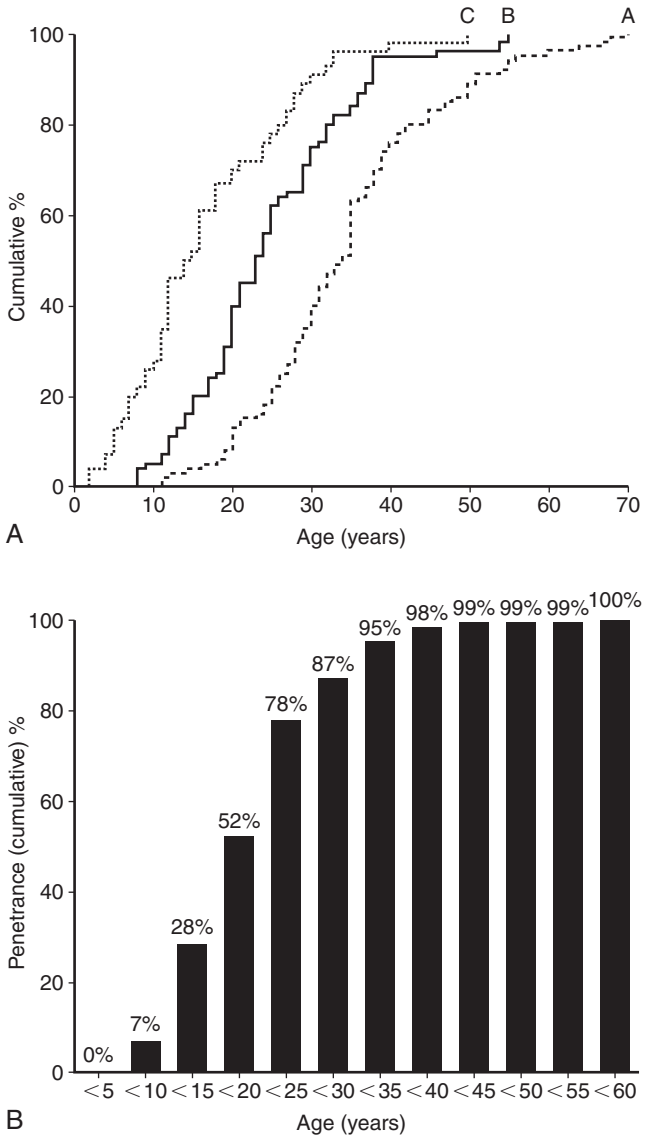


FIGURE 14-9 ■ Penetrance of the MEN1 phenotype. Age distributions (**A**) and age-related penetrance (**B**) of MEN1 clinical manifestations in 201 carriers of a mutated *MEN1* gene. Group A patients presented with clinical symptoms at the age depicted. Group B patients were asymptomatic but had positive biochemical screening at the age depicted. Group C patients were asymptomatic with negative biochemical screening with the age of their last biochemical testing shown. Groups B and C were significantly younger than Group A patients ($P < .001$). The age-related penetrance (B) is the proportion of mutation carriers who have developed a clinical manifestation by a given age. (From Bassett, J. H., Forbes, S. A., Pannett, A. A., et al. (1998). Characterization of mutations in patients with multiple endocrine neoplasia type 1. *Am J Hum Genet, 62,* 232–244.)

a malignant tumor process or sequelae of the disease.[216,236,237] In the current era, when medical management can effectively treat symptomatic gastrinoma, mortality due to the Zollinger-Ellison syndrome has declined. Currently, the greatest risk of mortality in MEN1 is chiefly from malignant enteropancreatic tumors and thymic carcinoids.[216,230,236]

The specific treatment for each type of MEN1-associated endocrine neoplasm is generally similar to that for the respective sporadic tumors occurring in patients without MEN1. This section will therefore focus more on the unique aspects of these disorders as they relate to MEN1 patients. As with most hereditary tumor syndromes, patients with MEN1 and their families should be managed by a multidisciplinary team consisting of relevant specialists with experience in the management of endocrine tumors.[216]

Primary Hyperparathyroidism

Primary hyperparathyroidism (PHPT) is the most common and earliest endocrine manifestation of MEN1.[216,226,230] MEN1 patients usually have multigland hyperplasia rather than single-gland adenomas. Symptoms of PHPT are due to the underlying hypercalcemia and can be nonspecific in children, including polyuria, difficulty concentrating, fatigue, headache, poor appetite, weight loss, abdominal pain, constipation, nausea, or emesis. Similar to adults, children can have end-organ damage (nephrolithiasis, nephrocalcinosis, acute pancreatitis, and bone involvement). Children with PHPT have been shown to have more severe symptoms, with 70% to 90% symptomatic compared with 20% to 50% of their adult counterparts.[238-240] Prolonged exposure to elevated PTH levels increases osteoclast activity, which can cause demineralization of bone, fractures, and eventually osteitis fibrosa cystica, the most severe skeletal manifestation of PHPT.[241] Compared with patients who

have sporadic PHPT, patients with MEN1 have lower bone density, a 1:1 male:female ratio (versus 1:3), lower PTH levels, and earlier age of onset (average age 20 to 25 years versus 55 years).[153,242,243] The youngest reported age of MEN1-associated PHPT is 8 years.[216]

The diagnosis of PHPT is based on an elevated calcium level in the setting of an inappropriately normal or frankly elevated PTH level. Because all parathyroid glands may be affected in MEN1 patients, preoperative imaging is generally not utilized.[244] However, in the case of reoperation, localization studies can be helpful to the surgeon.[245,246] Imaging modalities primarily include ultrasound, nuclear scintigraphy using technetium (^{99m}Tc) sestamibi, and CT/MRI.[247,248] Technetium (^{99m}Tc) sestamibi scans have a sensitivity of 85% to 100% in detecting single adenomas.[246] Sestamibi-SPECT imaging has a sensitivity of 90% for single adenomas, but only 55% for hyperplastic glands.[249] CT (sensitivity 50% to 70%), 4D-CT, or less commonly MRI, imaging can also help the surgeon preoperatively in those cases of reoperation.[246,250]

Surgical removal of the overactive parathyroid glands is the definitive treatment of PHPT in MEN1 patients, but the surgical approach remains controversial.[251] Subtotal (3.5 gland) parathyroidectomy with thymectomy or total (four gland) parathyroidectomy with thymectomy and parathyroid autotransplantation to the forearm are the primary options for surgical therapy. Resection of less than three glands results in the highest risk of persistent and recurrent disease (OR 3.11, 95% CI = 2.00 to 4.84).[252,253] A meta-analysis found that there was no significantly higher risk for persistent or recurrent HPT with a subtotal versus total procedure, but there was a significantly lower risk for permanent hypoparathyroidism with subtotal parathyroidectomy.[252,254] In studies that followed MEN1 patients beyond 10 years, the recurrence rates for either procedure range from 40% to 60%[255]; recurrent hypercalcemia can be as high as 50% in autotransplanted tissue.[216] Concomitant transcervical thymectomy is recommended to remove any potential ectopic parathyroid glands and to prophylactically remove the bulk of the thymus, which is at risk for the development of a thymic carcinoid.[252,253,255,256]

Surgical indications for treatment of asymptomatic PHPT in children with MEN1 are not clear,[251] especially given the potential for permanent hypoparathyroidism. Factors such as surgical experience, patient or parent preference, and availability of long-term monitoring of calcium levels should be considered in the timing of surgery.[216] In select MEN1 patients with PHPT, medical treatment with the calcimimetic agent, cinacalcet, appears to be effective.[257]

Gastroenteropancreatic-Neuroendocrine Tumors (GEP-NETs)

Gastroenteropancreatic neuroendocrine tumors (GEP-NETs) arise from neuroendocrine cells found in the pancreas, the gut, and its embryologic derivatives, including the thymus and bronchial tree. The term *carcinoid* is typically used to describe GEP-NETs arising in the gastrointestinal tract, lung, or thymus. Pancreatic NETs (pNETs), also known as pancreatic endocrine tumors (PETs), are GEP-NETs that originate in the neuroendocrine pancreas. The GEP-NETs most commonly encountered in MEN1 arise from the duodenum (gastrinomas) and the pancreatic islet cells (insulinoma, nonfunctioning tumors).[216,230]

GEP-NETs are the second most common manifestation of MEN1, occurring in 30% to 80% of patients.[153,216,230,258] GEP-NETs can be functional or nonfunctional. Gastrinomas primarily occur in the duodenum (within the so-called gastrinoma triangle), whereas other hormonally active tumors (such as insulinomas and VIPomas) arise from the pancreatic islet cells.[259-261] Nonfunctional pNETs are not associated with clinical symptoms caused by hypersecretion, but they can overproduce clinically silent hormones, such as pancreatic polypeptide and chromogranin A.[5,260,262-264]

Imaging modalities are similar for all GEP-NETs. Because GEP-NETs are often small and grow slowly, localization can be challenging and often multiple techniques are employed. Guidelines from the National Comprehensive Cancer Network (NCCN) recommend multiphasic CT or MRI as the first line for evaluation of pNETs, and MEN1 guidelines add endoscopic ultrasound as another modality.[216,262,265] The sensitivity for CT/MRI for pNET is 14% to 77% versus endoscopic ultrasound at 79% to 100%.[266] Endoscopic ultrasound (EUS) is especially helpful in the preoperative setting.[267] GEP-NETs generally possess somatostatin receptors. Thus, somatostatin receptor scintigraphy (i.e., an "octreotide scan") can be used as a second-line procedure.[262,268] FDG-PET is not routinely recommended due to the low proliferation rate of these neoplasms, but poorly differentiated tumors can be localized with FDG-PET.[262,269,270] Selective arterial injection of calcium or secretin with angiography or hepatic venous sampling can be helpful in tumor localization prior to surgery or identifying a functioning tumor among multiple nonfunctioning GEP-NETs. [271-273] A diagnosis of a malignant GEP-NET would be extraordinarily rare in childhood; guidelines regarding therapeutic approaches to malignant disease are available. [262]

Gastrinoma. Nearly 50% of GEP-NETs in MEN1 are gastrinomas, and 60% to 95% arise in the duodenum.[216,274] Gastrinomas are usually multiple, small (< 1 cm) tumors that result in elevated gastric acid production and recurrent peptic ulcer disease, referred to as the Zollinger-Ellison syndrome (ZES). Other symptoms can include diarrhea and steatorrhea.[216,266] The risk of malignancy to peripancreatic lymph nodes or, rarely, the liver is 50% to 60%.[255,266] The youngest reported age of diagnosis is 10 years old, but usually patients are older than 30 years, which is a decade earlier than sporadic cases.[230,275,276]

The diagnosis is established by an increased serum fasting gastrin concentration (usually > 1000 pg/mL) and elevated basal gastric acid secretion (gastric pH < 2).[5,262,277,278] Patients with borderline hypergastrinemia should be considered for the secretin stimulation test; after a 2 U/kg intravenous bolus,[279] a rise in gastrin ≥ 120 pg/mL from baseline affords the highest diagnostic sensitivity and specificity.[280] False-positive elevations of gastrin can occur due to use of proton pump inhibitors

or antacids, conditions of autoimmune chronic atrophic gastritis with achlorhydria, or chronic renal insufficiency.[281,282] Patients should therefore be off proton-pump inhibitors for at least 1 week, but this may not be possible in highly symptomatic patients.[262,278] Hypercalcemia can also stimulate gastrin secretion.[278]

Medical therapy with a potent oral proton pump inhibitor is the primary treatment of gastrinoma in MEN1 patients. Higher doses are generally needed compared with standard peptic ulcer disease.[216,273,283] Some patients may also require the addition of an H_2 receptor antagonist. The role of surgery in treating MEN1 patients with gastrinoma is controversial. Tumors are generally multiple and in the duodenum, so cure is less likely. If surgery is performed, both duodenal inspection and lymph node dissection are required.[259] For pancreatic gastrinomas or lesions > 2 cm, surgery should be considered to reduce risk of lymph node or hepatic metastasis.

Insulinoma. Insulinomas, β-islet cell tumors that oversecrete insulin, are the second most frequent functioning GEP-NET in MEN1. Most insulinomas are benign and occur in younger individuals as compared with sporadic cases.[216,275,284,285] Patients present with hypoglycemic symptoms that improve with glucose intake. The gold standard diagnostic test is a 72-hour fast. Diagnosis is established if serum glucose < 55 mg/dL with a concomitant insulin ≥ 3 μU/ml by immunochemiluminescent assay [ICMA], C-peptide ≥ 0.2 nmol/L and no detectable oral hypoglycemia in the blood.[286] One third of insulinoma patients will develop hypoglycemia at 12 hours, 80% at 24 hours, 90% at 48 hours, and 100% by 72 hours.[287]

Medical treatment with frequent carbohydrate meals, diazoxide, or octreotide is not always successful. Surgery is therefore considered the standard treatment.[287-289] In 10% of MEN1 patients, concomitant nonfunctioning pNETs are present, so preoperative localization can improve the success of surgery.[289] In addition to imaging modalities noted earlier for all GEP-NETs, intraoperative direct pancreatic ultrasonography is also likely to improve surgical success.[287] In contrast to other functioning GEP-NETs, insulinomas have a lower malignant potential, ranging from 0% to 20%.[275]

Glucagonoma. Glucagonomas secrete glucagon and are malignant in more than 70% of cases.[153] The syndrome produced is characterized by a skin rash in the groin and extremities (necrolytic migratory erythema) in 70% of patients, mild diabetes mellitus in 87%, weight loss in almost all patients, stomatitis, and anemia.[263] Glucagonomas commonly occur in the tail of the pancreas with liver metastases in 90% and lymph node metastases in 30% of cases.[262,263] Surgical resection is the treatment of choice but can be challenging given a metastatic presentation at diagnosis in 50% to 80% of cases.[284]

VIPoma. VIPomas are very rare tumors that secrete vasoactive intestinal peptide (VIP) and are associated with the Verner-Morrison syndrome of watery diarrhea, hypokalemia, and achlorhydria (i.e., pancreatic cholera).[263,290] Less frequent symptoms include glucose intolerance, hypercalcemia with normal parathyroid hormone levels, and episodes of cutaneous flushing. There can be co-secretion of VIP with calcitonin, serotonin, substance P, and some of the prostaglandins.[5] VIPomas are diagnosed by confirming plasma VIP levels > 60 pmol/L and stool volume > 0.5 to 1 L/day during a fast.[216] VIPomas are located usually in the tail of the pancreas. Surgical resection can be curative. However, VIPomas are often > 3 cm at presentation and metastatic spread to liver more than lymph nodes is present in about 50% at diagnosis, which may require systemic therapy or hepatic embolization.[5,291]

Nonfunctioning Pancreatic Neuroendocrine Tumors (NFpNETs). Nonfunctioning PETs (NFpNETs) were previously reported to occur in approximately 20% to 40% of MEN1 patients, but a prospective endoscopic ultrasound study found an incidence of almost 55%.[5,292] Thus, NFpNETs are becoming recognized as the most frequent GEP-NET in MEN1 patients. They are often sporadic with numerous microadenomas spreading throughout the pancreas,[293] and they have a significant potential for malignancy, especially with larger tumor size. Epidemiologic studies have shown as high as 27% of patients with NFpNETs between 2.1 to 3 cm have metastasis, compared with 11% of those ≤ 2 cm.[294] More important, NFpNETs are now reported to confer increased risk of death in MEN1 patients, comparable to thymic tumors and functioning NETs, except insulinomas.[236,237,295]

There is controversy on indications for surgical treatment. General consensus is that tumors ≥ 2 to 3 cm should be considered for resection because of increased risk of death and metastasis. Life expectancy of patients with NFpNETs ≤ 2 cm compared to MEN1 patients without pNET is not clearly different, so surgery may not be beneficial for smaller lesions.[294]

Pituitary Adenomas

Pituitary tumors occur in 15% to 50% of patients with MEN1.[296,297] The earliest reported age is 5 years old with a mean age of onset being 38 ± 15.3 years.[297,298] The clinical manifestations and treatment of pituitary tumors in MEN1 patients are similar to that for non-MEN1 patients. In MEN1, however, there does seem to be higher prevalence in women, higher rate of invasiveness and macroadenomas, more pluripotent tumors, and an overrepresentation of prolactinomas.[296,297,299] Approximately 60% of pituitary tumors in MEN1 patients secrete prolactin, < 25% secrete GH, < 10% are co-secreting, 5% secrete ACTH, and the remaining are nonfunctioning.[297,299] There is no increased prevalence of pituitary carcinoma or increased risk of death from having pituitary tumor.[236,299] Hypersecreting MEN1-associated pituitary tumors are reported to be more resistant to standard medical therapies.[297,299]

Other Clinical Manifestations

Carcinoid Tumors. In MEN1 patients, carcinoid tumors may be located in the bronchi, gastrointestinal (GI) tract,

or thymus. Carcinoids are generally diagnosed in the fifth decade of life, but the youngest reported case of thymic carcinoid in an MEN1 patient occurred in a patient who was 16 years old.[256] Thymic carcinoids are more prevalent in smokers and males (male:female ratio 20:1), whereas bronchial carcinoids are more prevalent in women (male:female ratio 1:4).[243,300] Bronchial carcinoids tend to behave indolently, whereas thymic carcinoids carry a significant increased risk for death in MEN1 patients.[236] Thymic carcinoids can still occur despite previous prophylactic transcervical thymectomy, presumably because the entire thymus is not removed via a cervical approach.[256]

Patients with thymic and bronchial carcinoids are generally asymptomatic at presentation, although thymic carcinoids have been associated with ectopic growth hormone releasing hormone and ACTH secretion.[301,302] CT (95% sensitivity) and MRI (100% sensitivity) are generally used for prospective screening, and MRI may be the preferred modality to avoid excessive exposure to ionizing radiation in a population already prone to tumor development.[256,303,304] Surgical resection is the treatment of choice.[303,305]

Type II gastric carcinoids are well-differentiated tumors with malignant potential that arise secondary to hypergastrinemia.[306-308] The incidence in patients with MEN1/ZES is as high as 30%.[309,310] Lesions are usually multiple, < 1 cm, and found by endoscopic surveillance. Treatment can include high doses of proton-pump inhibitors, removal of the gastrinoma as indicated, somatostatin analogs, and endoscopic excision for lesions < 1 cm or surgical resection for larger tumors.[306,311,312,306-308,310-314]

Adrenocortical Tumors. Unilateral and bilateral adrenocortical tumors are reported in 20% to 40% of MEN1 patients.[217,315,316] Adrenal hyperplasia, cortical adenomas, multiple adenomas, cysts, and adrenocortical carcinoma are the types of adrenal lesions reported. Most of these tumors are nonfunctioning, with 8% to 15% presenting with hypersecretion of aldosterone or cortisol.[315] The incidence of adrenocortical carcinoma is approximately 1% in MEN1 patients, but it increases to 13% for tumors larger than 1 cm.[254,315,316]

Cutaneous Manifestations. Often underappreciated as a component of the MEN1 phenotype, skin manifestations commonly occur in older MEN1 patients[317,318] (Figure 14-10). Multiple facial angiofibromas, benign tumors of blood vessels and connective tissue similar to those seen in tuberous sclerosis, occur in up to 88% of patients; other common cutaneous manifestations include collagenomas in 72% and lipomas in 34%.[317] Collagenomas are skin-colored cutaneous nodules, arranged symmetrically on the trunk, neck, and upper limbs. In general, these lesions do not require treatment, although lipomas can grow quite large and may need to be resected due to local symptoms.

Genetic Testing and Presymptomatic Screening

Many centers employ an integrated program of both genetic counseling and testing of at-risk individuals and clinical screening to detect the development of tumors in presymptomatic *MEN1* mutation carriers, with the hope that earlier diagnosis and treatment of these tumors may help to reduce morbidity and mortality.[216,319] However, at the present time, there is no specific treatment that will prevent an MEN1-associated disease, nor do any prophylactic therapies appear to be warranted. In addition, earlier identification of asymptomatic disease may potentially lead to earlier, possibly unnecessary treatment that may in fact have a negative long-term

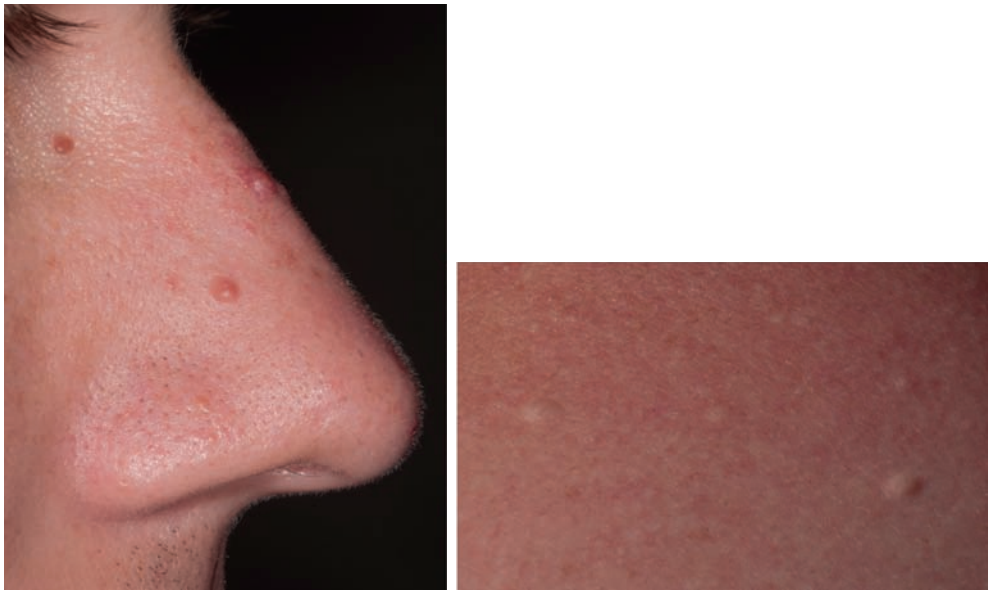


FIGURE 14-10 ■ Skin manifestations of MEN1. Angiofibromas (left panel) and collagenomas (right panel) are identified in the majority of adults with MEN1. Angiofibromas are telangiectatic papules characterized by fibrous tissue and vascular proliferation and distributed primarily on the nose and adjacent cheeks. Collagenomas are cutaneous stromal lesions that represent areas of excess collagen and tend to occur on the upper torso, neck, and shoulders. *(This image can be viewed in full color online at ExpertConsult.)*

impact. Thus, the goal of screening in MEN1 is primarily to detect a clinical manifestation at an earlier stage, specifically so that treatment can be rendered before malignancy develops in high-risk organs or serious ramifications from excess hormone secretion occur. Because of the long latency for disease manifestations, many unaffected individuals will potentially undergo years of episodic testing, which may have psychosocial and financial consequences, and this must be kept in mind as a prospective screening program is planned for any given MEN1 patient.

MEN1 germline mutation testing through an accredited clinical genetics laboratory should be offered to all index patients and first-degree relatives of those individuals with a known *MEN1* gene mutation, regardless of symptomatology.[216] Because relevant MEN1 disease has been diagnosed in children as young as 5 years of age (see Table 14-4), current practice is to offer testing at the earliest opportunity, but only after extensive genetic counseling. Mutational testing is also beneficial for the family members who do not end up carrying the mutation, so they can avoid unnecessary surveillance and relieve the burden of disease from their progeny.

Once an *MEN1* mutation is identified, the optimal ages to begin screening for disease have not been clearly established. MEN1 clinical practice guidelines based on systematic reviews of the medical literature and expert opinions were initially introduced in 2001 and updated in 2012.[153,216] Table 14-5 summarizes a suggested MEN1 screening program derived from the medical literature. Although most clinical centers employ annual laboratory evaluation, not all centers order radiographic screening for presymptomatic children and young adults as the guidelines have proposed, and the frequency of prospective radiographic imaging is an important area that warrants further research. In all cases, patient/parent preference, risk of screening (e.g., radiation exposure with CT), local resources, and clinical judgment should be considered.

Multiple Endocrine Neoplasia 2 (MEN2)

The original description of MEN2A is attributed to Sipple, when he reported the case of a 33-year-old man who died of intracranial hemorrhage and was found on autopsy to have bilateral pheochromocytomas, bilateral MTC, and probable parathyroid hyperplasia.[320] In 1962, Cushman proposed an association between these endocrine tumors,[321] and MEN2 was subsequently named as a distinct clinical syndrome.[322] Linkage analysis studies in large MEN2 kindreds led to the localization of the putative gene to chromosome 10.[323,324] Ultimately, mutations in the *RET* proto-oncogene were found to cause MEN2A.[137,138]

The original characterization of the MEN2B phenotype in the English literature was made by Williams and Pollock in 1966,[325] and MEN2B was further distinguished as a variant of hereditary MTC with a mucosal neuroma phenotype.[326] In 1994, MEN2B was reported to also be secondary to activating mutations in *RET*.[139,140]

As a result of the initial collaborative efforts of the International *RET* Mutation Consortium, it quickly became apparent that only a limited number of mutations were associated with MEN2 and that strong genotype-phenotype correlations were present[327](see Figure 14-5). Because of the high penetrance of MTC in individuals with a *RET* mutation, there was a rapid incorporation of genetic testing into management algorithms for patients with MTC and their families. Predictive genetic testing thus ushered in the era of performing total prophylactic thyroidectomies for presymptomatic individuals carrying a high-risk *RET* germline mutation.[146] As a result, much of the decision making and counseling of affected children and their families falls into the hands of the pediatric provider.[328,329]

TABLE 14-5 **Suggested Biochemical and Radiologic Screening for Asymptomatic MEN1 Patients (derived from references 216, 230, 256)**

Tumor	Age to Begin (years)	Biochemical Test Annually	Imaging Test*
Parathyroid	8	Intact PTH, calcium	None
Pituitary	5	Prolactin, IGF-1	MRI (every 3-5 years)
Enteropancreatic NET			
Gastrinoma	20	Gastrin	None
Insulinoma	5	Fasting glucose, insulin	None
Other pNETs	< 10	Chromogranin-A, pancreatic polypeptide, glucagon, VIP	MRI, CT, or EUS (every 1-3 years)
Carcinoid			
Gastric	NA	None	Gastric endoscopy (every 3 years if ZES present)
Thymic	15	None	CT or MRI chest (every 1-3 years)
Bronchial	15	None	CT or MRI chest (every 1-3 years)
Adrenal	< 10	If symptoms of functioning tumor or > 1 cm on imaging	CT or MRI abdomen (every 3 years)

*If available, MRI is the preferred screening modality to decrease lifetime exposure to ionizing radiation. Some centers will image less frequently than the above published recommendations and use individual risk factors for rarer MEN1 manifestations to guide imaging frequency.

MEN2A

MEN2A accounts for 90% to 95% of MEN2 cases and is a highly penetrant, autosomal-dominant endocrine tumor syndrome characterized by the development of MTC in > 90% of *RET* mutation carriers. Depending on the specific mutation, pheochromocytoma or primary hyperparathyroidism occurs in 0% to 50% and 0% to 20% of individuals with MEN2A, respectively.[330] Furthermore, activating mutations in *RET* are also associated with Hirschsprung disease[331-333] and cutaneous lichen amyloidosis,[334,335] a dermatologic disorder of intense pruritus and secondary skin changes that is typically located in the interscapular region of the back.

In MEN2A, mutations are located mostly in the extracellular cysteine-rich domain of the *RET* proto-oncogene, usually in exon 10 (codons 609, 611, 618, or 620) or exon 11 (codon 634)[160,327,336,337] (see Figure 14-5). Mutations in *RET* codon 634 account for ~50% of *RET*-positive MEN2A cases, whereas exon 10 mutations account for about 16%.[160,336] As molecular testing becomes widespread in patients with MTC, more *RET* mutations and DNA variants are being identified, which is contributing to an everchanging spectrum of the MEN2A genotype and phenotype.[338,339]

MTC in MEN2A. MEN2A-related MTC, when identified during childhood, is usually diagnosed after early thyroidectomy directed by genetic testing results and is usually a microcarcinoma < 1 cm in size.[150,169,170] Most children with MEN2A will have a positive family history; de novo *RET* mutations represent only 2% to 9% of cases.[340,341] A strong genotype-phenotype relationship is present, such that the rapidity with which a mutation carrier will develop MTC can be estimated.[2,327,330,339,342,343] Patients with mutations in *RET* codon 634 have the highest risk of malignant C-cell disease followed by those with mutations in codons 609, 611, 618, 620, or 630, whereas mutations in codons 768, 790, 804, or 891 impart the lowest risk for clinically aggressive MTC.[141,330] However, the natural history of MTC in MEN2A remains highly variable.

Pheochromocytoma in MEN2A. PHEO arising within the context of MEN2A is an adrenergic tumor that usually arises within a background of adrenal medullary hyperplasia.[147,344-347] The risk for PHEO is highest in codon 634 mutations and to a much lesser degree with mutations in codons 609, 611, 618, and 620. [327,348-350,351,352] Diagnosis is most likely during the fourth and fifth decades of life,[66,344,348,353,354] but PHEO have been rarely reported to occur in children < 10 years of age.[141,153] Typically, a PHEO develops only after MTC is identified or is diagnosed at the same time as the MTC, but in up to 30% of cases, it may be the initial clinical presentation.[344,347,352,354,355] Guidelines published in 2009 have given clear recommendations for screening of pheochromocytoma in children with MEN2,[141] but some centers screen children with high-risk *RET* mutations annually starting at the age of 5 years.[2,31]

Primary Hyperparathyroidism in MEN2A. Patients with MEN2A are at risk of developing PHPT due to parathyroid adenomas and hyperplasia. PHPT is chiefly associated with codon 634 mutations and less commonly described in other *RET* mutations (see Figure 14-1).[141,356,357] Onset during childhood is extraordinarily rare but has been described in children as young as 5 years of age.[358]

MEN2B

MEN2B is a highly penetrant disease with an autosomal dominant pattern of inheritance that accounts for 5% to 10% of MEN2 cases.[160] MEN2B is almost always (> 95%) due to a single mutation in exon 16 (M918T, which results in an amino acid substitution from methionine to threonine at codon 918) that is located in the intracellular tyrosine kinase domain of RET[327,336,337] (see Figure 14-5). Rare MEN2B cases can be attributed to double *RET* mutations involving codon 804[359] or a mutation in codon 883 (Ala883Phe, exon 15), which may be less aggressive than the Met918Thr mutation.[360] Unlike MEN2A, most cases of MEN2B arise as a result of a de novo mutation, with the child having unaffected parents.[152,361] For this reason and due to the rarity of the syndrome, the diagnosis of MEN2B is almost always delayed, even in the presence of obvious clinical features.[152]

MEN2B is characterized by the development of MTC (100% of cases), PHEO (up to 50% of cases), and a highly penetrant and characteristic clinical phenotype (100% of cases).[141,362-371] Although the clinical phenotype is present in all patients, individual manifestations have a variable presentation and are age dependent.[366] Ganglioneuromas are a major clinical manifestation and occur on the lips, tongue (Figure 14-11), or conjunctiva ("mucosal neuromas"), in addition to the urinary system and gastrointestinal tract. The symptoms of intestinal ganglioneuromatosis include constipation and feeding problems in infancy and the development of megacolon. A second component of the MEN2B phenotype is the presence of musculoskeletal abnormalities, including a Marfanoid body habitus (Figure 14-12), narrow long facies, pes cavus, pectus excavatum, high-arched palate, scoliosis, or slipped capital femoral epiphysis. Other clinical features include joint laxity, hypotonia or proximal muscle weakness, thickened lips, ophthalmologic findings (inability to make tears in infancy; thickened and everted eyelids; mild ptosis and prominent corneal nerves), and pubertal delay. The oral manifestations of MEN2B (see Figure 14-11) are highly penetrant and often lead to the clinical diagnosis. Primary hyperparathyroidism is not a feature of MEN2B.

MTC in MEN2B. MTC that occurs in MEN2B is a highly aggressive malignancy with very early onset of metastasis (lymph node metastases have been documented within the first year of life[171]), a high cancer stage (defined by the TNM classification system) at diagnosis, and an average age of MTC onset (second decade of life) that is about 10 years earlier than that observed in MEN2A cases.[150,153,348,366,372] Earlier MTC onset is observed in children with MEN2B who have severe intestinal manifestations, who also seem to have a poorer prognosis than those without these intestinal manifestations.[367] Morbidity and mortality rates are much higher in MEN2B, although the

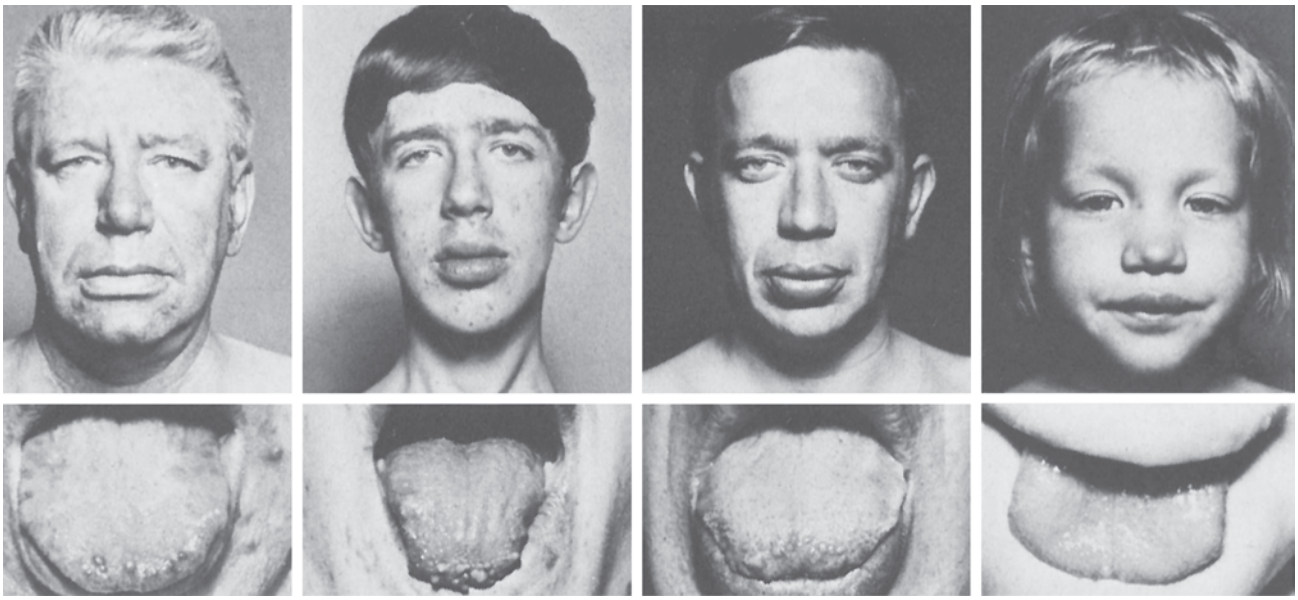


FIGURE 14-11 ■ Typical MEN2B facies. The typical MEN2B facies includes a narrow long face, eyelid eversion, thickened lips, and oral mucosal neuromas as demonstrated in several affected members of a single kindred. (From Sizemore, G. W., Health, H., 3rd, & Carney, J. A. (1980). Multiple endocrine neoplasia type 2. *Clin Endocrinol Metab, 9*, 299–315.)

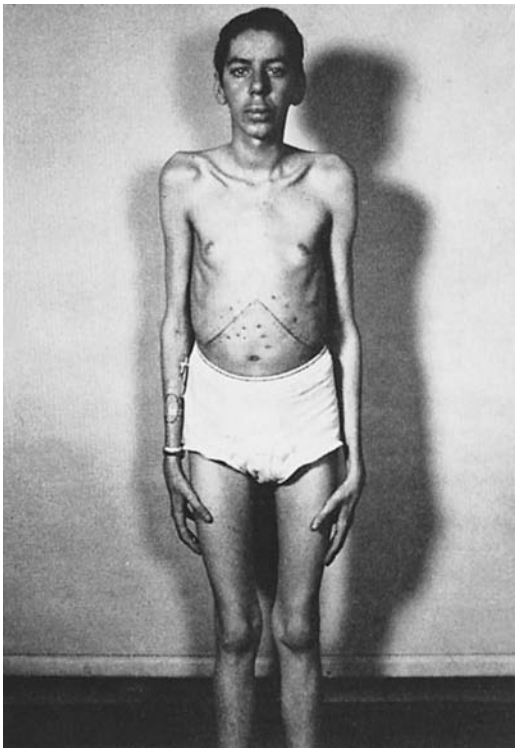


FIGURE 14-12 ■ Patient with typical features of MEN2B. Note the Marfanoid body habitus, thickened lips, and abdominal scars that reflect surgery for pheochromocytoma. (From Melvin, K. E., Tashjian, A. H., Jr, & Miller, H. H. (1972). Studies in familial (medullary) thyroid carcinoma. *Recent Prog Horm Res, 28*, 399–470.)

higher mortality rate may reflect the more advanced tumor stage at presentation rather than an intrinsically more aggressive carcinoma.[141,151]

Pheochromocytoma in MEN2B. Compared with the more heterogenous MEN2A population, patients with MEN2B have a higher lifetime risk (50%) of developing pheochromocytoma.[352] The clinical phenotype of the disease is not different than that seen in MEN2A. Despite an aggressive MTC phenotype and a constitutively activated RET tyrosine kinase, the rate of the development of malignant pheochromocytoma in patients with MEN2B does not seem higher,[125] nor does the age of onset seem different from patients with MEN2A, although some studies have suggested an earlier age of diagnosis.[347] Two notable differences between MEN2A and MEN2B are that PHEO is even less likely to be diagnosed before MTC and that historical mortality from PHEO is lower compared with death from MTC.[344,352]

Timing of Thyroidectomy in MEN2

The use of genetic testing to determine the timing of thyroid surgery in MEN2 has become increasingly complicated due to an improved understanding of the variable aggressiveness of MTC, even among family members with the same *RET* mutation.[373] Furthermore, novel and rare *RET* DNA variants that have not yet been fully established as pathogenic (the so-called variants of unknown significance) have been identified.[339,374,375] These considerations, plus the addition of routine ultrasonography and calcitonin screening,[141] have led to the contemporary dilemma of determining the optimal timing of prophylactic thyroidectomy for any individual patient. In any event, it is imperative for providers who care for children with MEN2 to recognize who needs early intervention to prevent morbidity and mortality from MTC and not to overtreat those children who are unlikely to develop clinically relevant disease over the short term.

In general, there is widespread agreement that the therapeutic goal in a child with MEN2 is to remove the at-risk thyroid before incurable MTC metastasis occurs

while minimizing potential medical and surgical morbidity. In experienced surgeons' hands, children who have a total thyroidectomy performed prior to the onset of metastatic disease have an excellent chance of remaining disease-free.[149,150,169,170,376-378] Cure is also possible even for children with high risk MEN2 despite surgery not being performed at the earliest prescribed ages.*

In the era before RET genetic testing, calcitonin response to the intravenous administration of potent calcitonin secretagogues (calcium, pentagastrin)[173,380] was used to identify mutation carriers and determine the timing of surgery. After the identification of RET as the gene responsible for MEN2, recommendations regarding the appropriate age for surgery were made on the basis of the specific *RET* mutation and the earliest age at which clinically relevant disease had been described for that particular mutation.[146,153] Arising from the 7th International Workshop on MEN in 1999, a consensus statement published in 2001 was the first to classify *RET* proto-oncogene mutations into three risk levels.[153] In 2009, the American Thyroid Association (ATA) published guidelines that expanded the previous consensus document, incorporating updated data on *RET* mutations and phenotypes and recognizing codon 634 mutations within a separate risk level (see Figure 14-5).[141] The ATA guidelines stratified all known *RET* mutations into one of four risk levels (ATA risk levels A through D), and the concept of safely delaying prophylactic thyroidectomy while offering careful expectant monitoring in children with lower risk level A and B mutations was introduced. The approach to care is evolving to incorporate knowledge regarding the RET genotype and clinical data such as thyroid ultrasound and calcitonin levels.[2,377,381,382]

Multiple Endocrine Neoplasia 4 (MEN4)

Multiple endocrine neoplasia type 4 (MEN4) is a newly recognized syndrome of multiple endocrine tumors that is caused by germline mutations in the *CDKN1B* gene on chromosome 12p13, which encodes the cell cycle inhibitor p27 and plays a critical role in regulating cell proliferation.[383] Homozygous mutations in *CDKN1B* were originally identified to cause MENX, a multitumor syndrome in the rat that has phenotypic features of both MEN1 and MEN2.[383,384] Subsequently, studies in humans without a proven MEN1 mutation confirmed that heterozygous mutations in the human homologue *CDKN1B* are also associated with an MEN1-like phenotype, subsequently called MEN4. Individuals with a germline *CDKN1B* mutation are at risk for developing tumors of the pituitary, parathyroid glands, adrenals, or GI neuroendocrine system, among others.[383-386] Although MEN4 is a rare cause of multiple endocrine neoplasia, mutational screening of the *CDKN1B* gene should be considered in patients with a clinical diagnosis of MEN1 but negative *MEN1* genetic testing.[216]

*See references 149, 150, 168-170, 329, 360, 366, 371, 377, and 379.

Von Hippel-Lindau Disease

VHL is an autosomal dominantly inherited disorder caused by germline mutations in the *VHL* tumor suppressor gene, first identified in 1993[387] and characterized by a clinical phenotype with variable penetrance that includes hemangioblastomas of the retina (also called retinal angiomas) and central nervous system; renal cysts and clear cell renal cell carcinoma; noradrenergic PHEO/PGL; pancreatic cysts, cystadenomas, and pancreatic NETs; endolymphatic sac tumors; and cystadenomas of the epididymis in males and broad ligament of the uterus in females.[388,389,390] About 20% of patients with VHL harbor a de novo mutation, and genetic testing is highly reliable, identifying a germline mutation in almost all clinically affected individuals.[390,391] The majority of VHL mutations are small deletions/insertions or point mutations, but about 25% are due to partial or whole gene deletion of the *VHL* gene, underscoring the importance of utilizing both sequence analysis and deletion/duplication analysis when performing genetic testing for VHL.[390]

PHEO/PGL occur in 10% to 20% of VHL patients,[388] (see Figure 14-1) primarily in individuals with missense mutations of the VHL gene.[388,389,392] PHEO/PGL can be one of the earliest clinical manifestations of VHL, having been diagnosed in patients as young as age 2 years,[393] and it can also be the sole clinical manifestation of VHL, as seen in VHL type 2C.[388,390,394] Prospective screening is generally recommended to commence by the age of 5 years in children with missense mutations or a family history of PHEO/PGL.[31,390,395] Although almost all VHL-related PHEO/PGL are functional noradrenergic tumors (see the previous section on PHEO and PGL), nonfunctional parasympathetic PGL occurring in the context of VHL have been described.[43,396,397]

Pancreatic NETs (pNETs) develop in 15% of VHL patients.[398] These tumors are clinically nonfunctional, although they can be malignant, especially with lesions > 3 cm, a mutation in exon 3, or a fast tumor doubling rate.[399,400] Prospective screening for pNET is performed as a component of screening for other intra-abdominal manifestations and includes intermittent ultrasound or MRI starting at age 16.[389,390,394]

Finally, testosterone-secreting lipid cell tumors have been described and so should be considered in women with VHL who present with an adnexal mass, amenorrhea, or hirsutism.[401]

OTHER TUMOR SYNDROMES ASSOCIATED WITH ENDOCRINE NEOPLASIA

APC-Associated Polyposis

The *APC*-associated polyposis disorders result from dominantly inherited germline mutations in the *APC* (adenomatous polyposis coli) tumor suppressor gene and include the clinical syndromes of familial adenomatous polyposis (FAP), attenuated FAP, Gardner syndrome, and Turcot syndrome.[402] Patients with an *APC* mutation are at greatest risk for colon cancer but may also develop endocrine neoplasia. Adrenocortical tumors (ACT) are two to four times as high in the *APC*-associated polyposis

syndromes compared with the general population.[403,404] Similar to sporadic adrenal neoplasia, *APC*-associated ACTs may be hormonally active or nonfunctioning and may rarely be malignant.[405] Patients with an *APC* mutation also have a 1% to 2% lifetime risk for thyroid malignancy,[403] with some studies suggesting a prevalence in FAP patients as high as 12%.[406] The primary subtype is papillary thyroid carcinoma, typically the cribriform-morular histologic variant, and it is a diagnosis almost exclusively made in young women during the third decade of life.[406-409]

Beckwith-Wiedemann Syndrome

The Beckwith-Wiedemann syndrome (BWS) is a congenital overgrowth disorder associated with abnormal regulation of gene transcription in an imprinted domain on chromosome 11p15.5 (also known as the BWS critical region).[410,411] Major findings include macrosomia and hemihyperplasia, macroglossia, visceromegaly, embryonal tumors (e.g., Wilms tumor, hepatoblastoma, neuroblastoma, pancreatoblastoma, and rhabdomyosarcoma), omphalocele, neonatal hypoglycemia, ear creases/pits, adrenocortical tumors/cytomegaly, and renal abnormalities (e.g., medullary dysplasia, nephrocalcinosis, medullary sponge kidney, and nephromegaly) (see Chapter 6). A variety of benign and malignant tumors, typically restricted to childhood onset, have been associated with BWS and occur in up to 7.5% of all cases.[411] ACTs, both benign and malignant, are among the more common neoplasia identified in BWS, representing up to 8% of benign and 7% of malignant tumors.[412] Fetal adrenocortical cytomegaly (a component of the underlying visceromegaly) and adrenal cystic lesions also occur.[412,413] Pheochromocytoma and thyroid carcinoma have also been reported,[412] but given only isolated cases, it remains unclear if these are truly associated with BWS.

Carney Triad

The Carney triad was originally described in 1977 as the association of gastric epithelioid leiomyosarcoma (later renamed GIST), paraganglioma, and pulmonary chondroma.[414] Over time, the phenotype has expanded to include clinically nonfunctioning adrenocortical tumors and esophageal leiomyomas.[415,416] The Carney triad affects primarily young women (85%) with a mean age of onset of 20 years.[7-48,416] Although it has been accepted to be a genetic disorder, the responsible gene(s) remains unknown. Due to incomplete expression of the phenotype, PGL is not present in all patients suspected to have the Carney triad.[415] When it occurs, PGL presents with catecholamine excess or due to tumor mass effects. PHEO can occur in a minority of patients, and the aortopulmonary body is a common site for development of PGL, although instances of PGL occur equally in the head and neck, thorax, and abdomen.[416]

Li-Fraumeni Syndrome

Li-Fraumeni syndrome (LFS) is a highly penetrant cancer-predisposition syndrome caused by dominantly inherited germline mutations in the tumor suppressor gene *TP53* (tumor protein p53).[417] Soft tissue and bone sarcomas, premenopausal breast cancer, adrenocortical carcinoma, and brain tumors represent the core tumors of LFS.[418,419] The major endocrine tumor associated with LFS is ACT, although several cases of thyroid carcinoma have also been reported.[420] The adrenal cortex is the third most common site of neoplasia development in LFS.[418,421] ACT have a very early age of onset, with the diagnosis most common in young children, who may represent the proband for a LFS kindred.[418,422,423] In southern Brazilian families, the *TP53* mutation Arg337His is associated with a higher susceptibility to isolated ACT in children (median age of diagnosis 3 years; range 4 months to 13.5 years).[424] Young children with an ACT typically present with virilization,[422,424] but at all ages, these tumors can present with other signs/symptoms of adrenocortical hyperfunction (such as Cushing syndrome) or with symptoms due to tumor mass effect, particularly in the case of nonfunctioning tumors.

Neurofibromatosis Type 1

Neurofibromatosis type 1 (NF1) is an autosomal dominant disorder caused by mutations in the *NF1* gene, a tumor suppressor gene located on chromosome 17.[425] Café-au-lait macules, neurofibromas, axillary and inguinal freckling, optic pathway gliomas, skeletal dysplasia, and Lisch nodules of the iris are the main clinical features.

NF1 patients are at an increased risk of developing PHEO/PGL and GEP-NETs. A catecholamine-producing tumor is diagnosed in 2% to 6% of NF1 patients.[275,426] PHEO (10% bilateral) represent the majority of NF1-associated cases, but intraabdominal and pelvic PGL are diagnosed in up to 6%.[426,427] Onset is typically in adulthood (mean age of presentation 41.6 years[427]), and NF1-associated PHEO are characterized by an adrenergic phenotype.[428] Malignancy is uncommon but does occur in about 10% of cases.[427] Rarely, a composite PHEO (a mixed tumor comprised of PHEO and neuroblastoma, ganglioneuroma, or ganglioneuroblastoma) may occur in the setting of NF1.[429] GEP-NET occur in $<$ 10% of NF-1 patients and are almost exclusively duodenal somatostatinomas that occur in the periampullary region.[275,430] NF1-associated somatostatinomas are typically diagnosed during the fifth decade of life.[430] Other types of GEP-NETs that have been reported in NF1 patients include insulinomas, gastrinomas, and nonfunctioning pancreatic endocrine tumors.[275,431,432]

Several case reports of primary hyperparathyroidism due to benign and malignant parathyroid tumors and adrenocortical tumors have also been reported to occur in NF1.[405,433,434] However, it is unclear if mutations in the *NF1* gene are directly involved in the pathogenesis of these tumors. Finally, a unique endocrinopathy associated with NF1 in children with infiltrating optic pathway gliomas is the clinical syndrome of gigantism due to unregulated growth hormone secretion.[435]

Peutz-Jeghers Syndrome

Peutz-Jeghers syndrome (PJS) is an autosomal dominant syndrome characterized by hamartomatous polyps in the gastrointestinal tract and mucocutaneous hyperpigmentation.[436,437] It is caused primarily by inactivating mutations in the *STK11* (*LKB1*) gene. Individuals with PJS are at an increased risk for various malignancies over the course of their lives,[438] chiefly luminal gastrointestinal cancers and breast cancer, followed by pancreatic cancer, cervical and ovarian malignancies, and lung cancer. Several case reports of differentiated thyroid cancer have been reported,[439] but it remains unclear if this is a random occurrence or a direct association with PJS.

Patients with PJS are at risk for unique gonadal tumors that may clinically present with an endocrinopathy during childhood: Sertoli cell tumors of the testis in males and ovarian sex cord tumors with annular tubules (SCTATs) in females. Testicular Sertoli cell tumors in PJS are clinically benign lesions that typically occur in prepubertal boys (mean age 6.8 years) who present with gynecomastia and bilateral (rarely unilateral) testicular enlargement.[189] The microscopic findings are distinctive and the term *intratubular large cell hyalinizing Sertoli cell neoplasia* has been proposed.[189] Calcifications are unusual and not extensive in these lesions, although large-cell calcifying Sertoli cell tumors (similar to those identified in the Carney complex) can also occur within the PJS spectrum.[440] The gynecomastia is due to estrogen overproduction secondary to high levels of aromatase activity,[189] making the use of an aromatase inhibitor a rational approach to medically treating the gynecomastia in such cases.[441] SCTAT is a distinctive neoplasm that has morphologic features that lie between those of a Sertoli cell tumor and a granulosa cell tumor.[442] When occurring in the PJS, these tumors are typically small, calcified, multifocal and bilateral lesions, as compared with non-PJS SCTATs. SCTAT is almost always a benign neoplasm with a mean age of diagnosis of 27 years (range 4 to 57 years).[442] Clinical presentation is often due to estrogen overproduction and manifested by menstrual irregularity in postmenarchal women and sexual precocity in prepubertal girls. Abdominal pain and a palpable adnexal mass can also be presenting features, and SCTAT can also be an incidental finding.[442]

PTEN Hamartoma Tumor Syndrome

The *PTEN* hamartoma tumor syndrome (PHTS) results from germline mutations in the *PTEN* (phosphatase and tensin homologue) tumor suppressor gene and is inherited in an autosomal dominant manner. The PHTS encompasses the clinical phenotypes of Cowden syndrome, Bannayan-Riley-Ruvalcaba syndrome, *PTEN*-related Proteus syndrome, and Proteus-like syndrome.[337,443,444] Cowden syndrome (CS), the most common PHTS phenotype, is a multiple hamartoma syndrome with an increased risk for both benign and malignant tumors arising from the thyroid follicular cell and, in women, the breast and endometrium.[337] Macrocephaly is a consistent finding, occurring in 94% of patients with a *PTEN* mutation,[445] and common skin manifestations include papillomatous papules, trichilemmomas, and acral keratoses. Thyroid neoplasia occurs in the majority of individuals with CS and primarily includes multiple adenomatous nodules, follicular adenomas, and differentiated thyroid carcinoma (papillary and follicular thyroid carcinomas).[446-448] Follicular thyroid carcinoma appears to be overrepresented in *PTEN* mutation positive patients, but papillary carcinomas are also common.[447-449] The risk of thyroid neoplasia begins during early childhood, with a reported case of follicular thyroid carcinoma as young as age 7 years.[447]

Tuberous Sclerosis Complex

The tuberous sclerosis complex (TSC) is an autosomal dominant, multiorgan hamartomatous disorder characterized by abnormalities of the skin (hypomelanotic macules, facial angiofibromas, shagreen patches, fibrous facial plaques, ungual fibromas); brain (cortical tubers, subependymal nodules and subependymal giant cell astrocytomas, seizures, intellectual disability/developmental delay); kidney (angiomyolipomas, cysts, renal cell carcinomas); heart (rhabdomyomas, arrhythmias); and lungs (lymphangioleiomyomatosis).[450] The two known genes that cause the TSC include *TSC1* and *TSC2*, which encode for the proteins hamartin and tuberin, respectively.[451,452] Various endocrine neoplasms occurring within the context of the TSC have been reported, including functional pituitary adenomas, parathyroid hyperplasia and adenomas, GEP-NETs, and a single case report of a PHEO.[453] The pNETs can be benign or malignant and functioning or nonfunctioning, with a large proportion of cases associated with *TSC2* gene mutations.[454] Insulinomas appear to be overrepresented in TSC patients, so a low threshold should exist for clinical evaluation in those with classic signs or symptoms of hypoglycemia or worsening neurologic symptoms.[453]

SUMMARY AND FUTURE DEVELOPMENTS

Although rare, neuroendocrine tumors diagnosed during childhood, especially PHEO and MTC, are frequently associated with a known tumor predisposition syndrome. Advances in genomic medicine have improved our understanding of the etiology and pathophysiology of these disorders, which in turn has changed the way physicians manage patients with the diseases discussed in this chapter. Because it is a rapidly evolving field, the reader should continue to seek the most current information for important clinical decisions regarding individual patient care. Future research will add to our knowledge regarding genotype-phenotype correlations, optimal screening strategies for asymptomatic children known to carry a disease-causing mutation, timing of interventions such as early thyroidectomy in MEN2 and parathyroidectomy in MEN1, and the treatment of advanced or inoperable disease with newer targeted therapies.

REFERENCES

1. Resta, R., Biesecker, B. B., Bennett, R. L., et al. (2006). A new definition of Genetic Counseling: National Society of Genetic Counselors' Task Force report. *J Genet Couns*, *15*, 77–83.
2. Waguespack, S. G., Rich, T. A., Perrier, N. D., et al. (2011). Management of medullary thyroid carcinoma and MEN2 syndromes in childhood. *Nat Rev Endocrinol*, *7*, 596–607.
3. Lenders, J. W., Eisenhofer, G., Mannelli, M., & Pacak, K. (2005). Phaeochromocytoma. *Lancet*, *366*, 665–675.
4. Mannelli, M., Dralle, H., & Lenders, J. W. (2012). Perioperative management of pheochromocytoma/paraganglioma: is there a state of the art? *Horm Metab Res*, *44*, 373–378.
5. DeLellis, R. A., Lloyd, R. V., Heitz, P. U., & Eng, C. (Eds.), (2004). *World Health Organization classification of tumours*. pathology and genetics of tumours of endocrine organs. Lyon: IARC Press.
6. Goodman, M. T., Gurney, J. G., Smith, M. A., & Olshan, A. F. (1999). Sympathetic nervous system tumors. In L. A. G. Ries, M. A. Smith, J. G. Gurney, et al. (Eds.), *Cancer incidence and survival among children and adolescents: United States SEER Program 1975–1995*. (pp. 65–72). Bethesda, MD: National Cancer Institute, SEER Program. NIH Pub. No. 99-4649.
7. Spoudeas, H. A. (Ed.), (2005). *Paediatric endocrine tumours*. West Sussex, United Kingdom: Novo Nordisk Ltd.
8. Barontini, M., Levin, G., & Sanso, G. (2006). Characteristics of pheochromocytoma in a 4- to 20-year-old population. *Ann N Y Acad Sci*, *1073*, 30–37.
9. Neumann, H. P., Bausch, B., McWhinney, S. R., et al. (2002). Germ-line mutations in nonsyndromic pheochromocytoma. *N Engl J Med*, *346*, 1459–1466.
10. Beltsevich, D. G., Kuznetsov, N. S., Kazaryan, A. M., & Lysenko, M. A. (2004). Pheochromocytoma surgery: epidemiologic peculiarities in children. *World J Surg*, *28*, 592–596.
11. Ross, J. H. (2000). Pheochromocytoma: special considerations in children. *Urol Clin North Am*, *27*, 393–402.
12. Stackpole, R. H., Melicow, M. M., & Uson, A. C. (1963). Pheochromocytoma in children. Report of 9 case and review of the first 100 published cases with follow-up studies. *J Pediatr*, *63*, 314–330.
13. Ciftci, A. O., Tanyel, F. C., Senocak, M. E., & Buyukpamukcu, N. (2001). Pheochromocytoma in children. *J Pediatr Surg*, *36*, 447–452.
14. Perel, Y., Schlumberger, M., Marguerite, G., et al. (1997). Pheochromocytoma and paraganglioma in children: a report of 24 cases of the French Society of Pediatric Oncology. *Pediatr Hematol Oncol*, *14*, 413–422.
15. King, K. S., Prodanov, T., Kantorovich, V., et al. (2011). Metastatic pheochromocytoma/paraganglioma related to primary tumor development in childhood or adolescence: significant link to SDHB mutations. *J Clin Oncol*, *29*, 4137–4142.
16. Deal, J. E., Sever, P. S., Barratt, T. M., & Dillon, M. J. (1990). Phaeochromocytoma: investigation and management of 10 cases. *Arch Dis Child*, *65*, 269–274.
17. Wyszynska, T., Cichocka, E., Wieteska-Klimczak, A., et al. (1992). A single pediatric center experience with 1025 children with hypertension. *Acta Paediatr*, *81*, 244–246.
18. Young, W. F., Jr. (2011). Endocrine hypertension. In S. Melmed, K. S. Polonsky, P. R. Larsen, & H. M. Kronenberg (Eds.), *Williams textbook of endocrinology* (12th ed.) (pp. 545–577). Philadelphia: Saunders Elsevier.
19. Armstrong, R., Sridhar, M., Greenhalgh, K. L., et al. (2008). Phaeochromocytoma in children. *Arch Dis Child*, *93*, 899–904.
20. Timmers, H. J., Pacak, K., Huynh, T. T., et al. (2008). Biochemically silent abdominal paragangliomas in patients with mutations in the succinate dehydrogenase subunit B gene. *J Clin Endocrinol Metab*, *93*, 4826–4832.
21. Cryer, P. E. (1980). Physiology and pathophysiology of the human sympathoadrenal neuroendocrine system. *N Engl J Med*, *303*, 436–444.
22. Tischler, A. S. (2008). Pheochromocytoma and extra-adrenal paraganglioma: updates. *Arch Pathol Lab Med*, *132*, 1272–1284.
23. Amar, L., Bertherat, J., Baudin, E., et al. (2005). Genetic testing in pheochromocytoma or functional paraganglioma. *J Clin Oncol*, *23*, 8812–8818.
24. Westfall, T. C., & Westfall, D. P. (2011). Neurotransmission: the autonomic and somatic motor nervous systems. In L. L. Brunton (Ed.), *Goodman & Gilman's the pharmacological basis of therapeutics* (12th ed.) (pp. 171–218). New York: McGraw-Hill.
25. Westfall, T. C., & Westfall, D. P. (2011). Catecholamines and sympathomimetic drugs. In L. L. Brunton (Ed.), *Goodman & Gilman's the pharmacological basis of therapeutics* (12th ed.) (pp. 277–333). New York: McGraw-Hill.
26. Sanders-Bush, E., & Hazelwood, L. (2011). 5-Hydroxytryptamine (serotonin) and dopamine. In L. L. Brunton (Ed.), *Goodman & Gilman's the pharmacological basis of therapeutics* (12th ed.) (pp. 335–361). New York: McGraw-Hill.
27. Eisenhofer, G., Kopin, I. J., & Goldstein, D. S. (2004). Catecholamine metabolism: a contemporary view with implications for physiology and medicine. *Pharmacol Rev*, *56*, 331–349.
28. Wurtman, R. J., & Pohorecky, L. A. (1971). Adrenocortical control of epinephrine synthesis in health and disease. *Adv Metab Disord*, *5*, 53–76.
29. Axelrod, J., & Reisine, T. D. (1984). Stress hormones: their interaction and regulation. *Science*, *224*, 452–459.
30. Pham, T. H., Moir, C., Thompson, G. B., et al. (2006). Pheochromocytoma and paraganglioma in children: a review of medical and surgical management at a tertiary care center. *Pediatrics*, *118*, 1109–1117.
31. Waguespack, S. G., Rich, T., Grubbs, E., et al. (2010). A current review of the etiology, diagnosis, and treatment of pediatric pheochromocytoma and paraganglioma. *J Clin Endocrinol Metab*, *95*, 2023–2037.
32. Kita, T., Imamura, T., Date, H., et al. (2003). Two cases of pheochromocytoma associated with tetralogy of Fallot. *Hypertens Res*, *26*, 433–437.
33. Rich, B. S., Moo, T. A., Mark, S., et al. (2013). Sympathetic paraganglioma in a patient with unrepaired tetralogy of Fallot: a case report and review of the literature. *J Clin Endocrinol Metab*, *98*, 7–12.
34. Manger, W. M. (2009). The protean manifestations of pheochromocytoma. *Horm Metab Res*, *41*, 658–663.
35. Karagiannis, A., Mikhailidis, D. P., Athyros, V. G., & Harsoulis, F. (2007). Pheochromocytoma: an update on genetics and management. *Endocr Relat Cancer*, *14*, 935–956.
36. Januszewicz, P., Wieteska-Klimczak, A., & Wyszynska, T. (1990). Pheochromocytoma in children: difficulties in diagnosis and localization. *Clin Exp Hypertens A*, *12*, 571–579.
37. Ein, S. H., Pullerits, J., Creighton, R., & Balfe, J. W. (1997). Pediatric pheochromocytoma: a 36-year review. *Pediatr Surg Int*, *12*, 595–598.
38. Sullivan, J., Groshong, T., Tobias, & J. D. (2005). Presenting signs and symptoms of pheochromocytoma in pediatric-aged patients. *Clin Pediatr* (Phila), *44*, 715–719.
39. Bissada, N. K., Safwat, A. S., Seyam, R. M., et al. (2008). Pheochromocytoma in children and adolescents: a clinical spectrum. *J Pediatr Surg*, *43*, 540–543.
40. Kassim, T. A., Clarke, D. D., Mai, V. Q., et al. (2008). Catecholamine-induced cardiomyopathy. *Endocr Pract*, *14*, 1137–1149.
41. Zelinka, T., Petrak, O., Turkova, H., et al. (2012). High incidence of cardiovascular complications in pheochromocytoma. *Horm Metab Res*, *44*, 379–384.
42. Prejbisz, A., Lenders, J. W., Eisenhofer, G., & Januszewicz, A. (2011). Cardiovascular manifestations of phaeochromocytoma. *J Hypertens*, *29*, 2049–2060.
43. Offergeld, C., Brase, C., Yaremchuk, S., et al. (2012). Head and neck paragangliomas: clinical and molecular genetic classification. *Clinics* (Sao Paulo), *67*(Suppl. 1), 19–28.
44. Pomares, F. J., Canas, R., Rodriguez, J. M., et al. (1998). Differences between sporadic and multiple endocrine neoplasia type 2A phaeochromocytoma. *Clin Endocrinol* (Oxf), *48*, 195–200.
45. Walther, M. M., Reiter, R., Keiser, H. R., et al. (1999). Clinical and genetic characterization of pheochromocytoma in von Hippel-Lindau families: comparison with sporadic pheochromocytoma gives insight into natural history of pheochromocytoma. *J Urol*, *162*, 659–664.
46. van Duinen, N., Steenvoorden, D., Bonsing, B. A., et al. (2010). Pheochromocytomas detected by biochemical screening in predisposed subjects are associated with lower prevalence of clinical and biochemical manifestations and smaller tumors than pheochromocytomas detected by signs and symptoms. *Eur J Endocrinol*, *163*, 121–127.

47. d'Herbomez, M., Forzy, G., Bauters, C., et al. (2007). An analysis of the biochemical diagnosis of 66 pheochromocytomas. *Eur J Endocrinol*, *156*, 569–575.
48. Lenders, J. W., Keiser, H. R., Goldstein, D. S., et al. (1995). Plasma metanephrines in the diagnosis of pheochromocytoma. *Ann Intern Med*, *123*, 101–109.
49. Weise, M., Merke, D. P., Pacak, K., et al. (2002). Utility of plasma free metanephrines for detecting childhood pheochromocytoma. *J Clin Endocrinol Metab*, *87*, 1955–1960.
50. Pacak, K., Eisenhofer, G., Ahlman, H., et al. (2007). Pheochromocytoma: recommendations for clinical practice from the First International Symposium, October 2005. *Nat Clin Pract Endocrinol Metab*, *3*, 92–102.
51. Havekes, B., Romijn, J. A., Eisenhofer, G., et al. (2009). Update on pediatric pheochromocytoma. *Pediatr Nephrol*, *24*, 943–950.
52. Sawka, A. M., Gafni, A., Thabane, L., & Young, W. F., Jr. (2004). The economic implications of three biochemical screening algorithms for pheochromocytoma. *J Clin Endocrinol Metab*, *89*, 2859–2866.
53. Grossman, A., Pacak, K., Sawka, A., et al. (2006). Biochemical diagnosis and localization of pheochromocytoma: can we reach a consensus? *Ann N Y Acad Sci*, *1073*, 332–347.
54. Sawka, A. M., Jaeschke, R., Singh, R. J., & Young, W. F., Jr. (2003). A comparison of biochemical tests for pheochromocytoma: measurement of fractionated plasma metanephrines compared with the combination of 24-hour urinary metanephrines and catecholamines. *J Clin Endocrinol Metab*, *88*, 553–558.
55. Eisenhofer, G., Goldstein, D. S., Walther, M. M., et al. (2003). Biochemical diagnosis of pheochromocytoma: how to distinguish true- from false-positive test results. *J Clin Endocrinol Metab*, *88*, 2656–2666.
56. de Jong, W. H., Eisenhofer, G., Post W. J., et al. (2009). Dietary influences on plasma and urinary metanephrines: implications for diagnosis of catecholamine-producing tumors. *J Clin Endocrinol Metab*, *94*, 2841–2849.
57. Brodeur, G. M., Hogarty, M. D., Mosse, Y. P., & Maris, J. M. (2011). Neuroblastoma. In P. A. Pizzo, & D. G. Poplack (Eds.), *Principles and practice of pediatric oncology* (6th ed.) (pp. 886–922). Philadelphia: Wolters Kluwer/Lippincott Williams & Wilkins.
58. Lawrence, A. M. (1967). Glucagon provocative test for pheochromocytoma. *Ann Intern Med*, *66*, 1091–1096.
59. Bravo, E. L., Tarazi, R. C., Fouad, F. M., et al. (1981). Clonidine-suppression test: a useful aid in the diagnosis of pheochromocytoma. *N Engl J Med*, *305*, 623–626.
60. Bravo, E. L., & Tagle, R. (2003). Pheochromocytoma: state-of-the-art and future prospects. *Endocr Rev*, *24*, 539–553.
61. Lenders, J. W., Pacak, K., Huynh, T. T., et al. (2010). Low sensitivity of glucagon provocative testing for diagnosis of pheochromocytoma. *J Clin Endocrinol Metab*, *95*, 238–245.
62. Eisenhofer, G., Lenders, J. W., Linehan, W. M., et al. (1999). Plasma normetanephrine and metanephrine for detecting pheochromocytoma in von Hippel-Lindau disease and multiple endocrine neoplasia type 2. *N Engl J Med*, *340*, 1872–1879.
63. Pacak, K., Eisenhofer, G., & Ilias, I. (2009). Diagnosis of pheochromocytoma with special emphasis on MEN2 syndrome. *Hormones* (Athens), *8*, 111–116.
64. Eisenhofer, G., Walther, M. M., Huynh, T. T., et al. (2001). Pheochromocytomas in von Hippel-Lindau syndrome and multiple endocrine neoplasia type 2 display distinct biochemical and clinical phenotypes. *J Clin Endocrinol Metab*, *86*, 1999–2008.
65. Lodish, M. B., Adams, K. T., Huynh, T. T., et al. (2010). Succinate dehydrogenase gene mutations are strongly associated with paraganglioma of the organ of Zuckerkandl. *Endocr Relat Cancer*, *17*, 581–588.
66. Eisenhofer, G., Timmers, H. J., Lenders, J. W., et al. (2011). Age at diagnosis of pheochromocytoma differs according to catecholamine phenotype and tumor location. *J Clin Endocrinol Metab*, *96*, 375–384.
67. van Duinen, N., Steenvoorden, D., Kema, I. P., et al. (2010). Increased urinary excretion of 3-methoxytyramine in patients with head and neck paragangliomas. *J Clin Endocrinol Metab*, *95*, 209–214.
68. Eisenhofer, G., Goldstein, D. S., Sullivan, P., et al. (2005). Biochemical and clinical manifestations of dopamine-producing paragangliomas: utility of plasma methoxytyramine. *J Clin Endocrinol Metab*, *90*, 2068–2075.
69. Timmers, H. J., Kozupa, A, Eisenhofer, G, et al. (2007). Clinical presentations, biochemical phenotypes, and genotype-phenotype correlations in patients with succinate dehydrogenase subunit B-associated pheochromocytomas and paragangliomas. *J Clin Endocrinol Metab*, *92*, 779–786.
70. Eisenhofer, G., Lenders, J. W., Timmers, H., et al. (2011). Measurements of plasma methoxytyramine, normetanephrine, and metanephrine as discriminators of different hereditary forms of pheochromocytoma. *Clin Chem*, *57*, 411–420.
71. Proye, C., Fossati, P., Fontaine, P., et al. (1986). Dopamine-secreting pheochromocytoma: an unrecognized entity? Classification of pheochromocytomas according to their type of secretion. *Surgery*, *100*, 1154–1162.
72. Dubois, L. A., & Gray, D. K. (2005). Dopamine-secreting pheochromocytomas: in search of a syndrome. *World J Surg*, *29*, 909–913.
73. Bilek, R., Safarik, L., Ciprova, V., et al. (2008). Chromogranin A, a member of neuroendocrine secretory proteins as a selective marker for laboratory diagnosis of pheochromocytoma. *Physiol Res*, *57*(Suppl. 1), S171–S179.
74. Algeciras-Schimnich, A., Preissner, C. M., Young, W. F., Jr., et al. (2008). Plasma chromogranin A or urine fractionated metanephrines follow-up testing improves the diagnostic accuracy of plasma fractionated metanephrines for pheochromocytoma. *J Clin Endocrinol Metab*, *93*, 91–95.
75. Grossrubatscher, E, Dalino, P, Vignati, F, et al. (2006). The role of chromogranin A in the management of patients with phaeochromocytoma. *Clin Endocrinol* (Oxf), *65*, 287–293.
76. Jacobson, A. F., Deng, H., Lombard, J., et al. (2010). 123I-metaiodobenzylguanidine scintigraphy for the detection of neuroblastoma and pheochromocytoma: results of a meta-analysis. *J Clin Endocrinol Metab*, *95*, 2596–2606.
77. Timmers, H. J., Taieb, D., & Pacak, K. (2012). Current and future anatomical and functional imaging approaches to pheochromocytoma and paraganglioma. *Horm Metab Res*, *44*, 367–372.
78. Blake, M. A., Cronin, C. G., & Boland, G. W. (2010). Adrenal imaging. *AJR Am J Roentgenol*, *194*, 1450–1460.
79. Sisson, J. C., & Wieland, D. M. (1986). Radiolabeled metaiodobenzylguanidine: pharmacology and clinical studies. *Am J Physiol Imaging*, *1*, 96–103.
80. Ilias, I., Divgi, C., & Pacak, K. (2011). Current role of metaiodobenzylguanidine in the diagnosis of pheochromocytoma and medullary thyroid cancer. *Semin Nucl Med*, *41*, 364–368.
81. Greenblatt, D. Y., Shenker, Y., & Chen, H. (2008). The utility of metaiodobenzylguanidine (MIBG) scintigraphy in patients with pheochromocytoma. *Ann Surg Oncol*, *15*, 900–905.
82. Lumachi, F., Tregnaghi, A., Zucchetta, P., et al. (2006). Sensitivity and positive predictive value of CT, MRI and 123I-MIBG scintigraphy in localizing pheochromocytomas: a prospective study. *Nucl Med Commun*, *27*, 583–587.
83. Berglund, A. S., Hulthen, U. L., Manhem, P., et al. (2001). Metaiodobenzylguanidine (MIBG) scintigraphy and computed tomography (CT) in clinical practice. Primary and secondary evaluation for localization of phaeochromocytomas. *J Intern Med*, *249*, 247–251.
84. Fonte, J. S., Robles, J. F., Chen, C. C., et al. (2012). False-negative (1)(2)(3)I-MIBG SPECT is most commonly found in SDHB-related pheochromocytoma or paraganglioma with high frequency to develop metastatic disease. *Endocr Relat Cancer*, *19*, 83–93.
85. Shulkin, B. L., & Shapiro, B. (1998). Current concepts on the diagnostic use of MIBG in children. *J Nucl Med*, *39*, 679–688.
86. Hoegerle, S., Nitzsche, E., Altehoefer, C., et al. (2002). Pheochromocytomas: detection with 18F DOPA whole body PET—initial results. *Radiology*, *222*, 507–512.
87. Ilias, I., Shulkin, B., & Pacak, K. (2005). New functional imaging modalities for chromaffin tumors, neuroblastomas and ganglioneuromas. *Trends Endocrinol Metab*, *16*, 66–72.
88. Jager, P. L., Chirakal, R., Marriott, C. J., et al. (2008). 6-L-18F-fluorodihydroxyphenylalanine PET in neuroendocrine tumors: basic aspects and emerging clinical applications. *J Nucl Med*, *49*, 573–586.
89. Ilias, I., Chen, C. C., Carrasquillo, J. A., et al. (2008). Comparison of 6-18F-fluorodopamine PET with 123I-metaiodobenzylguanidine and 111in-pentetreotide scintigraphy in localization of nonmetastatic and metastatic pheochromocytoma. *J Nucl Med*, *49*, 1613–1619.

90. Fiebrich, H. B., Brouwers, A. H., Kerstens, M. N., et al. (2009). 6-[F-18]Fluoro-L-dihydroxyphenylalanine positron emission tomography is superior to conventional imaging with (123)I-metaiodobenzylguanidine scintigraphy, computer tomography, and magnetic resonance imaging in localizing tumors causing catecholamine excess. *J Clin Endocrinol Metab*, *94*, 3922–3930.
91. Timmers, H. J., Chen, C. C., Carrasquillo, J. A., et al. (2009). Comparison of 18F-fluoro-L-DOPA, 18F-fluoro-deoxyglucose, and 18F-fluorodopamine PET and 123I-MIBG scintigraphy in the localization of pheochromocytoma and paraganglioma. *J Clin Endocrinol Metab*, *94*, 4757–4767.
92. Timmers, H. J., Kozupa, A., Chen, C. C., et al. (2007). Superiority of fluorodeoxyglucose positron emission tomography to other functional imaging techniques in the evaluation of metastatic SDHB-associated pheochromocytoma and paraganglioma. *J Clin Oncol*, *25*, 2262–2269.
93. Taieb, D., Tessonnier, L., Sebag, F., et al. (2008). The role of 18F-FDOPA and 18F-FDG-PET in the management of malignant and multifocal phaeochromocytomas. *Clin Endocrinol* (Oxf), *69*, 580–586.
94. Timmers, H. J., Chen, C. C., Carrasquillo, J. A., et al. (2012). Staging and functional characterization of pheochromocytoma and paraganglioma by 18F-fluorodeoxyglucose (18F-FDG) positron emission tomography. *J Natl Cancer Inst*, *104*, 700–708.
95. Ladroue, C., Carcenac, R., Leporrier, M., et al. (2008). PHD2 mutation and congenital erythrocytosis with paraganglioma. *N Engl J Med*, *359*, 2685–2692.
96. Yeh, I. T., Lenci, R. E., Qin, Y, et al. (2008). A germline mutation of the KIF1B beta gene on 1p36 in a family with neural and non-neural tumors. *Hum Genet*, *124*, 279–285.
97. Qin, Y, Yao, L, King, E. E., et al. (2010). Germline mutations in TMEM127 confer susceptibility to pheochromocytoma. *Nat Genet*, *42*, 229–233.
98. Burnichon, N., Briere, J. J., Libe, R, et al. (2010). SDHA is a tumor suppressor gene causing paraganglioma. *Hum Mol Genet*, *19*, 3011–3020.
99. Comino-Mendez, I., Gracia-Aznarez, F. J., Schiavi F, et al. (2011). Exome sequencing identifies MAX mutations as a cause of hereditary pheochromocytoma. *Nat Genet*, *43*, 663–667.
100. Hao, H. X., Khalimonchuk, O., Schraders, M., et al. (2009). SDH5, a gene required for flavination of succinate dehydrogenase, is mutated in paraganglioma. *Science*, *325*, 1139–1142.
101. Lorenzo F. R., Yang, C., Ng Tang Fui, M., et al. (2013). A novel EPAS1/HIF2A germline mutation in a congenital polycythemia with paraganglioma. *J Mol Med* (Berl), *91*, 507–512.
102. Zhuang, Z., Yang, C., Lorenzo, F., et al. (2012). Somatic HIF2A gain-of-function mutations in paraganglioma with polycythemia. *N Engl J Med*, *367*, 922–930.
103. Jafri, M., & Maher, E. R. (2012). The genetics of phaeochromocytoma: using clinical features to guide genetic testing. *Eur J Endocrinol*, *166*, 151–158.
104. Vanderveen, K. A., Thompson, S. M., Callstrom, M. R., et al. (2009). Biopsy of pheochromocytomas and paragangliomas: potential for disaster. *Surgery*, *146*, 1158–1166.
105. Gagner, M., Pomp, A., Heniford, B. T., et al. (1997). Laparoscopic adrenalectomy: lessons learned from 100 consecutive procedures. *Ann Surg*, *226*, 238–246; discussion 246–267.
106. Walz, M. K., Alesina, P. F., Wenger, F. A., et al. (2006). Posterior retroperitoneoscopic adrenalectomy: results of 560 procedures in 520 patients. *Surgery*, *140*, 943–948; discussion 948–950.
107. Ludwig, A. D., Feig, D. I., Brandt, M. L., et al. (2007). Recent advances in the diagnosis and treatment of pheochromocytoma in children. *Am J Surg*, *194*, 792–796; discussion 796–797.
108. Janetschek, G., Finkenstedt, G., Gasser, R., et al. (1998). Laparoscopic surgery for pheochromocytoma: adrenalectomy, partial resection, excision of paragangliomas. *J Urol*, *160*, 330–334.
109. Callender, G. G., Kennamer, D. L., Grubbs, E. G., et al. (2009). Posterior retroperitoneoscopic adrenalectomy. *Adv Surg*, *43*, 147–157.
110. Heloury, Y., Muthucumaru, M., Panabokke, G., et al. (2012). Minimally invasive adrenalectomy in children. *J Pediatr Surg*, *47*, 415–421.
111. St. Peter, S. D., Valusek, P. A., Hill, S., et al. (2011). Laparoscopic adrenalectomy in children: a multicenter experience. *J Laparoendosc Adv Surg Tech A*, *21*, 647–649.
112. Yip, L., Lee, J. E., Shapiro, S. E., et al. (2004). Surgical management of hereditary pheochromocytoma. *J Am Coll Surg*, *198*, 525–534; discussion 534–535.
113. Diner, E. K., Franks, M. E., Behari, A., et al. (2005). Partial adrenalectomy: the National Cancer Institute experience. *Urology*, *66*, 19–23.
114. Neumann, H. P., Bender, B. U., Reincke, M., et al. (1999). Adrenal-sparing surgery for phaeochromocytoma. *Br J Surg*, *86*, 94–97.
115. Asari, R., Scheuba, C., Kaczirek, K., & Niederl, B. (2006). Estimated risk of pheochromocytoma recurrence after adrenal-sparing surgery in patients with multiple endocrine neoplasia type 2A. *Arch Surg*, *141*, 1199–1205; discussion 1205.
116. Hack, H. A. (2000). The perioperative management of children with phaeochromocytoma. *Paediatr Anaesth*, *10*, 463–476.
117. Van Braeckel, P., Carlier, S, Steelant, P. J., et al. (2009). Perioperative management of phaeochromocytoma. *Acta Anaesthesiol Belg*, *60*, 55–66.
118. Goldstein, R. E., O'Neill, J. A. Jr., Holcomb, G. W., 3rd, et al. (1999). Clinical experience over 48 years with pheochromocytoma. *Ann Surg*, *229*, 755–764; discussion 764–766.
119. Kocak, S., Aydintug, S., & Canakci, N. (2002). Alpha blockade in preoperative preparation of patients with pheochromocytomas. *Int Surg*, *87*, 191–194.
120. Lebuffe, G., Dosseh, E. D., Tek, G, et al. (2005). The effect of calcium channel blockers on outcome following the surgical treatment of phaeochromocytomas and paragangliomas. *Anaesthesia*, *60*, 439–444.
121. Pacak, K. (2007). Preoperative management of the pheochromocytoma patient. *J Clin Endocrinol Metab*, *92*, 4069–4079.
122. Perry, R. R., Keiser, H. R., Norton, J. A., et al. (1990). Surgical management of pheochromocytoma with the use of metyrosine. *Ann Surg*, *212*, 621–628.
123. Linnoila, R. I., Keiser, H. R., Steinberg, S. M., & Lack, E. E. (1990). Histopathology of benign versus malignant sympathoadrenal paragangliomas: clinicopathologic study of 120 cases including unusual histologic features. *Hum Pathol*, *21*, 1168–1180.
124. Chrisoulidou, A., Kaltsas, G., Ilias, I., & Grossman, A. B. (2007). The diagnosis and management of malignant phaeochromocytoma and paraganglioma. *Endocr Relat Cancer*, *14*, 569–585.
125. Ayala-Ramirez, M., Feng, L. Johnson, M. M., et al. (2011). Clinical risk factors for malignancy and overall survival in patients with pheochromocytomas and sympathetic paragangliomas: primary tumor size and primary tumor location as prognostic indicators. *J Clin Endocrinol Metab*, *96*, 717–725.
126. Eisenhofer, G., Bornstein, S. R., Brouwers, F. M., et al. (2004). Malignant pheochromocytoma: current status and initiatives for future progress. *Endocr Relat Cancer*, *11*, 423–436.
127. Burnichon, N., Rohmer, V., Amar, L., et al. (2009). The succinate dehydrogenase genetic testing in a large prospective series of patients with paragangliomas. *J Clin Endocrinol Metab*, *94*, 2817–2827.
128. Neumann, H. P., & Eng, C. (2009). The approach to the patient with paraganglioma. *J Clin Endocrinol Metab*, *94*, 2677–2683.
129. Brouwers, F. M., Eisenhofer, G., Tao, J. J., et al. (2006). High frequency of SDHB germline mutations in patients with malignant catecholamine-producing paragangliomas: implications for genetic testing. *J Clin Endocrinol Metab*, *91*, 4505–4509.
130. Amar, L., Baudin, E., Burnichon, N., et al. (2007). Succinate dehydrogenase B gene mutations predict survival in patients with malignant pheochromocytomas or paragangliomas. *J Clin Endocrinol Metab*, *92*, 3822–3828.
131. Schiffman, J. D. (2011). No child left behind in SDHB testing for paragangliomas and pheochromocytomas. *J Clin Oncol*, *29*, 4070–4072.
132. Hazard, J. B., Hawk, W. A., & Crile, G., Jr. (1959). Medullary (solid) carcinoma of the thyroid; a clinicopathologic entity. *J Clin Endocrinol Metab*, *19*, 152–161.
133. Wolfe, H. J., Melvin, K. E., Cervi-Skinner, S. J., et al. (1973). C-cell hyperplasia preceding medullary thyroid carcinoma. *N Engl J Med*, *289*, 437–441.
134. Hogan, A. R., Zhuge, Y., Perez, E. A., et al. (2009). Pediatric thyroid carcinoma: incidence and outcomes in 1753 patients. *J Surg Res*, *156*, 167–172.

135. Waguespack, S., Wells, S., Ross, J., & Bleyer, A. (2006). Thyroid cancer. In A. Bleyer, M. O'Leary, R. Barr, & L. Ries (Eds.), *Cancer epidemiology in older adolescents and young adults 15 to 29 years of age, including SEER incidence and survival 1975-2000.* (pp. 143–154). Bethesda, MD: National Cancer Institute.
136. Raval, M. V., Sturgeon, C., Bentrem, D. J., et al. (2010). Influence of lymph node metastases on survival in pediatric medullary thyroid cancer. *J Pediatr Surg*, *45*, 1947–1954.
137. Donis-Keller, H., Dou, S., Chi, D., et al. (1993). Mutations in the RET proto-oncogene are associated with MEN 2A and FMTC. *Hum Mol Genet*, *2*, 851–856.
138. Mulligan, L. M., Kwok, J. B., Healey, C. S., et al. (1993). Germ-line mutations of the RET proto-oncogene in multiple endocrine neoplasia type 2A. *Nature*, *363*, 458–460.
139. Hofstra, R. M., Landsvater, R. M., Ceccherini, I., et al. (1994). A mutation in the RET proto-oncogene associated with multiple endocrine neoplasia type 2B and sporadic medullary thyroid carcinoma. *Nature*, *367*, 375–376.
140. Carlson, K. M., Dou, S., Chi, D., et al. (1994). Single missense mutation in the tyrosine kinase catalytic domain of the RET protooncogene is associated with multiple endocrine neoplasia type 2B. *Proc Natl Acad Sci U S A*, *91*, 1579–1583.
141. Kloos, R. T., Eng, C., Evans, D. B., et al. (2009). Medullary thyroid cancer: management guidelines of the American Thyroid Association. *Thyroid*, *19*, 565–612.
142. Jimenez, C., Hu, M. I., & Gagel, R. F. (2008). Management of medullary thyroid carcinoma. *Endocrinol Metab Clin North Am*, *37*, 481–496, x–xi.
143. Baloh, R. H., Enomoto, H., Johnson, E. M., Jr., & Milbrandt, J. (2000). The GDNF family ligands and receptors: implications for neural development. *Curr Opin Neurobiol*, *10*, 103–110.
144. Wagner, S. M., Zhu, S., Nicolescu, A. C., & Mulligan, L. M. (2012). Molecular mechanisms of RET receptor-mediated oncogenesis in multiple endocrine neoplasia 2. *Clinics* (Sao Paulo), *67*(Suppl. 1), 77–84.
145. Drosten, M., & Putzer, B. M. (2006). Mechanisms of disease: cancer targeting and the impact of oncogenic RET for medullary thyroid carcinoma therapy. *Nat Clin Pract Oncol*, *3*, 564–574.
146. Wells, S. A., Jr., Chi, D. D., Toshima, K., et al. (1994). Predictive DNA testing and prophylactic thyroidectomy in patients at risk for multiple endocrine neoplasia type 2A. *Ann Surg*, *220*, 237–247; discussion 247–250.
147. Gagel, R. F., Melvin, K. E., Tashjian, A. H., Jr., et al. (1975). Natural history of the familial medullary thyroid carcinoma-pheochromocytoma syndrome and the identification of preneoplastic stages by screening studies: a five-year report. *Trans Assoc Am Physicians*, *88*, 177–191.
148. Machens, A. (2004). Early malignant progression of hereditary medullary thyroid cancer. *N Engl J Med*, *350*, 943.
149. Skinner, M. A., Moley, J. A., Dilley, W. G., et al. (2005). Prophylactic thyroidectomy in multiple endocrine neoplasia type 2A. *N Engl J Med*, *353*, 1105–1113.
150. Rohmer, V., Vidal-Trecan, G., Bourdelot, A., et al. (2011). Prognostic factors of disease-free survival after thyroidectomy in 170 young patients with a RET germline mutation: a multicenter study of the Groupe Francais d'Etude des Tumeurs Endocrines. *J Clin Endocrinol Metab*, *96*, E509–E518.
151. de Groot, J. W., Plukker, J. T., Wolffenbuttel, B. H., et al. (2006). Determinants of life expectancy in medullary thyroid cancer: age does not matter. *Clin Endocrinol* (Oxf), *65*, 729–736.
152. Wray, C. J., Rich, T. A., Waguespack, S. G., et al. (2008). Failure to recognize multiple endocrine neoplasia 2B: more common than we think? *Ann Surg Oncol*, *15*, 293–301.
153. Brandi, M. L., Gagel, R. F., Angeli, A, et al. (2001). Guidelines for diagnosis and therapy of MEN type 1 and type 2. *J Clin Endocrinol Metab*, *86*, 5658–5671.
154. Guillem, J. G., Wood, W. C., Moley, J. F., et al. (2006). ASCO/SSO review of current role of risk-reducing surgery in common hereditary cancer syndromes. *J Clin Oncol*, *24*, 4642–4660.
155. Tuttle, R. M., Ball, D. W., Byrd, D, et al. (2010). Medullary carcinoma. *J Natl Compr Canc Netw*, *8*, 512–530.
156. Chen, H., Sippel, R. S., O'Dorisio, M. S., et al. (2010). The North American Neuroendocrine Tumor Society consensus guideline for the diagnosis and management of neuroendocrine tumors: pheochromocytoma, paraganglioma, and medullary thyroid cancer. *Pancreas*, *39*, 775–783.
157. Sosa, J. A., Tuggle, C. T., Wang, T. S., et al. (2008). Clinical and economic outcomes of thyroid and parathyroid surgery in children. *J Clin Endocrinol Metab*, *93*, 3058–3065.
158. Tuggle, C. T., Roman, S. A., Wang, T. S., et al. (2008). Pediatric endocrine surgery: who is operating on our children? *Surgery*, *144*, 869–877; discussion 877.
159. Carty, S. E., Cooper, D. S., Doherty, G. M., et al. (2009). Consensus statement on the terminology and classification of central neck dissection for thyroid cancer. *Thyroid*, *19*, 1153–1158.
160. Machens, A., Lorenz, K., & Dralle, H. (2009). Constitutive RET tyrosine kinase activation in hereditary medullary thyroid cancer: clinical opportunities. *J Intern Med*, *266*, 114–125.
161. Machens, A., Lorenz, K., & Dralle, H. (2009). Individualization of lymph node dissection in RET (rearranged during transfection) carriers at risk for medullary thyroid cancer: value of pretherapeutic calcitonin levels. *Ann Surg*, *250*, 305–310.
162. Chougnet, C., Brassard, M., Leboulleux, S., et al. (2010). Molecular targeted therapies for patients with refractory thyroid cancer. *Clin Oncol* (R Coll Radiol), *22*, 448–455.
163. Sherman, S. I. (2011). Targeted therapies for thyroid tumors. *Mod Pathol*, *24*(Suppl. 2), S44-S52.
164. Chau, N. G., & Haddad, R. I. (2012). Vandetanib for the treatment of medullary thyroid cancer. *Clin Cancer Res*, 19, 524-9.
165. Fox, E., Widemann, B. C., Whitcomb, P. O., et al. (2009). Phase I/II trial of vandetanib in children and adolescents with hereditary medullary thyroid carcinoma. *J Clin Oncol*, *27* (15s), (Abstract 10014).
166. Cupisti, K., Wolf, A., Raffel, A., et al. (2007). Long-term clinical and biochemical follow-up in medullary thyroid carcinoma: a single institution's experience over 20 years. *Ann Surg*, *246*, 815–821.
167. Boostrom, S. Y., Grant, C. S., Thompson, G. B., et al. (2009). Need for a revised staging consensus in medullary thyroid carcinoma. *Arch Surg*, *144*, 663–669.
168. Machens, A., Niccoli-Sire, P., Hoegel, J., et al. (2003). Early malignant progression of hereditary medullary thyroid cancer. *N Engl J Med*, *349*, 1517–1525.
169. Etit, D., Faquin, W. C., Gaz, R., et al. (2008). Histopathologic and clinical features of medullary microcarcinoma and C-cell hyperplasia in prophylactic thyroidectomies for medullary carcinoma: a study of 42 cases. *Arch Pathol Lab Med*, *132*, 1767–1773.
170. Dralle, H., Gimm, O., Simon, D., et al. (1998). Prophylactic thyroidectomy in 75 children and adolescents with hereditary medullary thyroid carcinoma: German and Austrian experience. *World J Surg*, *22*, 744–750, discussion 750–751.
171. Zenaty, D., Aigrain, Y., Peuchmaur, M., et al. (2009). Medullary thyroid carcinoma identified within the first year of life in children with hereditary multiple endocrine neoplasia type 2A (codon 634) and 2B. *Eur J Endocrinol*, *160*, 807–813.
172. Meijer, J. A., le Cessie, S., van den Hout, W. B., et al. (2010). Calcitonin and carcinoembryonic antigen doubling times as prognostic factors in medullary thyroid carcinoma: a structured meta-analysis. *Clin Endocrinol* (Oxf), *72*, 534–542.
173. Costante, G., Durante, C., Francis, Z., et al. (2009). Determination of calcitonin levels in C-cell disease: clinical interest and potential pitfalls. *Nat Clin Pract Endocrinol Metab*, *5*, 35–44.
174. Carney, J. A., Gordon, H., Carpenter, P. C., et al. (1985). The complex of myxomas, spotty pigmentation, and endocrine overactivity. *Medicine* (Baltimore), *64*, 270–283.
175. Shenoy, B. V., Carpenter, P. C., & Carney, J. A. (1984). Bilateral primary pigmented nodular adrenocortical disease: rare cause of the Cushing syndrome. *Am J Surg Pathol*, *8*, 335–344.
176. Stratakis, C. A., & Horvath, A. (2003). Carney complex. In R. A. Pagon, T. D. Bird, C. R. Dolan, et al. (Eds.), *GeneReviews*™ (2010/03/20 ed.). Seattle, WA: University of Washington, Seattle. (Last updated 22 June 2010).
177. Almeida, M. Q., & Stratakis, C. A. (2010). Carney complex and other conditions associated with micronodular adrenal hyperplasias. *Best Pract Res Clin Endocrinol Metab*, *24*, 907–914.
178. Kirschner, L. S., Carney, J. A., Pack, S. D., et al. (2000). Mutations of the gene encoding the protein kinase A type I-alpha regulatory

subunit in patients with the Carney complex. *Nat Genet*, *26*, 89–92.
179. Casey, M., Vaughan, C. J., He, J., et al. (2000). Mutations in the protein kinase A R1alpha regulatory subunit cause familial cardiac myxomas and Carney complex. *J Clin Invest*, *106*, R31–R38.
180. Stratakis, C. A., Carney, J. A., Lin, J. P., et al. (1996). Carney complex, a familial multiple neoplasia and lentiginosis syndrome: analysis of 11 kindreds and linkage to the short arm of chromosome 2. *J Clin Invest*, *97*, 699–705.
181. Bertherat, J., Horvath, A., Groussin, L., et al. (2009). Mutations in regulatory subunit type 1A of cyclic adenosine 5'-monophosphate-dependent protein kinase (PRKAR1A): phenotype analysis in 353 patients and 80 different genotypes. *J Clin Endocrinol Metab*, *94*, 2085–2091.
182. Lacroix, A., & Bourdeau, I. (2005). Bilateral adrenal Cushing's syndrome: macronodular adrenal hyperplasia and primary pigmented nodular adrenocortical disease. *Endocrinol Metab Clin North Am*, *34*, 441–458, x.
183. Stratakis, C. A. (2007). Adrenocortical tumors, primary pigmented adrenocortical disease (PPNAD)/Carney complex, and other bilateral hyperplasias: the NIH studies. *Horm Metab Res*, *39*, 467–473.
184. Stratakis, C. A., Sarlis, N., Kirschner, L. S., et al. (1999). Paradoxical response to dexamethasone in the diagnosis of primary pigmented nodular adrenocortical disease. *Ann Intern Med*, *131*, 585–591.
185. Doppman, J. L., Travis, W. D., Nieman, L., et al. (1989). Cushing syndrome due to primary pigmented nodular adrenocortical disease: findings at CT and MR imaging. *Radiology*, *172*, 415–420.
186. Stratakis, C. A., Kirschner, L. S., & Carney, J. A. (2001). Clinical and molecular features of the Carney complex: diagnostic criteria and recommendations for patient evaluation. *J Clin Endocrinol Metab*, *86*, 4041–4046.
187. Kirschner, L. S. (2010). PRKAR1A and the evolution of pituitary tumors. *Mol Cell Endocrinol*, *326*, 3–7.
188. Handley, J., Carson, D., Sloan, J., et al. (1992). Multiple lentigines, myxoid tumours and endocrine overactivity; four cases of Carney's complex. *Br J Dermatol*, *126*, 367–371.
189. Ulbright, T. M., Amin, M. B., & Young, R. H. (2007). Intratubular large cell hyalinizing Sertoli cell neoplasia of the testis: a report of 8 cases of a distinctive lesion of the Peutz-Jeghers syndrome. *Am J Surg Pathol*, *31*, 827–835.
190. Daly, A. F., Jaffrain-Rea, M. L., Ciccarelli, A., et al. (2006). Clinical characterization of familial isolated pituitary adenomas. *J Clin Endocrinol Metab*, *91*, 3316–3323.
191. Vierimaa, O., Georgitsi, M., Lehtonen, R., et al. (2006). Pituitary adenoma predisposition caused by germline mutations in the AIP gene. *Science*, *312*, 1228–1230.
192. Daly, A. F., Vanbellinghen, J. F., Khoo, S. K., et al. (2007). Aryl hydrocarbon receptor-interacting protein gene mutations in familial isolated pituitary adenomas: analysis in 73 families. *J Clin Endocrinol Metab*, *92*, 1891–1896.
193. Jaffrain-Rea, M. L., Daly, A. F., Angelini, M., et al. (2011). Genetic susceptibility in pituitary adenomas: from pathogenesis to clinical implications. *Expert Rev Endocrinol Metab*, *6*, 195–214.
194. Daly, A. F., Tichomirowa, M. A., Petrossians, P., et al. (2010). Clinical characteristics and therapeutic responses in patients with germ-line AIP mutations and pituitary adenomas: an international collaborative study. *J Clin Endocrinol Metab*, *95*, E373–E383.
195. Tichomirowa, M. A., Barlier, A., Daly, A. F., et al. (2011). High prevalence of AIP gene mutations following focused screening in young patients with sporadic pituitary macroadenomas. *Eur J Endocrinol*, *165*, 509–515.
196. Korbonits, M., Storr, H., & Kumar, A. V. (2012). Familial pituitary adenomas: who should be tested for AIP mutations? *Clin Endocrinol* (Oxf), *77*, 351–356.
197. Klein, R. D., Lloyd, R. V., & Young, W. F. (2008). Hereditary paraganglioma-pheochromocytoma syndromes. In R. A. Pagon, T. D. Bird, C. R. Dolan, et al. (Eds.), *GeneReviews*™ (2010/03/20 ed.). Seattle, WA: University of Washington, Seattle. (Last updated 3 September 2009).
198. Baysal, B. E., Ferrell, R. E., Willett-Brozick, J. E., et al. (2000). Mutations in SDHD, a mitochondrial complex II gene, in hereditary paraganglioma. *Science*, *287*, 848–851.
199. Niemann, S., & Muller, U. (2000). Mutations in SDHC cause autosomal dominant paraganglioma, type 3. *Nat Genet*, *26*, 268–270.
200. Astuti, D., Latif, F., Dallol, A., et al. (2001). Gene mutations in the succinate dehydrogenase subunit SDHB cause susceptibility to familial pheochromocytoma and to familial paraganglioma. *Am J Hum Genet*, *69*, 49–54.
201. Neumann, H. P., Pawlu, C., Peczkowska, M., et al. (2004). Distinct clinical features of paraganglioma syndromes associated with SDHB and SDHD gene mutations. *JAMA*, *292*, 943–951.
202. Benn, D. E., Gimenez-Roqueplo, A. P., Reilly, J. R., et al. (2006). Clinical presentation and penetrance of pheochromocytoma/paraganglioma syndromes. *J Clin Endocrinol Metab*, *91*, 827–836.
203. Baysal, B. E. (2004). Genomic imprinting and environment in hereditary paraganglioma. *Am J Med Genet C Semin Med Genet*, *129C*, 85–90.
204. Pasini, B., McWhinney, S. R., Bei, T., et al. (2008). Clinical and molecular genetics of patients with the Carney-Stratakis syndrome and germline mutations of the genes coding for the succinate dehydrogenase subunits SDHB, SDHC, and SDHD. *Eur J Hum Genet*, *16*, 79–88.
205. Stratakis, C. A., & Carney, J. A. (2009). The triad of paragangliomas, gastric stromal tumours and pulmonary chondromas (Carney triad), and the dyad of paragangliomas and gastric stromal sarcomas (Carney-Stratakis syndrome): molecular genetics and clinical implications. *J Intern Med*, *266*, 43–52.
206. Vanharanta, S., Buchta, M., McWhinney, S. R., et al. (2004). Early-onset renal cell carcinoma as a novel extraparaganglial component of SDHB-associated heritable paraganglioma. *Am J Hum Genet*, *74*, 153–159.
207. Srirangalingam, U., Walker, L., Khoo, B., et al. (2008). Clinical manifestations of familial paraganglioma and phaeochromocytomas in succinate dehydrogenase B (SDH-B) gene mutation carriers. *Clin Endocrinol* (Oxf), *69*, 587–596.
208. Xekouki, P., Pacak, K., Almeida, M., et al. (2012). Succinate dehydrogenase (SDH) D subunit (SDHD) inactivation in a growth-hormone-producing pituitary tumor: a new association for SDH? *J Clin Endocrinol Metab*, *97*, E357–E366.
209. Carpten, J. D., Robbins, C. M., Villablanca, A., et al. (2002). HRPT2, encoding parafibromin, is mutated in hyperparathyroidism-jaw tumor syndrome. *Nat Genet*, *32*, 676–680.
210. Rich, T. A., Hu, M. I., Martin, J. W., et al. (2008). CDC73-Related disorders. In R. A. Pagon, T. D. Bird, C. R. Dolan, et al. (Eds.), *GeneReviews*™ (2010/03/20 ed.). Seattle, WA: University of Washington, Seattle. (Last updated 24 May 2012).
211. Pichardo-Lowden, A. R., Manni, A., Saunders, B. D., & Baker, M. J. (2011). Familial hyperparathyroidism due to a germline mutation of the CDC73 gene: implications for management and age-appropriate testing of relatives at risk. *Endocr Pract*, *17*, 602–609.
212. Tan, M. H., & Teh, B. T. (2004). Renal neoplasia in the hyperparathyroidism-jaw tumor syndrome. *Curr Mol Med*, *4*, 895–897.
213. Bradley, K. J., Hobbs, M. R., Buley, I. D., et al. (2005). Uterine tumours are a phenotypic manifestation of the hyperparathyroidism-jaw tumour syndrome. *J Intern Med*, *257*, 18–26.
214. Marx, S. J. (2005). Molecular genetics of multiple endocrine neoplasia types 1 and 2. *Nat Rev Cancer*, *5*, 367–375.
215. Thakker, R. V. (2010). Multiple endocrine neoplasia type 1 (MEN1). *Best Pract Res Clin Endocrinol Metab*, *24*, 355–370.
216. Thakker, R. V., Newey, P. J., Walls, G. V., et al. (2012). Clinical practice guidelines for multiple endocrine neoplasia type 1 (MEN1). *J Clin Endocrinol Metab*, *97*, 2990–3011.
217. Giusti, F., Marini, F., & Brandi, M. L. (2005). Multiple endocrine neoplasia type 1. In R. A. Pagon, T. D. Bird, C. R. Dolan, et al. (Eds.), *GeneReviews*™. Seattle, WA: University of Washington, Seattle. (Last updated 6 September 2012).
218. Wermer, P. (1954). Genetic aspects of adenomatosis of endocrine glands. *Am J Med*, *16*, 363–371.
219. Nakamura, Y., Larsson, C., Julier, C., et al. (1989). Localization of the genetic defect in multiple endocrine neoplasia type 1 within a small region of chromosome 11. *Am J Hum Genet*, *44*, 751–755.
220. Thakker, R. V., Wooding, C., Pang, J. T., et al. (1993). Linkage analysis of 7 polymorphic markers at chromosome 11p11.2-11q13

in 27 multiple endocrine neoplasia type 1 families. *Ann Hum Genet*, *57*, 17–25.
221. Chandrasekharappa, S. C., Guru, S. C., Manickam, P., et al. (1997). Positional cloning of the gene for multiple endocrine neoplasia-type 1. *Science*, *276*, 404–407.
222. Agarwal, S. K., Kester, M. B., Debelenko, L. V., et al. (1997). Germline mutations of the MEN1 gene in familial multiple endocrine neoplasia type 1 and related states. *Hum Mol Genet*, *6*, 1169–1175.
223. Yang, Y., & Hua, X. (2007). In search of tumor suppressing functions of menin. *Mol Cell Endocrinol*, *265–266*, 34–41.
224. Lemos, M. C., & Thakker, R. V. (2008). Multiple endocrine neoplasia type 1 (MEN1): analysis of 1336 mutations reported in the first decade following identification of the gene. *Hum Mutat*, *29*, 22–32.
225. Kouvaraki, M. A., Lee, J. E., Shapiro, S. E., et al. (2002). Genotype-phenotype analysis in multiple endocrine neoplasia type 1. *Arch Surg*, *137*, 641–647.
226. Trump, D., Farren, B., Wooding, C., et al. (1996). Clinical studies of multiple endocrine neoplasia type 1 (MEN1). *QJM*, *89*, 653–669.
227. Knudson, A. G., Jr. (1971). Mutation and cancer: statistical study of retinoblastoma. *Proc Natl Acad Sci U S A*, *68*, 820–823.
228. Bassett, J. H., Forbes, S. A., Pannett, A. A. et al. (1998). Characterization of mutations in patients with multiple endocrine neoplasia type 1. *Am J Hum Genet*, *62*, 232–244.
229. Owens, M., Ellard, S., & Vaidya, B. (2008). Analysis of gross deletions in the MEN1 gene in patients with multiple endocrine neoplasia type 1. *Clin Endocrinol* (Oxf), *68*, 350–354.
230. Machens, A., Schaaf, L., Karges, W., et al. (2007). Age-related penetrance of endocrine tumours in multiple endocrine neoplasia type 1 (MEN1): a multicentre study of 258 gene carriers. *Clin Endocrinol* (Oxf), *67*, 613–622.
231. Lairmore, T. C., Piersall, L. D., DeBenedetti, M. K., et al. (2004). Clinical genetic testing and early surgical intervention in patients with multiple endocrine neoplasia type 1 (MEN 1). *Ann Surg*, *239*, 637–645, discussion 645–647.
232. Lourenco, D. M., Jr., Toledo, R. A., Coutinho, F. L., et al. (2007). The impact of clinical and genetic screenings on the management of the multiple endocrine neoplasia type 1. *Clinics* (Sao Paulo), *62*, 465–476.
233. Pieterman, C. R., Vriens, M. R., Dreijerink, K. M., et al. (2011). Care for patients with multiple endocrine neoplasia type 1: the current evidence base. *Fam Cancer*, *10*, 157–171.
234. Pieterman, C. R., Schreinemakers, J. M., Koppeschaar, H. P., et al. (2009). Multiple endocrine neoplasia type 1 (MEN1): its manifestations and effect of genetic screening on clinical outcome. *Clin Endocrinol* (Oxf), *70*, 575–581.
235. Thakker, R. V. (1993). The molecular genetics of the multiple endocrine neoplasia syndromes. *Clin Endocrinol* (Oxf), *38*, 1–14.
236. Goudet, P., Murat, A., Binquet, C., et al. (2010). Risk factors and causes of death in MEN1 disease: a GTE (Groupe d'Etude des Tumeurs Endocrines) cohort study among 758 patients. *World J Surg*, *34*, 249–255.
237. Dean P. G., van Heerden, J. A., Farley, D. R., et al. (2000). Are patients with multiple endocrine neoplasia type I prone to premature death? *World J Surg*, *24*, 1437–1441.
238. Hsu, S. C., & Levine, M. A. (2002). Primary hyperparathyroidism in children and adolescents: the Johns Hopkins Children's Center experience 1984–2001. *J Bone Miner Res*, *17*(Suppl 2), N44–N50.
239. Kollars, J., Zarroug, A. E., van Heerden, J., et al. (2005). Primary hyperparathyroidism in pediatric patients. *Pediatrics*, *115*, 974–980.
240. Lawson, M. L., Miller, S. F., Ellis, G., et al. (1996). Primary hyperparathyroidism in a paediatric hospital. *QJM*, *89*, 921–932.
241. Parisien, M., Silverberg, S. J., Shane, E., et al. (1990). Bone disease in primary hyperparathyroidism. *Endocrinol Metab Clin North Am*, *19*, 19–34.
242. Eller-Vainicher, C., Chiodini, I., Battista, C., et al. (2009). Sporadic and MEN1-related primary hyperparathyroidism: differences in clinical expression and severity. *J Bone Miner Res*, *24*, 1404–1410.
243. Goudet, P., Bonithon-Kopp, C., Murat, A., et al. (2011). Gender-related differences in MEN1 lesion occurrence and diagnosis: a cohort study of 734 cases from the Groupe d'etude des Tumeurs Endocrines. *Eur J Endocrinol*, *165*, 97–105.
244. Nilubol, N., Weinstein, L., Simonds, W. F., et al. (2012). Preoperative localizing studies for initial parathyroidectomy in MEN1 syndrome: is there any benefit? *World J Surg*, *36*, 1368–1374.
245. Grant, C. S., Thompson, G, Farley, D., & van Heerden, J. (2005). Primary hyperparathyroidism surgical management since the introduction of minimally invasive parathyroidectomy: Mayo Clinic experience. *Arch Surg*, *140*, 472–478 discussion 478–479.
246. Udelsman, R., Pasieka, J. L., Sturgeon, C, et al. (2009). Surgery for asymptomatic primary hyperparathyroidism: proceedings of the third international workshop. *J Clin Endocrinol Metab*, *94*, 366–372.
247. Gates, J. D., Benavides, L. C., Shriver, C. D., et al. (2009). Preoperative thyroid ultrasound in all patients undergoing parathyroidectomy? *J Surg Res*, *155*, 254–260.
248. Munk, R. S., Payne, R. J., Luria, B. J., et al. (2008). Preoperative localization in primary hyperparathyroidism. *J Otolaryngol Head Neck Surg*, *37*, 347–354.
249. Civelek, A. C., Ozalp, E., Donovan, P., Udelsman, R. (2002). Prospective evaluation of delayed technetium-99m sestamibi SPECT scintigraphy for preoperative localization of primary hyperparathyroidism. *Surgery*, *131*, 149–157.
250. Hunter, G. J., Schellingerhout, D., Vu, T. H., et al. (2012). Accuracy of four-dimensional CT for the localization of abnormal parathyroid glands in patients with primary hyperparathyroidism. *Radiology*, *264*, 789–795.
251. Giusti F., Tonelli, F., & Brandi, M. L. (2012). Primary hyperparathyroidism in multiple endocrine neoplasia type 1: when to perform surgery? *Clinics* (Sao Paulo), *67*(Suppl. 1), 141–144.
252. Schreinemakers, J. M., Pieterman, C. R., Scholten, A., et al. (2011). The optimal surgical treatment for primary hyperparathyroidism in MEN1 patients: a systematic review. *World J Surg*, *35*, 1993–2005.
253. Waldmann, J., Lopez, C. L., Langer, P., et al. (2010). Surgery for multiple endocrine neoplasia type 1-associated primary hyperparathyroidism. *Br J Surg*, *97*, 1528–1534.
254. Harach, H. R., & Jasani, B. (1992). Parathyroid hyperplasia in multiple endocrine neoplasia type 1: a pathological and immunohistochemical reappraisal. *Histopathology*, *20*, 305–313.
255. Tonelli, F., Marcucci, T., Fratini, G., et al. (2007). Is total parathyroidectomy the treatment of choice for hyperparathyroidism in multiple endocrine neoplasia type 1? *Ann Surg*, *246*, 1075–1082.
256. Goudet, P., Murat, A., Cardot-Bauters, C., et al. (2009). Thymic neuroendocrine tumors in multiple endocrine neoplasia type 1: a comparative study on 21 cases among a series of 761 MEN1 from the GTE (Groupe des Tumeurs Endocrines). *World J Surg*, *33*, 1197–1207.
257. Moyes, V. J., Monson, J. P., Chew, S. L., & Akker, S. A. (2010). Clinical use of cinacalcet in MEN1 hyperparathyroidism. *Int J Endocrinol*, *2010*. doi:10.1155/2010/906163.
258. Carty, S. E., Helm, A. K., Amico, J. A., et al. (1998). The variable penetrance and spectrum of manifestations of multiple endocrine neoplasia type 1. *Surgery*, *124*, 1106–1113; discussion 1113–1114.
259. Dickson, P. V., Rich, T. A., Xing, Y, et al. (2011). Achieving eugastrinemia in MEN1 patients: both duodenal inspection and formal lymph node dissection are important. *Surgery*, *150*, 1143–1152.
260. Ehehalt, F., Saeger, H. D., Schmidt, C. M., & Grutzmann, R. (2009). Neuroendocrine tumors of the pancreas. *Oncologist*, *14*, 456–467.
261. Kloppel, G., Perren, A., & Heitz, P. U. (2004). The gastroenteropancreatic neuroendocrine cell system and its tumors: the WHO classification. *Ann N Y Acad Sci*, *1014*, 13–27.
262. Kulke, M. H., Benson, A. B., 3rd, Bergsland, E., et al. (2012). Neuroendocrine tumors. *J Natl Compr Canc Netw*, *10*, 724–764.
263. Tomassetti, P., Migliori, M., Lalli, S., et al. (2001). Epidemiology, clinical features and diagnosis of gastroenteropancreatic endocrine tumours. *Ann Oncol*, *12*(Suppl. 2), S95–S99.
264. Oberg, K., & Eriksson, B. (2005). Endocrine tumours of the pancreas. *Best Pract Res Clin Gastroenterol*, *19*, 753–781.

265. Thoeni, R. F., Mueller-Lisse, U. G., Chan, R., et al. (2000). Detection of small, functional islet cell tumors in the pancreas: selection of MR imaging sequences for optimal sensitivity. *Radiology*, *214*, 483–490.
266. Niina, Y., Fujimori, N., Nakamura, T., et al. (2012). The current strategy for managing pancreatic neuroendocrine tumors in multiple endocrine neoplasia type 1. *Gut Liver*, *6*, 287–294.
267. Kann, P. H., Balakina, E., Ivan, D., et al. (2006). Natural course of small, asymptomatic neuroendocrine pancreatic tumours in multiple endocrine neoplasia type 1: an endoscopic ultrasound imaging study. *Endocr Relat Cancer*, *13*, 1195–1202.
268. Krausz, Y., Keidar, Z., Kogan, I., et al. (2003). SPECT/CT hybrid imaging with 111In-pentetreotide in assessment of neuroendocrine tumours. *Clin Endocrinol* (Oxf), *59*, 565–573.
269. Nakamoto, Y., Higashi, T., Sakahara, H., et al. (2000). Evaluation of pancreatic islet cell tumors by fluorine-18 fluorodeoxyglucose positron emission tomography: comparison with other modalities. *Clin Nucl Med*, *25*, 115–119.
270. Rufini, V., Calcagni, M. L., & Baum, R. P. (2006). Imaging of neuroendocrine tumors. *Semin Nucl Med*, *36*, 228–247.
271. Jackson, J. E. (2005). Angiography and arterial stimulation venous sampling in the localization of pancreatic neuroendocrine tumours. *Best Pract Res Clin Endocrinol Metab*, *19*, 229–239.
272. Lo, C. Y., Chan, F. L., Tam, S. C., et al. (2000). Value of intra-arterial calcium stimulated venous sampling for regionalization of pancreatic insulinomas. *Surgery*, *128*, 903–909.
273. Metz, D. C., & Jensen, R. T. (2008). Gastrointestinal neuroendocrine tumors: pancreatic endocrine tumors. *Gastroenterology*, *135*, 1469–1492.
274. Hoffmann, K. M., Gibril, F., Entsuah, L. K., et al. (2006). Patients with multiple endocrine neoplasia type 1 with gastrinomas have an increased risk of severe esophageal disease including stricture and the premalignant condition, Barrett's esophagus. *J Clin Endocrinol Metab*, *91*, 204–212.
275. Jensen, R. T., Berna, M. J., Bingham, D. B., & Norton, J. A. (2008). Inherited pancreatic endocrine tumor syndromes: advances in molecular pathogenesis, diagnosis, management, and controversies. *Cancer*, *113*, 1807–1843.
276. Roy, P. K., Venzon, D. J., Shojamanesh, H., et al. (2000). Zollinger-Ellison syndrome: clinical presentation in 261 patients. *Medicine* (Baltimore), *79*, 379–411.
277. Berna, M. J., Hoffmann, K. M., Serrano, J., et al. (2006). Serum gastrin in Zollinger-Ellison syndrome: I. Prospective study of fasting serum gastrin in 309 patients from the National Institutes of Health and comparison with 2229 cases from the literature. *Medicine* (Baltimore), *85*, 295–330.
278. Norton, J. A., Cornelius, M. J., Doppman, J. L., et al. (1987). Effect of parathyroidectomy in patients with hyperparathyroidism, Zollinger-Ellison syndrome, and multiple endocrine neoplasia type I: a prospective study. *Surgery*, *102*, 958–966.
279. McGuigan, J. E., & Wolfe, M. M. (1980). Secretin injection test in the diagnosis of gastrinoma. *Gastroenterology*, *79*, 1324–1331.
280. Berna, M. J., Hoffmann, K. M., Long, S. H., et al. (2006). Serum gastrin in Zollinger-Ellison syndrome: II. Prospective study of gastrin provocative testing in 293 patients from the National Institutes of Health and comparison with 537 cases from the literature: evaluation of diagnostic criteria, proposal of new criteria, and correlations with clinical and tumoral features. *Medicine* (Baltimore), *85*, 331–364.
281. Arnold, R. (2007). Diagnosis and differential diagnosis of hypergastrinemia. *Wien Klin Wochenschr*, *119*, 564–569.
282. Goldman, J. A., Blanton, W. P., Hay, D. W., & Wolfe, M. M. (2009). False-positive secretin stimulation test for gastrinoma associated with the use of proton pump inhibitor therapy. *Clin Gastroenterol Hepatol*, *7*, 600–602.
283. Termanini, B., Gibril, F., Stewart. C. A., et al. (1996). A prospective study of the effectiveness of low dose omeprazole as initial therapy in Zollinger-Ellison syndrome. *Alimentary Pharmacol Ther*, *10*, 61–71.
284. Akerstrom, G., Hessman, O., Hellman, P., & Skogseid, B. (2005). Pancreatic tumours as part of the MEN-1 syndrome. *Best Pract Res Clinical Gastroenterol*, *19*, 819–830.
285. Marx, S., Spiegel, A. M., Skarulis, M. C., et al. (1998). Multiple endocrine neoplasia type 1: clinical and genetic topics. *Ann Intern Med*, *129*, 484–494.
286. Cryer, P. E., Axelrod, L., Grossman, A. B., et al. (2009). Evaluation and management of adult hypoglycemic disorders: an Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab*, *94*, 709–728.
287. Grant, C. S. (2005). Insulinoma. *Best Pract Res Clin Gastroenterol*, *19*, 783–798.
288. Norton, J. A., Fang, T. D., & Jensen, R. T. (2006). Surgery for gastrinoma and insulinoma in multiple endocrine neoplasia type 1. *J Natl Compr Canc Netw*, *4*, 148–153.
289. O'Riordain, D. S., O'Brien, T, van Heerden, J. A., et al. (1994). Surgical management of insulinoma associated with multiple endocrine neoplasia type I. *World J Surg*, *18*, 488–493, discussion 493–494.
290. Perry, R. R., & Vinik, A. I. (1995). Clinical review 72: diagnosis and management of functioning islet cell tumors. *J Clin Endocrinol Metab*, *80*, 2273–2278.
291. Kirkwood, K. S., & Debas, H. T. (1995). Neuroendocrine tumors: common presentations of uncommon diseases. *Compr Ther*, *21*, 719–725.
292. Thomas-Marques, L., Murat, A., Delemer, B., et al. (2006). Prospective endoscopic ultrasonographic evaluation of the frequency of nonfunctioning pancreaticoduodenal endocrine tumors in patients with multiple endocrine neoplasia type 1. *Am J Gastroenterol*, *101*, 266–273.
293. Heymann, M. F., Moreau, A., Chetritt, J, et al. (1996). [Pathologic study with immunohistochemistry of 61 pancreatic endocrine tumors in 16 patients suffering from multiple endocrine neoplasia type I (MEN I). Review of the literature]. *Ann Pathol*, *16*, 167–173.
294. Triponez, F., Goudet, P., Dosseh, D., et al. (2006). Is surgery beneficial for MEN1 patients with small (< or = 2 cm), nonfunctioning pancreaticoduodenal endocrine tumor? An analysis of 65 patients from the GTE. *World J Surg*, *30*, 654–662, discussion 663–664.
295. Geerdink, E. A., Van der Luijt, R. B., & Lips, C. J. (2003). Do patients with multiple endocrine neoplasia syndrome type 1 benefit from periodical screening? *Eur J Endocrinol*, *149*, 577–582.
296. Trouillas, J., Labat-Moleur, F., Sturm, N., et al. (2008). Pituitary tumors and hyperplasia in multiple endocrine neoplasia type 1 syndrome (MEN1): a case-control study in a series of 77 patients versus 2509 non-MEN1 patients. *Am J Surg Pathol*, *32*, 534–543.
297. Verges, B., Boureille, F., Goudet, P., et al. (2002). Pituitary disease in MEN type 1 (MEN1): data from the France-Belgium MEN1 multicenter study. *J Clin Endocrinol Metab*, *87*, 457–465.
298. Stratakis, C. A., Schussheim, D. H., Freedman, S. M., et al. (2000). Pituitary macroadenoma in a 5-year-old: an early expression of multiple endocrine neoplasia type 1. *J Clin Endocrinol Metab*, *85*, 4776–4780.
299. Beckers, A., Betea, D., Valdes Socin, H., & Stevenaert, A. (2003). The treatment of sporadic versus MEN1-related pituitary adenomas. *J Intern Med*, *253*, 599–605.
300. Sakurai, A., Imai, T., Kikumori, T., et al. (2013). Thymic neuroendocrine tumor in multiple endocrine neoplasia type 1: female patients are not rare exceptions. *Clin Endocrinol* (Oxf), *78*, 248–254.
301. Boix, E., Pico, A., Pinedo, R., et al. (2002). Ectopic growth hormone-releasing hormone secretion by thymic carcinoid tumour. *Clin Endocrinol* (Oxf), *57*, 131–134.
302. Takagi, J., Otake, K., Morishita, M., et al. (2006). Multiple endocrine neoplasia type I and Cushing's syndrome due to an aggressive ACTH producing thymic carcinoid. *Intern Med*, *45*, 81–86.
303. Gibril, F., Chen, Y. J., Schrump, D. S., et al. (2003). Prospective study of thymic carcinoids in patients with multiple endocrine neoplasia type 1. *J Clin Endocrinol Metab*, *88*, 1066–1081.
304. Mayo, J. R., Aldrich, J., & Muller, N. L. (2003). Radiation exposure at chest CT: a statement of the Fleischner Society. *Radiology*, *228*, 15–21.
305. Filosso, P. L., Actis Dato, G. M., Ruffini, E, et al. (2004). Multidisciplinary treatment of advanced thymic neuroendocrine carcinoma (carcinoid): report of a successful case and review of the literature. *J Thorac Cardiovasc Surg*, *127*, 1215–1219.
306. Kidd, M., & Gustafsson, B. I. (2012). Management of gastric carcinoids (neuroendocrine neoplasms). *Curr Gastroenterol Rep*, *14*, 467–472.

307. Delle Fave, G., Capurso, G., Milione, M., & Panzuto, F. (2005). Endocrine tumours of the stomach. *Best Pract Res Clinical Gastroenterol*, *19*, 659–673.
308. Norton, J. A., Melcher, M. L., Gibril, F., & Jensen, R. T. (2004). Gastric carcinoid tumors in multiple endocrine neoplasia-1 patients with Zollinger-Ellison syndrome can be symptomatic, demonstrate aggressive growth, and require surgical treatment. *Surgery*, *136*, 1267–1274.
309. Lehy, T., Cadiot, G., Mignon, M., et al. (1992). Influence of multiple endocrine neoplasia type 1 on gastric endocrine cells in patients with the Zollinger-Ellison syndrome. *Gut*, *33*, 1275–1279.
310. Berna, M. J., Annibale, B., Marignani, M., et al. (2008). A prospective study of gastric carcinoids and enterochromaffin-like cell changes in multiple endocrine neoplasia type 1 and Zollinger-Ellison syndrome: identification of risk factors. *J Clin Endocrinol Metab*, *93*, 1582–1591.
311. Richards, M. L., Gauger, P., Thompson, N. W., & Giordano, T. J. (2004). Regression of type II gastric carcinoids in multiple endocrine neoplasia type 1 patients with Zollinger-Ellison syndrome after surgical excision of all gastrinomas. *World J Surg*, *28*, 652–658.
312. Tomassetti, P., Migliori, M., Caletti, G. C., et al. (2000). Treatment of type II gastric carcinoid tumors with somatostatin analogues. *N Engl J Med*, *343*, 551–554.
313. Modlin, I. M., & Nangia, A. K. (1992). The pathobiology of the human enterochromaffin-like cell. *Yale J Biol Med*, *65*, 775–792, discussion 827–829.
314. Hakanson, R., & Sundler, F. (1990). Proposed mechanism of induction of gastric carcinoids: the gastrin hypothesis. *Eur J Clin Invest*, *20*(Suppl. 1), S65–S71.
315. Gatta-Cherifi, B., Chabre, O., Murat, A., et al. (2012). Adrenal involvement in MEN1: analysis of 715 cases from the Groupe d'etude des Tumeurs Endocrines database. *Eur J Endocrinol*, *166*, 269–279.
316. Langer, P., Cupisti, K., Bartsch, D. K., et al. (2002). Adrenal involvement in multiple endocrine neoplasia type 1. *World J Surg*, *26*, 891–896.
317. Darling, T. N., Skarulis, M. C., Steinberg, S. M., et al. (1997). Multiple facial angiofibromas and collagenomas in patients with multiple endocrine neoplasia type 1. *Arch Dermatol*, *133*, 853–857.
318. Vidal, A., Iglesias, M. J., Fernandez, B., et al. (2008). Cutaneous lesions associated to multiple endocrine neoplasia syndrome type 1. *J Eur Acad Dermatol Venereol*, *22*, 835–838.
319. Newey, P. J., & Thakker, R. V. (2011). Role of multiple endocrine neoplasia type 1 mutational analysis in clinical practice. *Endocr Pract*, *17*(Suppl. 3), 8–17.
320. Sipple, J. H. (1961). The association of pheochromocytoma with carcinoma of the thyroid gland. *Am J Med*, *31*, 163–166.
321. Cushman, P., Jr. (1962). Familial endocrine tumors; report of two unrelated kindred affected with pheochromocytomas, one also with multiple thyroid carcinomas. *Am J Med*, *32*, 352–360.
322. Steiner, A. L., Goodman, A. D., & Powers, S. R. (1968). Study of a kindred with pheochromocytoma, medullary thyroid carcinoma, hyperparathyroidism and Cushing's disease: multiple endocrine neoplasia, type 2. *Medicine* (Baltimore), *47*, 371–409.
323. Simpson, N. E., & Kidd, K. K. (1987). Where is the locus for multiple endocrine neoplasia type 2A? *Henry Ford Hosp Med J*, *35*, 168–171.
324. Mathew, C. G., Chin, K. S., Easton, D. F., et al. (1987). A linked genetic marker for multiple endocrine neoplasia type 2A on chromosome 10. *Nature*, *328*, 527–528.
325. Williams, E. D., & Pollock, D. J. (1966). Multiple mucosal neuromata with endocrine tumours: a syndrome allied to von Recklinghausen's disease. *J Pathol Bacteriol*, *91*, 71–80.
326. Chong, G. C., Beahrs, O. H., Sizemore, G. W., & Woolner, L. H. (1975). Medullary carcinoma of the thyroid gland. *Cancer 35*, 695–704.
327. Eng, C., Clayton, D., Schuffenecker, I., et al. (1996). The relationship between specific RET proto-oncogene mutations and disease phenotype in multiple endocrine neoplasia type 2: international RET mutation consortium analysis. *JAMA*, *276*, 1575–1579.
328. Szinnai, G., Sarnacki, S., & Polak, M. (2007). Hereditary medullary thyroid carcinoma: how molecular genetics made multiple endocrine neoplasia type 2 a paediatric disease. *Endocr Dev*, *10*, 173–187.
329. Waguespack, S. G. (2009). A perspective from pediatric endocrinology on the hereditary medullary thyroid carcinoma syndromes. *Thyroid*, *19*, 543–546.
330. Frank-Raue, K., Rondot, S., & Raue, F. (2010). Molecular genetics and phenomics of RET mutations: impact on prognosis of MTC. *Mol Cell Endocrinol*, *322*, 2–7.
331. Decker, R. A., Peacock, M. L., & Watson, P. (1998). Hirschsprung disease in MEN 2A: increased spectrum of RET exon 10 genotypes and strong genotype-phenotype correlation. *Hum Mol Genet*, *7*, 129–134.
332. Moore, S. W., & Zaahl, M. G. (2008). Multiple endocrine neoplasia syndromes, children, Hirschsprung's disease and RET. *Pediatr Surg Int*, *24*, 521–530.
333. Moore, S. W., & Zaahl, M. (2010). Familial associations in medullary thyroid carcinoma with Hirschsprung disease: the role of the RET-C620 "Janus" genetic variation. *J Pediatr Surg*, *45*, 393–396.
334. Gagel, R. F. (2002). When "the 7-year itch" is indicative of an endocrine malignant condition. *Endocr Pract*, *8*, 72–74.
335. Verga, U., Fugazzola, L., Cambiaghi, S., et al. (2003). Frequent association between MEN 2A and cutaneous lichen amyloidosis. *Clin Endocrinol* (Oxf), *59*, 156–161.
336. Romei, C., Mariotti, S., Fugazzola, L., et al. (2010). Multiple endocrine neoplasia type 2 syndromes (MEN 2): results from the ItaMEN network analysis on the prevalence of different genotypes and phenotypes. *Eur J Endocrinol*, *163*, 301–308.
337. Zbuk, K. M., & Eng, C. (2007). Cancer phenomics: RET and PTEN as illustrative models. *Nat Rev Cancer*, *7*, 35–45.
338. Frank-Raue, K., Rondot, S., Schulze, E., & Raue, F. (2007). Change in the spectrum of RET mutations diagnosed between 1994 and 2006. *Clin Lab*, *53*, 273–282.
339. Margraf, R. L., Crockett, D. K., Krautscheid, P. M., et al. (2009). Multiple endocrine neoplasia type 2 RET protooncogene database: repository of MEN2-associated RET sequence variation and reference for genotype/phenotype correlations. *Hum Mutat*, *30*, 548–556.
340. Wohllk, N., Cote, G. J., Bugalho, M. M., et al. (1996). Relevance of RET proto-oncogene mutations in sporadic medullary thyroid carcinoma. *J Clin Endocrinol Metab*, *81*, 3740–3745.
341. Schuffenecker, I., Ginet, N., Goldgar, D., et al. (1997). Prevalence and parental origin of de novo RET mutations in multiple endocrine neoplasia type 2A and familial medullary thyroid carcinoma. Le Groupe d'Etude des Tumeurs a Calcitonine. *Am J Hum Genet*, *60*, 233–237.
342. Machens, A., Gimm, O., Hinze, R., et al. (2001). Genotype-phenotype correlations in hereditary medullary thyroid carcinoma: oncological features and biochemical properties. *J Clin Endocrinol Metab*, *86*, 1104–1109.
343. Kouvaraki, M. A., Shapiro, S. E., Perrier, N. D., et al. (2005). RET proto-oncogene: a review and update of genotype-phenotype correlations in hereditary medullary thyroid cancer and associated endocrine tumors. *Thyroid*, *15*, 531–544.
344. Modigliani, E., Vasen, H. M., Raue, K., et al. (1995). Pheochromocytoma in multiple endocrine neoplasia type 2: European study. The Euromen Study Group. *J Intern Med*, *238*, 363–367.
345. DeLellis, R. A., Wolfe, H. J., Gagel, R. F., et al. (1976). Adrenal medullary hyperplasia: a morphometric analysis in patients with familial medullary thyroid carcinoma. *Am J Pathol*, *83*, 177–196.
346. Carney, J. A., Sizemore, G. W., & Tyce, G. M. (1975). Bilateral adrenal medullary hyperplasia in multiple endocrine neoplasia, type 2: the precursor of bilateral pheochromocytoma. *Mayo Clin Proc*, *50*, 3–10.
347. Lairmore, T. C., Ball, D. W., Baylin, S. B., & Wells, S. A., Jr. (1993). Management of pheochromocytomas in patients with multiple endocrine neoplasia type 2 syndromes. *Ann Surg*, *217*, 595–601, discussion 601–603.
348. Yip, L., Cote, G. J., Shapiro, S. E., et al. (2003). Multiple endocrine neoplasia type 2: evaluation of the genotype-phenotype relationship. *Arch Surg*, *138*, 409–416, discussion 416.
349. Quayle, F. J., Fialkowski, E. A., Benveniste, R., & Moley J. F. (2007). Pheochromocytoma penetrance varies by RET mutation in MEN 2A. *Surgery*, *142*, 800–805, discussion 805, e801.
350. Nguyen, L., Niccoli-Sire, P., Caron, P., et al. (2001). Pheochromocytoma in multiple endocrine neoplasia type 2: a prospective study. *Eur J Endocrinol*, *144*, 37–44.

351. Frank-Raue K, Rybicki L. A., Erlic Z, et al. (2011). Risk profiles and penetrance estimations in multiple endocrine neoplasia type 2A caused by germline RET mutations located in exon 10. Hum Mutat 32, 51–58.
352. Machens, A., Brauckhoff, M., Holzhausen, H. J., et al. (2005). Codon-specific development of pheochromocytoma in multiple endocrine neoplasia type 2. *J Clin Endocrinol Metab*, *90*, 3999–4003.
353. Iihara, M., Yamashita, T., Okamoto, T., et al. (1997). A nationwide clinical survey of patients with multiple endocrine neoplasia type 2 and familial medullary thyroid carcinoma in Japan. *Jpn J Clin Oncol*, *27*, 128–134.
354. Bryant, J., Farmer, J., Kessler, L. J., et al. (2003). Pheochromocytoma: the expanding genetic differential diagnosis. *J Natl Cancer Inst*, *95*, 1196–1204.
355. Calmettes, C., Rosenberg-Gourgin, M., Caron, J., & Feingold, N. (1992). Pheochromocytoma: a frequent indicator for MEN 2. *Henry Ford Hosp Med J*, *40*, 276–277.
356. Schuffenecker, I., Virally-Monod, M., Brohet, R., et al. (1998). Risk and penetrance of primary hyperparathyroidism in multiple endocrine neoplasia type 2A families with mutations at codon 634 of the RET proto-oncogene. Groupe D'etude des Tumeurs a Calcitonine. *J Clin Endocrinol Metab*, *83*, 487–491.
357. Carling, T., & Udelsman, R. (2005). Parathyroid surgery in familial hyperparathyroid disorders. *J Intern Med*, *257*, 27–37.
358. Magalhaes, P. K., Antonini, S. R., de Paula, F. J., et al. (2011). Primary hyperparathyroidism as the first clinical manifestation of multiple endocrine neoplasia type 2a in a 5-year-old child. *Thyroid*, *21*, 547–550.
359. Cranston, A. N., Carniti, C., Oakhill, K., et al. (2006). RET is constitutively activated by novel tandem mutations that alter the active site resulting in multiple endocrine neoplasia type 2B. *Cancer Res*, *66*, 10179–10187.
360. Jasim, S., Ying, A. K., Waguespack, S. G., et al. (2011). Multiple endocrine neoplasia type 2B with a RET proto-oncogene A883F mutation displays a more indolent form of medullary thyroid carcinoma compared with a RET M918T mutation. *Thyroid*, *21*, 189–192.
361. Carlson, K. M., Bracamontes, J., Jackson, C. E., et al. (1994). Parent-of-origin effects in multiple endocrine neoplasia type 2B. *Am J Hum Genet*, *55*, 1076–1082.
362. Jones, B. A., & Sisson, J. C. (1983). Early diagnosis and thyroidectomy in multiple endocrine neoplasia, type 2b. *J Pediatr*, *102*, 219–223.
363. Kaufman, F. R., Roe, T. F., Isaacs, H., Jr., & Weitzman, J. J. (1982). Metastatic medullary thyroid carcinoma in young children with mucosal neuroma syndrome. *Pediatrics*, *70*, 263–267.
364. Saltzman, C. L., Herzenberg, J. E., Phillips, W. A., et al. (1988). Thick lips, bumpy tongue, and slipped capital femoral epiphysis—a deadly combination. *J Pediatr Orthop*, *8*, 219–222.
365. O'Riordain, D. S., O'Brien, T, Crotty, T. B., et al. (1995). Multiple endocrine neoplasia type 2B: more than an endocrine disorder. *Surgery*, *118*, 936–942.
366. Brauckhoff, M., Machens, A., Hess, S., et al. (2008). Premonitory symptoms preceding metastatic medullary thyroid cancer in MEN 2B: an exploratory analysis. *Surgery*, *144*, 1044–1050, discussion 1050–1053.
367. Brauckhoff, M., Gimm, O., Weiss, C. L., et al. (2004). Multiple endocrine neoplasia 2B syndrome due to codon 918 mutation: clinical manifestation and course in early and late onset disease. *World J Surg*, *28*, 1305–1311.
368. Camacho, C. P., Hoff, A. O., Lindsey, S. C., et al. (2008). Early diagnosis of multiple endocrine neoplasia type 2B: a challenge for physicians. *Arq Bras Endocrinol Metabol*, *52*, 1393–1398.
369. Parker, D. G., Robinson, B. G., & O'Donnell, B. A. (2004). External ophthalmic findings in multiple endocrine neoplasia type 2B. *Clin Experiment Ophthalmol*, *32*, 420–423.
370. Vasen, H. F., van der Feltz, M., Raue, F., et al. (1992). The natural course of multiple endocrine neoplasia type IIb: a study of 18 cases. *Arch Intern Med*, *152*, 1250–1252.
371. Leboulleux, S., Travagli, J. P., Caillou, B., et al. (2002). Medullary thyroid carcinoma as part of a multiple endocrine neoplasia type 2B syndrome: influence of the stage on the clinical course. *Cancer*, *94*, 44–50.
372. O'Riordain, D. S., O'Brien, T., Weaver, A. L., et al. (1994). Medullary thyroid carcinoma in multiple endocrine neoplasia types 2A and 2B. *Surgery*, *116*, 1017–1023.
373. Frohnauer, M. K., & Decker, R. A. (2000). Update on the MEN 2A c804 RET mutation: is prophylactic thyroidectomy indicated? *Surgery*, *128*, 1052–1057, discussion 1057–1058.
374. Erlic, Z., Hoffmann, M. M., Sullivan, M., et al. (2010). Pathogenicity of DNA variants and double mutations in multiple endocrine neoplasia type 2 and von Hippel-Lindau syndrome. *J Clin Endocrinol Metab*, *95*, 308–313.
375. Crockett, D. K., Piccolo, S. R., Ridge, P. G., et al. (2011). Predicting phenotypic severity of uncertain gene variants in the RET proto-oncogene. *PLoS One*, *6*, e18380.
376. Waguespack, S. G., & Rich, T. A. (2010). Multiple endocrine neoplasia [corrected] syndrome type 2B in early childhood: long-term benefit of prophylactic thyroidectomy. *Cancer*, *116*, 2284.
377. Grubbs, E. G., Waguespack, S. G., Rich, T. A., et al. (2010). Do the recent American Thyroid Association (ATA) guidelines accurately guide the timing of prophylactic thyroidectomy in MEN2A? *Surgery*, *148*, 1302–1310.
378. Frank-Raue, K., Buhr, H., Dralle, H. et al. (2006). Long-term outcome in 46 gene carriers of hereditary medullary thyroid carcinoma after prophylactic thyroidectomy: impact of individual RET genotype. *Eur J Endocrinol*, *155*, 229–236.
379. Rodriguez, G. J., Balsalobre, M. D., Pomares, F, et al. (2002). Prophylactic thyroidectomy in MEN 2A syndrome: experience in a single center. *J Am Coll Surg*, *195*, 159–166.
380. Elisei, R., & Pinchera, A. (2012). Advances in the follow-up of differentiated or medullary thyroid cancer. *Nat Rev Endocrinol*, *8*, 466–475.
381. Morris, L. F., Waguespack, S. G., Edeiken-Monroe, B. S., et al. (2013). Ultrasonography should not guide the timing of thyroidectomy in pediatric patients diagnosed with multiple endocrine neoplasia syndrome 2A through genetic screening. *Ann Surg Oncol*, *20*, 53–59.
382. Elisei, R., Romei, C., Renzini, G., et al. (2012). The timing of total thyroidectomy in RET gene mutation carriers could be personalized and safely planned on the basis of serum calcitonin: 18 years experience at one single center. *J Clin Endocrinol Metab*, *97*, 426–435.
383. Pellegata, N. S. (2012). MENX and MEN4. *Clinics* (Sao Paulo), *67*(Suppl. 1), 13–18.
384. Pellegata, N. S., Quintanilla-Martinez, L., Siggelkow, H., et al. (2006). Germ-line mutations in p27Kip1 cause a multiple endocrine neoplasia syndrome in rats and humans. *Proc Natl Acad Sci U S A*, *103*, 15558–15563.
385. Georgitsi, M., Raitila, A., Karhu, A., et al. (2007). Germline CDKN1B/p27Kip1 mutation in multiple endocrine neoplasia. *J Clin Endocrinol Metab*, *92*, 3321–3325.
386. Agarwal, S. K., Mateo, C. M., & Marx, S. J. (2009). Rare germline mutations in cyclin-dependent kinase inhibitor genes in multiple endocrine neoplasia type 1 and related states. *J Clin Endocrinol Metab*, *94*, 1826–1834.
387. Latif, F., Tory, K., Gnarra, J., et al. (1993). Identification of the von Hippel-Lindau disease tumor suppressor gene. *Science*, *260*, 1317–1320.
388. Lonser, R. R., Glenn, G. M., Walther, M, et al. (2003). von Hippel-Lindau disease. *Lancet*, *361*, 2059–2067.
389. Maher, E. R., Neumann, H. P., & Richard, S. (2011). von Hippel-Lindau disease: a clinical and scientific review. *Eur J Hum Genet*, *19*, 617–623.
390. Frantzen, C., Links, T. P., & Giles, R. H. (2000). Von Hippel-Lindau disease. In R. A. Pagon, T. D. Bird, C. R. Dolan, et al. (Eds.), *GeneReviews*™ (2010/03/20 ed.). Seattle, WA: University of Washington, Seattle. (Last updated 21 June 2012).
391. Stolle, C., Glenn, G., Zbar, B., et al. (1998). Improved detection of germline mutations in the von Hippel-Lindau disease tumor suppressor gene. *Hum Mutat*, *12*, 417–423.
392. Maher, E. R., Webster, A. R., Richards, F. M., et al. (1996). Phenotypic expression in von Hippel-Lindau disease: correlations with germline VHL gene mutations. *J Med Genet*, *33*, 328–332.
393. Sovinz, P., Urban, C., Uhrig, S., et al. (2010). Pheochromocytoma in a 2.75-year-old-girl with a germline von Hippel-Lindau mutation Q164R. *Am J Med Genet A*, *152A*, 1752–1755.

394. VHL Family Alliance, Boston (2012). *The VHL handbook: what you need to know about VHL* (4th ed.) .
395. Prasad, R., Johnston, L. B., Savage, M. O., et al. (2011). Pediatric endocrine screening for von Hippel-Lindau disease: benefits and the challenge of compliance. *J Endocrinol Invest*, *34*, 296–299.
396. Boedeker, C. C., Erlic, Z., Richard, S., et al. (2009). Head and neck paragangliomas in von Hippel-Lindau disease and multiple endocrine neoplasia type 2. *J Clin Endocrinol Metab*, *94*, 1938–1944.
397. Gaal, J., van Nederveen, F. H., Erlic, Z., et al. (2009). Parasympathetic paragangliomas are part of the Von Hippel-Lindau syndrome. *J Clin Endocrinol Metab*, *94*, 4367–4371.
398. Charlesworth, M., Verbeke, C. S., Falk, G. A., et al. (2012). Pancreatic lesions in von Hippel-Lindau disease? A systematic review and meta-synthesis of the literature . *J Gastrointest Surg*, *16*, 1422–1428.
399. Blansfield, J. A., Choyke, L, Morita, S. Y., et al. (2007). Clinical, genetic and radiographic analysis of 108 patients with von Hippel-Lindau disease (VHL) manifested by pancreatic neuroendocrine neoplasms (PNETs). *Surgery*, *142*, 814–818, discussion 818, e811-e812.
400. Corcos, O., Couvelard, A., Giraud, S., et al. (2008). Endocrine pancreatic tumors in von Hippel-Lindau disease: clinical, histological, and genetic features. *Pancreas*, *37*, 85–93.
401. Wagner, M., Browne, H. N., Marston Linehan, W., et al. (2010). Lipid cell tumors in two women with von Hippel-Lindau syndrome. *Obstet Gynecol*, *116*(Suppl. 2), 535–539.
402. Jasperson, K. W., & Burt, R. W. (1998). APC-associated polyposis conditions. In R. A. Pagon, T. D. Bird, C. R. Dolan, et al. (Eds.), *GeneReviews*™. Seattle, WA: University of Washington, Seattle. (Last updated 27 October 2011).
403. Groen, E. J., Roos, A., Muntinghe F. L., et al. (2008). Extra-intestinal manifestations of familial adenomatous polyposis. *Ann Surg Oncol*, *15*, 2439–2450.
404. Marchesa, P., Fazio, V. W., Church, J. M., & McGannon, E. (1997). Adrenal masses in patients with familial adenomatous polyposis. *Dis Colon Rectum*, *40*, 1023–1028.
405. Else, T. (2012). Association of adrenocortical carcinoma with familial cancer susceptibility syndromes. *Mol Cell Endocrinol*, *351*, 66–70.
406. Herraiz, M., Barbesino, G., Faquin, W., et al. (2007). Prevalence of thyroid cancer in familial adenomatous polyposis syndrome and the role of screening ultrasound examinations. *Clin Gastroenterol Hepatol*, *5*, 367–373.
407. Plail, R. O., Bussey, H. J., Glazer, G., & Thomson, J. P. (1987). Adenomatous polyposis: an association with carcinoma of the thyroid. *Br J Surg*, *74*, 377–380.
408. Cetta, F., Montalto, G., Gori, M., et al. (2000). Germline mutations of the APC gene in patients with familial adenomatous polyposis-associated thyroid carcinoma: results from a European cooperative study. *J Clin Endocrinol Metab*, *85*, 286–292.
409. Truta, B., Allen, B. A., Conrad, P. G., et al. (2003). Genotype and phenotype of patients with both familial adenomatous polyposis and thyroid carcinoma. *Fam Cancer*, *2*, 95–99.
410. Shuman, C., Beckwith, J. B., Smith, A. C., & Weksberg, R. (2000). Beckwith-Wiedemann syndrome. In R. A. Pagon, T. D. Bird, C. R. Dolan, et al. (Eds.), *GeneReviews*™. Seattle, WA: University of Washington, Seattle. (Last updated 14 December 2010).
411. Cohen, M. M., Jr. (2005). Beckwith-Wiedemann syndrome: historical, clinicopathological, and etiopathogenetic perspectives. *Pediatr Dev Pathol*, *8*, 287–304.
412. Lapunzina, P. (2005). Risk of tumorigenesis in overgrowth syndromes: a comprehensive review. *Am J Med Genet C Semin Med Genet*, *137C*, 53–71.
413. Ribeiro, R. C., Pinto, E. M., & Zambetti, G. P. (2010). Familial predisposition to adrenocortical tumors: clinical and biological features and management strategies. *Best Pract Res Clin Endocrinol Metab*, *24*, 477–490.
414. Carney, J. A., Sheps, S. G., Go, V. L., & Gordon, H. (1977). The triad of gastric leiomyosarcoma, functioning extra-adrenal paraganglioma and pulmonary chondroma. *N Engl J Med*, *296*, 1517–1518.
415. Carney, J. A. (1999). Gastric stromal sarcoma, pulmonary chondroma, and extra-adrenal paraganglioma (Carney triad): natural history, adrenocortical component, and possible familial occurrence. *Mayo Clin Proc*, *74*, 543–552.
416. Carney, J. A. (2009). Carney triad: a syndrome featuring paraganglionic, adrenocortical, and possibly other endocrine tumors. *J Clin Endocrinol Metab*, *94*, 3656–3662.
417. Malkin, D., Li, F. P., Strong, L. C., et al. (1990). Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. *Science*, *250*, 1233–1238.
418. Gonzalez, K. D., Noltner, K. A., Buzin, C. H., et al. (2009). Beyond Li Fraumeni syndrome: clinical characteristics of families with p53 germline mutations. *J Clin Oncol*, *27*, 1250–1256.
419. Schneider, K., & Garber, J. (1999). Li-Fraumeni syndrome. In R. A. Pagon, T. D. Bird, C. R. Dolan, et al. (Eds.), *GeneReviews*™. Seattle, WA, University of Washington, Seattle. (Last updated 9 February 2010).
420. Masciari, S., Van den Abbeele, A. D., Diller, L. R., et al. (2008). F18-fluorodeoxyglucose-positron emission tomography/computed tomography screening in Li-Fraumeni syndrome. *JAMA*, *299*, 1315–1319.
421. Olivier, M., Hollstein, M., & Hainaut, P. (2010). TP53 mutations in human cancers: origins, consequences, and clinical use. *Cold Spring Harb Perspect Biol*, *2*, a001008.
422. Ribeiro, R. C., Pinto, E. M., Zambetti, G. P., & Rodriguez-Galindo, C. (2012). The International Pediatric Adrenocortical Tumor Registry initiative: contributions to clinical, biological, and treatment advances in pediatric adrenocortical tumors. *Mol Cell Endocrinol*, *351*, 37–43.
423. Choong, S., Latiff, Z., Mohamed, M., et al. (2012). Childhood adrenocortical carcinoma as a sentinel cancer for detecting families with germline TP53 mutations. *Clin Genet*, *82*, 564–568.
424. Ribeiro, R. C., Sandrini, F., Figueiredo, B., et al. (2001). An inherited p53 mutation that contributes in a tissue-specific manner to pediatric adrenal cortical carcinoma. *Proc Natl Acad Sci U S A*, *98*, 9330–9335.
425. Friedman, J. M. (1998). Neurofibromatosis 1. In R. A. Pagon, T. D. Bird, C. R. Dolan, et al. (Eds.), *GeneReviews*™. Seattle, WA: University of Washington, Seattle. (Last updated 3 May 2012).
426. Walther, M. M., Herring, J., Enquist, E., et al. (1999). von Recklinghausen's disease and pheochromocytomas. *J Urol*, *162*, 1582–1586.
427. Welander, J., Soderkvist, P., & Gimm, O. (2011). Genetics and clinical characteristics of hereditary pheochromocytomas and paragangliomas. *Endocr Relat Cancer*, *18*, R253-R276.
428. Eisenhofer, G., Pacak, K., Huynh, T. T., et al. (2011). Catecholamine metabolomic and secretory phenotypes in phaeochromocytoma. *Endocr Relat Cancer*, *18*, 97–111.
429. Kimura, N., Watanabe, T., Fukase, M., et al. (2002). Neurofibromin and NF1 gene analysis in composite pheochromocytoma and tumors associated with von Recklinghausen's disease. *Mod Pathol*, *15*, 183–188.
430. Relles, D., Baek, J., Witkiewicz, A., & Yeo, C. J. (2010). Periampullary and duodenal neoplasms in neurofibromatosis type 1: two cases and an updated 20-year review of the literature yielding 76 cases. *J Gastrointest Surg*, *14*, 1052–1061.
431. Perren, A., Wiesli, P., Schmid, S., et al. (2006). Pancreatic endocrine tumors are a rare manifestation of the neurofibromatosis type 1 phenotype: molecular analysis of a malignant insulinoma in a NF-1 patient. *Am J Surg Pathol*, *30*, 1047–1051.
432. Lee, W. S., Koh, Y. S., Kim J. C., et al. (2005). Zollinger-Ellison syndrome associated with neurofibromatosis type 1: a case report. *BMC Cancer*, *5*, 85.
433. Kodama, H., Iihara, M., Okamoto, T., & Obara, T. (2007). Water-clear cell parathyroid adenoma causing primary hyperparathyroidism in a patient with neurofibromatosis type 1: report of a case. *Surg Today*, *37*, 884–887.
434. Demirjian, A. N., Grossman, J. M., Chan, J. L., & Parangi, S. (2008). Parathyroid carcinoma and neurofibromatosis. *Surgery*, *144*, 827–829.
435. Josefson, J., Listernick, R., Fangusaro, J. R., et al. (2011). Growth hormone excess in children with neurofibromatosis type 1-associated and sporadic optic pathway tumors. *J Pediatr*, *158*, 433–436.
436. Beggs, A. D., Latchford, A. R., Vasen, H. F., et al. (2010). Peutz-Jeghers syndrome: a systematic review and recommendations for management. *Gut*, *59*, 975–986.

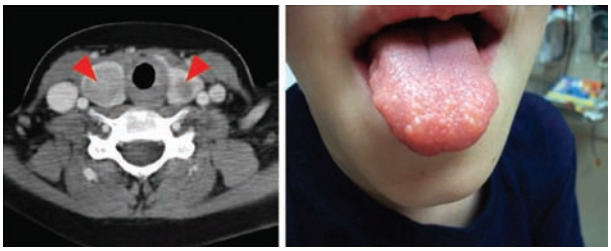
437. Amos, C. I., Frazier, M. L., Wei, C., & McGarrity, T. J. (2001). Peutz-Jeghers syndrome. In R. A. Pagon, T. D. Bird, C. R. Dolan, et al. (Eds.), *GeneReviews*™. Seattle, WA: University of Washington, Seattle. (Last updated 22 February 2011).
438. van Lier, M. G., Wagner, A., Mathus-Vliegen, E. M., et al. (2010). High cancer risk in Peutz-Jeghers syndrome: a systematic review and surveillance recommendations. *Am J Gastroenterol*, *105*, 1258–1264, author reply, 1265.
439. Triggiani, V., Guastamacchia, E., Renzulli, G., et al. (2011). Papillary thyroid carcinoma in Peutz-Jeghers syndrome. *Thyroid*, *21*, 1273–1277.
440. Dreyer, L., Jacyk, W. K., & du Plessis, D. J. (1994). Bilateral large-cell calcifying Sertoli cell tumor of the testes with Peutz-Jeghers syndrome: a case report. *Pediatr Dermatol*, *11*, 335–337.
441. Lefevre, H., Bouvattier, C., Lahlou, N., et al. (2006). Prepubertal gynecomastia in Peutz-Jeghers syndrome: incomplete penetrance in a familial case and management with an aromatase inhibitor. *Eur J Endocrinol*, *154*, 221–227.
442. Young, R. H., Welch, W. R., Dickersin, G. R., & Scully, R. E. (1982). Ovarian sex cord tumor with annular tubules: review of 74 cases including 27 with Peutz-Jeghers syndrome and four with adenoma malignum of the cervix. *Cancer*, *50*, 1384–1402.
443. Eng, C. (2001). PTEN hamartoma tumor syndrome (PHTS). In R. A. Pagon, T. D. Bird, C. R. Dolan, et al. (Eds.), *GeneReviews*™. Seattle, WA: University of Washington, Seattle. (Last updated 19 April 2012).
444. Liaw, D., Marsh, D. J., Li, J., et al. (1997). Germline mutations of the PTEN gene in Cowden disease, an inherited breast and thyroid cancer syndrome. *Nat Genet*, *16*, 64–67.
445. Mester, J. L., Tilot, A. K., Rybicki, L. A., et al. (2011). Analysis of prevalence and degree of macrocephaly in patients with germline PTEN mutations and of brain weight in Pten knock-in murine model. *Eur J Hum Genet*, *19*, 763–768.
446. Harach, H. R., Soubeyran, I., Brown, A., et al. (1999). Thyroid pathologic findings in patients with Cowden disease. *Ann Diagn Pathol*, *3*, 331–340.
447. Smith, J. R., Marqusee, E., Webb, S., et al. (2011). Thyroid nodules and cancer in children with PTEN hamartoma tumor syndrome. *J Clin Endocrinol Metab*, *96*, 34–37.
448. Laury, A. R., Bongiovanni, M., Tille, J. C., et al. (2011). Thyroid pathology in PTEN-hamartoma tumor syndrome: characteristic findings of a distinct entity. *Thyroid*, *21*, 135–144.
449. Ngeow, J., Mester, J., Rybicki, L. A., et al. (2011). Incidence and clinical characteristics of thyroid cancer in prospective series of individuals with Cowden and Cowden-like syndrome characterized by germline PTEN, SDH, or KLLN alterations. *J Clin Endocrinol Metab*, *96*, E2063–E2071.
450. Northrup, H., Koenig, M. K., & Au, K. S. (1999). Tuberous sclerosis complex. In R. A. Pagon, T. D. Bird, C. R. Dolan, et al. (Eds.), *GeneReviews*™. Seattle, WA: University of Washington, Seattle. (Last updated 23 November 2011).
451. European Chromosome 16 Tuberous Sclerosis Consortium. (1993). Identification and characterization of the tuberous sclerosis gene on chromosome 16. *Cell*, *75*, 1305–1315.
452. van Slegtenhorst, M., de Hoogt, R., Hermans, C., et al. (1997). Identification of the tuberous sclerosis gene TSC1 on chromosome 9q34. *Science*, *277*, 805–808.
453. Dworakowska, D., & Grossman, A. B. (2009). Are neuroendocrine tumours a feature of tuberous sclerosis? A systematic review. *Endocr Relat Cancer*, *16*, 45–58.
454. Arva, N. C., Pappas, J. G., Bhatla, T, et al. (2012). Well-differentiated pancreatic neuroendocrine carcinoma in tuberous sclerosis: case report and review of the literature. *Am J Surg Pathol*, *36*, 149–153.
455. Skinner, M. A., DeBenedetti, M. K., Moley, J. F., et al. (1996). Medullary thyroid carcinoma in children with multiple endocrine neoplasia types 2A and 2B. *J Pediatr Surg*, *31*, 177–181, discussion 181–182.
456. Hernandez, F. C., Sanchez, M., Alvarez, A., et al. (2000). A five-year report on experience in the detection of pheochromocytoma. *Clin Biochem*, *33*, 649–655.
457. Schiavi, F., Boedeker, C. C., Bausch, B., et al. (2005). Predictors and prevalence of paraganglioma syndrome associated with mutations of the SDHC gene. *JAMA*, *294*, 2057–2063.
458. Ricketts, C. J., Forman, J. R., Rattenberry, E., et al. (2010). Tumor risks and genotype-phenotype-proteotype analysis in 358 patients with germline mutations in SDHB and SDHD. *Hum Mutat*, *31*, 41–51.
459. Stratakis, C. A., Tichomirowa, M. A., Boikos, S., et al. (2010). The role of germline AIP, MEN1, PRKAR1A, CDKN1B and CDKN2C mutations in causing pituitary adenomas in a large cohort of children, adolescents, and patients with genetic syndromes. *Clin Genet*, *78*, 457–463.
460. Newey, P. J., Jeyabalan, J., Walls, G. V., et al. (2009). Asymptomatic children with multiple endocrine neoplasia type 1 mutations may harbor nonfunctioning pancreatic neuroendocrine tumors. *J Clin Endocrinol Metab*, *94*, 3640–3646.
461. Fabbri, H. C., Mello, M. P., Soardi, F. C., et al. (2010). Long-term follow-up of an 8-year-old boy with insulinoma as the first manifestation of a familial form of multiple endocrine neoplasia type 1. *Arq Bras Endocrinol Metabol*, *54*, 754–760.

QUESTIONS

1. A previously healthy 2-year-old boy presents with gonadotropin-independent precocious puberty. Pubic hair is Tanner 3, penis is enlarged, and testes are prepubertal.Congenital adrenal hyperplasia is ruled out with cosyntropin stimulation testing, and there is no known exogenous testosterone exposure. Adrenal neoplasia is suspected. Which of the following statements is correct?
   a. Adrenal imaging is likely to be negative.
   b. Ophthalmologic examination might reveal retinal angiomas.
   c. Genetic testing for a *TP53* mutation should be undertaken.
   d. A GnRH agonist is the treatment of choice.
   e. There is likely to be a family history of thyroid cancer.

Answer: c

2. A 10-year-old boy presents for evaluation of thyroid nodules:



Which of the following statements is *incorrect*?
   a. The lifetime chance of developing primary hyperparathyroidism is ~20%.
   b. Postoperative ileus may be problematic in this patient.
   c. His parents are unlikely to have the same diagnosis.
   d. The patient may have a history of failure to make tears and feeding difficulties.
   e. The patient is likely to have an activating mutation in codon 918 (M918T) of the *RET* proto-oncogene.

Answer: a

3. A 15-year-old girl is diagnosed with primary hyperparathyroidism (PHPT). Family history is positive for PHPT and prolactinoma in the mother and a maternal uncle with PHPT, a nonfunctioning pituitary adenoma, and a gastroenteropancreatic neuroendocrine tumor (GEP-NET). Which of the following statement is *incorrect?*
   a. The patient has a higher likelihood of developing a GEP-NET than a pituitary tumor.
   b. The chance of finding a mutation is > 80%.
   c. She should be screened for other endocrine neoplasia, even if asymptomatic.
   d. An activating mutation in *MEN1* is likely responsible.
   e. Her children have a 50% chance of having the same condition.

Answer: d

4. An 8-year-old boy with a history of ADHD is found to be hypertensive at a routine office visit. A pheochromocytoma is diagnosed after elevated plasma metanephrines are documented and a 3-cm left adrenal mass is identified on imaging. Which of the following genes is most likely to be mutated in this patient's case?
   a. *MEN1*
   b. *SDHB*
   c. *NF1*
   d. *RET*
   e. *VHL*

Answer: e

5. The management of a child with a pheochromocytoma includes all of the following *except*:
   a. Consultation with a genetic counselor
   b. Biopsy to confirm the diagnosis prior to surgery
   c. Long-term follow-up to assess for recurrence and the development of metachronous tumors
   d. Fluid expansion via salt loading prior to surgery
   e. Treatment with an α-adrenergic receptor blocker prior to surgery

Answer: b

6. The management of a child with medullary thyroid carcinoma includes all of the following *except*:
   a. Consultation with a genetic counselor
   b. Surgery performed by an experienced thyroid cancer surgeon
   c. Thyroid-stimulating hormone (TSH)-suppressive therapy
   d. Periodic assessment of calcitonin and CEA levels
   e. Testing for a germline mutation in the *RET* proto-oncogene

Answer: c

7. Which of the following hereditary tumor syndromes are associated with endocrine neoplasia?
   a. Beckwith-Wiedemann syndrome
   b. Tuberous sclerosis complex
   c. Neurofibromatosis type 1
   d. None of the above
   e. All of the above

Answer: e

CHAPTER 15

Puberty and its Disorders in the Female

Robert L. Rosenfield, MD • David W. Cooke, MD • Sally Radovick, MD

CHAPTER OUTLINE

INTRODUCTION

Puberty is the stage of development during which secondary sexual characteristics appear and there is a transition from the sexually immature to the sexually mature stage. *Adolescence* is widely used as a generally synonymous term for puberty, but it is often used to convey an added cultural connotation as a psychosocial coming of age.

By the mid-1960s, a general concept of the major factors involved in the initiation of puberty was established (Figure 15-1).[1,2] A decrease in sensitivity of the brain "gonadostat" to sex hormone negative feedback was thought to be the primary event. This signaled the hypothalamus to discharge neurohumors (then unidentified), which in turn stimulated the pituitary to release gonadotropins. The resultant rise in secretion of the gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), was thought to account directly for increased estrogen production by the ovary. A mature relationship was thought to develop in which the blood levels of estrogen and gonadotropins were regulated reciprocally via the gonadostat,[3] much as a furnace is regulated by a thermostat. The pineal was identified as having gonadal suppressive properties. The increased adrenocortical secretion of 17-ketosteroids (17-KS), which becomes apparent at about the time of puberty ("adrenarche"), was thought to be due to a pituitary factor stimulating adrenal androgens in synergism with adrenocorticotropic hormone (ACTH).[4]

The rapid scientific advances since 1965 have permitted this concept to be tested in increasingly sophisticated ways. In the subsequent decade, radioimmunoassay (RIA), originally developed by Yalow and Berson, was applied to the measurement of gonadotropins and sex steroids; the gonadotropin-releasing hormone (GnRH) for both LH and FSH was isolated, identified, and synthesized by Guillemin's and Schally's groups. Cyclic adenosine-3′,5′-monophosphate (cAMP), postulated by Sutherland to mediate the action of peptide hormones, was found to mediate gonadotropin effects on the ovarian follicle. Jensen, Gorski, and their groups defined the initial steps in the mechanism of action of steroid hormones. The awarding of Nobel Prizes in medicine to Sutherland in 1971 and to Yalow, Schally, and Guillemin in 1977 marked the recognition of the landmark nature of many of these discoveries.

Our present view of the mechanisms controlling puberty is more refined and complex than it once was, although the previously discussed schema is correct in a general sense. The gonadostat is a patently oversimplistic concept for a complex system that regulates the hypothalamic GnRH pulse generator, a functionally interconnected and synchronized network of GnRH neurons.[5] The gonadostat setting seems to change throughout childhood in a biphasic

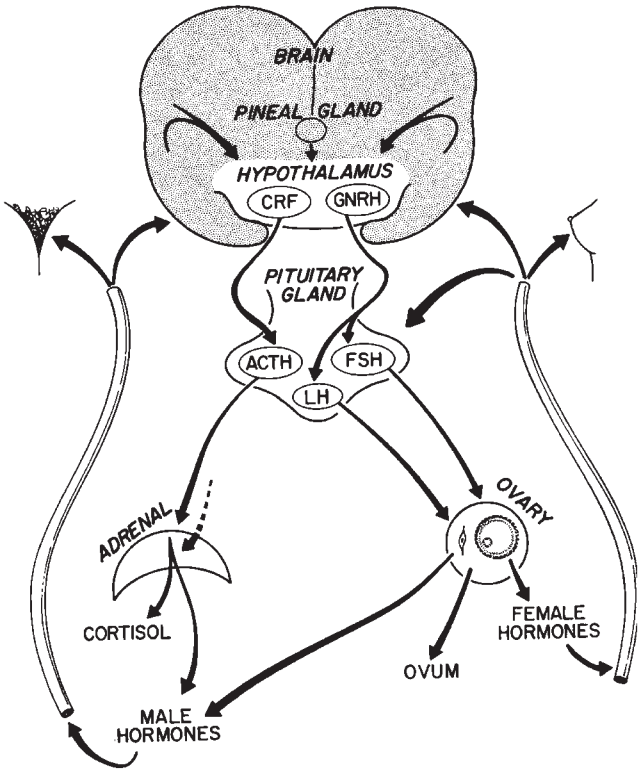


FIGURE 15-1 ■ Schematic representation of the neuroendocrine-ovarian axis involved in normal pubertal development. ACTH, adrenocorticotropic hormone; CRF, corticotropin-releasing factor; GnRH, gonadotropin-releasing hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone.

manner. This concept is illustrated in Figure 15-2.[6,7] During fetal and perinatal life, the gonadostat is insensitive to negative feedback by sex steroid hormones; at this time the nascent neuroendocrine-gonadal axis functions at a pubertal level. The gonadostat becomes increasingly sensitive to negative feedback during infancy, but it does not become highly sensitive until mid-childhood, at which time GnRH pulse generator activity is minimal. During late prepuberty the gonadostat begins to relinquish its inhibition. This permits the onset of puberty. The changing set point initially permits increasing, episodic secretion of GnRH. Increasing sensitivity of the pituitary gonadotropic cells to GnRH follows. The change in LH and FSH secretion is first detectable during sleep. Gradually the gonads become increasingly sensitized to gonadotropin stimulation, grow at an increased rate, and bring about sustained rises in plasma sex steroid hormone levels. Some of these phenomena synergize with others, so that autoamplification occurs and the pace of change accelerates. Eventually the set point for gonadotropin release comes to vary sufficiently to encompass a positive feedback mechanism.

The data on which this model is based are presented here. The most recent data on the hormonal milieu and accompanying physical stages of normal puberty are then presented. Abnormal puberty is subsequently discussed: the causes, differential diagnosis, and management.

DEVELOPMENT OF THE FEMALE REPRODUCTIVE SYSTEM

Maturation of the Neuroendocrine-Ovarian Axis

Fetus

Neuroendocrine Unit. The anterior lobe of the pituitary gland, of stromal ectodermal origin, and the posterior lobe, of neural origin, differentiate by 11 weeks' gestational age.[8] By this time, GnRH neurons have migrated from the olfactory placode into place in the medial basal hypothalamus.[9] Hypothalamic GnRH subsequently rises in parallel with fetal pituitary and serum LH and FSH.[10] All peak at about 20 to 24 weeks, as the connections of the pituitary portal system become complete, to levels not again seen until menopause.[11]

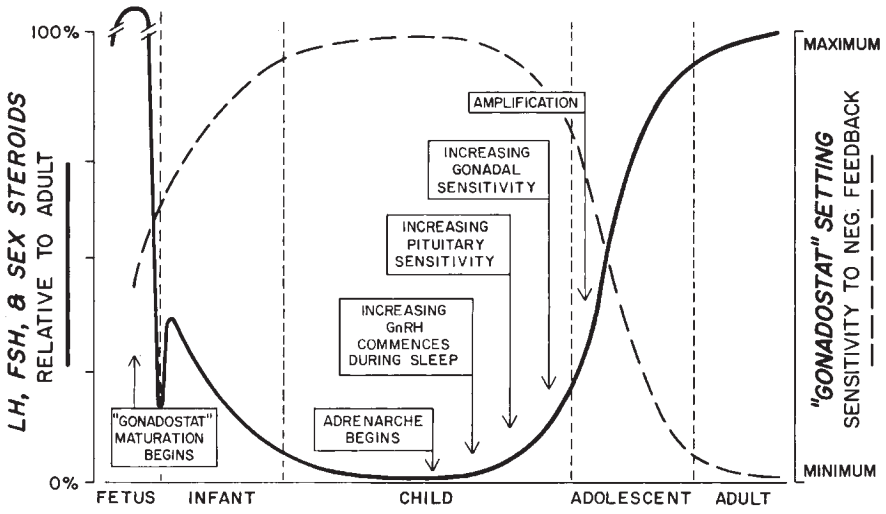


FIGURE 15-2 ■ The changing pattern of serum gonadotropins and sex hormones from fetal life to maturity in relationship to the apparent sensitivity of the central nervous system "gonadostat" to the negative feedback effect of sex hormones and the underlying hormonal events. FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone. (Modified from M. Grumbach, C. Grave, & F. Mayer (Eds.), *The control of the onset of puberty.* New York: John Wiley & Sons.)

Serum LH and FSH levels are higher in human female than male fetuses.[11] In rats, GnRH-containing neurons develop earlier in females than in males,[12] and there are sexual dimorphisms in the degree of synapsing of specific tracts with dendritic spines in the preoptic nucleus, one of the major GnRH-containing areas of the hypothalamus.[13,14] These differences may be determined by gonadal sex steroid hormone output. In all species studied, fetal secretion of LH, particularly LH pulse frequency, is permanently desensitized to estradiol (E2)-progesterone negative feedback by fetal virilization.[15] In the rat this has been demonstrated to be mediated by permanent impairment of estradiol-induced progesterone receptor gene expression.[16]

In late gestation, fetal hypothalamic GnRH and pituitary gonadotropin secretion fall to low levels. These changes are likely explicable by the negative feedback effect of the high sex steroids produced by the fetoplacental unit. Meanwhile, maturation of the central nervous system (CNS) tracts that inhibit hypothalamic GnRH secretion and mediate gonadal negative feedback signals appears to progress throughout gestation.[17,18]

The production of gonadotropins by the fetal pituitary seems to facilitate normal ovarian development. Hypophysectomy of rhesus fetuses has been reported to reduce the number of germ cells and oocytes as well as the integrity of the rete ovarii.[19] Therefore, it seems that the survival of gametes depends on the secretions of the fetal pituitary.

Ovary. The ovaries differentiate in the urogenital ridge adjacent to the anlage of the adrenal cortex and the kidney. The granulosa cells are the homologues of the Sertoli cells of the testes. The theca, interstitial, and hilus cells are the homologues of the Leydig cells; hilus cells may even contain crystalloids like Leydig cells. Adrenocortical rests occasionally have been found in the hilus of the ovary.[20] Conversely, ovarian rests have been identified in the adrenal glands.[21]

The primitive germ cells migrate into the ovary from the yolk sac endoderm during the first month of gestation. The ovaries begin to become distinguishable from testes by 8 weeks of gestation[22] in the absence of testicular development being switched on by signaling cascade initiated by the *SRY* gene on the Y chromosome.[23] *Wnt-4* and forkhead (*Fox*) family transcription factor signaling by germ cells are critical for ovarian differentiation by sustaining oocyte and granulosa cell development and suppressing Sertoli and Leydig cell differentiation; they also support later aspects of follicle develoment.[24] Steroidogenic factor-1 (*SF-1*) *WT-1*, *LIM-1*, and possibly *DAX-1* genes play roles in the formation of the ovaries.[25] Germ cell bone morphogenetic proteins (BMPs) are necessary for primordial germ cell proliferation. Mitotic division of oogonia is maximal before the third month and comes to an end by the seventh month. Oogonia then undergo oogenesis, entering the prophase of meiosis to become primary oocytes during the final 5 to 6 months of gestation.[26] The number of oocytes reaches a peak at the fifth month when there are 6.8 million germ cells, of which 80% appear to be viable (Figure 15-3).[27] When oocytes enter the diplotene stage of meiotic prophase, they must be furnished with granulosa cells to form a primordial follicle, or else they undergo atresia.[28]

Primordial follicles appear in the fourth month, when the epithelium of the secondary sex cords provides granulosa cells to the oocytes, and they peak in number between the fifth and ninth months. They become primary follicles when the encircling granulosa cells become cuboidal. Primordial and small primary follicles (Figure 15-4)[29,30] are resting follicles, which are the major repository of germ cells.[31] This stock of germ cells is depleted only very slowly during childhood (see Figure 15-3). Secondary follicles and preantral follicles, characterized, respectively, by organization of theca and a larger granulosa cell population, then appear successively. After the seventh month, antral (graafian) follicles appear and those granulosa cells enveloping the oocyte become the cumulus.[30,32,33] Typically one or two antral follicles of 1 to 2 mm in diameter are present in the ovary by term, at which time the number of small

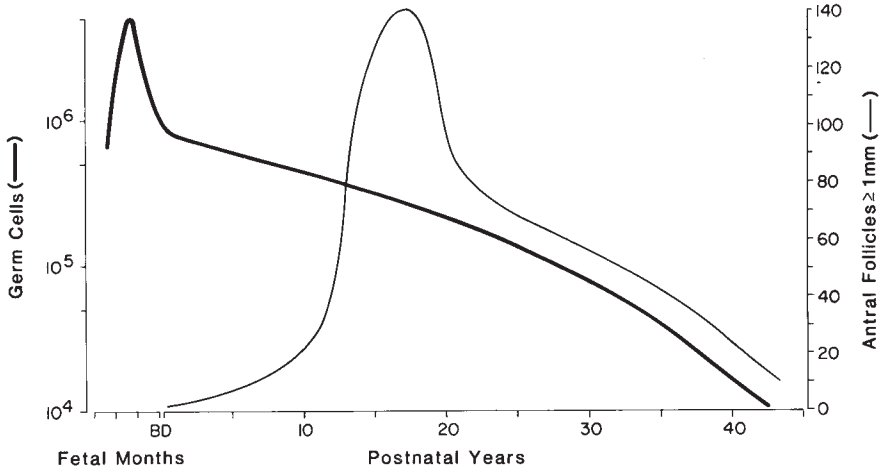


FIGURE 15-3 ■ The development of ovarian follicles from fetal life to maturity. Curves for total number of viable germ cells (thick line) and large antral follicles (thin line) smoothed from the data of Baker and Block. The number of germ cells is maximal at the fifth month of fetal life. The loss of germ cells is exponential throughout postnatal life. At puberty, a marked shift occurs in the pattern of development of follicles. An increased fraction grows to large antral size.

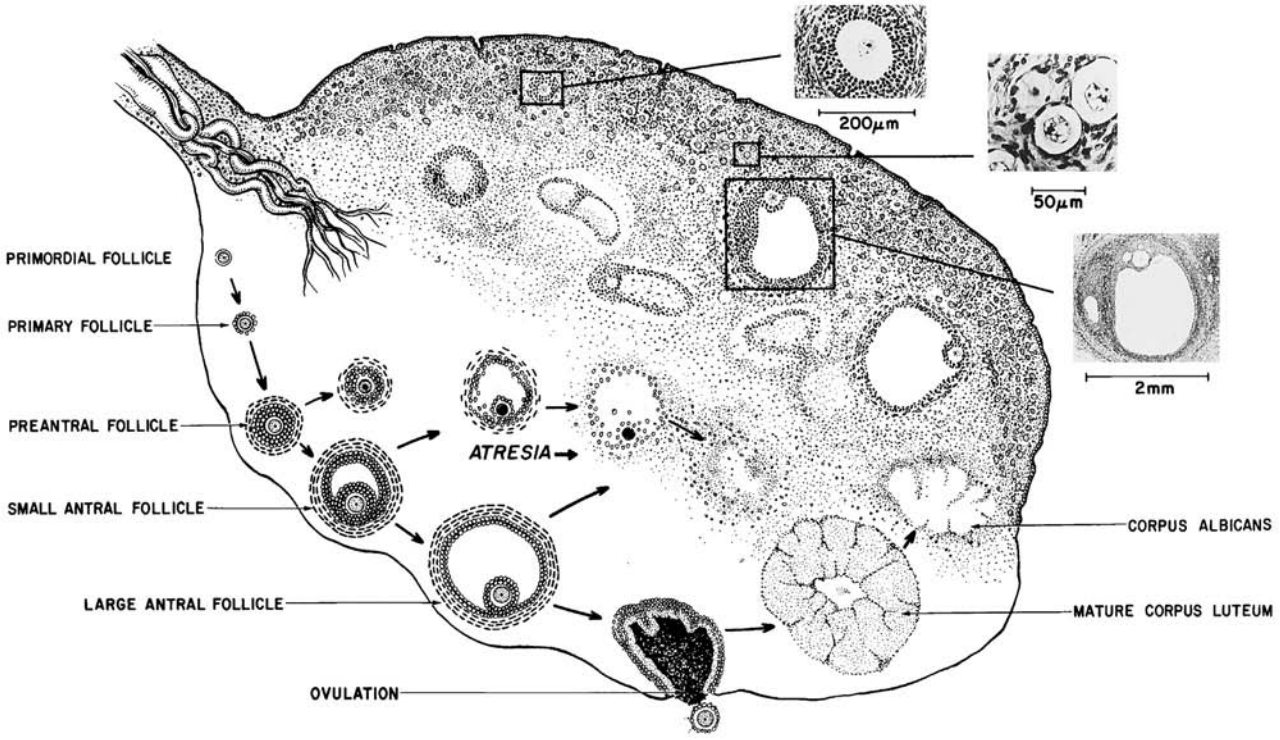


FIGURE 15-4 ■ The human ovary. The lower portion of the figure shows the classification of follicles. Preantral follicles contain as many as 300 granulosa cells, and their diameter ranges from 50 to 200 μm. The oocyte diameter increases from 25 or less to 80 μm. Antral (graafian, tertiary, or vesicular) follicles have a fluid-filled antrum and a full-grown oocyte, are lined with more than 300 granulosa cells, and have a well-developed theca. They are greater than 200 μm in diameter. The dimensions of the mature ovary are approximately 1.25 × 2.75 × 4 cm. The upper portion of the figure illustrates the histologic appearance of the perimenarcheal ovary. (Photomicrographs of ovarian details are reproduced from Peters, H. (1979). The human ovary in childhood and early maturity. *Eur J Obstet Gynecol Reprod Biol, 9,* 137; modified from Ross, G. T., Schreiber, J. R. (1978). The ovary. In S. S. C. Yen, & R. Jaffe (Eds.), *Reproductive endocrinology* (p. 63). Philadelphia: WB Saunders.)

preantral follicles is at its peak. At term, ovarian follicle development is complete,[30,32,33] and the complement of ova is greater than at any other time during postnatal life (see Figure 15-3), totaling 2 million, of which half appear atretic.[27,34]

Both X chromosomes are active in oocytes,[35] and these are necessary for the induction of the granulosa cell layer necessary for oocyte survival.[32,36] Several oocyte transcription factors then mediate the transition from primordial to primary follicles.[24] Growth differentiation factor-9 is critical for signaling the granulosa cells of primary follicles to induce the theca cell layer. Other autosomally determined paracrine factors, prominently involving TGF-β superfamily signaling, are necessary for the survival of oocytes and the further differentiation and growth of follicles. The forkhead transcription factor FOXL2, expressed specifically in granulosa cells, both mediates the GDF9 effect and restrains it from prematurely activating oocytes.[37]

Follicle number is determined by the balance between the number of ovarian germ cells and the rate of atresia. The endowment of ovarian germ cells has been thought to be determined during fetal life, as the germ cells of the ovary, unlike those of the testes, seem to be a non-renewing population; however, this concept has been challenged by research with germline stem cells that suggests a process of slow neogenesis.[38] Regardless, the regulation of atresia by the balance of cell survival and programmed cell death signals is a critical determinant of follicle number.[39] The endowment of follicles may be influenced by environmental factors, such as toxins,[40] thymic factors,[41] and placental insufficiency.[42] Fetal undernutrition has been suspected of temporarily or permanently altering ovarian follicular development.[43-45]

Theca-interstitial cells of the fetal primordial follicle form androstenedione and dehydroepiandrosterone as early as 3 months gestation in humans, but E2 formation probably does not occur until antral follicular development begins at 34-38 weeks gestation.[18,22,46] Thus, the contribution of the ovaries to fetal sex steroid levels is probably relatively low.

Placenta. The fetoplacental unit becomes the major source of sex hormones in the female fetus in the latter half of pregnancy: the fetal adrenal gland provides 17-ketosteroids as substrate for the formation of potent sex steroids by the placenta. Excess androgen, from any source, in the female fetus masculinizes genital differentiation, as discussed in other chapters. This also programs for LH elevation and insulin resistance in adult life.[15] Placental insufficiency, via hypoxemia and the resultant overactivation of fetal prostaglandin production and cortisol secretion, is another factor predisposing to postnatal insulin resistance.[47]

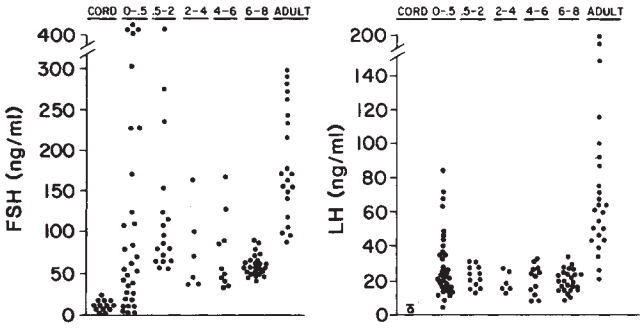


FIGURE 15-5 ■ The distribution of serum gonadotropin levels according to early generation radioimmunoassays from birth to adulthood (age in years) Left, Follicle-stimulating hormone (FSH). Right: Luteinizing hormone (LH). Umbilical cord level of LH measured by beta-subunit-specific radioimmunoassay. Standard LER-907: 100 ng equivalent to 2 mIU FSH and 6 mIU LH of the First International Reference Preparation of human pituitary gonadotropin for bioassay. (Data from Winter, J., Faiman, C., Hobson, W., et al. (1975). Pituitary-gonadal relations in infancy: I. Patterns of serum gonadotropin concentrations from birth to four years of age in the human and the chimpanzee. *J Clin Endocrinol Metab, 40*, 545; from Kaplan, S., Grumbach, M., & Aubert, M. (1976). The ontogenesis of pituitary hormones and hypothalamic factors in the human fetus. *Recent Prog Horm Res, 32*, 161.)

Infant and Child

Neuroendocrine Unit. The hypothalamic-pituitary-gonadal axis is transiently active during the neonatal period. This is sometimes termed the *minipuberty of the newborn*; unlike true puberty the clinical manifestations are only nascent and do not progress. The regulation of neonatal gonadotropin secretion, like that during puberty, is incompletely known.

Serum FSH and LH are low in cord blood and remain low until estrogen concentrations fall from inhibitory levels upon disruption of the fetoplacental unit at birth. Then the LH and FSH levels of neonates promptly begin to rise in pulsatile fashion to early pubertal levels in the first week of life (Figure 15-5).[7,11,48-51] This pituitary activity wanes in older infants, which may be related to the maturation of neural tracts that conduct inhibitory signals from the CNS.

Serum LH and FSH levels rise higher in female than male premature infants, reaching into the postmenopausal range.[17,52] This sexual dimorphism seems to be related to a lack of negative feedback due to lagging ovarian follicular development: antral follicle development begins near term gestational age.[18] There is parallel hyperprolactinemia without sexual dimorphism.[53]

By 40 weeks' gestational age, serum gonadotropins and LH/FSH ratios fall to lower levels in girls than in boys,[17] apparently because girls lack androgen-programmed accentuation of GnRH pulsatility.[16,54,55] Responses to GnRH and GnRH agonist are similar to those of early puberty (Figure 15-6).[56-59] In congenital agonadism, gonadotropins reach postmenopausal levels during the neonatal period.[60]

After about 4 months of age, gonadotropin and prolactin levels gradually begin to fall into the prepubertal range (see Figure 15-5). FSH is higher in girls than in boys, a tendency that tends to persist into early childhood.[48,61] This appears in part related to negative feedback by the higher activin-A and lower inhibin-B serum levels of girls than boys.[62] GnRH secretion also appears to be greater in girls than in boys at this time.[63]

The subsequent decline in gonadotropins may be related to an increase in hypothalamic sex steroid receptors. Hypothalamic estrogen receptors increase in a pattern reciprocal to the fall in serum gonadotropins in the rat (Figure 15-7),[64] as do hypothalamic dihydrotestosterone (DHT) receptors.[65] Increasing sensitivity of the hypothalamus to sex steroid hormone negative feedback could account for the inhibitory effect of the small amounts of circulating E2 and testosterone.

A nadir in both serum gonadotropins occurs by about 6 years of age (see Figures 15-2 and 15-5). At this age the LH and FSH response to GnRH is minimal, apparently

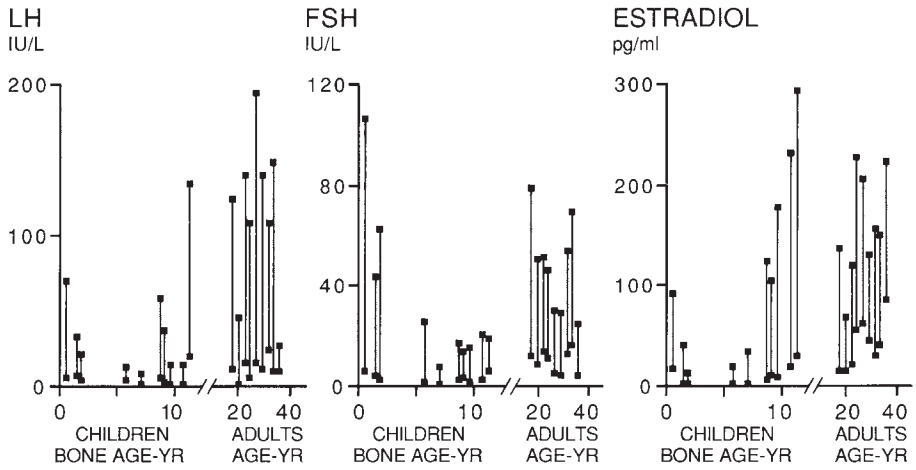


FIGURE 15-6 ■ Basal and peak responses to the gonadotropin-releasing hormone agonist nafarelin (1 μg/kg subcutaneously) during development. Lines connect the basal and peak responses in control children. The responses are related to bone age in children and chronologic age in adults. Note the biphasic pattern of the responses. They are high in infancy, lower in mid-childhood, and rise again during puberty. The peak gonadotropin responses occur at approximately 4 hours, and peak estradiol responses occur at 20 hours. FSH, follicle-stimulating hormone; LH, luteinizing hormone. (From Rosenfield, R. L., Burstein, S., Cuttler, L., et al. (1989). Use of nafarelin for testing pituitary-ovarian function. *J Reprod Med, 34*, 1044.)

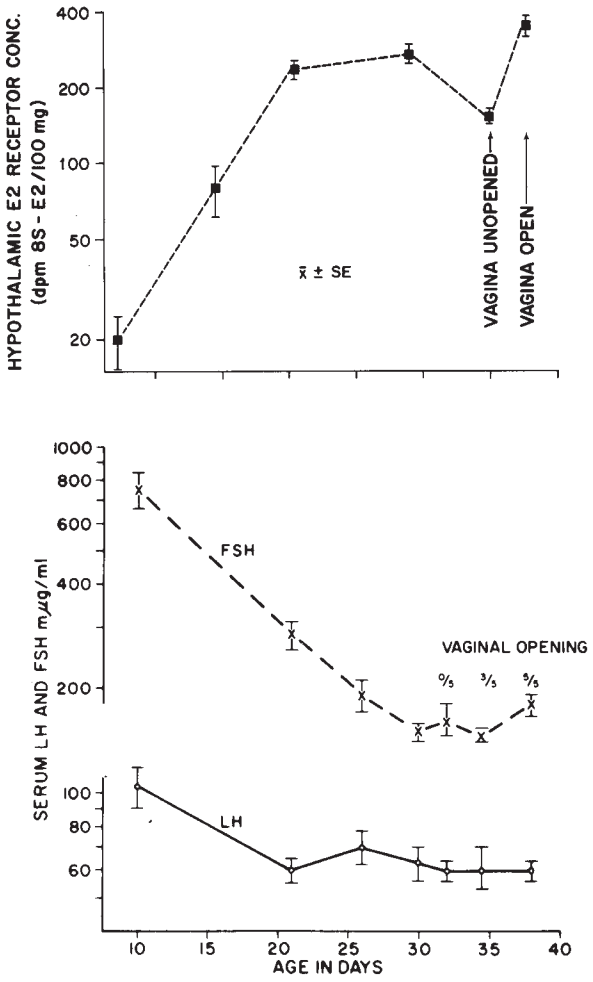


FIGURE 15-7 ■ Relationship of maturation of hypothalamic estrogen receptors (top) to serum gonadotropin levels (bottom) in the developing female rat. FSH, follicle-stimulating hormone; LH, luteinizing hormone. (From Rosenfield, R. L. [1977]. Hormonal events and disorders of puberty. In J. R. Givens (Ed.), *Gynecologic endocrinology*. Chicago: Year Book Medical. By permission of Mosby-Year Book.)

from lack of GnRH stimulation. Furthermore, at this stage agonadism is seldom reflected in a rise in serum gonadotropins or gonadotropin reserve.[60]

However, gonadotropin production is not completely suppressed in mid-childhood. Gonadotropins have been detected in the urine of young prepubertal children, at the limits of sensitivity of classic bioassays: LH excretion averaged 3% and FSH 15% of the adult amounts.[66] Specific monoclonal antibody-based assays have revealed that LH falls to less than 0.2 U/L during the day, whereas FSH remains detectable, and that the gonadotropins produced at this stage are secreted in micropulses that approximately double in association with sleep.[67] The gonadotropins also appear to be bioactive judging from their sensitivity to E2 negative feedback in the primate[68] and the active formation of antral follicles during childhood, which indicates gonadotropin stimulation, as discussed in the following section on the adult.

Between 7 and 10 years of age, even prepubertal girls experience subtle but significant increases in gonadotropin levels.[69] This change corresponds with rising secretion of GnRH.[63] These data indicate that the hormonal secretory pattern of the prepubertal 10-year old child is different from that of the 7-year old and indicate that the hormonal changes signaling the development of puberty are found late in the first decade of life, antedating by some time the development of secondary sex characteristics.

Ovary. The ovary of the infant and child is not quiescent. Initiation of growth and development of resting follicles occurs throughout childhood. The neonatal ovary typically contain an antral follicle with thecal luteinization,[70,71] and the number of antral follicles approximately doubles over that in infancy by 7 years and quadruples by 9 years (see Figure 15-3).[28] All these antral follicles normally undergo atresia in childhood, and this augments the amount of stroma.[28] As a result, by mid-childhood the ovaries of normal girls have up to 5 antral follicles 4 to 9 mm in diameter, and ovarian volume increases up to approximately 3.5 cc. Ovarian morphologic development begins to accelerate just before the onset of puberty.[28,72-76] Small follicle growth is reflected in serum anti-Müllerian hormone (AMH), which achieves adult levels by the time puberty begins.[77]

During the first few months of life, early pubertal blood levels of ovarian hormones are found as part of the transient activation of the hypothalamic-pituitary-gonadal axis that occurs in the newborn. Serum E2 and inhibin-B levels parallel those of FSH. In the neonatal period they begin rising to early pubertal levels, remain there for the first few months of life, and fall gradually thereafter (Figure 15-8).[56,59] In premature infants ovarian function is delayed until near term gestational age, and it is then exaggerated and prolonged.[18] The high gonadotropins of prematurity sometimes cause ovarian hyperstimulation in preterm infants.[78] Breast diameter peaks at 2 to 4 weeks of age in term infants, later in premature infants, and commonly persists for several months.[79] According to an ultrasensitive recombinant

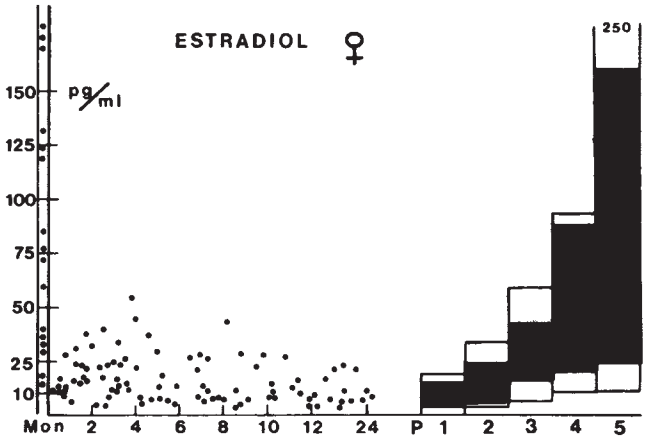


FIGURE 15-8 ■ The distribution of plasma estradiol levels in infant females compared with pubertal and adult female levels. The columns represent the normal ranges for the various stages of puberty. The area between 10th and 90th percentiles is dark. Stage P1 includes all prepubertal girls older than 2 years. The values between the ordinates were found between 2 and 5 days of age. (From Bidlingmeier, F., & Knorr, D. (1978). Oestrogens: physiological and clinical aspects. *Pediatr Adolesc Endocrinol, 4,* 43.)

cell bioassay, girls' estrogen levels in late infancy are several-fold greater than those of boys, averaging 1 pg/mL and ranging up to 3 pg/mL.[80] On occasion there may be subclinical but detectable estrogen effects on urogenital cytology.[81]

In mid-childhood, gonadotropin secretion in response to GnRH agonist testing elicits a prompt small rise in E2 secretion.[82,83] As girls begin to experience increasing diurnal production of gonadotropins in late prepuberty, E2 levels rise in diurnal fashion to approximate 10 pg/mL in midmorning.[69,84]

Adolescent

The endocrinologic changes of puberty actually begin in late preadolescence before secondary sex characteristics appear, as just reviewed. The underlying basic event is increasing secretion of hypothalamic GnRH. Puberty is the consequence of the hypothalamus releasing GnRH with increasing frequency and amplitude, first only at night, then gradually throughout the day.

Increased GnRH secretion in humans was initially deduced when Kastin, Job, Grumbach, and their collaborators demonstrated that preadolescent children had GnRH-releasable pituitary stores of LH and FSH (Figure 15-9; see Figure 15-6).[85] Subsequently, it was

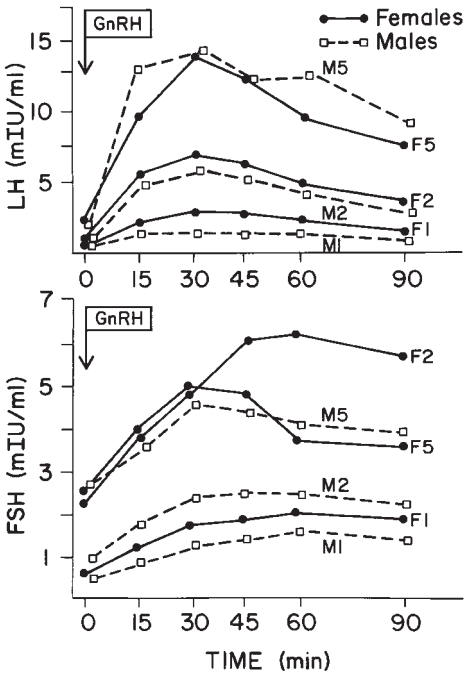


FIGURE 15-9 ■ The luteinizing hormone (LH) and follicle-stimulating hormone (FSH) responses to gonadotropin-releasing hormone (GnRH) bolus (50 μg/kg/day) in males (M) and females (F) in prepuberty (age 5 to 6 years: F1, M1), early puberty (F2, M2), and later puberty (F5, M5). The responses to GnRH tend to progress with advancing puberty. However, early pubertal girls have a readily releasable FSH pool that is greater than that of more advanced adolescents. The peak responses of girls tend to be somewhat greater than those of boys at comparable stages. (Data from Dickerman, Z., Prager-Lewin, R., & Laron, Z. (1976). Response of plasma LH and FSH to synthetic LHRH in children at various pubertal stages. *Am J Dis Child, 130,* 634.)

reported that in humans the output of an immunoreactive fragment of GnRH increases to adult levels during puberty.[63,86] Studies in the rat suggest that hypothalamic GnRH increases through puberty.[87]

Knobil subsequently showed that puberty can be induced in the immature female rhesus monkey by administering GnRH in hourly pulses that yield blood levels of about 2000 pg/mL.[88] Prolonged administration of GnRH according to this regimen first gradually brings about transient increases in LH and FSH. This then induces cyclic follicular development. The resultant moderate E2 surge is of such magnitude as to result in menarche due to withdrawal menstrual bleeding in an anovulatory cycle (Figure 15-10). Continuation of the same GnRH regimen leads to the development of normal monthly ovulatory menstrual periods. Physiologic pulses of GnRH in humans probably attain lower concentrations (200 pg/mL) and occur at slightly wider intervals than in monkeys.[89] Consequently, LH pulses in mature women occur at intervals of approximately 1 to 1.5 hour during the follicular phases, slowing during the luteal phase.[90]

Puberty begins when GnRH secretion increases. Serum LH then begins to rise disproportionately to FSH; this LH-FSH disparity is particularly evident during sleep, which is reflected in responses to GnRH or GnRH agonist (Table 15-1). Puberty becomes clinically apparent as thelarche when E2 levels are sustained $>$ 10 pg/mL.[83] It seems likely that a rise in inhibin-B as increasing ovarian follicles develop plays a key negative-feedback role in limiting further increase in FSH levels during puberty. FSH levels become less GnRH dependent during puberty.[67] The mechanisms for differential regulation of FSH and LH are discussed later in this chapter.

Pubertal gonadotropin cycles seem to develop well before menarche[7,91] and are capable of inducing cyclic estrogen production.[81,91] Our working model of the nature of pituitary-ovarian dynamics in early puberty is illustrated in Figure 15-11.

Puberty progresses as LH rises. Whereas serum FSH levels rise about 2.5-fold over the course of puberty, LH levels rise 25-fold or more.[67] The initial change in LH secretion at the beginning of puberty is a nightly increase in LH secretion that begins within 20 minutes of the onset of sleep. Subsequently, LH increases more with the onset of sleep, stays up longer, and falls less during waking hours. As the child approaches menarche, the daytime LH levels continue to increase until the diurnal rhythm is typically lost. FSH levels follow a similar pattern, although the FSH changes are less striking. The gonadotropin diurnal rhythm during puberty seems entirely related to sleep, unlike the cortisol circadian rhythm.[92] There is a delay of about 12 hours between the peak LH level during sleep and the E2 zenith, such that E2 levels are maximal between late morning and early afternoon.[84,93] The gonadotropin and E2 rhythms in an early pubertal girl are shown in Figure 15-12.[93]

Augmentation of the bioactivity of serum LH occurs during pubertal progression. Plasma LH bioactivity rises nearly fivefold more during the course of puberty than does LH as measured by polyclonal RIA (Figure 15-13).[94,95]

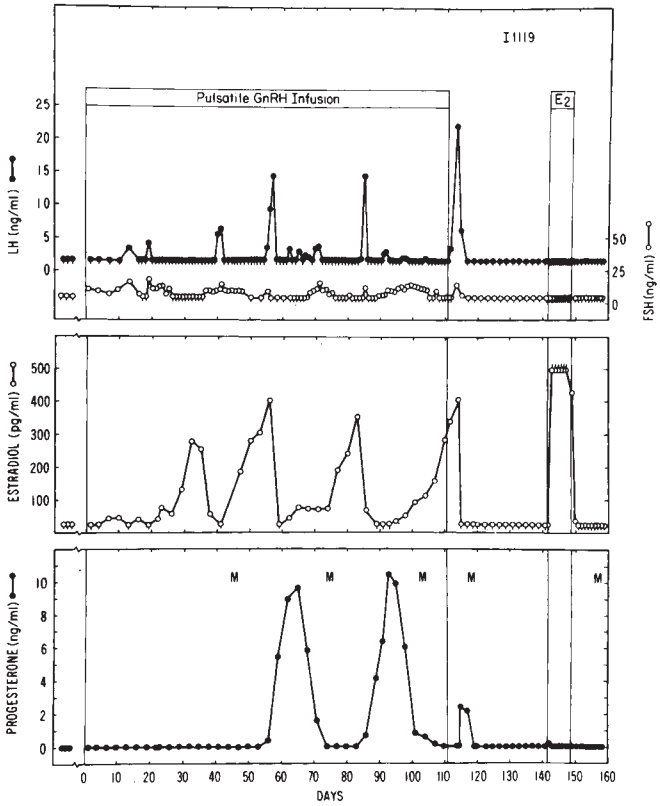


FIGURE 15-10 ■ Induction of puberty in a 13-month-old prepubertal rhesus monkey by an unvarying pulsatile gonadotropin-releasing hormone (GnRH) regimen (1 μg/min × 6 min hourly). Luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol (E2), and progesterone were undetectable before the GnRH infusion. On GnRH infusion, a rise in FSH was the first change detectable by midmorning sampling midway between GnRH pulses. A substantial E2 surge occurred approximately 1 month later. The subsequent LH surge was too modest to elicit ovulation, but menses (M) occurred a few days after subsidence of the week-long E2 surge—menarche resulting from an anovulatory cycle. Continuation of the GnRH led to the sustained occurrence of ovulatory menstrual cycles at 28-day intervals. An identical outcome results if an arcuate-lesioned adult animal undergoes this GnRH regimen. The third of the LH surges occurred 2 days after GnRH was discontinued. Progesterone secretion from the corpus luteum was blunted and transient in the absence of sustained LH secretion. A subsequent increase in plasma E2 produced by E2 implantation subcutaneously failed to elicit a gonadotropin surge, indicating that the animal had reverted to an immature state. Menarche eventually spontaneously recurred in such animals at the usual age (approximately 27 months). Small vertical lines beneath data points indicate values below the sensitivity of the assay. Note that gonadotropins and E2 were often undetectable (prepubertal range) during the induced puberty. (From Knobil, E. (1980). The neuroendocrine control of the menstrual cycle. *Recent Prog Horm Res, 36*, 53.)

The change in bioactive LH is mirrored well by the "third generation" monoclonal antibody-based immunometric ("pediatric") assays that have very high specificity for bioactive LH epitopes. However, disparities in the ratio of bioactive to immunoreactive LH (B/I) persist with these assays, for reasons related to the molecular microheterogeneity of gonadotropins, which is discussed later. Serum FSH rises during puberty according to immunoassay more so than by bioassay.[96]

E2 output increases rapidly in the year approaching menarche.[97] This seems to be the result of a variety of autoamplification phenomena that facilitate puberty, maturation of the dominant follicle, and ovulation. These are summarized in Box 15-1.[98-111] These phenomena occur at all levels of the axis. The CNS is stimulated by preovulatory levels of E2 to increase GnRH pulse amplitude. At the pituitary level there is the self-priming effect of GnRH, whereby a pulse of GnRH sensitizes the pituitary to have a greater LH response to a subsequent identical GnRH pulse. Critical patterns of E2 and progesterone secretion enhance the pituitary LH and FSH responsiveness to GnRH. At the gonadal level, the cascade of events is augmented by the FSH induction of aromatase activity and progestin production in granulosa cells, phenomena in which androgens play a synergistic role. Furthermore, FSH stimulates granulosa cell mitosis and induces LH receptors, phenomena in which E2 may play a synergistic role. Subsequently, LH is able to further enhance the aromatase and progesterone effects. Progesterone itself plays a synergistic role in stimulating granulosa cell progesterone and prostaglandin synthesis in concert with both FSH and LH. In the rat, ovarian GnRH receptor sites also diminish just before ovulation,[112] and at about this time the ovary changes its pattern of metabolism so that the secretion of androstanediol-3β-monosulfate decreases to levels that are no longer inhibitory to LH secretion.[113]

The preovulatory gonadotropin surge occurs when all these cascading processes culminate in activation of

BOX 15-1 Autoamplification Processes Involved in Pubertal Progression, Follicle Maturation, and Ovulation

Central nervous system GnRH secretion increases[98,99] via the following:
- E2-induced progesterone receptors[100]
- Progesterone synergization with E2[101]

Pituitary LH and FSH responsiveness to GnRH increases via:
- GnRH self-priming[102]
- Critical patterns of E2 secretion-stimulating LH/FSH responsiveness[103-105]
- Progesterone synergization with E2[104-106]
- LH bioactivity increases[94]

Gonadal responsiveness to FSH and LH increases via:
- FSH-induced aromatase and progesterone in granulosa cells: androgens and progesterone synergization with this effect[107-109]
- FSH-stimulated granulosa meiosis[31]and FSH-induced granulosa LH receptors; IGF-1 synergization[110,111]

E2, estradiol; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; IGF-1, insulin-like growth factor-1; and LH, luteinizing hormone.

TABLE 15-1 **Typical Normal Ranges for LH, FSH, and Ovarian Steroids, at Baseline and in Response to ACTH and GnRH Agonist Tests**[a]

	LH (U/L)	FSH (U/L)	Estradiol (pg/mL)	Estrone (pg/mL)	Testosterone (ng/dL)	Androstenedione (ng/dL)	DHEA (ng/dL)	17PROG (ng/dL)	17PREG (ng/dL)	DHEAS (μg/dL)
Baseline (8:00 a.m.)										
Term infants, 1 day old	—	—	300-500	300-500	15-75	100-410	300-2600	150-850	110-3000	20-410
Children, 1-5 yr old	<0.15	<0.15-3.5	<10	<20	<20	10-50	20-130	5-115	10-105	5-35
Children, 6-10 yr old	<0.15-0.3	<0.5-2.9	<10	<30	<20	10-75	20-345	5-115	10-200	10-115
Premenarcheal pubertal, 9-13 yr old	<0.15-7.2	1.1-9.0	<10-55	10-35	10-35	40-175	40-600	15-220	35-350	35-130
Postmenarcheal,early follicular phase	1.5-5.6	3.6-7.9	20-85	20-50	20-60	50-200	100-850	≤130[b]	55-360	75-255
Peak After $ACTH_{1-24}$ (30-60 minutes after ≥10 μg/m^2 IV)										
Children, 1-5 yr old			—	—	<20	15-70	25-100	50-270	45-350	5-35
Children, 6-10 yr old			—	—	<20	25-100	70-320	85-300	60-650	10-115
Premenarcheal pubertal, 9-13 yr old			—	—	10-35	55-230	70-725	90-400	150-750	35-130
Postmenarcheal, early follicular phase			—	—	20-60	60-250	250-1470	35-160[b]	150-1070	75-255
Peak After GnRH agonist (leuprolide acetate 10 μg/kg SC)										
Prepubertal, 6-9 yr old	1.2-8.9	9.3-37	<10-55	—	<20[c]	25-50[c]	25-70[c]	<25[c]	—	—
Premenarcheal pubertal, 9-13 yr old	2.8-99	14-40	30-350	—	10-45[c]	25-165[c]	60-185[c]	<155[c]	—	—
Postmenarcheal, early follicular phase	30-135	15-60	65-260	—	10-60[c]	50-180[c]	60-450[c]	30-145[c]	—	—
Conversion multipliers to SI units			3.67 (pmol/L)	3.70 (pmol/L)	0.0347 (nmol/L)	0.0349 (nmol/L)	0.0347 (nmol/L)	0.0303 (nmol/L)	0.0316 (nmol/L)	0.0271 (μmol/L)

[a]5th-95th percentile for third generation gonadotropin immunoassays and high-specificity assays after preparatory chromatography, except for DHEAS. Values differ slightly among laboratories.
[b]17-hydroxyprogesterone early follicular phase baseline levels. 130 ng/dL are found in women who are heterozygous for 21-hydroxylase deficiency, and they often have responses to ACTH greater than those shown. 17PROG begins rising during the late follicular phase and peaks as high as 400 ng/dL in the luteal phase of the cycle.
[c]At 1600 hours after dexamethasone administration (0.5 mg po at 1200 hours) to blunt coincidental adrenocortical secretion.
[d]Baseline early morning serum LH: Prepubertal # 0.15 to 0.6, premenarcheal 0.1 to 7.2, postmenarcheal 1.4 to 5.3 U/L.
[e]Baseline early morning serum FSH: Prepubertal 0.5 to 2.9, premenarcheal 1.1 to 9, postmenarcheal 3.8 to 9.2 U/L.
DHEA, dehydroepiandrosterone; 17PREG, 17-hydroxypregnenolone; 17PROG, 17-hydroxyprogesterone.
Data from Rosenfield, R. L. (2007). Identifying children at risk of polycystic ovary syndrome. J Clin Endocrinol Metab, 92, *787–791; Rosenfield, R. L., Bordini, B., & Yu, C. (2013). Comparison of detection of normal puberty in girls by a hormonal sleep test and a gonadotropin-releasing hormone agonist test.* J Clin Endocrinol Metab, 98, *1591–1601; Mortensen, M., Ehrmann, D. A., Littlejohn, E., & Rosenfield, R. L. (2009). Asymptomatic volunteers with a polycystic ovary are a functionally distinct but heterogeneous population.* J Clin Endocrinol Metab, 94, *1579–1586; Forest, M. (1979). Function of the ovary in the neonate and infant.* Eur J Obstet Gynecol Reprod Biol, 9, *145–160; de Peretti, E., & Forest, M. G. (1982). Pitfalls in the etiological diagnosis of congenital adrenal hyperplasia in the early neonatal period.* Horm Res, 16, *10–22.*

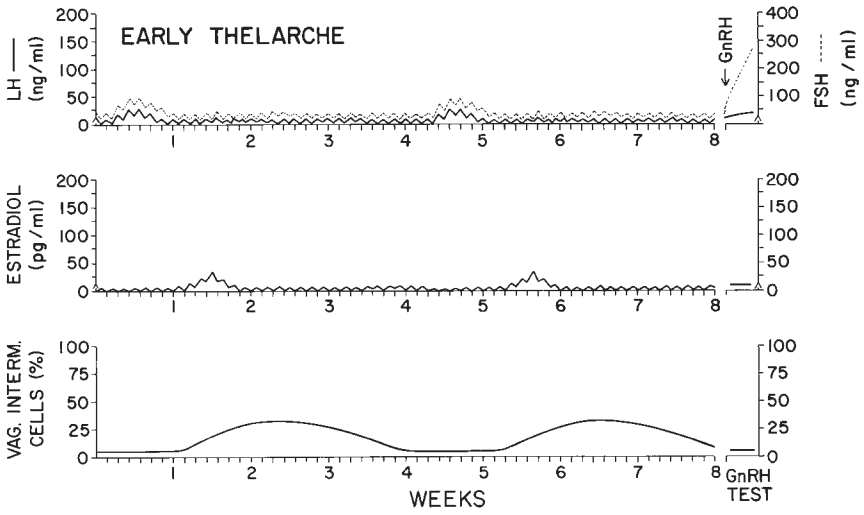


FIGURE 15-11 ■ Diagram depicting our working hypothesis of the hormonal patterns in girls during very early puberty. We conceptualize this pattern as occurring both cyclically in the earliest stage of normal puberty and occasionally in unsustained sexual precocity (i.e., most U.S. cases of idiopathic premature thelarche). Daytime and nighttime serum concentrations of hormones (gonadotropins relative to the LER-907 standard) and the percentage of intermediate cells on vaginal smear are shown. The typical response to a gonadotropin-releasing hormone (GnRH) test is illustrated. Subclinical hormonal cycles lasting approximately 1 month result from a few days of increased follicle-stimulating hormone (FSH) and luteinizing hormone (LH) secretion. Because the drive to gonadotropin release is relatively weak, FSH and LH production are suppressed promptly and for long periods of time by the resultant modest amounts of estradiol (E2) secretion. Estradiol is detectable in plasma for only a few days a month. Maturation of the vaginal mucosa, however, is detectable for approximately 2 weeks after E2 production has waned.

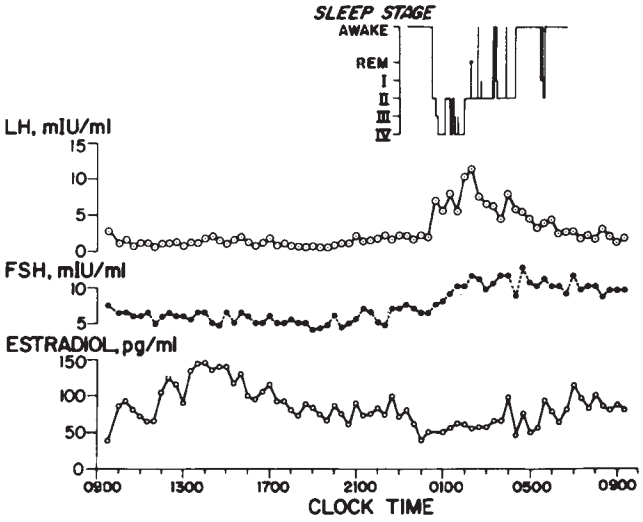


FIGURE 15-12 ■ The patterns of serum luteinizing hormone (LH), follicle-stimulating hormone (FSH), and estradiol (E2) typical of early female puberty. Note that daytime gonadotropin levels are in the prepubertal range. Note also the episodic nature of LH release at intervals of 1 to 3 hours. Estradiol levels are seen to fluctuate considerably in the course of the daytime, rising to peak levels about 12 hours after the maximum nocturnal gonadotropin surges. (From Boyar, R. M., Wu, R. H. K., Roffwarg, H., et al. (1976). Human puberty: 24-hour estradiol patterns in pubertal girls. *J Clin Endocrinol Metab*, *43*, 1418.)

the positive feedback mechanism, the hallmark of sexual maturity in the female. "Positive feedback" refers to the neuroendocrine system acquiring the ability to secrete a midcycle surge of LH when the ovary signals via increasing estrogen secretion that it is prepared for ovulation.

Menarche does not necessarily indicate full maturation of the neuroendocrine-ovarian axis. As the studies of Knobil illustrate (see Figure 15-10), menarche can be due to estrogen-withdrawal bleeding—and it is about half of the time—but ovulatory cycles may follow in short order. General characteristics of the mature ovary are shown in Figure 15-4.

The morphology of the normal adolescent ovary has long been considered polycystic, and histologic examination typically has shown thecal luteinization.[70,114] In the perimenarcheal period, the combination of a high number of follicles and mature gonadotropin stimulation leads to a greater number of large antral follicles than at any other stage (see Figure 15-3),[27] which often leads to a "multifollicular" ultrasonographic appearance.[72,115,116] By 1 year after menarche, at approximately 14 years of age, the ovaries normally achieve adult functional and anatomic characteristics. Many exceed normal adult ultrasonographic size or follicle count criteria, the largest reaching approximately a volume of 10.8 to 11.8 mL with 10-17 small (2-9 mm) antral follicles in the maximum plane.[73,116-119] Thus, many eumenorrheic normal postmenarcheal adolescents meet current ultrasonographic criteria for polycystic ovary morphology (see the "polycystic ovary syndrome" section),[120] often transiently.[121]

Adult

The follicular phase of each menstrual cycle recapitulates puberty in many respects. Gonadotropin and sex hormone levels are low during the premenstrual phase of the mature cycle (Figure 15-14A).[122,123] Gonadotropin concentrations then increase at the time of menstruation, FSH predominating in the early follicular phase while nocturnal LH pulsation is slow[124] (Figure 15-14B). Luteinizing hormone pulsation increases to a circhoral pattern around a stable baseline, and E2 production slowly begins as antral follicles

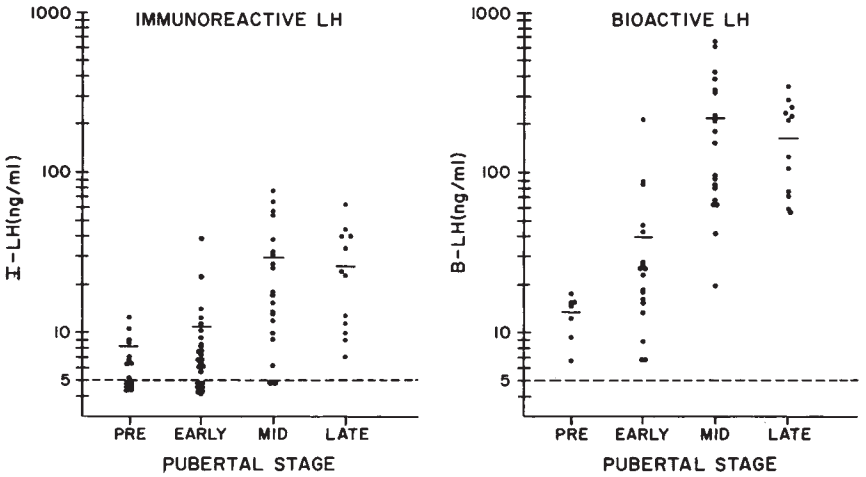


FIGURE 15-13 ■ Bioactive luteinizing hormone (B-LH) (right) and immunoreactive LH (I-LH) (left) in the same daytime serum samples of girls 10 to 16 years of age at various pubertal stages. The dashed lines indicate the limit of sensitivity of the assays. B-LH rises relatively more than I-LH in the course of puberty. The peak in the apparent biopotency of LH, estimated from the ratio of B-LH to I-LH, occurs at about the time of menarche. Late indicates early follicular phase normally menstruating postmenarcheal girls. Standard LER-907: 100 ng equivalent to 6 mIU LH of First International Reference Preparation (IRP) of human pituitary gonadotropin. The disparity between immunoreactivity and bioactivity is principally due to the presence of different proportions of immunoreactive and bioactive LH moieties in serum and standards. The ratio of bioactivity to immunoreactivity is closer to unity when sera are assayed against highly purified standards (like the first and second IRP of human luteinizing hormone), which have about fivefold higher specific activity and yield dose response relationships with serum LH that are more linear. (Data from Lucky, A. W., Rich, B. H., Rosenfield, R. L., et al. (1980). LH bioactivity increases more than immunoreactivity during puberty. *J Pediatr*, *97*, 205; Rosenfield, R. L., Helke, J. (1992). Is an immunoassay available for the measurement of bioactive LH in serum? *J Androl*, *13*, 1.)

develop (Figure 15-14C). E2 levels gradually increase and serum FSH levels fall reciprocally (Figure 15-14D). Upon formation of a dominant follicle, serum E2 concentrations increase geometrically. This selectively begins to amplify the pituitary's LH response to GnRH as E2 reaches about 90 pg/mL for over 3 days[104,105,125] (Figure 15-14E).

When the serum estradiol rises to over 200 to 300 pg/mL for 36 hours, the positive feedback mechanism is activated and the midcycle gonadotropin surge commences (Figure 15-14F). E2 then appears to induce progesterone receptor (PR) expression in the hypothalamus and pituitary.[126] An increase in progesterone to 100 ng/dL facilitates the LH surge, shortens the duration of time over which E2 is required for the surge to 24 hours, and brings about an FSH surge. The mechanism of progesterone action involves inhibition of GnRH cleavage.[106] Androgens may also play a role in facilitating FSH and GnRH release.[127,128] The LH surge is then primarily responsible for luteinizing the preovulatory ovarian follicle (see Figure 15-14F). At this time, LH pulses become larger in amplitude but slower in frequency and their apparent bioactivity increases. Ovulation then results.

As the follicle is disrupted by ovulation, estrogen levels fall (Figure 15-14G). As the corpus luteum begins to form, progesterone increases steadily to be sustained at very high levels for several days, along with lesser but substantial increases in E2 and 17-hydroxyprogesterone levels.[122,123,129] In response to the high progesterone level, LH pulses become slow and large.[124,129] In the absence of increasing human chorionic gonadotropin (hCG) from a conceptus, the corpus luteum's life span is exhausted and its production of progesterone and E2 wanes. Subsequently, FSH begins to rise out of

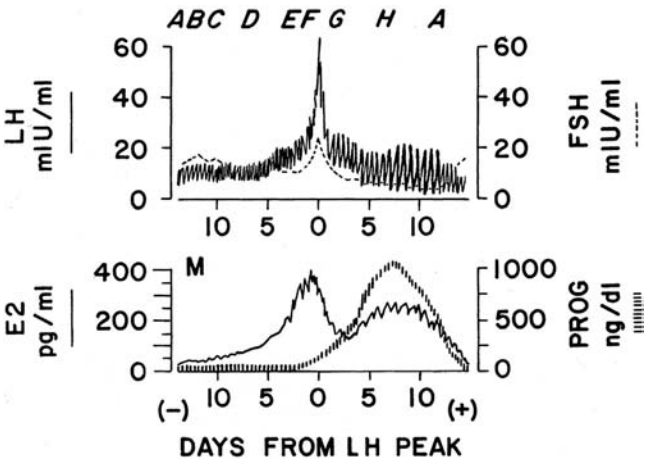


FIGURE 15-14 ■ Diagram of gonadotropin and female hormone levels during the normal menstrual cycle. The levels are centered in reference to the day of the midcycle luteinizing hormone (LH) peak (day 0). Letters A through F above the top panel correspond to stages of follicular development in Figure 15-15. G and H are discussed in the text. M (bottom panel) shows time of menses. E2, estradiol; FSH, follicle-stimulating hormone; PROG, progesterone. (Based on data of Abraham, G. E. (1974). Ovarian and adrenal contributions to peripheral androgens during the menstrual cycle. *J Clin Endocrinol Metab*, *39*, 340; Ross, G. T., Cargille, C. M., Lipsett, M. B., et al. (1970). Pituitary and gonadal hormones in women during spontaneous and induced ovulatory cycles. *Recent Prog Horm Res*, *26*, 1; Soules, M., Steiner, R., Clifton, D., et al. (1984). Progesterone modulation of pulsatile luteinizing hormone secretion in normal women. *J Clin Endocrinol Metab*, *58*, 378.)

proportion to LH. Shortly after the sex steroids withdraw from the scene, the endometrium sloughs, giving rise to menstrual flow. Meanwhile, the follicular growth induced earlier by FSH begins to gain momentum and the next cycle begins.

Follicular (Proliferative) Phase Ovary. The hormonal functions of the follicle have dual purposes that must be closely coordinated: to change the milieu of the ovum to prepare for ovulation and to signal the pituitary to send the signal to ovulate (i.e., the LH surge). Thus, the ovary is the zeitgeber for the cycle: the normal cyclic pattern of ovarian hormone secretion induces the midcycle surge of pituitary gonadotropins, even in the presence of unchanging circhoral pulses of GnRH.[88] Ovarian hormones also augment the amplitude of the GnRH response,[98-101] which is a fail-safe mechanism that "guarantees" a preovulatory gonadotropin surge.

Ovarian follicular development and steroid secretion in relationship to changing gonadotropin levels are illustrated in Figure 15-15.[31,130-132] FSH and LH play major roles in granulosa and thecal cell differentiation, respectively, whereas a host of local factors modulate gonadotropin action. For example, follicular maturation in response to gonadotropins is enhanced by insulin-like growth factors (IGFs), transforming growth factor (TGF)-β and fibroblast growth factor, whereas it is inhibited by TGF-α, epidermal growth factor, and other factors.

Primordial follicle growth and development is gonadotropin independent. Subsequently, granulosa cells of preantral follicles develop FSH receptors, and theca cells, which encircle granulosa cells, develop LH receptors (Figure 15-15A). Activin causes FSH-independent up-regulation of FSH receptors in preantral follicles,[111] although it opposes FSH stimulation of antral follicle development.[31] Primordial follicle growth is constitutively repressed by nuclear forkhead transcription factor Foxo3; when Foxo3 is released in response to stimulation of the PTEN-PI3K-Akt pathway, follicular growth progresses to the point where follicles become responsive to FSH.[133]

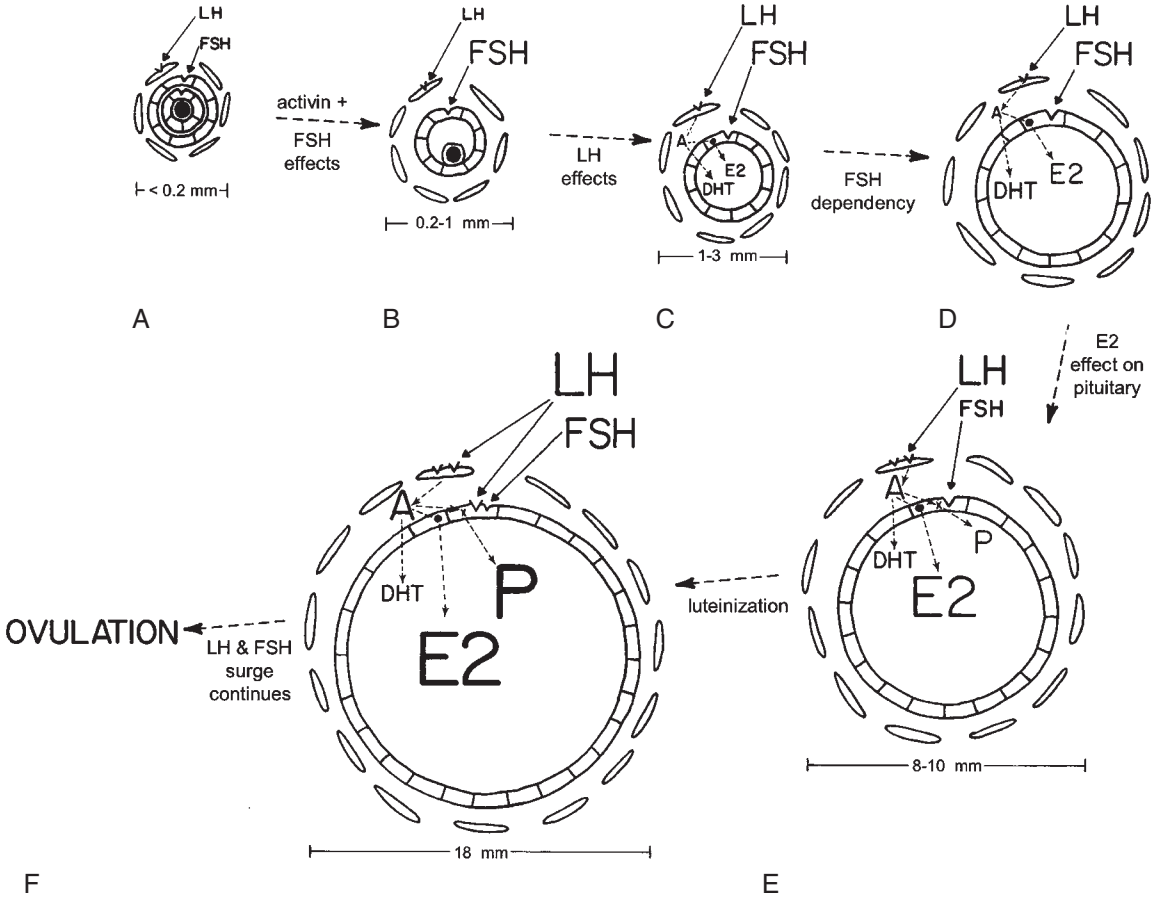


FIGURE 15-15 ■ Relationships among gonadotropins, the ovarian follicle, and ovarian steroids according to the two-cell two-gonadotropin model of ovarian steroidogenesis. **A** through **F,** Stages of ovarian follicular development found during the times of the menstrual cycle designated by the corresponding letters on Figure 15-14. The size of the letters designating hormones relates to the magnitude of their serum and/or follicular concentrations. **A,** Preantral follicle with luteinizing hormone (LH) and follicle-stimulating hormone (FSH) receptors in theca and granulosa cells, respectively. There is no antrum surrounding the ovum (stippled in center). **B,** Small antral follicle. Activin up-regulates FSH receptors, and the FSH receptor activation is required to initiate antrum formation. **C,** Large antral follicle (1 mm or larger). Aromatase activity (.) has been induced in granulosa cells. Interactions between theca and granulosa cells, the former producing androgens (androstenedione: A), result in increasing estradiol (E2) and dihydrotestosterone (DHT) synthesis. **D,** FSH-dependent follicular growth results in more E2 synthesis. **E,** Estradiol enhances pituitary LH secretion in response to GnRH, while at the same time inhibiting pituitary FSH secretion. The increased LH induces more theca LH receptors and stimulates androgen production. Androgens serve as substrate for E2 formation and synergize with FSH to stimulate progesterone (P) secretion, initiating luteinization of granulosa cells. **F,** In the preovulatory follicle, FSH induces LH receptors on the granulosa cell—which completes luteinization of granulosa cells. Steroid secretion is augmented further. Then, increasing progesterone amplifies the positive feedback effect of E2 to initiate the preovulatory gonadotropin surge.

Antrum formation requires a trace (prepubertal) amount of FSH receptor activation (Figure 15-15B).[31,134-137] FSH stimulates androgen receptor expression in primary follicles, and androgens in turn stimulate further expression of FSH receptors and the early stages of follicular growth.[138] Androgen action is also necessary for the development of a full complement of follicles, and androgen excess stimulates excessive follicle number.[139,140] Luteinizing hormone stimulates the appearance in thecal cells of the enzymes necessary for androgen biosynthesis.[141] Evidence that theca cells of small antral follicles form E2 is meager.[142]

As antral follicles grow over 2.5 mm diameter, their granulosa cells begin to form E2 from androgen supplied by theca cells (Figure 15-15C).[143-147] Androgen production at low levels may synergize with FSH to stimulate aromatase activity within the granulosa cells.[107,148,149]

At this stage, follicles are increasingly FSH dependent and are consequently uniformly FSH responsive.[31,134] IGF-1 is required for follicular growth beyond the early antral stage in response to FSH.[150] Antral follicles do not grow over 5 mm in diameter without a pubertal degree of FSH stimulation.[136] By the midfollicular phase, the proliferation of FSH-responsive granulosa cells results in an accelerating rate of E2 production and preferential conversion of androstenedione to E2 rather than DHT by these cells (Figure 15-l5D).[143-145,147,151,152] Estradiol itself clearly stimulates proliferation of granulosa cells and oocyte survival in rodents.[153] In humans, E2 appears to promote antral growth independently of LH[154] and is synergistic with FSH in bringing about the development of the dominant follicle.[155,156]

A dominant follicle is selected at the beginning of the menstrual cycle from a crop of follicles that were recruited 2.5 months prior.[31] Recruitment of a group of follicles is normally promoted by the midcycle FSH surge and regresses with increasing corpus luteum progesterone secretion. Another wave of follicle growth in the late luteal phase is promoted by the rise of FSH as luteal progesterone and E2 secretion wanes. The selected follicle is the one that is the most sensitive to FSH (lowest "FSH threshold"). FSH is critically important during the follicular phase for optimal development of this dominant follicle. By the midfollicular phase of the cycle this follicle becomes virtually the sole source of E2 (Figure 15-15E). Typically, there is only one such follicle. Only this follicle continues to grow so as to reach a diameter of 10 mm or more. All other gonadotropin-dependent follicles undergo atresia.

At this stage the rising E2 level is suppressing FSH secretion and augmenting pituitary LH responsiveness to GnRH. FSH is more bioactive in the dominant follicle because it is more efficiently concentrated[151] and because local factors increase ovarian responsiveness to FSH. The increased LH causes further proliferation of thecal cells and an increase in their LH receptor content.[108] Consequently, androgen production increases. This synergizes with FSH to both augment aromatase activity and bring about increasing progesterone secretion by the well-estrogenized granulosa cells of these follicles. Progesterone then enhances the synthesis of both itself and E2.[108,110] The increased thecal androstenedione production is diverted much more to E2 than to dihydrotestosterone biosynthesis. Antral fluid steroid concentrations reflect these changes (Figure 15-16).[143,144,151] Activin acts so as to prevent premature luteinization of granulosa cells, and activin tone seems to wane as the preovulatory phase approaches.[31,111]

FSH next induces LH receptors in the granulosa cells (Figure 15-15F).[110] These luteinized granulosa cells are capable of augmenting E2 and progestin production in response to LH as well as FSH.

The LH and FSH surge then occurs in response to the positive feedback action of E2 at both the CNS and pituitary levels, an effect amplified by the rising levels of progesterone. The final steps in follicle maturation ensue rapidly: the LH surge induces granulosa cell PR and prostaglandin synthase while inhibiting cyclin gene transcription,[24,157] and the FSH surge up-regulates vascular endothelial growth factor.[158] In the absence of these critical steps, ovulation and follicular rupture do not occur. Then the follicle promptly becomes desensitized to LH and FSH and ceases to grow.[159] This is followed by

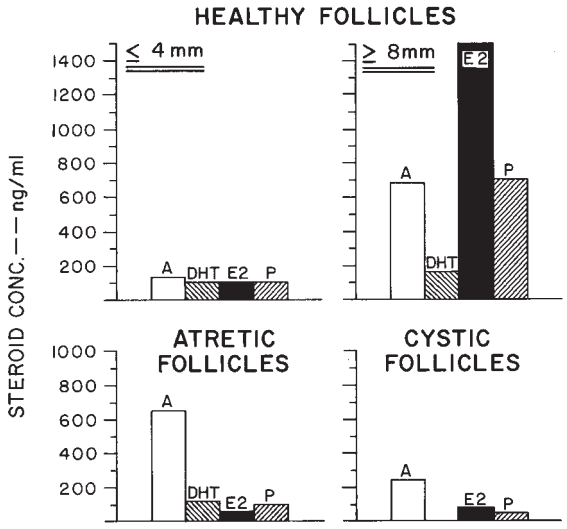


FIGURE 15-16 ■ Normal human antral fluid steroid concentrations. Healthy follicles are well populated by granulosa cells (50% or more of maximal complement). Healthy follicles seem capable of further development because many of them (75%) contain healthy-appearing oocytes (histologically intact germinal vesicles), 96% of which are viable in culture. Moderately large follicles (8 mm or larger in diameter) make their appearance only in the midfollicular phase of the cycle and contain follicle-stimulating hormone. Data are shown only for those large follicles well populated by granulosa cells, only one of which usually arises in the follicular phase of each menstrual cycle. Atretic follicles are small follicles beginning to show degenerative changes in the number of granulosa cells and appearance of the oocyte. Cystic follicles tend to be larger follicles with only a sparse granulosa cell lining. The testosterone content of antral fluid is about a third that of dihydrotestosterone (DHT) owing to the pattern of granulosa cell metabolism of androstenedione (A). E2, estradiol; P, progesterone. (Interpolation based on the data of McNatty, K. P., Makris, A., Reinhold, V. N., et al. (1979). Steroids, 34, 429; McNatty, K. P., Smith, D. M., Makris, A., et al. (1979). The microenvironment of the human antral follicle: interrelationships among the steroid levels in antral fluid, the population of granulosa cells, and the status of the oocyte in vivo and in vitro. *J Clin Endocrinol Metab, 49*, 851.)

an inflammatory-type response. Protease activity, prostaglandin production, and vascular permeability increase, cell junctions loosen, and cumulus cells form a mucopolysaccharide envelope around the oocyte (cumulus expansion).

Oocyte meiotic maturation resumes in response to a specific phosphodiesterase,[160] forming the haploid gamete (secondary oocyte) and the first polar body in response to the LH surge.[161] Ovulation of the cumulus-oocyte complex then occurs. The presence of a favorable follicular steroidal milieu is necessary both for ovulation (a premature LH surge in a subject with an unripe follicle will not result in ovulation) and subsequent developmental competence of the oocyte.[162,163] Meiosis will complete and the second polar body will be extruded only in response to contact with a sperm.

The processes stimulating dominant follicle emergence are delicately balanced by those preventing it. It seems critical that the intraovarian concentration of androgens not become excessive or no follicles will remain viable beyond about the 8-mm stage.[145] Androgen excess seems to prevent the emergence of dominant follicles by antagonizing granulosa cell proliferation and development.[164] The mechanisms involved include inhibition of aromatase in situations of low FSH activity[107,148] and antagonism of LH receptor formation and action.[109,165] Follicles arrested in their growth become atretic, and atretic follicles contain relatively high concentrations of androgens (see Figure 15-16). Progesterone also suppresses further differentiation of nondominant follicles[166] by some of the same mechanisms.[167] High concentrations of estrogen play a critical role in inhibiting selection of the dominant follicles in primates.[168] If there is interference with estrogenization, multiple large cystic follicles develop that are impaired in their ability to ovulate and undergo androgen-dependent atresia.[169-171]

AMH and inhibins have emerged as other follicular factors important in the direct and indirect regulation of follicular development. Granulosa cells of preantral and small antral follicles produce AMH, which regulates growth of follicles by exerting a paracrine negative feedback effect on the recruitment of precursor (primordial) follicles and inhibits aromatase activity.[172,173] AMH levels do not fluctuate during the normal menstrual cycle[174] but are indirectly inhibited by FSH during ovulation induction in response to estradiol produced by large antral follicles.[175] AMH serum levels index the size of the oocyte pool ("ovarian reserve") and gradually fall from young adulthood to become undetectable after menopause.[176] Granulosa cells also produce inhibins, which are regulated by FSH in a negative feedback loop and up-regulate thecal steroidogenesis, as discussed later: inhibin-B is the predominant form of inhibin: inhibin-A is a product of the preovulatory follicle (and corpus luteum) that responds to both LH and FSH.[177,178]

Atresia is the fate of all except the few hundred follicles chosen for ovulation during an individual's life span. Most follicles beyond the primordial stage become atretic. Atresia occurs by the process of programmed cell death.[31] This apoptotic process has diverse determinants, including cell death inducer and repressor genes.[39,132] FSH support becomes increasingly necessary for survival as the follicle matures, and normally only the follicle that has the lowest FSH threshold escapes atresia.

Luteal (Secretory) Phase Ovary. Histologically, luteinization is a process of lipid accumulation and begins as the preovulatory follicle forms. The biochemical hallmark of luteinization is the capacity for progesterone biosynthesis in response to LH; this is accompanied by increased secretion of estrogen and 17-hydroxyprogesterone in man.[179-181] Following ovulatory rupture of the Graafian follicle, capillaries and fibroblasts from the theca proliferate and break down the separating basement membrane. The luteinized granulosa and theca cells then intermingle and complete the luteinization process by forming the corpus luteum.[182]

During its functional life span, the corpus luteum is normally the major source of the sex hormones secreted by the ovary. Corpus luteum function reaches its peak about 4 days after ovulation and begins to wane about 4 days before menstruation (Figure 15-14H). Loss of sensitivity to LH and estradiol heralds luteal senescence. Regression of the corpus luteum—luteolysis—occurs if pregnancy does not provide hCG. Luteolysis is probably mediated by prostaglandin. Transformation of the corpus luteum into an avascular scar, the corpus albicans, then occurs.

Early luteal phase increases in secretion of both E2 and progesterone cause secretory transformation and hyperplasia of the endometrium. A significant rise in basal body temperature, averaging 0.35° C, occurs when serum progesterone reaches an average of 400 ng/dL and continues as long as that level is maintained.[183] Later fall-off in secretion of female hormones to a level insufficient to maintain the endometrium results in menstruation. Withdrawal of progesterone is specifically responsible for constriction of spiral arteries, local prostaglandin accumulation, and subsequent ischemic necrosis of the endometrium. Normal menstrual flow then results from a complete slough of the secretory endometrium.

A major determinant of normal corpus luteum formation and function is optimal development of the corpus luteum predecessor, the dominant follicle. Experimental lowering of FSH levels in the early follicular phase has been shown to impair subsequent corpus luteum function.[184]

Regulation of the Neuroendocrine-Ovarian Axis

Factors Controlling the Onset of Puberty

Pubertal onset is under the control of a complex regulatory network that is able to dynamically respond to numerous endogenous and environmental signals. GnRH neurons play a critical hierarchical role in the direct and indirect integration of these central and peripheral signals. Reproductive development is coupled with metabolic cues that influence the maturational process. The mechanisms by which neuroendocrine and genetic factors control pubertal development remain unknown. Epidemiologic studies indicate that nutrition, ethnicity, and genetic factors are normally important in the pubertal

process.[185] Environmental chemicals and chronic inflammatory disease can disrupt the process.[185-188]

Evidence that there are genetic factors involved at the time of puberty comes from multiple studies.[189-199] It has been estimated that between 50% to 80% of the variation in the timing of puberty is genetically determined. Genes involved in GnRH signaling, pituitary development, hormonal regulation, fatty acid biosynthesis, and energy homeostasis have been implicated.[200-204] Although mutations in these genes have been shown to cause physiologic interruptions in development, their role in the initiation of puberty remains unknown. Specifically, single nucleotide polymorphisms (SNPs) in the GnRH and GnRH receptor genes have not been associated with variations in the timing of puberty in the general population.[205]

The key in the initiation of puberty is the activation of the hypothalamic GnRH pulse generator. The molecular events that control the pulse generator include a complex interplay between both inhibitory and stimulatory factors. The mechanism of central activation of puberty first appears to be a consequence of a removal of a restraint mechanism, with a rise in gonadotropin secretion (initially during sleep).[206] This restraint in the GnRH pulse generator is independent of the presence of gonads[60] and more intense in males.[207] However, the high levels of testosterone to which the male fetus is exposed during the period of sexual differentiation may be responsible for the more prolonged suppression of GnRH release in males than females. A role for decreased estrogen feedback sensitivity by the hypothalamic pulse generator near the time of puberty has also been shown.[208]

Evidence points to an important role for GPR54, a G-protein-coupled receptor, and its ligand, kisspeptin, as a signal for pubertal GnRH release. Expression of both proteins has been found to increase prior to pubertal onset in association with the increase in GnRH pulse generator activity in the hypothalamus.[209] Kisspeptin binding to GPR54 on GnRH neurons stimulates GnRH secretion. Leptin and androgen synergistically up-regulate this system, and estrogen antagonizes it.[210] Mutations in the GPR54 gene result in hypogonadotropic hypogonadism.[201,211,212] However, mutations in GPR54 have not been found in boys with pubertal delay, nor have polymorphic sequences been associated with delay of pubertal development.[213] Elegant studies in primates have demonstrated an increase in kisspeptin during pubertal development with a corresponding increase in GPR54 associated with an increase in LH. The maximum level of expression of kisspeptin and GPR54 in the hypothalamus in both males and females occurs at puberty.[214,215] Chronic administration of kisspeptin to immature female rats induces precocious activation of the central axis.[214] In addition, chronic treatment with kisspeptin restores pubertal development in a rat model of undernutrition.[216] Kisspeptin may thus not only influence the priming of puberty but also the integration of nutritional and energy status.[217] Although it is clear that kisspeptin activation of GnRH neurons occurs at puberty and that GnRH is increasingly sensitive to kisspeptin activation during development,[218,219] other pathways contribute to GnRH activation, as the hypogonadism associated with deficiency of Kiss1 or Gpr54 is not complete.[220]

Neurokinin B signaling seems to be critical for the initiation of puberty.[221] Some kisspeptin neurons coexpress neurokinin B, dynorphin A, and their receptors (TAC3R and KOR), the primary function of which seems to be synchronizing kisspeptin neuron pulsatility.[222] Receptors for neurokinin B are also located on GnRH neurons, where they seem to modulate GnRH release or transport.

Disrupting mutations in makorin ring finger protein 3 (MKRN3), a paternally expressed, imprinted gene located in the Prader-Willi syndrome locus, were discovered to be associated with central precocious puberty.[223] This indicates the presence of a previously unrecognized GnRH release-inhibiting pathway centered in the arcuate nucleus.

Initiation of puberty involves coordinated changes in transsynaptic and glial-neuronal communication.[224] The major inhibitory systems are GABAergic and opioidergic, whereas the major excitatory systems involve glutamate and kisspeptin signaling, with glial cells facilitating GnRH secretion in diverse ways (Figure 15-17).[222,224] It appears that gamma-aminobutyric acid (GABA) receptor signaling develops in advance of glutamate signaling.[225] Increased signaling via glutamate receptors of several types (iontropic and metabotropic) appears to be the major proximate change in neurotransmission involved in puberty onset.[206,207,224] At puberty, however, seemingly as a consequence of glutamate receptor signaling, GABA-A receptor signaling on GnRH neurons increases GnRH secretion.[206,209,226,227] Glial cells facilitate the process through elaboration of transforming growth factors (TGFs), other growth factors, prostaglandin E2, and the elaboration of enzymes that control the concentration of glutamate (glutamic dehydrogenase, which catalyzes the synthesis of glutamate, and glutamine synthase, which converts glutamate to glutamine).

The basis of the change in neurotransmitter balance is becoming clearer. A second tier of control seems to be modulation of these processes by increased hypothalamic expression at puberty of tumor-suppressor genes that integrate glial-neuronal interactions. A yet higher echelon of candidate hypothalamic genes has been identified, made up of transcriptional regulators of the second-tier genes. These genes include Oct-2, a regulator of the POU-domain homeobox genes, EAP1 (enhanced at puberty 1), the knockout of which delays puberty and decreases fertility of mice, thyroid transcription factor I (TTF1), yin yang 1 (YY1), and CUX1.[228] Genes contiguous to elastin appear to be involved in the pace of puberty: deletion of chromosome 7q11.23 in Williams syndrome typically leads to an early normal onset but rapid pace of puberty with an abbreviated pubertal growth spurt.[229] Substantial redundancy of these networks and the signaling neurochemicals exists because the onset of puberty depends on the expression of many genes, likely arranged in a coordinated network. The gene products may function as activators or repressors of targets important for pubertal onset and progression. Sex steroids have been implicated as important modulators in pubertal onset.[225]

Thus, the onset of puberty is controlled by an opposing increase in excitatory and a corresponding decrease

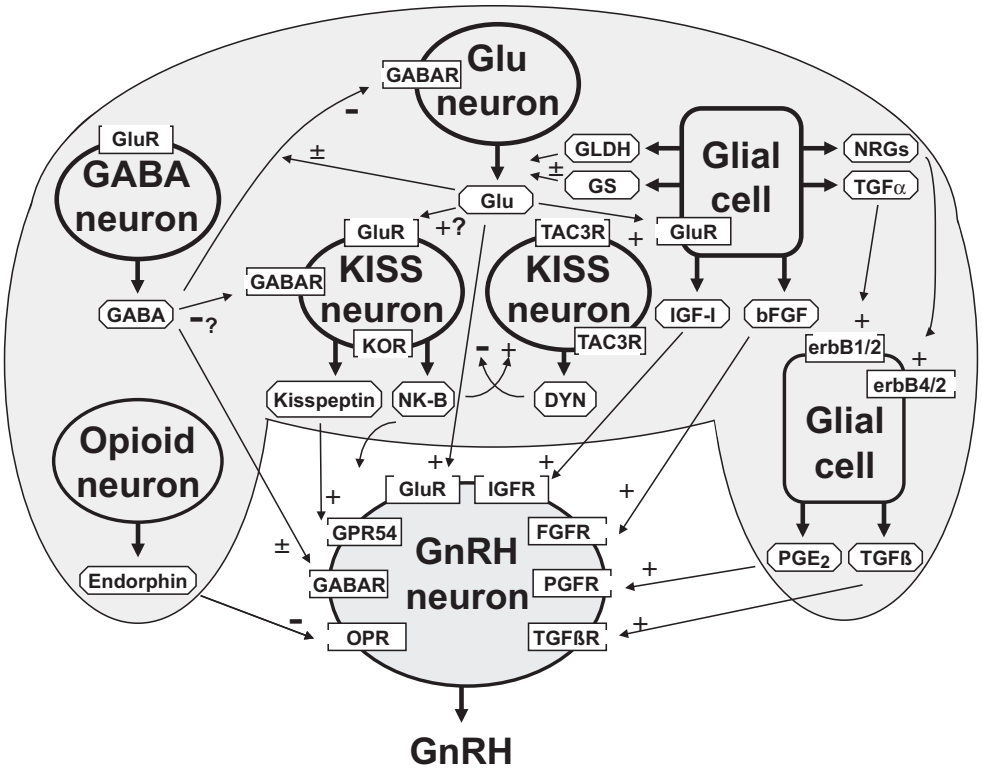


FIGURE 15-17. ■ The molecular biologic basis for the major known proximate hypothalamic pathways regulating GnRH secretion. The left-hand column depicts the major inhibitory pathways, which involve GABA signaling through the GABA receptor and opioidergic signaling through the endorphin receptor (OPR). The central column depicts the major excitatory pathways, which involve glutamate (Glu) signaling through the family of glutamate receptors and kisspeptin (KISS) signaling through GPR54. The right column shows the major glial factors that facilitate GnRH release. These include the elaboration of the enzymes glutamic dehydrogenase (GLDH) and glutamine synthase (GS), which regulate the concentration of glutamate and the elaboration of a variety of growth factors. Most kisspeptin neurons co-express neurokinin B (NKB), dynorphin A (DYN), and their receptors (TAC3R and KOR), the primary function of which seems to be synchronizing kisspeptin neuron pulsatility; receptors for NKB are also located on GnRH neurons. BFGF, basic fibroblast growth factor; erbB 1-4, subunits for the TGFα and NRG receptors; IGF-1, insulin-like growth factor; NRG, neuroregulins; PGE, prostaglandin E; R, receptor; TGFα, tumor growth factor-α; TGFβ, tumor growth factor β; +, positive stimulation; −, inhibition; ± =, either; ?, unknown. (Modified from Ojeda, S. R., Lomniczi, A., Mastronardi, C., et al. (2006). Minireview: the neuroendocrine regulation of puberty: is the time ripe for a systems biology approach? *Endocrinology, 147*, 1166–1174).

in inhibitory signaling from neural networks targeting the GnRH neuron. Lesioning studies indicate that inhibitory tracts mainly seem to be routed through the posterior hypothalamus and stimulatory ones through the anterior hypothalamic preoptic area.[1,230] These studies have been complemented by studies in genetically engineered mouse models. In one such model, the anteroventral periventricular nucleus (AVPV) population of kisspeptin neurons was shown to be the site of estrogen positive feedback in the control of pubertal progression, and kisspeptin cells in the arcuate nucleus of the hypothalamus were shown to be critical for E2 negative feedback.[231] Neonatal androgenization, which ablates the ability to generate a midcycle LH surge, was shown to selectively inhibit development of the AVPV population of kisspeptin neurons.[222]

An overview of the systems involved in regulating the initiation of puberty is shown in Figure 15-18. Pubertal maturation and skeletal maturation seem to have common determinants. Abundant clinical evidence indicates that sex steroid hormones are among these determinants.[232,233] Thus, genes involved in sex steroid hormone metabolism and action are candidate regulators of the onset of puberty. Evidence has begun to appear that early childhood exposures to hormonally active chemicals are among the "environmental disruptors" that may influence the onset of puberty,[185-187] and experience with diethylstilbestrol indicates that fetal exposures can have epigenetic effects.[234] The growth hormone (GH)-IGF system is another determinant. GH facilitates the onset and tempo of puberty.[235] Experimental studies suggest that this occurs through GH or IGF actions at all levels of the neuroendocrine-ovarian axis.[236,237] Girls generally enter puberty when they achieve a pubertal bone age. Pubertal stage normally correlates better with the bone age (r = 0.82) than with the chronologic age (r = 0.72; Rosenfield, unpublished data), particularly as menarche approaches.[238] Skeletal age correlates better with menarche than chronologic age, height, or weight, and its variance at menarche is half that of chronologic age.[239] The bone age at the onset of breast development averages about 10.75 years, and that at menarche averages about 13 years. Disorders that accelerate bone maturation, such as congenital adrenal hyperplasia or hyperthyroidism, tend to advance the age of the onset of true puberty.[240] Disorders that retard skeletal maturation,

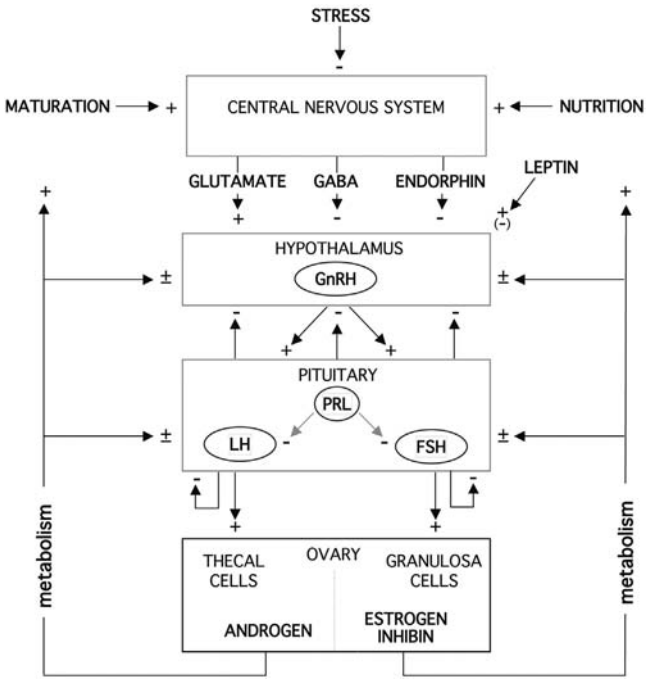


FIGURE 15-18 ■ Diagram of the major mechanisms controlling the development and function of sex hormone secretion by the unripe antral follicle. Regulation may be either stimulatory (+) or inhibitory (−). The central nervous system (CNS) influences gonadotropin-releasing hormone (GnRH) secretion both negatively and positively. For the CNS to relinquish its inhibitory control over GnRH secretion, it must achieve a high level of maturity. Even after this is achieved, psychological or physical stress may negatively influence the system. Nutrition must be optimal. Leptin is a critical mediator of the nutrition effect. Sex steroids have a maturing effect. Whether efferent tracts from the hypothalamus to the cerebrum play a role in reproductive function is unknown. Pineal secretion of melatonin and other substances are known to exert inhibitory influences on GnRH in lower animals (not shown). GnRH stimulates luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Paracrine and autocrine feedback of the gonadotropins on GnRH release and on their own release, respectively, is shown. Prolactin (PRL) has multiple effects on gonadotropin secretion. In unripe antral follicles, LH acts on thecal and interstitial cells and FSH acts on granulosa cells. Androstenedione and testosterone secreted by the theca cells are aromatized by the granulosa cell, under the influence of FSH, to estradiol. The granulosa cell is also the site of production of the FSH inhibitor inhibin B. Estradiol has a biphasic effect on the mature pituitary and on hypothalamic GnRH release as well. Androgens seem normally to be of minor importance in regulating gonadotropin release in females. Intraovarian mechanisms seem to modulate LH action so as to coordinate thecal formation of androgens with granulosa cell formation of estrogens. Paracrine and autocrine factors, including insulin-like growth factors, are involved. GABA, gamma-aminobutyric acid.

such as GH deficiency, hypothyroidism, or anemia, tend to delay the onset of puberty.[241] On the other hand, some data suggest that factors linked to intrauterine growth retardation, although not necessarily the growth retardation itself, predispose to sexual precocity.[185]

Optimal nutrition is clearly necessary for initiation and maintenance of normal menstrual cycles. The hypothesis that body fat is the weight-related trigger for pubertal development originated with the discovery by Frisch and coworkers that weight correlated with initiation of the pubertal growth spurt, peak growth velocity, and menarche better than chronologic age or height.[242] Mid-childhood may be a critical period for weight to influence the onset of puberty.[185] Suboptimal nutrition related to socioeconomic factors is an important factor in the later onset of puberty in underdeveloped when compared to developed countries.[185] Conversely, obesity appears to be an important factor in advancing the onset of puberty in the United States.[243] Some of the obesity effect may be mediated by IGF-1 and adrenal androgen.[244]

Leptin appears to be an important link between nutrition and the attainment and maintenance of reproductive competence.[207,245,246] Leptin deficiency causes obesity and gonadotropin deficiency. Paradoxically, prolonged leptin excess can down-regulate the leptin receptor and GnRH release.[247] Leptin is secreted by fat cells. It acts on the hypothalamus to reduce appetite and stimulate gonadotropin secretion. A critical threshold level appears to signal that nutritional stores are sufficient for mature function of the GnRH pulse-generator and, thus, to be permissive for puberty. Blood leptin levels rise throughout childhood and puberty to reach higher levels in girls than boys.[248] Leptin binding protein, a truncated form of the leptin receptor, falls as puberty begins, which suggests that circulating leptin becomes more bioavailable. Whether leptin has a direct role in the pubertal activation of the GnH pulse generator is unknown. In models of leptin insufficiency, the administration of kisspeptin induced LH secretion.[214] Conversely, leptin's effect on puberty did not require signaling in kisspeptin neurons in other mouse models.[249]

Other factors also link nutrition and gonadotropic function. Part of the leptin effect is mediated by inhibition of hypothalamic neuropeptide Y (NPY) formation.[250] NPY is a potent appetite-stimulating member of the pancreatic polypeptide family that directly inhibits GnRH release during food deprivation.[250] However, in the preovulatory state it stimulates GnRH release,[251] an effect mediated by a different neural network acting on a

different NPY receptor subtype on the GnRH neuron.[252] NPY is also inhibited by the anorexigenic peptide YY (PYY), a gut hormone secreted in response to food and inhibited by growth hormone; the pubertal fall in PYY has been postulated to permit the coordinated pubertal rise in appetite and gonadotropins.[253]

Other cues that provide information on nutritional status to the central reproductive axis may include glucose,[254] ghrelin,[255] and insulin.[256] The effect of these factors on LH pulsatility may be mediated directly at the level of the gonadotroph or indirectly by changes in GnRH secretion. There is little evidence for the role of pineal secretions in human reproduction that is found in lower animals.[257,258]

The essential element for the onset of puberty is an increase in pulsatile hypothalamic GnRH secretion that is regulated by a complex interplay of excitatory and inhibitory signals that have yet to be fully understood or elucidated.[224] During childhood the activity of the GnRH pulse generating system is restrained, an awakening of the pulse generator occurs gradually during late childhood, and the tempo of GnRH neuronal activation increases during puberty. The underlying mechanisms for all these changes are unclear. The pubertal diminution in tone of the CNS centers that inhibit hypothalamic GnRH secretion during childhood has traditionally been considered to result from decreasing sensitivity of a "gonadostat" to negative feedback by sex steroids.[6,259,260] However, this now seems an overly simplistic concept for a mechanism that seems to involve a change in the balance of neural inhibitory and stimulatory signals that impinge on the GnRH neuron.

Many studies have been performed to help explain the initiating developmental events or the "trigger" for pubertal onset. In fact, it is becoming increasingly clear that there is no single trigger for puberty, but instead there is a gradual increase in GnRH pulsatility associated with a complex interplay of factors and hypothalamic developmental programs. Thus, the apparent "sensitivity of the gonadostat" seems increasingly likely to reflect the degree of activity of the GnRH neuron. That is, when GnRH secretory activity is attenuated, the pulse generator is easily inhibited; when the GnRH neuron is active, the pulse generator is relatively insensitive to negative feedback.

The integration of hypothalamic signaling systems along with the developmental changes in the control of GnRH neuronal function seem to converge to trigger the onset of puberty. In the rat, structural remodeling of the GnRH neuron was demonstrated during pubertal progression by an increase in the density of dendritic and somal spines, the percentage of total neurons with spines being lowest at birth and increased gradually postnatally until puberty.[261] The spiny processes of neurons are the location of excitatory synapses important in neuronal plasticity. The greatest percentage of complex neurons is in the peripubertal period, with the percentage decreasing after completion of puberty.[262] These developmental changes are correlated with an increase in excitatory synaptic input to the GnRH neuron triggering the onset of puberty in mice.[262,263] Which excitatory synaptic input (e.g., glutamatergic, kisspeptinergic, or yet unknown neurochemical signals) plays a role in the pubertal increase in GnRH secretion is unknown. Whether primate or human GnRH neurons undergo synaptic excitatory remodeling during development is also unknown.[264]

Regulation of Gonadotropin Secretion

An essential feature of the mature hypothalamic-pituitary-gonadal axis is the long-loop, negative-feedback control of gonadotropin secretion by gonadal secretory products, as depicted in Figure 15-18. The generally tonic nature of gonadotropin secretion is punctuated by two prominent types of periodicity: two- to threefold pulsations of LH above trough levels at 1.5 to 2 hours intervals and, in the sexually mature female, a transient, midcycle, preovulatory gonadotropin surge. The latter is characterized by a greater than 10-fold, rapid rise of LH and a lesser rise of FSH. This surge is brought about by positive feedback when a critical level of E2, facilitated by a modest rise in progesterone, is achieved for a critical period of time, as discussed in relation to Figure 15-14.

E2 in concert with inhibin, reciprocally regulates FSH in a sensitive, log-dose, negative-feedback loop.[265] Progesterone in high (luteal phase) concentrations is a major negative regulator of GnRH-LH pulse frequency.[126] Androgens have a biphasic long-loop feedback relationship with gonadotropins: at modest elevations they stimulate gonadotropin release, and at very high levels they inhibit it.[266]

E2 exerts triphasic, and progesterone biphasic, effects on gonadotropin secretion. As E2 rises after the midpoint of the follicular phase, it selectively reduces the FSH response to GnRH, and when it reaches preovulatory levels, it transiently exerts positive feedback effects on LH and, to a lesser extent, FSH.[267] At sustained high levels, E2 suppresses both gonadotropins. As progesterone reaches a preovulatory level, it enhances the E2 positive feedback effect, but at the higher levels that ensue during the luteal phase, it suppresses LH pulse frequency while enhancing LH pulse amplitude.[126]

The GnRH neurons primarily responsible for maintenance of the reproductive cycle are those of the arcuate (infundibular) nucleus (Figure 15-19).[88] GnRH neurons are inherently pulsatile.[268] Synchrony is promoted by fluxes of ionic calcium into these cells and autocrine GnRH inhibitory feedback. GnRH secretion is modulated by the variety of neurotransmitters and growth factors involved in initiating puberty.[224] Synchrony of the network of GnRH neurons that accounts for pulsatility is conferred when the hypothalamic concentration of GABA periodically falls from levels inhibitory to GABAA receptors in the presence of an excitatory neurotransmitter.[269,270] EAP1, a hypothalamic protein shown to be important for pubertal onset, has also been implicated in the control of menstrual cyclicity in primates.[271]

Sex steroid signals are in part conveyed to GnRH neurons indirectly. Regulation of GnRH secretion by estrogen involves in part induction of PRs in the hypothalamus.[126,272] GnRH neuronal cell lines have been studied in which E2 directly stimulates and inhibits GnRH gene expression under different experimental

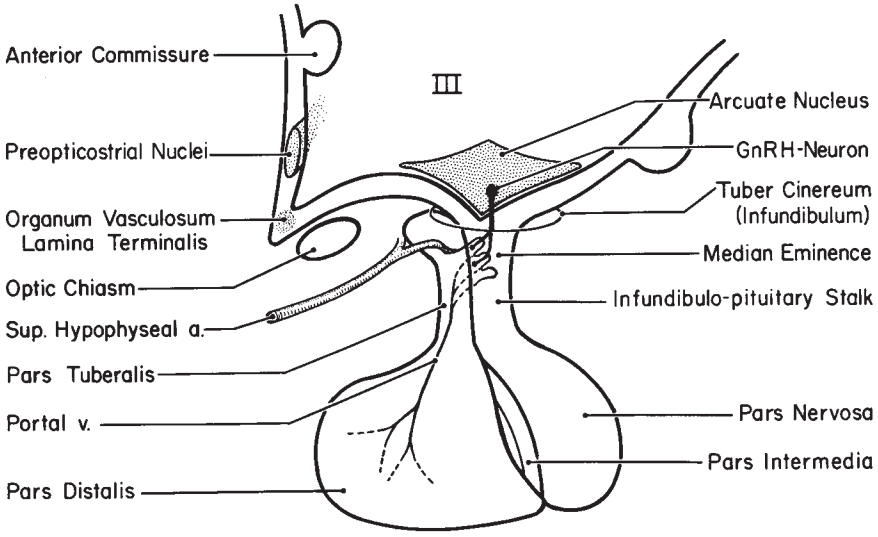


FIGURE 15-19 ■ The location of major gonadotropin-releasing hormone (GnRH)-containing neurons (shaded) in relation to the hypothalamus and pituitary gland. The neurons are of greatest density in the arcuate nuclei and in the periventricular wall of the medial basal hypothalamus. These neurons project to the adjacent median eminence; the second most dense population of GnRH neurons lies in the preopticostrial area. The development of some is altered by early androgenization. Some are connected by the stria terminalis to the amygdalae. Other projections from this area appear to connect indirectly with the median eminence, perhaps via the organum vasculosum lamina terminalis—a midline structure that resembles the median eminence. The pituitary portal veins transport blood rich in releasing factors to sinusoids engulfing anterior pituitary cells.

conditions.[273,274] Although progesterone exerts its main inhibitory effect on GnRH secretion, it has effects at higher CNS levels and at the pituitary level.[275-277] Prolactin suppresses both hypothalamic and gonadotropin GnRH receptor expression.[278,279]

Other clinically relevant factors affecting GnRH release are sleep, endorphins (endogenous opioids), and interleukins. In sexually mature women, sleep inhibits GnRH pulse frequency, and female hormones seem to amplify this effect.[280] Endorphins are important physiologic regulators of GnRH release after puberty has begun. Hypothalamic β-endorphin suppresses oophorectomy-initiated GnRH secretion, and opiate antagonists reverse this effect, as well as the sleep effect. The inhibitory effect of stress on gonadotropin release appears to be mediated by β-endorphin released from proopiomelanocortin in response to corticotropin-releasing hormone.[281] Interleukins also inhibit gonadotropin release.[282] Serotonin seems to modulate LH pulsatility and facilitate the LH surge.[283]

GnRH receptors on the gonadotroph are maintained in an optimally active state only when GnRH is delivered in pulses approximately 1 to 2 hours apart in humans.[88,284] Pulses substantially less frequent result in a hypogonadotropic state. Paradoxically, continuous administration of an initially stimulatory dose of GnRH results in down-regulation of gonadotropin production, after an initial burst of gonadotropin release.[285] This is the physiologic basis for the success of long-acting gonadotropin agonists in suppressing puberty in children with true central precocious puberty. However, whereas gonadotropins are down-regulated, free alpha-subunit production is elevated and responsive to GnRH.

Hypothalamic GnRH receptor function is modulated by autocrine and paracrine factors, including GnRH itself and kisspeptin.[5] Pituitary GnRH receptors appear to be directly and indirectly down-regulated by GnRH, gonadotropins, and inhibins, as well as sex steroids.[286] LH and FSH themselves inhibit GnRH release (short-loop feedback) and inhibit their own release (autocrine feedback).[286,287]

How is differential regulation of gonadotroph LH and FSH release accomplished in response to a single GnRH pulse? The frequency of the GnRH pulse is one determinant. Speeding this signal stimulates LH β-subunit gene expression, whereas slowing this signal stimulates FSH β-subunit and suppresses follistatin gene expression, altering the FSH/LH ratio.[288] Pituitary adenylate cyclase activating polypeptide amplifies LH responses to GnRH while blocking its effect on FSH.[289]

The sex hormone milieu is also clearly a major differential modulator of gonadotroph LH and FSH release.[88,277,290-292] FSH is more sensitive than LH to inhibition by estrogen; this effect of modest levels of E2 is of rapid onset and sustained. LH is the more sensitive to the stimulatory effects of higher E2 levels; this effect is of later onset and short lived. Similar relationships pertain in aromatase null mice. Estrogen receptor (ER) null mice have identified ER-alpha as the predominant receptor isoform that conveys negative feedback regulation to the gonadotroph.[293] Progesterone exerts both negative and positive feedback effects at the pituitary level, and these effects are antagonized by androgen. The progesterone metabolite 3α-hydroxyprogesterone suppresses FSH release.[294]

Inhibins of gonadal origin seem to be the major nonsteroid-specific negative feedback regulator of pituitary FSH synthesis and secretion.[295,296] Inhibins inhibit FSH release at the pituitary level, but they may act at a higher level as well.[297] Serum levels of both inhibins rise upon FSH stimulation.[177,178] Inhibin-B, produced by small antral follicles in response to FSH, is virtually the

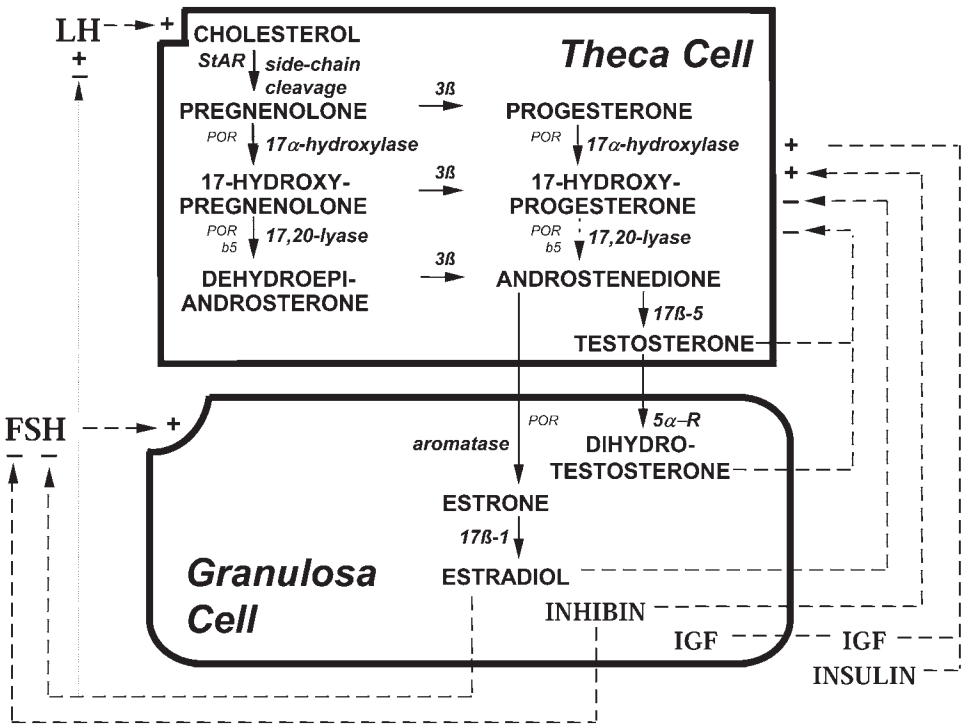


FIGURE 15-20 ■ Major factors regulating ovarian androgen and estrogen biosynthesis during the early follicular phase depicted according to the two-cell two-gonadotropin model. Luteinizing hormone (LH) stimulates androgen formation within theca cells by means of the steroidogenic pathway common to the gonads and adrenal zona reticularis. Follicle-stimulating hormone (FSH) regulates estradiol biosynthesis from androgen by granulosa cells. Estradiol at early to midfollicular phase levels does not exert a long-loop negative feedback effect on LH. Androgen formation in response to LH appears to be modulated by intraovarian and endocrine feedback at multiple levels, prominently including 17-hydroxylase and 17,20-lyase—both of which are activities of cytochrome P450c17. Androgen (via dihydrotestosterone) and estradiol inhibit (–), and inhibin, insulin, and insulin-like growth factors (IGFs) stimulate (+) enzyme activities. The sites of aromatase and IGF gene expression appear to vary with the stage of follicular development. Other peptides probably also modulate the steroidogenic response to LH. 3β-hydroxysteroid dehydrogenase (HSD): 17β-HSD5, type 5 17β-HSD; 5α-R, 5α-reductase. (Modified from Ehrmann, D. A., Barnes, R. B., & Rosenfield, R. L. (1995). Polycystic ovary syndrome as functional ovarian hyperandrogenism due to dysregulation of androgen secretion. *Endocr Rev, 16,* 322.)

only inhibin moiety in blood during puberty. Its blood levels rise during the early follicular phase and then fall thereafter except for a small postovulatory peak, generally paralleling the changes in serum FSH; the latter peak may attenuate the FSH surge. Serum inhibin-A, a marker of the preovulatory follicle and corpus luteum, begins to rise in the late follicular phase and thereafter parallels the levels of progesterone; its fall late in the luteal phase appears to contribute to the early follicular phase rise in the FSH level.

The structurally related activins seem to be important as regulators of both pituitary and ovary function.[298] Activin is formed by gonadotrophs themselves and its primary role is to stimulate FSH release. It also upregulates the activin binding protein follistatin, which arises within folliculostellate cells of the anterior pituitary.[250] Follistatin, by competitively inhibiting binding of activin to its receptor, specifically inhibits activin stimulation of FSH secretion.[265]

Regulation of Ovarian Secretion

Ovarian secretion results from the combined actions of LH and FSH, as discussed previously with regard to Figures 15-14 and 15-15. The early follicular phase follicle functions according to the two-cell, two-gonadotropin model illustrated in Figure 15-20.[141,299,300] In response to LH, androstenedione, the most abundant steroid formed in the ovary, is secreted by the theca-interstitial-stromal (thecal) cell compartment. In response to FSH regulation, aromatase then forms estrogen from precursor androstenedione in granulosa cells. FSH also stimulates granulosa cells to secrete inhibins. As by-products of the secretion of both ovarian E2 and adrenal cortisol, androgens do not normally contribute to negative feedback regulation of gonadotropins. However, they have a biphasic effect on gonadotropin secretion: modest elevations increase GnRH pulse frequency by interfering with progesterone negative feedback, and very high levels directly inhibit gonadotropin secretion.[266]

The regulation of the intraovarian androgen concentration is critical to ovarian function.[141,301] Androgens are obligate substrates for E2 biosynthesis and promote the growth of small follicles. However, in excess they interfere with the process of follicular maturation, preventing the emergence of the dominant follicle, thus committing the follicle to atresia, as well as interfering with LH action on luteinized granulosa cells. Therefore, androgen synthesis must be kept to the minimum necessary to optimize follicular development. This means that the synthesis of ovarian androgens must be coordinated with the needs of the follicle. This is achieved by

intraovarian intracrine, autocrine, and paracrine modulation of LH action.

LH stimulates theca cell development and steroidogenesis and is necessary for the expression of gonadal steroidogenic enzymes and sex hormone secretion. However, once the adult LH levels are achieved, further LH increase normally has little further effect on androgen levels because excess LH causes homologous desensitization of theca cells.[141,301] Desensitization involves down-regulation of LH receptor expression and steroidogenesis. Because steroidogenic down-regulation is primarily exerted on 17,20-lyase activity, which converts 17α-hydroxysteroids to 17-ketosteroids, 17-hydroxyprogesterone levels rise in response to increased LH levels, but there is only a limited rise in androgens.[266]

A model of the intraovarian interaction among the major factors regulating steroidogenesis is shown in Figure 15-20.[141] Stimulation of androgen secretion by LH appears to be augmented by specific intraovarian FSH-dependent factors, such as inhibins and IGFs. These processes seem to normally be counterbalanced by other FSH-dependent processes that down-regulate androgen formation as LH stimulation increases. Androgens and estrogens themselves seem to mediate at least a portion of this desensitization to LH, with estrogens being critical through an ERα-dependent mechanism.[293,302]

Insulin and insulin-like growth factors are important co-regulators of ovarian function. The entire IGF system is represented in the ovary and is essential to full FSH action; indeed, IGF-1 augments FSH receptor expression.[150,303] Insulin is a potent augmentor of both androgen and estrogen biosynthesis in response to gonadotropins, and insulin receptor mRNA is ubiquitous in all ovarian compartments at all phases of the cycle. In addition, insulin may exert its effects indirectly by its interactions with the IGF system in multiple ways. These include binding to the IGF-1 receptor, up-regulating that receptor, and lowering the serum concentration of IGF binding protein-1. GH also promotes granulosa cell steroidogenesis.[304,305]

Many other peptides modulate ovarian cell growth or function in response to gonadotropins.[141,301] Inhibin stimulates ovarian androgen production, whereas androgens reciprocally stimulate ovarian inhibin production. Activin opposes the inhibin effect. A variety of other ovarian peptides are also capable of modulating thecal androgen synthesis.[141] Stimulators include catecholamines, for which an intraovarian system exists,[306] prostaglandin, and angiotensin. Inhibitors include leptin, corticotropin-releasing hormone, epidermal growth factor, tumor necrosis factor, TGF-β, and growth differentiation factor-9.[307] Leptin antagonizes IGF-1 effects.[308] TGF-β is particularly interesting because it suppresses androgen biosynthesis and stimulates aromatase activity; it also stimulates meiotic maturation of the oocyte.[309] Other peptides acting on granulosa cells include cytokines, which have diverse effects,[303] and AMH, which inhibits aromatase.[173] GnRH is also capable of modulating thecal steroidogenesis. A GnRH-like protein has been described in the ovary that may act through ovarian GnRH receptors to suppress steroidogenesis in the human ovary.[310,311] It inhibits FSH induction of progesterone secretion, aromatase activity, and LH receptors in granulosa cells, down-regulates LH receptors, and inhibits the hCG stimulation of progesterone secretion by luteal cells.[110,131]

Prolactin has complex effects on steroidogenesis. In low concentrations, it enhances ovarian E2 and progesterone secretion by increasing LH receptors.[312] On the other hand, high levels of prolactin inhibit ovarian E2 and progesterone biosynthesis.[313] Prolactin also stimulates adrenal androgen production.[314]

Adrenarche and the Regulation of Adrenal Androgen Secretion

Adrenarche denotes the onset of the adrenal androgen production that gradually begins in mid-childhood well before the pubertal maturation of the neuroendocrine-gonadal axis.[15,315] It represents a change in the pattern of adrenal secretory response to ACTH (Figure 15-21). It is characterized by disproportionate rises in the responses to ACTH of the Δ^5-3β-hydroxysteroids 17-hydroxypregnenolone and dehydroepiandrosterone (DHEA), whereas cortisol secretion does not change. Dehydroepiandrosterone sulfate (DHEAS) is the predominant marker for adrenarche. A DHEAS level over 40 μg/dL is usually considered adrenarchal. Other serum androgens and precursors are ordinarily at the upper end of the prepubertal range at the onset of adrenarche (Table 15-1).

Adrenarche reflects the development of the adrenocortical zona reticularis. This zone becomes continuous at about 5 years of age and enlarges steadily over the subsequent decade. Its increasing development correlates

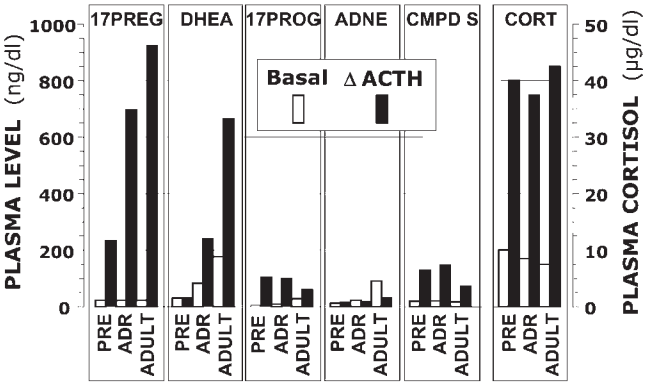


FIGURE 15-21 ■ Changing pattern of adrenal steroidogenic response to adrenocorticotropic hormone with maturation. Shown are plasma steroid levels before (basal, 8:00 a.m. after dexamethasone 1 mg/m²) and the rise (Δ) 30 minutes after cosyntropin (ACTH) administration (10 μg/m²) in healthy prepubertal children, children with premature adrenarche as an isolated phenomenon, and follicular phase adult women. Note that 17-hydroxypregnenolone (17PREG) and dehydroepiandrosterone (DHEA) responses of children with premature adrenarche are intermediate between prepubertal and adult responses. 17PROG, 17-hydroxyprogesterone; ADIONE, androstenedione; CMPD S, 11-deoxycortisol; DHEAS, DHEA sulfate. (Based on data from Rich, B. H., Rosenfield, R. L., Lucky, A. W., et al. (1981). Adrenarche: changing adrenal response to adrenocorticotropin. *J Clin Endocrinol Metab, 52*, 1129.)

with DHEAS levels. This zone's secretion pattern results from a unique enzyme expression profile: it expresses low 3β-hydroxysteroid dehydrogenase type 2 (HSD3B2) but high cytochrome b5 (an enhancer of the 17,20-lyase activity of cytochrome P450c17) and steroid sulfotransferase (SULT2A1) activities.[316]

Although the zona reticularis resembles the fetal zone of the adrenal cortex in its location and function, it appears to originate from stem cells located in the outer definitive zone of the fetal adrenal gland. A pituitary hormone ("adrenarche factor") may well be required to bring about their adrenarchal development.[4] It has been postulated to be an ACTH-related hormone distinct from ACTH, because adrenal androgen production is more sensitive to glucocorticoid suppression than is cortisol production,[317] falls more slowly than cortisol after dexamethasone administration[122], and rises more sluggishly after its withdrawal.[318] Candidates for a dexamethasone-suppressible adrenarchal factor include pro-ACTH related peptides and corticotropin-releasing hormone (CRH), but the data have not been convincing.[319] Prolactin may be involved.[320] Currently the only established adrenal androgen-stimulating hormone in postnatal life is ACTH. Because the adrenarchal secretion pattern represents a change in the pattern of steroidogenic response to ACTH, an adrenarche factor need only control the growth and differentiation of zona reticularis cells or regulate their unique pattern of steroidogenic enzyme expression. A number of factors are known to enhance adrenal androgen output. Insulin and IGF-1 particularly stimulate expression of adrenal P450c17 activities. Leptin has been reported to stimulate P450c17 activity.[321] Intraadrenal cortisol may participate in the regulation of adrenal DHEA secretion through inhibition of 3β-hydroxysteroid dehydrogenase activity.[322] In addition, interleukin-6 is strongly expressed in the zona reticularis and stimulates DHEA secretion.[323] Although gonadal dysgenesis is associated with earlier adrenarche,[324] paradoxically, ovariectomy precipitates an early decline in DHEAS levels that estrogen replacement does not reverse.[325]

Adrenarchal levels of androgens suffice to successively initiate sebaceous gland development, apocrine gland development, and the growth of pubic hair. They may promote stronger cortical bone and mid-childhood growth. Sulfation of DHEA within the adrenal cortex prevents adrenal hyperandrogenism.[326] Whether adrenarche plays a more fundamental role in normal puberty is unknown. DHEAS and its precursor, pregnenolone sulfate, have been found to be stimulatory neuroactive steroids.[327] DHEAS has been suspected of having a number of other functions, but these seem inconsistent; whether they differ from those of low-dose testosterone remains to be established.

Hormonal Secretion, Transport, Metabolism, and Action

Peptide Hormones

Peptide hormones act after binding to specific receptors located in the plasma membranes of target cells. GnRH receptors and gonadotropin receptors are members of the seven-transmembrane receptor family. These receptors are necessary for the actions of their cognate hormones. Receptors expressed in nonclassical sites are not necessarily functionally mature.[328] Mature receptors signal after coupling to a guanine nucleotide (G-protein) subunit (Figure 15-22).[5,284,288,329-331] Gs signaling activates adenylate cyclase and acts via phosphodiesterase-regulated cAMP to activate protein kinase A. Gq signaling activates phospholipase C, which acts via protein kinase C and Ca^{2+}; Ca^{2+} may also be mobilized by other factors that influence ion channels. Phosphorylation of various cytoplasmic and nuclear proteins ultimately mediates the action of the peptide hormones and secondarily involves the RAS and epidermal growth factor (EGF) signaling cascades in the case of gonadotropins.[24] The diversity among target cells in their responses to the action of protein kinases in part relates to diversity and type of kinase, intracellular compartmentalization, substrate availability, and other differences in gene expression that are specific to each type of target cell.

Gonadotropin-releasing hormone is a decapeptide [pyro]Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH2.[284] One gene encodes the single precursor protein for both GnRH and prolactin release-inhibiting factor.[332] Gonadotropin-releasing hormone not only effects prompt release of preformed gonadotropins (the "readily releasable pool"), but it also stimulates

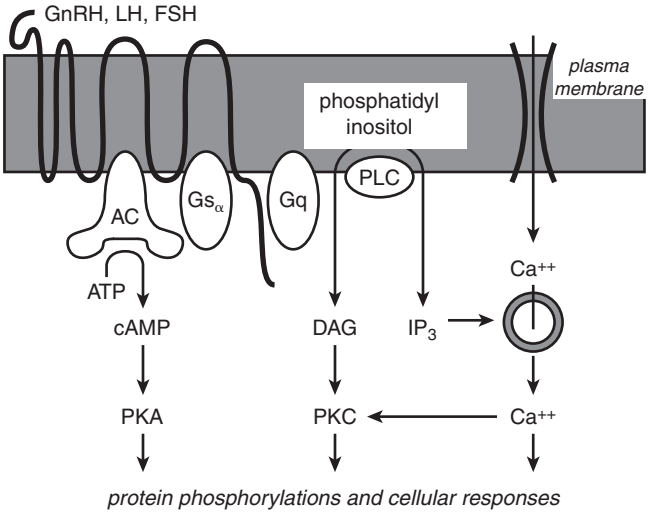


FIGURE 15-22 ■ Overview of the pathways established to mediate gonadotropin-releasing hormone (GnRH) and gonadotropin action. The receptors for these hormones are members of the seven-transmembrane family of receptors. Hormone-receptor binding alters receptor configuration. One consequence is to couple the receptor to adenylate cyclase (AC) via the stimulatory alpha-subunit of G protein (GS_a). This permits the efficient generation of cyclic adenosine monophosphate (cAMP) from adenosine triphosphate (ATP). Another consequence is to couple phospholipase C (PLC) to the receptor through Gq. PLC is a phosphodiesterase that hydrolyzes phosphatidylinositol to diacylglycerol (DAG) and inositol-1,4,5-triphosphate (IP3). DAG stimulates the calcium-sensitive protein kinase C (PKC). IP3 mobilizes ionic calcium (Ca^{2+}) from intracellular organelles and stimulates Ca^{2+} influx through calcium ion channels. PKA, PKC, and Ca^{2+} then bring about cellular responses through protein phosphorylations. FSH, follicle-stimulating hormone; and LH, luteinizing hormone.

the synthesis of gonadotropins (the "reserve pool").[333] Repeated administration of GnRH augments the pituitary responsiveness to subsequent GnRH pulses ("self-priming").[102] This has been ascribed partly to up-regulation of GnRH receptors. Gonadotropin-releasing hormone has an important paradoxical effect. As discussed earlier, it acutely stimulates gonadotropin secretion, but upon protracted, continuous administration, it down-regulates pituitary gonadotropin secretion. The significance of the expression of GnRH and its receptor in nonhypothalamic reproductive tissues[334] is unclear.

An evolutionarily conserved form of GnRH (GnRH-II) acts primarily through the type 2 GnRH receptor[335]; GnRH-II and the type 2 GnRH receptor are products of unique genes, rather than being modified products of the GnRH or type 1 GnRH receptor genes.[336] The cell bodies of the GnRH-II neurons lie predominantly in the midbrain and only a minority project to the hypothalamic-pituitary area. GnRH-II function in humans is unknown; there is speculation that it is a neurotransmitter involved in sexual behavior.

Luteinizing hormone and FSH are synthesized in a single type of cell, and both are sometimes identified within the same cell.[337] A vestigial population of hCG-secreting pituitary cells has been described.[338] Luteinizing hormone, FSH, and hCG are glycoprotein hormones that consist of two chains.[339] After synthesis of these hormones on the ribosomes, the carbohydrate moieties, which constitute about 16% of the weight, are added in the rough endoplasmic reticulum and Golgi apparatus. The α chains of LH, FSH, hCG, and thyroid-stimulating hormone (TSH) are identical in amino acid sequence (92 amino acids). Although the β chain of each hormone is different in both primary amino acid sequence and length, these β chains nevertheless share 30% to 80% amino acid homology. Biologic activity is conferred when an α and β chain are glycosylated and assemble within the cell. The α/β dimer is stabilized by a β subunit derived "seat belt" that wraps around the α subunit. Neither the isolated α nor the β glycosylated protein subunit exhibits biologic activity unless noncovalently bound to one another.

The gonadotropins exhibit considerable molecular heterogeneity.[95,340-342] The major basis for this is variation in the relative degree of glycosyl sialylation or sulfonation, steps which occur reciprocally in the pituitary gland.[343] These differences affect in vitro and in vivo bioactivity. Polymorphisms in the amino acid sequence of the LH-β and hCG-β gene also may affect the expression or bioactivity of LH or hCG.[344] Reproductive status affects isoform distribution, with sialylation predominating in the hypogonadal state.[345] Androgens increase and estrogens decrease in vitro LH biopotency by altering LH sialylation.[346,347] Thus, the pituitary gland contains multiple isoforms of LH and FSH that vary in bioactivity. Consequently, different pituitary LH and FSH standards as well as serum contain variable proportions of immunoreactive material of varying bioactivity.

One corollary of the molecular heterogeneity is that the antibodies generated from these gonadotropin moieties detect heterogeneous epitopes that are not necessarily bioactive; indeed some may even act as gonadotropin antagonists.[348] These factors combine to cause the ratio of bioactive to immunoreactive (B/I) gonadotropin to vary in a wide variety of circumstances.[95,340] The purest of standards, even recombinant ones, interact very differently in the diverse immunoassay systems. Likewise, the putative level of LH or FSH in a serum sample differs substantially among immunoassays. Furthermore, bioactivity assessments vary with the bioassay model system.[95,349] Monoclonal antibody-based immunometric assays yield results that correlate with, but are not necessarily equivalent to, those by bioassay.[95,350] The "third generation" immunometric assays have the advantage of being more sensitive and specific for low levels of gonadotropins in serum than polyclonal antiserum-based RIA, but B/I discrepancies remain.

The major determinant of in vivo gonadotropin bioactivity is the serum half-life. Terminal sialic acid residues retard clearance by the liver, the primary site of metabolism, whereas sulfonated ones facilitate clearance.[343] About 10% to 15% of gonadotropins are excreted in urine according to radioimmunoassay[351]; only about one third of this is in a biologically active form.[352]

Luteinizing hormone is cleared more rapidly from the blood than FSH or hCG.[353,354] Luteinizing hormone disappears from blood in an exponential pattern: radioimmunoassay indicates that the half-life of the first component is about 20 minutes and that of the second component is about 4 hours. The bioactive LH half-life is about 25% to 50% shorter.[355] The respective components for immunoreactive FSH are 4 and 70 hours; those for hCG are 11 and 23 hours. Hormone production rates in follicular phase women, which approximate midpubertal values, are given in Table 15-2.[356-362]

Prolactin has structural and functional similarities to GH and placental lactogen. Prolactin has a considerable degree of structural heterogeneity, which results from genetic and posttranslational events within pituitary cells as well as modifications such as glycosylation in the periphery.[363] Lactotrope growth and prolactin secretion are stimulated by estrogens. Prolactin release from the anterior pituitary is primarily under the control of hypothalamic inhibition, probably mediated by dopamine[364] and prolactin release-inhibiting factor.[332] The latter is contained within the same precursor protein as GnRH, thus providing a potential mechanism for reciprocal control of these two peptides. Prolactin secretion also is inhibited by thyroxine and is directly responsive to thyrotropin releasing hormone (TRH). Estrogen and suckling are stimulatory. These signals may be positively mediated by α-melanocyte stimulating hormone.

Inhibins and activins are members of the TGF-β superfamily and signal accordingly.[298,365] Inhibin was discovered as the result of the search for the nonsteroidal gonadal hormone capable of specifically suppressing FSH. Activin was serendipitously discovered as the FSH-stimulating activity in the side fractions in these studies. These hormones are formed by the differential disulfide-linked dimerization of two of three subunits (α– β_A, and β_B), each encoded by a distinct gene. The combination of an α– and β-subunit yields the inhibins, inhibin-A ($\alpha\beta_A$) and inhibin-B ($\alpha\beta_B$). Activins are dimers of β-subunits,

TABLE 15-2 **Average Hormone Blood Production Rates in Midfollicular-Phase Women***

Hormone	Production Rate	Pertinent References
Luteinizing hormone	615 IU/day†	356
Follicle-stimulating hormone	215 IU/day†	357
Androstenedione	3.4 mg/day	358
Dehydroepiandrosterone	7 mg/day	358
Dehydroepiandrosterone sulfate	7 mg/day‡	359, 360
Dihydrotestosterone	0.06 mg/day	358
Estradiol	0.1 mg/day	361
Estrone	0.1 mg/day	361
Progesterone	1.1 mg/day	362
17-Hydroxyprogesterone	1.2 mg/day	362
Testosterone	0.2 mg/day	358

*These production rates are roughly equivalent to those in midpuberty. The average daily production of those hormones that fluctuate cyclically is substantially greater. For example, E_2 production transiently peaks to about 0.5 mg/day, and thus the average production over the monthly cycle is about 0.2 mg/day or 6 mg/month.
†In terms of second International Reference Preparation, human menopausal gonadotropin.
‡Approximate urinary production rate, expressed as unconjugated steroid.

$\beta_A\beta_A$, $\beta_B\beta_B$, and $\beta_A\beta_B$ (activin-A, B, and AB). Inhibin antagonizes all known actions of activin. The genes for all three subunits are differentially expressed in a wide variety of tissues. Furthermore, these factors, particularly activin, have proven to exert effects not only on gonadotrophs but within other pituitary cells, the gonads, and in nonsexual target tissues.

Steroid Hormones

The ovary and adrenocortical zona reticularis share the core of the steroid biosynthesis pathway (Figure 15-23).[301,366,367] Gonadal cholesterol seems to be derived more from low-density than high-density lipoprotein in humans, and cholesterol can also be formed de novo. Most steroidogenic steps are mediated by cytochrome P450 family members. These are the terminal enzymes in electron transfer chains, which include P450 oxidoreductase (POR) as the clinically relevant electron donor for all in the endoplasmic reticulum. The initial step in the biosynthesis of all steroid hormones is the conversion of cholesterol to pregnenolone. This is a two-stage process. The rapidity of the process depends on the transport of cholesterol from the outer to the inner mitochondrial membrane by the steroidogenic acute regulatory protein (StAR). The conversion itself is carried out by the cholesterol side chain cleavage activity (scc) of cytochrome P450scc. The next steps are either the 3β-hydroxysteroid dehydrogenase/Δ^5-isomerase (3β-HSD) step or 17α-hydroxylation. 3β-HSD converts Δ^5-3β-hydroxysteroids to steroids with the Δ^4-3-keto configuration—that is, pregnenolone to progesterone, 17-hydroxypregnenolone to 17-hydroxyprogesterone, and DHEA to androstenedione. This step is obligatory for the synthesis of all potent steroid hormones. The type 2 3β-HSD isozyme accounts for the majority of the 3β activity in the human ovary and adrenal; the type 1 isozyme accounts for 3β-HSD activity in liver and skin. Pregnenolone alternatively undergoes a two-step conversion to the 17-ketosteroid DHEA along the Δ^5-steroid pathway: this conversion is accomplished via cytochrome P450c17. P450c17 is a single enzyme with both 17α-hydroxylase and 17, 20-lyase activities, the latter being less efficient and critically dependent on electron transfer from cytochrome *b*. Progesterone undergoes a parallel transformation to androstenedione in the Δ^4-steroid path: 17α-hydroxylation of progesterone by P450c17 forms 17-hydroxyprogesterone, but in humans P450c17 does not efficiently utilize 17-hydroxyprogesterone as a substrate for 17,20-lyase activity, so P450c17 seems to form little if any androstenedione. There is some evidence for the existence of a P450c17-independent Δ^4-pathway to androstenedione, but most seems to be formed from DHEA by the action of 3β-HSD.[368] Sulfotransferase 2A1 is uniquely expressed in the adrenal zona reticularis and requires the co-factor 3′-phosphoadenosine-5′-phosphosulfate synthase type 2.[326] Other sulfotransferases (e.g., for formation of estrone sulfate) and steroid sulfatase (for the reverse reaction) are widely expressed.[369]

17β-Hydroxysteroid dehydrogenase (17β-HSD) and aromatase activities are required for the formation of potent sex steroids. In the ovary, androstenedione is the major precursor for sex steroids. The conversion of 17-ketosteroids to 17β-hydroxysteroids by 17β-HSDs is essential for the formation of both androgen and estrogen: testosterone is formed in the ovary by 17β-HSD type 5 (also termed aldo-keto reductase, AKR,1C3), whereas E2 formation requires 17β-HSD type 1.[370] Aromatase activity, effected by P450arom, is essential for E2 formation. Alternate promoters are utilized by the P450arom gene in the gonads, placenta, and adipose tissue, which yields alternatively spliced forms of aromatase. The organization and regulation of steroidogenesis in the developing follicle is depicted in Figure 15-20.

The ovary normally accounts for about 25% of testosterone secretion in the mature female (0.06 mg daily),

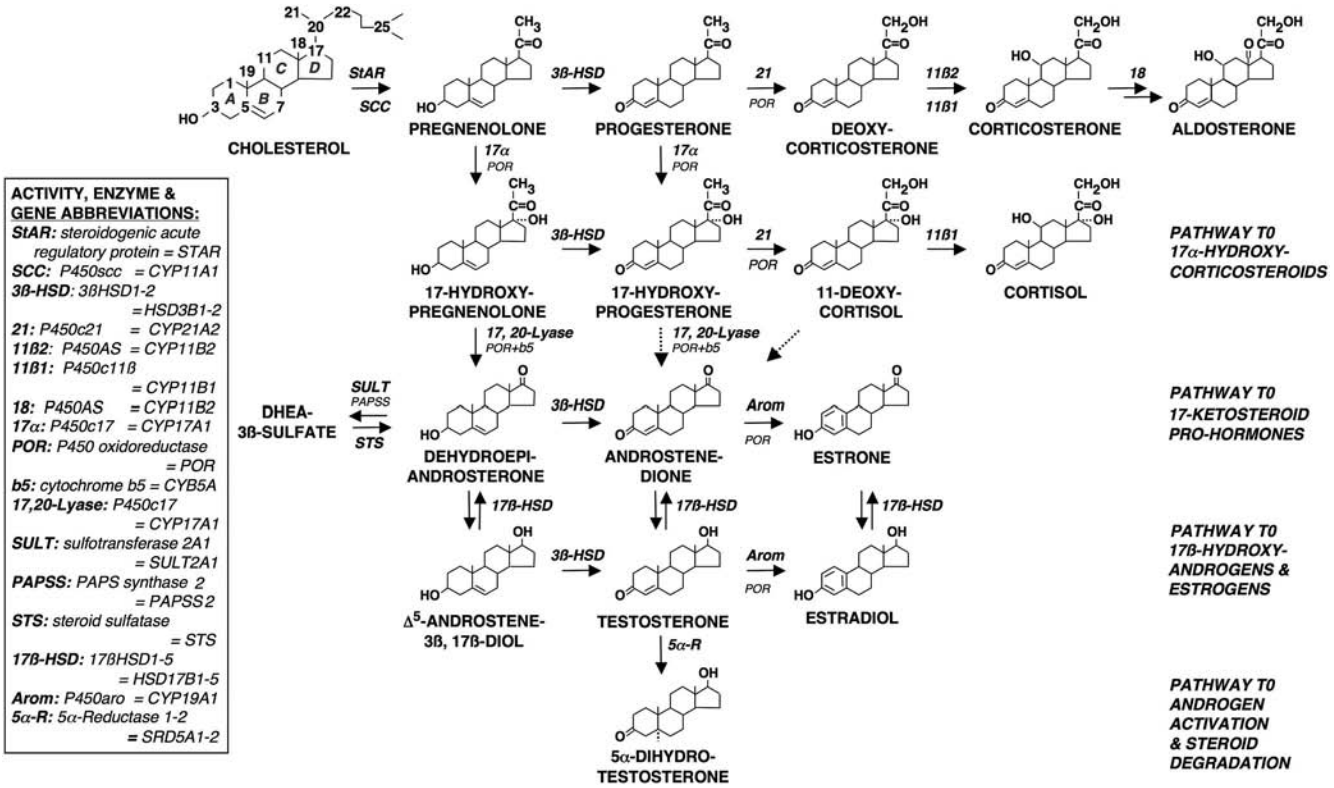


FIGURE 15-23 ■ Major pathways of steroid hormone biosynthesis from cholesterol. Carbon atoms of cholesterol are designated by conventional numbers and rings by conventional letters. The flow of hormonogenesis is generally downward and to the right. The top row shows the pathway to progesterone and mineralocorticoids, the second row the pathway to glucocorticoids, the third row the 17-ketosteroid pro-hormones, the fourth row the potent 17β-hydroxysteroids, and the bottom row the activation of androgen. The steroidogenic enzymes are italicized. Abbreviations for enzymes include the following cytochrome P450 enzyme activities: cholesterol side-chain cleavage (scc), 17α-hydroxylase (17α), 21-hydroxylase (21), 11β-hydroxylase (11β1), aldosterone synthase (11β2, 18-hydroxylase/oxidase), and aromatase (Arom). Non-P450 enzyme activity abbreviations include Δ^5-isomerase-3β-hydroxysteroid dehydrogenase (3β) and 17β-hydroxysteroid dehydrogenase (17β-HSD). Clinically relevant electron transfer enzymes include P450 oxidoreductase (POR), cytochrome b5 (b5), and 3′-phosphoadenosine-5′-phosphosulfate synthase type 2 (PAPSS). (Modified from Rosenfield, R. L., Lucky, A. W., & Allen, T. D. (1980). The diagnosis and management of intersex. *Curr Prob in Pediatr, 10,* 1.)

but it secretes about 30 times as much androstenedione (1.6 mg daily).[358] These amounts are similar to those secreted by the adrenal. However, the ovary secretes less than one tenth as much DHEA as the adrenal.

The "production rate" of a hormone equals its secretion rate plus the rate of formation of the hormone by peripheral conversion of secreted precursors outside of endocrine glands. The "blood production rate" is calculated as metabolic clearance rate x serum concentration; in the steady state, the amount of hormone irreversibly leaving the plasma compartment equals the amount entering it. Because of extensive steroid interconversions, the quantity of these hormones excreted in urine is not necessarily indicative of the amount reaching target tissues.[358] For example, so large a fraction of urinary testosterone glucuronide is formed directly from androstenedione by compartmentalized metabolism within the liver that the range of urinary excretion of testosterone in women overlaps that in men (Figure 15-24).[371] Estrone sulfate, like DHEAS in the androgen pathway, forms a circulating reservoir of inactive estrogen that can be returned to the active pool by hepatic sulfatase activity.[372] The blood production rates of representative steroid hormones are given in Table 15-2 and are shown for estrogens in Figure 15-25. During the luteal phase of the menstrual cycle, E2 production doubles[361] and progesterone production rises 15-fold or more.[373]

Sex hormones also have environmental origins. Structurally distinct biologic estrogens include equine estrogens and plant-derived phytoestrogens.[372] Synthetic estrogens include pharmacologic compounds such as ethinyl E2, diethylstilbestrol, selective estrogen receptor modulators (SERMs), and some industrial chemicals such as organochlorines and plasticizers. Environmental estrogens act as "endocrine disrupters," environmental chemicals that interfere with normal endocrine signaling systems by mimicking or blocking hormone signaling through its receptor, or by modulating the synthesis, release, transport, metabolism, binding, or elimination of natural hormones.[187]

Peripheral conversion of secreted prehormones by nonendocrine organs accounts for a major portion of sex hormone production. The ovary and the adrenal cortex are sources of prehormones as well as secreted hormones. About 50% of serum testosterone (0.1 mg daily) normally is formed indirectly by peripheral conversion. Although 85% of normal estrogen production in women

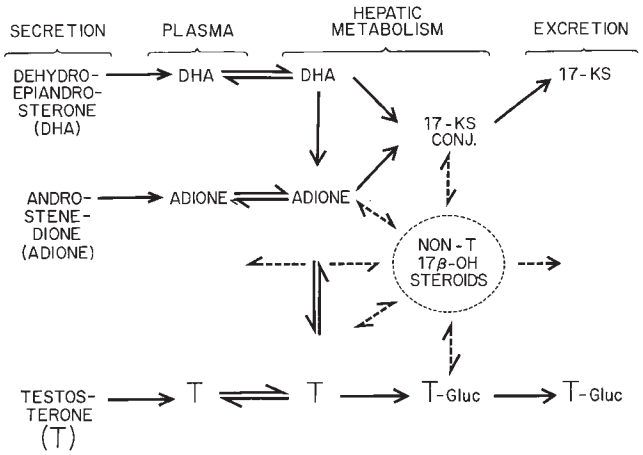


FIGURE 15-24 ■ Diagram illustrating the relationship among secreted, plasma, and urinary steroids. 17-ketosteroid (17-KS) excretion does not reflect accurately the excretion of the most important plasma androgens. Only 25% or less of testosterone is excreted as 17-KS metabolites. Therefore, important changes in testosterone production may not appreciably affect urinary 7-KS excretion. Furthermore, even the major 17-KS (DHA-sulfate) is excreted poorly until its production rate becomes quite high. On the other hand, about half 17-KS will not be identified by the standard colorimetric test and 2 mg daily of 17-KS in adults results from hydrocortisone metabolism. In addition, testosterone glucuronide excretion does not accurately reflect the plasma testosterone level: less than 2% of testosterone appears in the urine as such. Furthermore, the plasma 17-KS androstenedione may be converted to testosterone glucuronide without ever circulating as unconjugated testosterone. (From Rosenfield, R. L. (1973). Relationship of androgens to female hirsutism and infertility. *J Reprod Med, 11*, 87.)

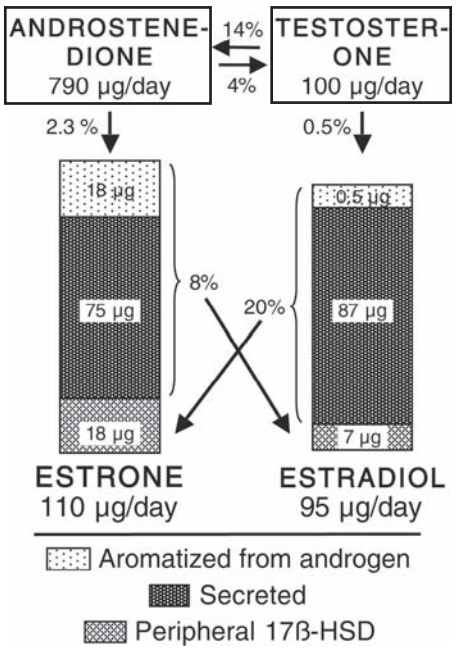


FIGURE 15-25 ■ Sources of estrone and estradiol in blood of follicular phase premenopausal women. Estrogen is derived from direct secretion by the gonad, aromatization of androgen, or conversion of an estrogen precursor by 17β-HSD activities. The percentage of substrate converted per day and total approximate production in micrograms per day are noted for each source. (Modified from Alonso, L. C., & Rosenfield, R. L. (2002). Oestrogens and puberty. *Best Pract Res Clin Endocrinol Metab, 16*, 13.)

arises by secretion in midcycle, 50% of estrogen production can arise from extraglandular sources during the low-estrogen phases of the menstrual cycle.[374] Peripheral formation of active steroids occurs in a wide number of sites, including liver, fat, and target organs.[301,375] For example, hepatic 17β-hydroxysteroid dehydrogenase and 5α-reductase activities are involved in steroid degradation (Figure 15-23).

Peripheral steroid metabolism is not tightly regulated. It seems determined to some extent by the perinatal androgenic milieu,[376] the effect of which is possibly mediated by GH.[377] Postnatally, it is influenced by the sex hormone binding globulin level (SHBG) and the state of nutrition. Adipose tissue becomes a major site of conversion of androstenedione to both estrone and testosterone in the obese.[266,378] Cytochrome P450 mixed function oxidases, the most important of which is CYP3A4, affect steroid efficacy by forming hydroxylated steroid metabolites of varying potency.[379,380] They are subject to induction or inhibition by numerous drugs. Phytoestrogens increase E2 bioavailability by inhibiting hepatic sulfotransferase.[381]

Plasma steroids appear to reach their sites of action and metabolism by simple diffusion from the vascular compartment.[382] The bioactive portion of serum testosterone seems to be the free testosterone and a portion of the albumin-bound testosterone that differs among tissues according to the diffusion characteristics of the vascular bed.[383] About 98% of serum testosterone and E2 are bound to albumin and SHBG. The SHBG concentration determines the fraction of serum testosterone and other ligands (e.g., E2, dihydrotestosterone) that are free or bound to albumin. It is also a major determinant of ligand egress from plasma (Figure 15-26).[384] Some sex steroid effects may be mediated by SHBG binding to membrane receptors and activation of adenylate cyclase.[385,386] A number of physiologic and pathologic states affect the SHBG level: it is increased by estrogen and thyroid hormone excess; it is decreased by androgen, insulin-resistant obesity, glucocorticoid, GH, and inflammatory cytokines.[387-389]

Target cell metabolism influences the cell's response to the steroid hormones that reach it (Figure 15-27).[390] The intracellular conversion of testosterone to dihydrotestosterone by one of the two isozymes of 5α–reductase is important for many but not all effects of testosterone,[391] dependent on the tissue-specific pattern of steroid metabolism. An important mode of testosterone action is via E2, notably within the brain. Although transformation is not fundamental to the mode of action of E2, its effectiveness is influenced by target cell metabolism: the induction of 17β-hydroxysteroid oxidation in target tissues by progesterone, resulting in conversion of E2 to the less potent estrogen estrone, counterbalances estrogenization.[392] There is also evidence that novel steroid metabolites exert tissue-specific effects.[393,394]

Within target cells, all steroid hormones regulate the genome similarly, starting with binding to high-affinity intracellular receptors (Figure 15-28).[395-397] The steroid hormone receptors belong to the superfamily of nuclear hormone receptors. The estrogen, progesterone, and androgen receptors are, thus, homologous. Classic sex

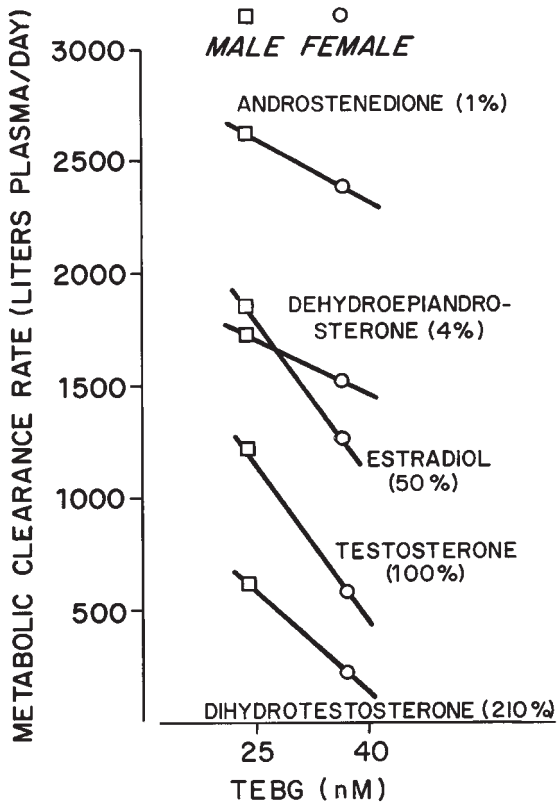


FIGURE 15-26 ■ The relationship between the metabolic clearance rate (MCR) and binding of sex hormones to sex hormone binding globulin (SHBG = testosterone-estradiol binding globulin, TEBG). The MCR of each steroid has been related to the mean SHBG levels of men and women. The approximate affinity of each steroid for SHBG relative to testosterone is indicated in parentheses. (From Rosenfield, R. L. (1975). Studies of the relation of plasma androgen levels to androgen action in women. *J Steroid Biochem, 6,* 695.)

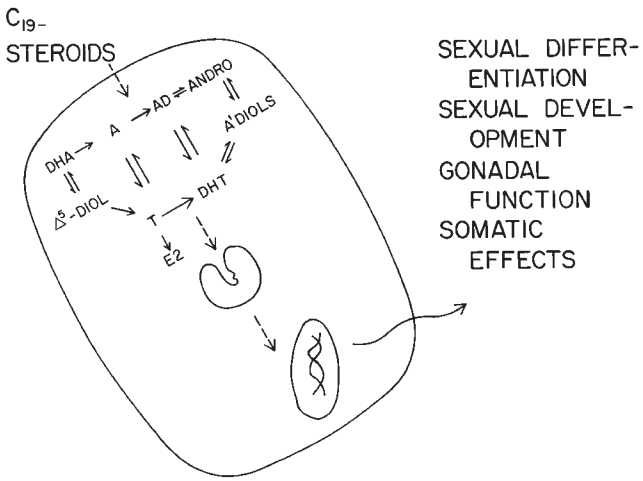


FIGURE 15-27 ■ Model of the mechanism of androgen action emphasizing the effect of steroid metabolism within a target cell on the mode of action. Solid arrows indicate pathways of steroid metabolism from 17-ketosteroid precursors as laid out in Figure 15-23. Broken arrow indicates transport. The cell-specific intracellular pattern of C19-steroid metabolism determines the relative availability of testosterone or dihydrotestosterone (DHT) to the cytosol receptor for translocation to the nucleus. In cells such as the rat granulosa cell in which Δ^5,3β-hydroxysteroid dehydrogenase activity is high, androstenediol (Δ^5-diol) is as potent as testosterone. The human sebaceous gland has a similar pattern of steroid metabolism. A, androstenedione; AD, androstanedione; DHA, dehydroepiandrosterone. (From Nimrod, A., Rosenfield, R. L., & Otto, P. (1980). Relationship of androgen action to androgen metabolism in isolated rat granulosa cells. *J Steroid Biochem, 13,* 1015, with permission from Elsevier Science.)

hormone effects are exerted by the interaction of steroid with receptor, not by either alone. Steroid binding triggers the dissociation of inhibitory chaperone heat shock proteins from the receptor.[398] The active receptor-ligand complex then undergoes noncovalent dimerization and binding to its specific hormone response element on the gene. The DNA-bound steroid-receptor complex acts as a transcriptional regulator of the target gene promoter. Sensitivity to steroids is also modulated by molecular chaperone proteins that influence receptor configuration, intracellular trafficking, and receptor turnover, all of which are determinants of steroid action.[399,400]

The binding properties of steroids to their cognate receptors are the initial determinants of classical steroid action.[395,396] Ligand-based selectivity is one element of this interaction. E2 is a more potent estrogen than estrone and estriol, partly because it binds best to the steroid binding domain of the estrogen receptor.[401] Dihydrotestosterone is an inherently more potent androgen than testosterone mainly because of its higher association rate constant and its lower dissociation rate constant.[402] The antiestrogens tamoxifen and clomiphene and the antiandrogens cyproterone acetate and spironolactone competitively inhibit the active ligands from binding to their specific receptor sites by weakly and transiently occupying receptor sites. These differences result from potent agonists snugly fitting into the binding pocket, which induces a receptor conformation different than that of antagonist-bound receptor. One such change is the C-terminal tail of the receptor flipping over to close the "door" when a potent agonist enters; this simultaneously provides a different outer surface for interaction with co-regulator proteins.

Thus, ligand-based selectivity arises not only because of tighter ligand binding but because alternative ligands produce both intermediate and unique conformational changes in the receptor, which in turn induce altered receptor interactions with co-regulator proteins that result in a spectrum of activities.[403] Thus, steroids do not simply switch receptors on; they induce selective functions that depend on the nature of the co-regulators that are recruited to the complex.[404-407] In part, this selectivity arises because different domains of these receptors mediate these different functions. For example, the AF-1 domain of the ER mediates interactions with mitogen-activated protein (MAP) kinase and TGF-β3, whereas the AF-2 domain mediates interactions with co-regulator proteins.[408] Coactivators, in turn, regulate alternative splicing, gene activation and repression (in some cases via their dual enzymatic functions), ubiquitin-proteosome-mediated turnover of the receptor–co-regulator complex,[409] and also determine cell-specific, site-based actions,[396] as discussed later.

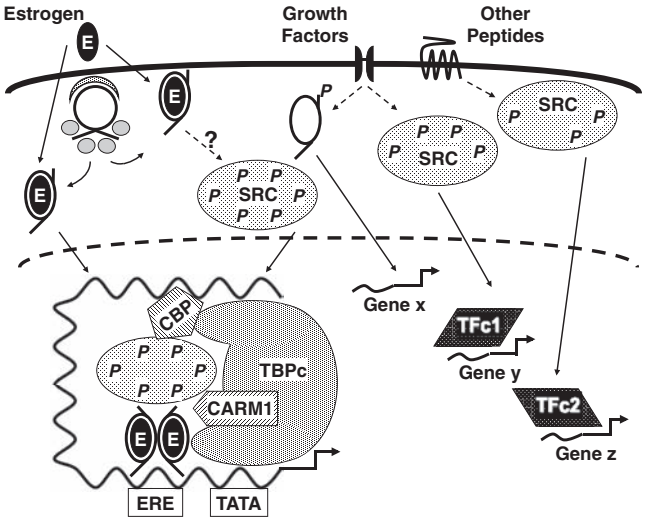


FIGURE 15-28 ■ A model for the mechanism of estrogen (E) action that emphasizes the role of interactions of the estrogen receptor with steroid receptor co-regulator (SRC) and phosphorylation signaling. Estrogen causes the 4S subunit and heat-shock proteins to dissociate from the unliganded estrogen receptor. Then estrogen entry into the binding pocket causes a conformational change in the receptor. Estrogen also stimulates phosphorylation of SRC in a specific pattern (*P*s), possibly via liganded membrane-bound ER as it does some transcription factors, and recruits it to the nuclear DNA steroid-receptor complex with the estrogen response element (ERE). SRC in turn recruits other co-activators, such as the cAMP response element binding protein-binding protein (CBP) and coactivator-associated methyltransferase (CARM1) to the hormone binding complex. This aggregate then interacts with the TATA binding protein initiation complex (TBPc) to initiate estrogen-specific gene transcription. The genomic E2 effect is modulated by the effects of environmental signals on other cell-specific transcription factors (TF), some of which involve differentially phosphylated SRC complexes (TFc) in gene activation, others of which involve ligand-independent ER. Dotted lines indicate diverse kinase pathways. ER recycling is not shown. (Based on Katzenellenbogen, B. S., Montano, M. M., Ediger, T. R., et al. (2000). Estrogen receptors: selective ligands, partners, and distinctive pharmacology. *Recent Prog Horm Res*, *55*, 163–193; O'Malley, B. W. (2005). A life-long search for the molecular pathways of steroid hormone action. *Mol Endocrinol*, *19*, 1402–1411; McDevitt, M. A., Glidewell-Kenney, C., Jimenez, M. A., et al. (2008). New insights into the classical and non-classical actions of estrogen: evidence from estrogen receptor knock-out and knock-in mice. *Mol Cell Endocrinol*, *290*, 24–30.)

Receptor-based selectivity is a second element in steroid action. There are now known to be two isoforms of each of the sex steroid receptors. The α and β forms of the estrogen receptor, though homologous, are coded by separate genes.[170] A and B forms of the progesterone and androgen receptors exist.[410,411] These forms of the PR arise by transcription from alternate promoters within the same gene, whereas those of the androgen receptor arise from posttranslational modification of a single mRNA. These isoforms have variously been shown to manifest a differential tissue expression pattern and respond differentially to antagonists. Interactions of a steroid with different forms of receptor can regulate some target genes differentially. One role of ERβ is apparently to modulate ERα activity: ERα and ERβ can have opposite actions at AP-1 and SP-1 sites, and studies of transcriptional activity in bone and breast tissue of mice indicate a restraining effect of ERβ on responses to E2.[170,412] Thus, different target tissues exposed to the same hormone may respond selectively because of a distinct repertoire of receptor isoform expression. Some examples are notable. Although both forms of estrogen receptor are expressed in most target tissues, the classic form of the estrogen receptor, ERα, plays the key role in regulation of LH and estrogen actions on the uterus, breasts, sex-specific behavior, and bone.[170,413,414] In the mouse ovary, knockout experiments show that ERα is expressed in thecal cells where it prevents androgen excess in response to LH. In contrast, ERβ is expressed only in granulosa cells, where its inhibition of androgen receptor expression is critical to prevent premature follicular atresia.[171] Both are necessary for oocyte survival and the ability of preovulatory follicles to rupture. Furthermore, loss of both causes transdifferentiation of granulosa cells to Sertoli-like cells and massive oocyte death.[153] Liganded progesterone receptor$_A$ is essential for ovulation[415] and is the more effective antagonist of estrogen receptor action.[395] In addition, sequence variation in hormone response elements contributes to differential gene regulation.[416]

Effector site-based selectivity is a third variable in classical sex hormone action. In other words, the potency and character of a response to a ligand-receptor complex are not simply inherent properties of the complex. Rather, they depend on the array of effector molecules present in the site of action. Thus, the array of genes expressed locally and the relative expression level of co-regulators (coactivators and corepressors) are extremely important in the determination of appropriate and graded responses to a ligand by a target cell.[395,417] Heterodimerization of the estrogen receptor with other nuclear receptors can modulate its action.[418] Androgens appear to exert some of their genomic effects by directly complexing with transcription factors other than the androgen receptor.[419] Both estrogen and androgen appear to exert antiapoptotic effects in osteoblasts and osteocytes by activating a ligand-dependent, but nongenomic, kinase-mediated signaling pathway.[420]

Nuclear receptor coactivators are critical for sensing cell-specific environmental signals and coordinating signals emanating from membrane receptors with nuclear receptor action.[396] Surface receptors send signals through kinase pathways that result in specific serine/threonine phosphorylation patterns of coactivators. These phosphorylation patterns serve as a code for the coactivator to preferentially bind and activate distinct sets of downstream transcription factors (see Figure 15-28). Overexpression of steroid receptor coactivator-3 (SRC-3) is as important in the pathogenesis of some breast cancer as is estrogen receptor positivity.

The effects of an alternative ligand-ER complex often differ from those of the E2-ER complex among cell types. This is the basis for the development of selective estrogen receptor modulators (SERMs): these compounds exert effects in a tissue-specific manner depending on the cell context.[395,403] The chemical structure of a SERM—or any ER ligand, for that matter—determines the configuration of the ER, resulting in a spectrum of activities from agonist to antagonist, depending on which co-regulators are

available for recruitment in the target cell. Raloxifene is an E2 agonist in bone and epiphyseal cartilage but is antiestrogenic in uterus and breast; tamoxifen is estrogenic in uterus but antiestrogenic in breast and bone. Both appear to retain neural and endothelial estrogenic activity.[421,422]

Nonclassical mechanisms play a role in sex steroid action.[397] The nonclassical mechanisms are mainly of two general types: (1) genotropic estrogen response element (ERE)-independent signaling, in which liganded ER acts as a co-regulator of other transcription factors that act through their specific DNA response elements; and (2) nongenotropic signaling, in which E2 binding to membrane-associated receptors, including ERα, rapidly stimulates phosphorylation pathways.[423,424]

The nongenomic effects via membrane signaling occur rapidly (within minutes) and can mediate cell proliferation, apoptosis, and migration in cell-specific ways.[420,425]Nongenomic E2 actions account for most of the LH-inhibitory and energy balance effects of E2.[424,426] These effects can be mediated by binding to nuclear ER in plasma membrane domains provided by scaffolding proteins such as caveolin. On such platforms, the E2-ER complex acts like a membrane receptor, coupling with G proteins and activating cytoplasmic pathways involving SRC and MAP kinase. Androgens appear to act similarly. Nongenomic actions of nuclear PR have also been reported. Some nongenomic effects seem to involve the activation of novel G-protein coupled transmembrane receptors for E2 and progesterone that interact with either steroids or their metabolites.

Genomic ER signaling may also be ligand independent. For example, cell membrane signaling by growth factors or other peptides stimulates ER phosphorylation. Epidermal growth factor activates phosphorylation of the ER and simulates diverse estrogen effects.[427] Activation of unliganded ERα seems to be involved in repressing expression of the androgenic 17β-HSD testicular isoform in the ovary.[293]

Steroids that act by binding to membrane-bound receptors in the brain are termed *neuroactive*.[327] Neuroactive steroids synthesized in the brain are termed *neurosteroids*.[428-430] The best documented of these effects are on neurotransmitters that control ion channels. Allopregnanolone (3α-hydroxy-5α-tetrahydroprogesterone) and 3α-androstanediol are GABAA receptor agonists and so have sedative and antiepileptic properties.[431] Pregnenolone sulfate and DHEAS have the opposite effect, the former also stimulating the glutamate receptors. Receptors for 5-hydroxytryptamine have been implicated in mediating some of effects of sex steroids and certain of their metabolites.[432,433] Some estrogen effects in brain are membrane mediated.[434]

The tissue-specific posttranscriptional events involved in sex steroid signaling are poorly understood. E2 and progesterone modulate the actions of each other through effects on their specific receptors: increased estrogens in the preovulatory phase of the cycle up-regulate target organ receptors for both E2 and progesterone; luteal phase levels of progesterone then suppress the production of both receptors.[435,436] Estrogen prevents bone loss by blocking the production of proinflammatory cytokines.[437] Androgen action seems to involve the arachidonic acid cascade in genitalia[438] and peroxisome proliferator-activated receptors in sebaceous cells,[439] whereas in epiphyseal cartilage testosterone stimulates the IGF-1 system.[440]

Maturation of Sex Hormone Target Organs

Genital Tract

The Müllerian system of the embryo gives rise to the uterus, cervix, upper vagina, and fallopian tubes in the absence of AMH secretion by fetal testes during the first trimester of gestation.[366] Genital swelling develops to engulf the base of the penis-like clitoris between 11 and 20 weeks' gestation in parallel with the development of the ovarian follicular system.[441] Estrogen receptors are expressed in the labia minora, prepuce, and glans in females, but not in the homologous structures of males.[442] An association between antiestrogen and genital ambiguity has been reported.[443] Diethylstilbestrol induces dysplasia of the genital tracts.[444] These data suggest that estrogen may play a direct role in female genital tract differentiation. However, knockout of estrogen receptors has no obvious effect on genital tract differentiation.[153]

The infantile uterus and cervix enlarge under the influence of estrogen during puberty. The endometrium and cervical glands then undergo cyclical changes in concert with cyclic ovarian function. In response to rising estrogen during the follicular phase of the cycle, the endometrial epithelium and stroma proliferate. The uterine glands increase in number and lengthen. Endometrial hyperplasia is prevented by progestin[445] and androgen excess.[446] In response to progesterone secretion after ovulation, the endometrium increases in thickness: stromal edema occurs, and the uterine glands enlarge, become sacculated, and secrete a glycogen-rich mucoid fluid. The coiled arteries lengthen further during this time and become increasingly spiral. These changes are critical to permit implantation. High-dose progestin is an effective postcoital contraceptive because it prevents implantation when taken within 3 days of unprotected intercourse.[447]

Endocervical gland secretions lubricate the vaginal vault. The endocervical mucus is scanty and relatively thin during the low-estrogen phase of the cycle. The increase in mucus flow with advancing follicular development seems to require tissue-specific stimulation of the cystic fibrosis transmembrane regulator by estrogen.[448] Cervical mucus becomes more viscous and elastic as estrogens rise in the later follicular phase of the cycle—the extent to which it can be stretched into a long spindle, *spinnbarkeit*, is a function of the estrogen level.

The mucosa of the vagina and the urogenital tract is comprised of hormone-responsive stratified squamous epithelium (Figure 15-29).[2] The basal layer is the regenerative area. In the absence of estrogen, there is only a parabasal layer of cells over this, and the vagina is thin, with a tendency to alkalinity, which predisposes it to local infection (nonspecific vaginitis).[449] In response to

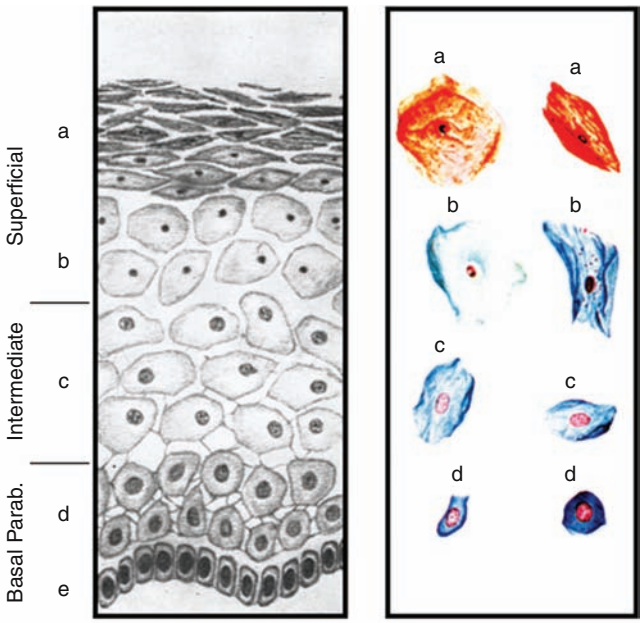


FIGURE 15-29 ■ The layers of vaginal epithelium of the well-estrogenized adult. The superficial layer contains surface cells that are cornified (squamous) with eosinophilic cytoplasm and pyknotic nuclei (a) as well as large intraepithelial cells that are also karyopyknotic but basophilic (b). The intermediate zone contains basophilic cells that have less cytoplasm and intermediate-size nuclei (c). Parabasal and basal cells have successively smaller amounts of basophilic cytoplasm and more vesicular nuclei (d, e). *This image can be viewed in full color online at ExpertConsult.* (Modified from Wilkins, L. (1968). *The diagnosis and treatment of endocrine disorders in childhood and adolescence.* Springfield, IL: Charles C Thomas.)

estrogen, epithelial proliferation occurs, with formation of successive intermediate and superficial layers. With this maturation the cytoplasm of each cell first expands, leading to formation of small intermediate cells. With further estrogenization, the nuclei become pyknotic and large intermediate cells form. Greater estrogenization brings about their transformation to cornified squamous superficial cells: the cytoplasm changes from basophilic to acidophilic with the accumulation of glycogen. Resistance to infection of the fully developed vaginal mucosa results from its thickness and from its acid pH, which occurs from the fermentation of the glycogen of the superficial cells. In response to luteal phase progesterone, degenerative changes appear in vaginal mucosal cells: superficial cells decrease, the cytoplasm assumes a "crinkled" appearance, cells degenerate, and bacterial proliferation increases.

Vaginal smears show the characteristic cyclic changes in the cell types comprising the vaginal epithelium (see Figure 15-29).[450] In the prepubertal years parabasal cells predominate, and characteristically 10% or less are small intermediate cells. A pattern consisting entirely of intermediate cells is typical of early puberty. The early follicular phase of the menstrual cycle is characterized by the predominance of large intermediate cells with few, if any, superficial cells. Peak maturation is reached at midcycle, at which time 35% to 85% of the cells seen on vaginal smear are superficial; the remainder are large intermediate cells. This cornification develops over a 1-week period in response to E2 levels of about 70 pg/mL and persists 1 to 2 weeks after estrogen withdrawal (see Figure 15-11).[451]

Progesterone antagonizes estrogen effects on the vaginal epithelium and cervix. Inhibition of cervical ripening by progestins is used to prevent recurrent spontaneous preterm delivery.[452,453]

Many normal variations have been recognized in the appearance of the hymen. The transverse diameter increases with age.[454,455]

Mammary Glands

Multiple rudimentary branching mammary ducts are found beneath the nipple in infancy; they grow and branch very slowly during the prepubertal years.[456] Estrogen stimulates the nipples to grow, mammary terminal duct branching to progress to the stage at which ductules are formed, and fatty stromal growth to increase until it constitutes about 85% of the mass of the breast. GH (via IGF-1) and glucocorticoids play a permissive role.[457,458] These hormones interact with breast stroma and local growth factors to stimulate the development of breast epithelium. Lobulation appears around menarche, when multiple blind saccular buds form by branching of the terminal ducts. These effects are due to the presence of progesterone. The breast stroma swells cyclically during each luteal phase. Full alveolar development normally only occurs during pregnancy under the influence of additional progesterone and prolactin. Prolactin does not play a role in breast growth without priming by female hormones.[459]

Estrogen and progesterone also play a role in breast cancer susceptibility.[460] Earlier than average age at menarche is a modest risk factor for breast cancer, regardless of BRCA status[461,462]; notably, however, breast cancer risk has not been shown to be increased in precocious puberty, the *BRCA1* gene normally restrains mammary growth, at least in part, by inhibiting expression of ERα and PRs, and cancer-related mutations reverse these processes.[463]

Pilosebaceous Unit

The pilosebaceous unit (PSU), with but few exceptions, consists of both a piliary and a sebaceous component.[464] Androgens are a prerequisite for the growth and development of PSUs in their characteristic pattern. Androgens exert their effects both on the dermal papilla, which regulates the hair growth cycle, and on PSU epithelium. Before puberty, the androgen-dependent PSU consists of a prepubertal vellus follicle in which the hair and sebaceous gland components are virtually invisible to the naked eye (Figure 15-30). Under the influence of androgens, in the *sexual hair areas* the PSU switches to producing a medullated terminal hair follicle that expresses a unique type of keratin that is androgen responsive.[465] The difference in the apparent density of sexual hair between men and women is due to differences in the density of terminal hairs that develop in response to androgen. In the *balding-prone area* of scalp in individuals genetically predisposed to pattern alopecia, androgens weakly attenuate the hair

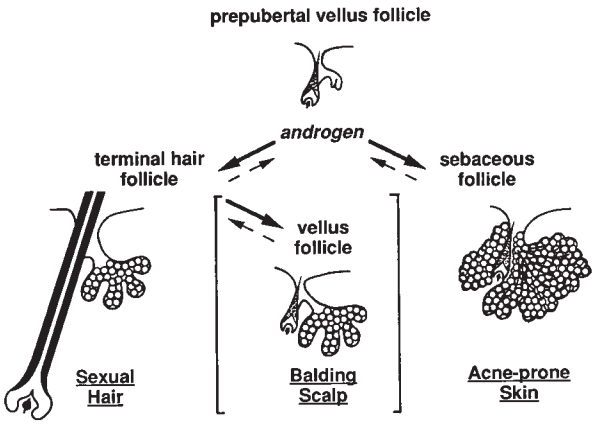


FIGURE 15-30 ■ Role of androgen in the development of the pilosebaceous unit. Androgens (solid lines) are responsible for the patterned differentiation of the pilosebaceous unit at puberty. Dotted lines indicate effects of antiandrogens. Hairs are depicted only in the anagen (growing) phase of the growth cycle. In balding scalp (bracketed area), terminal hairs not previously dependent on androgen regress to vellus hairs under the influence of androgen. (From Rosenfield, R. L., & Deplewski, D. (1994). Role of androgens in the developmental biology of the pilosebaceous unit. *Am J Med, 97*, 80.)

growth cycle, so that the PSU gradually generates only vellus follicles.[466] In *acne-prone areas*, androgen causes the prepubertal vellus follicle to develop into a sebaceous follicle, in which the sebaceous epithelium develops and the hair remains vellus. Adrenarchal levels of androgens suffice to successively initiate sebaceous gland development and the growth of pubic hair. Progressively greater amounts of androgen are in general required to stimulate terminal hair development along a pubic to cranial gradient. All these effects of androgen are to some extent reversible by antiandrogens.

Estrogens modestly stimulate hair growth, probably by inhibiting the catagen (resting) phase of the hair cycle[467]; this may well be due to induction of androgen receptors by estrogen. Estrogens also directly inhibit sebum secretion. GH synergizes with androgen action on the PSU, in part by acting through IGF-1. Retinoic acid receptor agonists antagonize the effects of androgen on the sebaceous gland by inhibiting sebocyte proliferation and differentiation. Insulin, prolactin, glucocorticoids, thyroxine, and catecholamines also play roles in PSU growth, development, and function.

Bone

Increased secretion of sex hormones clearly initiates the pubertal growth spurt. About half of this effect of sex hormones is due to their stimulation of the GH-IGF axis.[468] The remaining effects of sex steroids on skeletal growth are direct.[469,470]

Differences between the actions of sex hormones contribute to women's bones being shorter and narrower than men's.[471,472] The basis for these differences are diverse and involve interactions with IGF-1 and effects on cortical, cancellous, and periosteal bone formation.

Estrogen and androgen both stimulate epiphyseal growth. E2 is the critical hormone that brings about epiphyseal closure.[473] Estrogen also is particularly effective in reducing bone turnover. To some extent these effects may be prenatally programmed.[474]

Bone accrual during puberty is a major determinant of adult fracture risk. Menarche after 15 years carries a 1.5-fold increase in fracture risk, and the risk rises with age of menarche.[475]

Adipose Tissue

Women have a greater percentage of body fat than men.[476] During puberty they develop both more and larger fat cells than men in the lower body. Serum levels of leptin rise throughout puberty to reach higher levels in females than males,[248] whereas levels of the antilipolytic adipocytokine adiponectin remain stable in females but fall in males.[477]

The reasons for this sexual dimorphism are complex. Androgens inhibit adipogenic differentiation of human mesenchymal stem cells and pluripotent cells reciprocally to their stimulation of the myogenic lineage, in a dose-dependent fashion.[478,479] The role of estrogen itself in body fat accrual is unclear. In young women estrogen promotes lipogenesis and a lower-body (gluteofemoral) fat distribution; this contrasts with androgen, which generally promotes lipolysis and upper-body (visceral) fat accumulation. However, hypogonadal adults become obese. Studies in rodents suggest that estrogen deficiency causes obesity and that estrogen reverses this effect; the data are compatible with estrogen stimulating energy expenditure and weight loss by a hypothalamic effect.[426] In rats, progesterone counters the estrogen effect. In humans, use of the progesterone analogue megestrol acetate is approved to stimulate appetite and weight gain; use of progestins is associated with insulin resistance.[480]

Central Nervous System

Genetic, epigenetic, biologic, social, and psychological factors are all important determinants of gender-related aspects of human development and behavior. Sex chromosomes directly affect sexually dimorphic neuronal differentiation.[481] The maternally inherited X chromosome is preferentially expressed in glutamatergic neurons of the cerebral cortex, and sex-specific imprinting of autosomal genes of the hypothalamus is common in females.[482] These findings suggest sex-specific parent-of-origin influences over neurologic and hypothalamic function of offspring.

Considerable evidence supports the concept that sex hormone exposure prenatally or during the period of transient activation of the hypothalamic-pituitary-gonadal axis of the neonate organizes gene expression and neural substrates in a sexually dimorphic manner. This predisposes to sex-typical behavior or function that is activated when the child is exposed to the sex-specific pubertal hormonal milieu.[483,484]

Several human brain structures are sexually dimorphic, some becoming so at puberty.[485-487] In lower species, sexually dimorphic nuclei develop only if the brain is exposed to androgen during a critical time in the newborn period, but not if the androgen exposure is too late or too little.

For example, in rats the preoptic nucleus of the hypothalamus is larger in males, and treatment of newborn females with testosterone or E2 permanently increases neuronal development and causes subsequent masculinized sexual behavior and anovulation.[13,488]

This and many other such effects appear to be mediated by the intraneuronal aromatization of testosterone to E2 in a manner that is regulated in a site-specific fashion by androgen and estrogen.[489,490] Thus, it has been postulated that low levels of E2 promote the development of the brain and greater amounts masculinize it. These higher levels of estradiol are generated in the male brain by neuronal aromatization of circulating testosterone. Estrogen receptor-α knockout in female mice reduces sexual behavior and parenting behavior while increasing aggressiveness.[491] On the other hand, some masculinizing effects of testosterone on behavior are androgen specific.[492]

Androgen has been shown to up-regulate brain 5α-reductase and aromatase only in the perinatal period.[493,494] There is also sexual dimorphism in cerebral progestin receptors, and progesterone attenuates the testosterone effect on the brain and reduces the number of estrogen receptors in the cerebral cortex.[495,496]

Sex hormones also have diverse effects on areas of the adult brain that variously exhibit apoptosis-mediated morphologic plasticity in synaptic patterns or are involved in reproductive behavior and function and nonreproductive functions such as memory and learning. Estrogen, for example, alters the pattern of synaptic connections in spatially specific and precise patterns that appear to fine-tune the sensitivity of certain regions of the brain to excitatory and inhibitory amino acids.[497,498] Hypothalamic changes in synaptic remodeling have been correlated with the preovulatory surge of GnRH. Androgen up-regulation of androgen receptor levels and the nuclear size of sexually dimorphic hypothalamic areas of the brain has been shown to occur in adult animals.[499,500] Progestins seem to protect against brain injury in adults, but studies indicate that they act oppositely in immature animals, possibly in relation to maturational changes in GABA action.[501,502]

On average, women tend to perform better than men on tasks that involve verbal skills, processing speed and accuracy, and fine-motor skills, whereas men tend to excel in visual-spatial memory; the sexes do not differ in vocabulary or math skills.[484,503-505] These differences are quantitatively modest, of the order of 0.4 to 1 SD, leading, therefore, to large overlaps in these skills between the sexes. The male advantage in visual-spatial skills is established by 4.5 years of age. Because both boys and girls who are congenitally sex hormone deficient are relatively poor in visual-spatial abilities, and sex hormone treatment at puberty does not ameliorate these deficits, this difference seems to be the result of estrogen-mediated patterning in both sexes. The extent to which these differences are innate or due to sociocultural factors is a subject of considerable debate.

A wide variety of sexual behaviors can be found in young children, but normally they have a different character than in adults.[506] Gender identity is established by 3 years of age.[486] Sexual orientation is established by 10 years of age; it has been postulated that this is dependent on adrenarche rather than true puberty.[507] Early pubertal amounts of androgen or estrogen have little effect on sexual behaviors but increase some aspects of aggressive behavior.[508,509] Only later in puberty is there activation of the sex drive, which has been programmed in early development.

A male level of androgen acting through the androgen receptor pre- or perinatally seems to be an important determinant of male gender role behavior and mildly disruptive to female gender identity.[486,510] Differences in the size of specific brain structures in men with gender identity disorders has raised the possibility that these disorders similarly have a biologic basis. In homosexuals, the human homologue of the rat preoptic nucleus is smaller than that of heterosexual men, as is the case in females,[511] and the anterior commissure is larger than that of heterosexual men or women.[512] In male-to-female transsexuals, a nucleus within the stria terminalis is small, female sized.[513] Male homosexuals have a pattern of nuclear activation in response to pheromone-like chemosignals like heterosexual women rather than like heterosexual men, and homosexual women have an intermediate type of activation.[514]

Androgen and estrogen metabolites in sweat and urine, which contain unusual steroids such as androst-4,14-diene-3-one,[515] have been found to exert sexually dimorphic activation of the anterior hypothalamus that is independent of their odor.[514] Therefore, they appear to act as pheromone-equivalent chemosignals. Human pheromones appear to modulate the timing of ovulation[516] and mood.[517] It is likely that a dedicated population of olfactory receptors that project to GnRH neurons act as pheromone receptors.[518]

Other Targets of Sex Hormone Action

Sex steroid hormones affect a wide variety of tissues in ways that are often unrecognized. An estrogen effect on stabilizing muscle integrity has been noted in muscular dystrophy.[519] Female and male sex steroids exert both complementary and antagonistic effects on immune function, but the extent to which differences in sex steroid levels underlie sex differences in immune function is unclear. Estrogen down-regulates blood levels of the inflammatory cytokine interleukin-6.[520]

The cardiovascular effects of estrogen include up-regulation of estrogen and progesterone receptors in vascular tissue and nongenomic effects on endothelial nitric oxide synthase.[521] Estrogens and progestins also exert hemostatic effects that are associated with increased resistance to the anticoagulant action of activated protein C.[522] Although estrogen improves the disturbed endothelial dysfunction of young hypogonadal women and is necessary for the cardioprotective effect of exercise,[523] oral conjugated estrogen use with and without medroxyprogesterone have been found to increase cardiovascular risk in postmenopausal women. However, such cardiovascular risks were not found in users of transdermal E2 doses $\leq$ 50 μg daily, whether or not combined with progestin.[524,525] Whether these results apply to premenopausal females or to other forms of estrogen is an area of

active research. Current evidence suggests that estrogen protects against the development of atherosclerosis but may be harmful after atherosclerosis is established.[526,527]

Oral contraceptives containing estrogen carry about a four-fold increased risk of venous thromboembolism in first-time users.[528,529] The risk falls with decreasing dose of estrogen and duration of use and may double in those containing the third-generation progestins drospirenone and desogestrel. Nevertheless, the risk is less than that of pregnancy. Progestin-only contraceptives are not associated with any increased risk of venous or arterial thrombosis.[528,530]

The differences between the sexes in lipid levels are not explained by physiologic differences in estrogen levels.[531] Although oral estrogens raise triglycerides, this is due to a first-pass hepatic effect. Differences in androgen (lowers high-density lipoprotein [HDL] cholesterol) and progesterone (lowers triglycerides and HDL-cholesterol) levels only explain part of the difference.

NORMAL SEXUAL MATURATION: HORMONAL AND PHYSICAL STAGES

The Fetus and Neonate

Steroid hormone levels from birth through puberty are shown in Table 15-1.[15,83,532-534] The fetus grows in a richer steroidal milieu than the pubertal female owing to the function of the fetoplacental unit. Concentrations of estrogens in fetal serum are extremely high. The umbilical cord plasma free testosterone level is modestly greater than that in normal adult females.[358] Dehydroepiandrosterone sulfate is at an adrenarchal level in the neonate. Pubertal hormone levels fall to a nadir within the first week of withdrawal from the intrauterine environment and then rise to maximal values in the early pubertal range at 3 to 4 months of age, as discussed in reference to Figures 15-5 and 15-8.

In premature infants, the "neonatal minipuberty" evolves according to a developmental program based on gestational age. Gonadotropin and sex steroid levels become normal for term infants when term gestational age is achieved. Adrenal contributions to steroid levels are higher in premature infants due to the persistence of the fetal adrenocortical zone and immaturity of the definitive adrenocortical zones.[535-537] Meanwhile, ovarian development and function lag, whereas compensatory gonadotropin levels are higher in premies.[18,538]

The newborn shows some signs of the pubertal degree of hormonal stimulation from the intrauterine environment. Hypertrophic labia minora and superficial cell transformation of the urogenital epithelium are consistently observed estrogen effects, and a palpable breast bud is present at term in one third of babies.[532] Menstrual bleeding and colostrum production sometimes occur as the newborn is withdrawn from the estrogenic environment. Sebaceous gland hypertrophy results from the androgenic state,[539] and the clitoral shaft sometimes is prominent, particularly in small premature babies.[441]

The activation of the hypothalamic-pituitary-gonadal axis of the newborn seems to be sufficient to sustain breast development through the first several months of life.[79] These phenomena then regress gradually over the first 2 years of life as the inhibitory tone of the neuroendocrine-gonadal axis undergoes juvenile maturation. Whether the transient activity of the neuroendocrine-gonadal axis in the newborn ("minipuberty of the newborn") has a programming influence on subsequent behaviors remains unclear.[486]

Childhood

As the neuroendocrine-gonadal axis becomes quiescent and the fetal zone of the adrenal cortex regresses, steroid hormone levels fall through infancy to reach a nadir in mid-childhood (see Table 15-1). Although gonadotropin levels are low, there is considerable evidence that secretion of bioactive gonadotropins is occurring. Though there is seldom obvious sexual development as a consequence of prepubertal gonadotropin production, there is a low level of bioactive gonadotropin production (see Figure 15-13) and ovarian follicular development[28,76,77] and occasional evidence of transient estrogen secretion.[540]

Adolescence

Hormonal

The earliest hormonal change during the preadolescent years is the adrenarchal rise in DHEAS (see Table 15-1). The earliest hormonal changes of true puberty occur gradually during late preadolescence—clinically prepubertal 10-year-olds develop greater average gonadotropin and sex hormone levels than do prepubertal 7-year-olds.[69]

In the average girl, serum gonadotropins achieve pubertal levels after 8 years of age. However, the chronologic age at which puberty begins varies considerably among children. Therefore, the pubertal rise in gonadotropins is best appreciated by relating gonadotropin levels to pubertal stage. Daytime serum LH rises 25-fold from prepuberty to late puberty according to bioassay, but this rise is underestimated by polyclonal radioimmunoassay (see Figure 15-13).[94] "Third-generation" immunometric assays, using monoclonal antibodies and a more purified standard than earlier radioimmunoassays, show a rise similar to that found by bioassay.[67,69]

The hallmark of early puberty is an increase in the sleep-related rise in LH (see Figure 15-12).[69,93] Daytime sampling underestimates the rise in gonadotropins in early puberty because it does not detect most of this sleep-related increase. In early puberty, third-generation assays show that LH rises during sleep to reach peaks in the lower adult range, generally above 1 U/L, and then typically falls during the day to 0.6 U/L or less.[69,83,541,542] A single daytime sample does not necessarily truly represent a child's pubertal status because it does not account for episodic and cyclic changes in gonadotropin secretion (see Figure 15-12).[91,543] The serum LH response to GnRH is slightly more indicative of the pubertal status than a morning basal sample (see Figure 15-9) (see Table 15-1).[85,541,542] An LH level 1-hour post-GnRH agonist of $\geq$ 3.2 U/L is 90% sensitive and $\geq$5.5 U/L is 95% specific for the onset of puberty in girls.[83]

The response of LH to GnRH or GnRH agonist administration increases more than that of FSH during puberty, with a resultant increase in the LH:FSH ratio.[67,544] GnRH agonists add a dimension to GnRH testing: they provide a sufficiently potent and prolonged stimulus to LH and FSH release to bring about an increase in ovarian E2 secretion in pubertal girls.[545] These responses likewise increase characteristically with sexual maturation (see Figure 15-6).[58]

Sex hormone levels rise further as the consequence of ovarian and adrenal maturation. Pubertal levels are intermediate between those of prepubertal and sexually mature individuals. Table 15-1 shows typical normal ranges for serum levels of the major steroid hormones. Once pubertal levels of estrogens and androgens are achieved, their effects ordinarily become obvious within 6 months.

Serum prolactin rises moderately in females at about 14 years of age.[546] This may be a response to estrogen secretion, because it does not occur in boys.

Clinical

The first physical sign of puberty is breast development (thelarche). In a minority of girls, pubic hair development (pubarche) occurs before thelarche. Thelarche represents a response to estrogen, and pubarche a response to androgen. When pubarche precedes thelarche, it is a reflection of adrenal androgen production (adrenarche) rather than a sign of puberty. The stages of breast and pubic hair development are shown in Figure 15-31.[547,548] Tanner stage 1 is prepubertal. The initial stage of breast development (stage 2, B2) is appreciated as a palpable subareolar bud before it can be seen as an elevation. Stage B3 is obvious enlargement and elevation of the whole breast. Stage B4, the phase of areolar mounding, is very transient and may not necessarily appear. Stage B5 is the stage of attainment of mature breast contour. Pubic hair first starts as presexual pubic hair development (PH2)—hairs which are shorter, lighter, and straighter than sexual pubic hairs but longer than vellus body hair. Hypertrichosis is sometimes mistaken for stage 2 pubic hair; these can be differentiated by comparing genital hair to hair on the forearm. Sexual pubic hair development (PH3)—curly terminal (long, dark) hair—usually commences on the labia majora before spreading to the pubis. Pubic hair then gradually progresses to the mature female escutcheon (inverted triangle pattern, stage 5). Axillary hair usually appears about a year later than pubic hair and passes through similar stages.

The age at which pubertal milestones are normally attained is not known with certainty. There has been considerable debate about the normalcy of pubertal changes between 6 and 8 years of age.[185,198,549] The debate stems from office practice observations that breast and pubic hair development were found more frequently than expected in black girls in this age range, whereas the age of menarche was unremarkable. The increasing prevalence of obesity interacting with ethnic factors now appears to be a major factor in this early onset of puberty.[243] The prevalence of pubertal milestones has been estimated for the general U.S. population by modeling data collected on children 8 years of age and older by the National Health and Nutrition Examination Survey III (NHANES III).[193,194,243] Although NHANES data has the advantage of nationally representative sampling, the quality of the data for early breast and pubic hair development is questionable, and modeling assumptions that permit extrapolation to younger ages may not be valid.[243,550,551] The ages at which the major pubertal milestones are currently attained in normal-weight U.S. girls according to this database are given in Table 15-3.[243] It appears that puberty begins before 8 years in $< 5\%$ of the normal general female population, although breasts may normally appear during the 7th year in blacks and Mexican Americans.

An important normal variable is the span of time between the onset of breast development (B2) and menarche. The concept that this is constant at 2.3 ± 1 year regardless of the age at which B2 occurs[547] has been challenged. In several normal populations of girls observed longitudinally, the onset of puberty was only weakly correlated with the age at menarche, and the earlier puberty began, the longer its duration and the greater the incremental growth so that height potential was preserved.[552-554] These studies suggest that the factors governing the onset

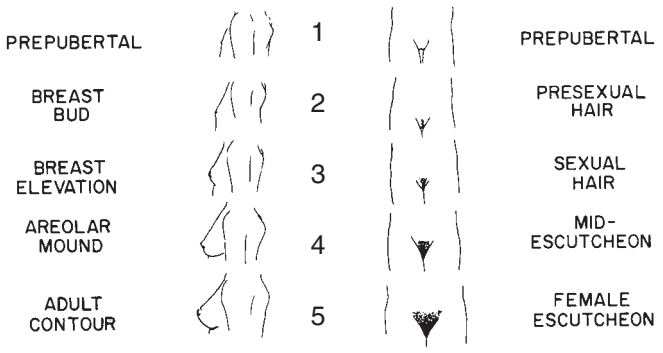


FIGURE 15-31 ■ The stages of breast and pubic hair development. (Redrawn from Ross, G. T., Vande Wiele, R. (1974). The ovary. In R. H. Williams (Ed.), *Textbook of endocrinology* (5th ed.) Philadelphia, WB Saunders.)

TABLE 15-3 **Pubertal Milestone Attainment in Normal BMI Girls in the General U.S. Population (Estimated Age in Years)***

Stage	5%	50%	95%
Breast stage 2	8.25	10.2	12.1
Pubic hair stage 3	9.25	11.6	13.9
Menarche	11.0	12.6	14.1

*Breasts appear before age 8 years in 12% to 19% and sexual pubic hair (stage 3) in ≤ 3% of normal-BMI non-Hispanic black and Mexican-American girls. Menarcheal milestones are attained at similar ages in these ethnic groups except for the 5th percentile being significantly earlier in blacks (10.5 years) than non-Hispanic whites (11.3 years).

Adapted from Rosenfield, R. L., Lipton, R. B., & Drum, M. L. (2009). Thelarche, pubarche, and menarche attainment in children with normal and elevated body mass index. Pediatrics, 123, *84–88.*

of puberty and its tempo differ. Longitudinal and hormonal data are compatible, with excess adiposity slowing the tempo of puberty, although less so than prepubertal adiposity advances puberty onset.[552,554-556] A subgroup of early maturers with a history of intrauterine growth retardation seem to have an unusually rapid tempo of puberty and lose height potential.[557]

The onset of puberty is more closely related to an individual's bone age than to chronologic age. This is particularly important in the case of subjects who are later than average in entering puberty, as discussed previously. The great majority of girls can be expected to begin puberty by the time their skeletal age reaches 12.5 years and to experience menarche by the time skeletal age reaches 14 years.

The pubertal growth spurt in girls occurs during early adolescence. The peak of linear growth velocity corresponds most closely with stage B2,[558] and the increase in serum alkaline phosphatase levels corresponds with B3.[559] Fat accumulation increases, and fat distribution changes as well.[552] As a consequence of these pubertal changes occurring out of phase with chronologic age, girls begin to differ considerably in size and habitus during the ninth year of life.

Adult Menstrual Cycle

The menstrual cycle of young adults averages 28 days in length (90% population limits, 22 to 42 days after the fifth gynecologic year).[560] The variation in cycle length is almost entirely due to differences in the duration of the follicular phase. The luteal phase, the time between ovulation and the onset of menses, invariably lasts 14 ± 1 (SD) days.[123]

The cyclic changes of LH, FSH, E2, and progesterone serum levels during the menstrual cycle are shown in Figure 15-14. Diurnal and episodic fluctuations are superimposed upon these diurnal changes. Because testosterone and androstenedione have both adrenal and ovarian origins, their levels fluctuate to some extent in cyclic, diurnal, and episodic patterns. For example, testosterone levels tend to be 20% greater in the morning than in the evening and tend to rise 50% to 100% in midcycle.[122,561] The normal range for most of the important ovarian sex hormone levels of women during the early follicular phase of the menstrual cycle is given in Table 15-1. Progesterone levels are below 100 ng/dL until the periovulatory phase of the cycle and then peak to over 500 ng/dL in the midluteal phase. Hormonal production rates for the midfollicular phase in women are given in Table 15-2. Serum prolactin increases transiently in midcycle with maximum ovarian E2 secretion.[562] Prolactin levels also transiently rise in response to mammary stimulation and psychological factors.[563]

Normal Variations in Pubertal Development

Although the onset of breast development (stage B2) characteristically precedes the appearance of sexual pubic hair (PH3) and the onset of menses substantially (see Table 15-3), there is normally considerable variation in the sequence of these events. Pubic hair may appear before breasts begin to develop, a situation arising from lack of direct linkage between adrenarche and gonadarche. Menarche may occur within months after the appearance of breasts; however, this is so unusual that its occurrence demands exclusion of an abnormal hyperestrogenic state.

A common normal variant is the unilateral onset of breast development. Unilateral breast development may exist up to 2 years before the other breast becomes palpable. This phenomenon seems related to an asymmetry that normally persists into adulthood. Excisional biopsy of a normal unilateral breast papilla in search of a nonexistent tumor should be avoided, because such a procedure excises the entire breast anlage.

Two extreme variations of normal are the most common causes of premature sexual development.[564] These are the isolated appearance of breast development *(premature thelarche)* and the isolated appearance of sexual hair *(premature pubarche)*.

Premature Thelarche

Breast development before 8 years usually seems to be due to idiopathic subtle overfunction of the pituitary-ovarian axis, occurring in girls whose FSH levels tend to be sustained about the upper end of the prepubertal normal range.[540] Average serum levels of FSH at baseline and in response to GnRH are significantly increased, whereas those of LH are not. E2 levels are generally below the level of detection in most standard assays, but they are significantly elevated according to ultrasensitive assay,[565] and intermittent low-grade estrogenization of the urogenital mucosa is sometimes found (see Figure 15-11). Ovarian ultrasound examination shows an increased prevalence of antral follicles ("microcysts"). Nevertheless, a growth spurt does not occur, the bone age does not advance abnormally, and menses does not appear until the usual age.

In infants the syndrome seems to be due to a lag in inhibition of the transient activation of the hypothalamic-pituitary-gonadal axis of the newborn and is usually unsustained. In older children the breast development is more likely to persist. A subgroup with "exaggerated thelarche" has an increased growth rate with relatively proportionate bone age advancement. The unsustained or intermittent neuroendocrine activation seems to lie on a spectrum between ordinary premature thelarche and true sexual precocity (Figure 15-32).[566] However, the McCune-Albright syndrome mutation is found in the peripheral blood of about 25% of such patients.[567] Premature thelarche may be the first sign of feminizing disorders (see "Precocious Puberty"). Therefore, follow-up of these patients is indicated.

Premature Pubarche

Pubarche before 8 years of age usually is due to "premature adrenarche," which is indicated by adrenarchal androgen levels—that is, DHEAS 40 to 130 μg/dL with other androgen levels in the marginally early pubertal range (see Table 15-1).[15,568] However, it may occur at androgen levels that are less ("idiopathic premature pubarche") or more ("exaggerated adrenarche"). The androgen excess is ordinarily so subtle that the only other

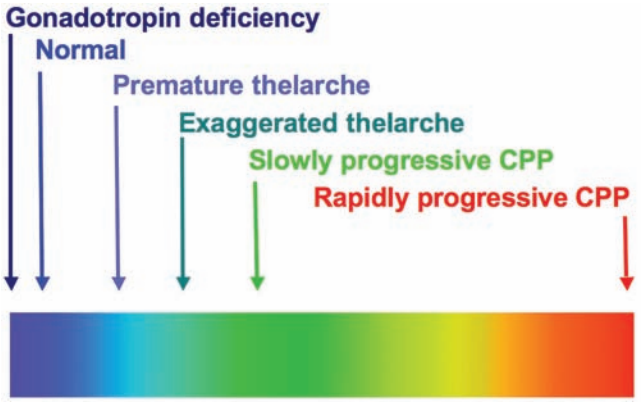


FIGURE 15-32 ■ The spectrum of gonadotropin secretion in girls. Normal girls are conceptualized as having a small amount of pituitary-ovarian axis activation, which is more than that of congenitally hypogonadotropic girls. Premature thelarche, exaggerated thelarche, slowly progressive precocity, and rapidly progressive precocity fall along a spectrum of increasing activation of the axis—with deterioration of height potential occurring only in those near the most activated end. *This image can be viewed in full color online at ExpertConsult.* (Modified from Kreiter, M. L., Cara, J. F., & Rosenfield, R. L. (1993). Modifying the outcome of complete precocious puberty: to treat or not to treat. In G. D. Grave, & G. B. Cutler (Eds.), *Sexual precocity: etiology, diagnosis, and management* (pp. 109–120). New York: Raven Press.)

sign of increased androgen production may be microcomedonal acne and apocrine body odor. There is no obvious growth spurt, the bone age typically does not advance abnormally, and there are no other signs of sexual maturation.

Exaggerated adrenarche is an extreme type of premature adrenarche. These girls have clinical features that suggest subtle androgen excess (e.g., significant bone age advancement, but not clitoromegaly) or insulin resistance (e.g., central adiposity or acanthosis nigricans). Such children generally have a slightly advanced onset of true puberty, but height potential is not compromised. Adrenal steroid levels are in the mid- or late pubertal range (e.g., DHEAS > 130 to 185 μg/dL, androstenedione > 75 to 99 ng/dL, or post-ACTH 17-hydroxypregnenolone > 750 ng/dL). Testosterone does not exceed the lower end of the adult female range.

It is unclear whether premature adrenarche is simply an extreme normal variant due to advanced onset of normal zona reticularis development or an early manifestation of the steroidogenic dysregulation of polycystic ovary syndrome. Carriers for congenital adrenal hyperplasia may be overrepresented in this group.[569,570] Premature adrenarche appears to carry about a 10% to 20% risk of developing polycystic ovary syndrome; it is unclear whether those with exaggerated adrenarche are primarily at risk. The differential diagnosis includes virilizing disorders, of which nonclassic congenital adrenal hyperplasia is the most common. Girls with premature adrenarche should be followed through puberty.

Constitutional Delay of Pubertal Development

By statistical definition, delayed puberty occurs in 3% of girls. Most of these girls are otherwise normal, in which case this is termed *constitutional delay of growth and pubertal development*. It is familial[195,196]; 80% in a large series had a parent with objectively delayed puberty.[571] Although its inheritance likely is complex, some predisposing genetic factors seem to have a dominant effect. Delayed puberty seems overrepresented in families with idiopathic hypogonadotropic hypogonadism or hypothalamic amenorrhea cases, and rare variants in genes underlying these conditions appear to contribute to the etiology.[572,573] Girls with constitutional delay are generally more slight in habitus and have lower bone mineral density upon entering puberty than earlier maturing girls.[574] Girls do not usually become concerned about delayed puberty until they enter high school at 14 years of age and realize that not only has pubertal development not begun but most of their friends are menstruating. When puberty does ensue in such girls, it is perfectly normal in tempo. Endocrinologic status is normal for the stage of puberty. The differential diagnosis includes chronic endocrine, metabolic, and systemic disease of almost any kind, as well as gonadotropin deficiency, which it closely resembles and from which it is distinguished with difficulty (see "Gonadotropin Deficiency" and the discussion of functional hypothalamic amenorrhea presented in "Hypothalamic Anovulation").

Physiologic Adolescent Anovulation

Because of immaturity of the hypothalamic-pituitary-ovarian axis, about half of menstrual cycles during the first 2 years after menarche are anovulatory or have attenuated ovulation that results in luteal insufficiency.[575-577] This accounts for the greater menstrual irregularity and longer average intermenstrual length in the early postmenarcheal years than in adults (Figure 15-33).[560,578] Although about half of these seemingly "anovulatory" cycles are irregular, half are of normal length by adult standards, so menstrual regularity is greater than ovulatory frequency would

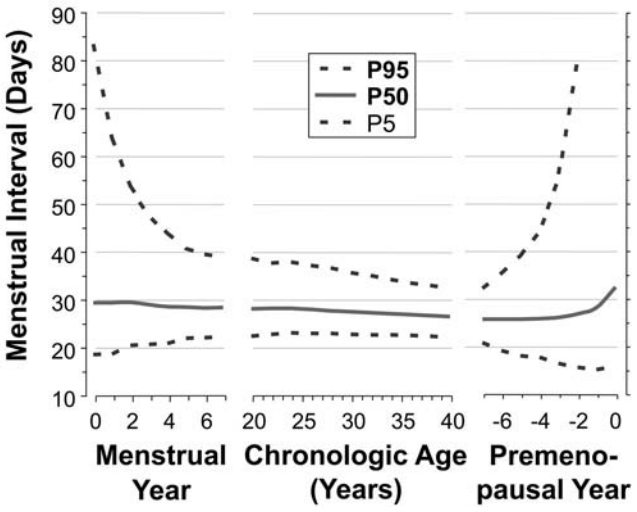


FIGURE 15-33 ■ Normal range for interval between menstrual cycles. Note that cycle intervals averaging more than 90 days or fewer than 22 days are abnormal at any age. P5 and P95 = 5th and 95th percentiles, respectively. (Modified from Treloar, A., Boynton, R., Benn, B., & Brown, B. (1967). Variation of human menstrual cycle through reproductive life. *Int J Fertil, 12,* 77–84.)

suggest. Although there is considerable variation in the time it takes for menstrual cycles to mature, menstrual regularity approximates adult standards in most girls within a year of menarche: three quarters have a cycle length between 21 and 45 days, and 5% more fall within these bounds by each of the next 3 years.[575,576] By the fifth gynecologic year onward, 90% of menstrual cycles last 21 to 40 days, and about 75% of cycles are ovulatory, though ovulatory adequacy continues to mature for several years thereafter.

Menstrual abnormalities in adolescence can be defined similarly to those in adults.[578,579] *Primary amenorrhea* is failure to begin menses at a normal age (by 15 years of age or within 3 years of thelarche). *Secondary amenorrhea* is the absence of menstrual periods for 90 days or more after initially menstruating. *Oligomenorrhea* is defined during the first postmenarcheal year as having fewer than four periods a year; during the second year as fewer than six periods a year; and from three until five years postmenarche it is defined as fewer than eight periods per year (i.e., missing more than four periods per year, which amounts to > 45 days between menstrual periods). Thereafter it is defined according to the adult criterion of having less than 9 periods a year. Anovulatory bleeding may also be excessive, as discussed in the later section on *Dysfunctional uterine bleeding*.

Menstrual abnormalities in adolescents are more appropriately considered "symptomatic" rather than being considered "physiologic" adolescent anovulation. By 1 year postmenarche, failure to establish and sustain a normal adult menstrual pattern carries approximately a 50% risk of persistent oligo-ovulation, and failure to do so by 2 years after menarche carries approximately a two-thirds risk (Figure 15-34).[574] Thus, persistence of menstrual irregularity for 1 year or more is a strong indication for investigation (see "Abnormal Puberty").

Serum LH, testosterone, and androstenedione levels are significantly higher in adolescents with anovulatory than those with ovulatory cycles.[580,581] It is unclear whether this is the cause or the result of the anovulation; however, if hyperandrogenemia is found, it seldom regresses.[116] A polycystic ovary is common in adolescents and is usually a variant of normal, unless associated with menstrual abnormality or hyperandrogenism.[73,116-118,121,534]

Other Normal Adolescent Variations

There is considerable variation in the amount of *acne*, *hirsutism*, and *adiposity* among normal adolescent girls, most of which is related to familial factors. Three quarters of adolescent girls experience some degree of acne, with one quarter experiencing mild inflammatory acne.[582,583] The initiation of acne is more closely related to blood levels of DHEAS than of other androgens.

Profound *psychological* changes occur during adolescence. Sexually immature girls tend to be socially immature, and the onset of puberty is associated with increased

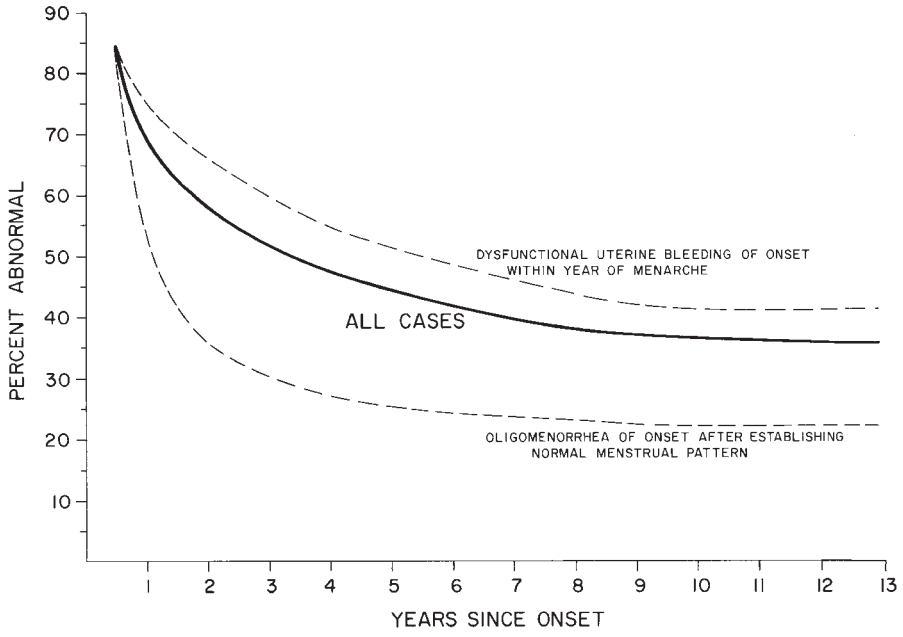


FIGURE 15-34 ■ Probability that an adolescent with a menstrual abnormality severe enough to require gynecologic consultation will have continued menstrual abnormality. The lines show the cumulative rates at which subjects with menstrual abnormalities converted to normal patterns. The heavy curve shows the overall incidence of continued menstrual abnormality in adolescents when considering both types of anovulatory manifestation (dysfunctional uterine bleeding and oligomenorrhea) regardless of time of onset. The dotted lines show those subgroups that differ most from the overall pattern. Other subgroups fall within approximately 5% of the mean for the entire group. Note that dysfunctional uterine bleeding of onset within 1 year of menarche carries the worst prognosis for continuing menstrual abnormality (upper dotted line). Note also that oligomenorrhea of relatively short duration occurring after a normal menstrual pattern has been established carries the best overall prognosis. Nevertheless, it can be seen that if the menstrual abnormality persists for 1 year there is about a 50% probability, and if for 2 years there is nearly a 67% probability, that the patient will not spontaneously evolve to normal cycles. Similarly, if the problem persists for 5 years, there is an 80% likelihood of persistence of the abnormality. (Redrawn from Southam, A. L., & Richart, E. M. (1966). The prognosis for adolescents with menstrual abnormalities. *Am J Obstet Gynecol*, *94*, 637.)

independence and profound changes in outlook on life and intellectual capacities. The extent to which these developments occur in reaction to the physical changes of puberty and the extent to which they are direct effects of sex hormones are unknown. Masculine tomboyish traits usually have no clear hormonal basis, although there is some evidence that they may have prenatal hormonal determinants. Social interactions have effects on these aspects of development. They affect even the synchrony of the menstrual cycle.[584]

Despite the popular notion that adolescence is inherently a period of turmoil, the majority of teenagers do not develop significant social, emotional, or behavioral difficulties.[585] Occasional experimentation and risk-taking are normal, as are withdrawal from and conflict with parents. Adolescent behavior must be understood in the context of individual susceptibility, family upbringing and interactions, peer group interactions, changes in brain maturation, and adolescents' reaction to their perception of the bodily changes and to the sexual urges that are the direct consequences of puberty. Simply because a problem is displayed during adolescence does not mean that it is a direct consequence of puberty.

Many behavioral problems that emerge during adolescence have earlier roots. Although the prevalence of depression increases during puberty, many children who develop depression during adolescence have had preexisting symptoms of psychological distress. Likewise, most delinquent teenagers have had antecedent problems at home and school.

Early-maturing girls in Western cultures are more popular, but they have more emotional problems, lower self-image, and higher rates of depression, anxiety, and disordered eating than their peers. Early maturation appears particularly to be a risk factor for problem behavior among girls who have had a history of difficulties prior to adolescence, when they have more opposite-sex friendships and relationships, and when they attend coeducational schools.

Short-term administration of testosterone or estrogen has minimal effects on behavior or mood in adolescents.[508,586] Thus, variation in hormone levels accounts for only a small fraction of adolescents' affective issues, and social influences account for considerably more. Although there is little evidence that psychological difficulties stem directly from hormonal changes during normal puberty, it is likely that the bodily changes of adolescence play a role in the development of a negative body image when they occur out of synchrony with sociocultural norms.

Problems with initiating and maintaining sleep are common in adolescents and contribute a small amount to poor school performance.[587] Although insufficient sleep might be due to environmental factors (e.g., social and academic pressures), intrinsic factors clearly play an important role. A 50% decline in the intensity of deep (slow wave, delta) sleep occurs during adolescence, and half of this change occurs between 12 and 14 years of age.[588] Evidence indicates that this change is related to age and sex, beginning earlier in girls, but not to pubertal stage. It has been proposed that this shift is a manifestation of the widespread synaptic pruning that is related to the emergence of adult cognitive capacity.

The causal direction of the link between pubertal development and the quality of family relationships has come into question. Several studies have indicated that family dynamics may affect the timing and course of puberty, with earlier and faster maturation observed among adolescents raised in homes characterized by more conflict and among girls from homes in which the biologic father is not present.[585]

ABNORMAL PUBERTY

Abnormal Development

Disorders of Sex Development

Patients with disorders of sexual development (DSD) (formerly termed *intersex*)—those whose genitalia are ambiguous or inappropriate for their gonadal sex as a result of endocrinopathy—may come to a physician's attention for the first time at puberty. These syndromes have been categorized as 46, XX DSD, which encompasses cases formerly termed *female pseudohermaphroditism* and including 46, XX testicular DSD (formerly termed *XX sex reversal*); 46, XY DSD, which encompasses cases formerly termed *male pseudohermaphroditism* and including 46, XY complete gonadal dysgenesis (XY sex reversal, Swyer syndrome); and sex chromosome DSD, which includes Turner syndrome, Klinefelter syndrome, mixed gonadal dysgenesis, and chimeric ovotesticular DSD.[486] In the absence of chromosomal mosaicism, ovotesticular DSD, formerly termed *true hermaphroditism*, is categorized as either 46, XX DSD or 46, XY DSD. Patients with any of these disorders may undergo inappropriate puberty. They may present with clitoromegaly and be found upon examination to have a degree of genital ambiguity that was previously overlooked. Virilization beginning at puberty is sometimes the presenting complaint. Ovotesticular DSD and 46, XX DSD due to congenital adrenal hyperplasia are compatible with fertility.[366] Androgen insensitivity syndrome in a genetic male may present as primary amenorrhea in an otherwise phenotypically normal adolescent girl. The disorders of sexual differentiation are reviewed in detail in Chapter 5.

Congenital virilization of the female developmentally programs the emergence of polycystic ovary syndrome at puberty. These observations are consistent with studies of fetal androgenization of the female in several species, including primates.[15,16,589] There is a persistent increase in LH pulse frequency and impairment of the negative feedback effect of progesterone on LH release that appear to be related to suppression of hypothalamic PR in response to E2. Experimental animals exposed to androgen excess early in gestation develop classic polycystic ovary syndrome features: these animals have elevated LH levels, ovarian and adrenal hyperandrogenism, oligomenorrhea, and polyfollicular ovaries. They also have abdominal obesity, insulin resistance, impaired glucose tolerance, and dyslipidemia, which likewise appear to result from developmental programming.

Genetic males with androgen insensitivity have low LH levels and poor LH responsiveness to GnRH in the neonatal period.[54] Gonadotropin levels are normal to high at puberty, yet these individuals lack the capacity for positive feedback, which therefore appears to be mediated by estrogen metabolites of testosterone.[590]

Other Dysgenetic Disorders

Failure of the onset of menses can result from structural abnormalities of the genital tract that do not have an endocrinologic basis. The hymen may be imperforate, which results in hydrocolpos if the vagina is intact. The vagina may be aplastic, which will result in hydrometrocolpos if the uterus is intact. The uterus may be congenitally aplastic. Uterine synechiae develop as the consequence of endometritis, which may result from infection or irradiation (Asherman syndrome). Congenital absence of the vagina may be associated with varying degrees of uterine aplasia; this is the Rokitansky-Kustner-Hauser syndrome.[591] This syndrome seems to occur as a single gene defect or as an acquired teratogenic event involving mesodermal development and the mesonephric kidney, the latter resulting in abnormalities of the genital tract and sometimes the urinary tract. A subtype due to *Wnt4* gene defects is associated with hyperandrogenism.[592]

Precocious Puberty

Causes

When breast or sexual pubic hair development begins before the age of 8 years or when menses begins before the age of 9.5, puberty is traditionally considered precocious, or premature. It should be kept in mind that breast development during the 7th year is within normal limits in ethnic minority girls. In addition, presexual pubic hair (stage 2) may be normal in 6- and 7-year-old ethnic minority girls.

Puberty can occur prematurely as an extreme variation of normal, because of a disturbance in the hypothalamic-pituitary-gonadal (HPG) axis normally involved in sexual maturation, or because of a disturbance outside the HPG axis. Depending on which part of this hormonal axis is involved, different forms of precocious puberty are distinguished. A classification of the causes of premature puberty, together with typical findings, is given in Table 15-4.

It is important to distinguish between *true* precocious puberty and pseudoprecocious puberty. True precocious puberty is gonadotropin dependent; thus, *central* is another term applied to this type of precocity. Maturation is *complete*: both breasts and pubic hair develop as the result of CNS activating pituitary secretion of the respective gonadotropins FSH and LH (although breast development may be the sole manifestation of early complete precocity for as long as 6 to 12 months). Patients with true precocious puberty have "isosexual" precocity because the secondary sexual characteristics are appropriate for the sex of the child. Pseudoprecocious puberty is gonadotropin independent; it is not mediated by pubertal pituitary gonadotropin secretion and is sometimes termed *peripheral*. Maturation is *incomplete*, with only one type of secondary sexual characteristic developing early. Peripheral precocity has diverse causes. In some patients with pseudoprecocity, pubertal development is isosexual; in others it is *contrasexual*, meaning that characteristics of the opposite sex are manifested.

Complete Precocious Puberty. True isosexual precocity results from pubertal function of the hypothalamic-pituitary-gonadal axis. About 95% of true precocity in girls is idiopathic. *Idiopathic* true sexual precocity appears to be due to premature triggering of the normal pubertal mechanism. Pubertal development usually is qualitatively and quantitatively normal except for its early occurrence. The predominance of the idiopathic cases in females and its benign nature are compatible with the likelihood that this disorder is an extreme exaggeration of the normal tendency of girls to have relatively high gonadotropin levels. Most cases are sporadic, a few familial. The majority of these patients seem to go on to have normal menstrual cycles and fertility.[593] Indeed, pregnancy has been documented to occur as early as 4 years of age.

Rapidly progressive puberty with a growth spurt ensues when activation of the pituitary-ovarian axis is sustained. However, precocious puberty is not necessarily sufficiently intense or sustained to cause inexorable progression or bring about deterioration of height potential.[594] Precocity in the 6- to 8-year age range usually is not rapidly progressive and most commonly seems to be due to excessive adiposity.[243]

Any type of intracranial disturbance can cause true isosexual precocity. These *neurogenic* disturbances are presumed to cause true sexual precocity by increasing the prevalence of excitatory inputs or by interfering with CNS inhibition of hypothalamic GnRH secretion.[595] These include congenital brain dysfunction, such as cerebral palsy or hydrocephalus, or acquired disorders, such as irradiation,[596] trauma, chronic inflammatory disorders, or masses in the region of the hypothalamus. The activation of GnRH release by hypothalamic injury may be mediated by TGF-α elaborated by reactive astrocytes. An empty sella is occasionally found.[597] The precocity of neurofibromatosis type 1 (von Recklinghausen disease) usually results from an optic glioma, which is often of low-grade malignancy, or from a hamartoma,[598] though occasionally with neither.[599] Hamartoma of the hypothalamus may cause central sexual precocity; this effect is most likely caused by an anatomic effect on hypothalamic structures rather than by acting as an "accessory hypothalamus" that releases pulses of GnRH into the pituitary portal circulation.[600,601] Figure 15-35 shows a hypothalamic hamartoma.

A small proportion of pineal tumors cause true sexual precocity.[602,603] The incidence of sexual precocity is about 3.5 times as great in nonparenchymatous neoplasms (such as gliomas and teratomas) as in parenchymatous pineal tumors. This suggests that these tumors cause sexual precocity via the absence of a normal pineal inhibitory factor rather than by destructive effects on inhibitory tracts. Although pineal masses may cause paralysis of upward gaze by pressure on the corpora quadrigemina, this sign is present in only a minority of cases.

TABLE 15-4 **Typical Findings in Female Sexual Precocity**

Locus	Type	HA*	BA*	Estrogens†	Androgens†	LH/FSH†	Pathology	Characteristics
Complete Precocity (Central, Gonadotropin-Dependent)								
Hypothalamic	Isosexual	+	++	+	+	+	Idiopathic Neurogenic Advanced somatic maturation	95% of female cases
Incomplete Precocity (Peripheral, Gonadotropin-Independent)								
Normal variant	Isosexual	−	−	±	−	±	None	Thelarche
	Isosexual	−	−	−	±	−	None	Pubarche/adrenarche
Neuroendocrine	Contrasexual	+	++	−	+	+/+++	LH/hCG excess	Familial or tumor
	Isosexual	Low	Low	−	−	+	Hypothyroid	Growth arrest
Ovary	Isosexual	+	++	+/+++	−	±	McCune-Albright	Bone lesions ± nevi ± ovarian cysts
	Isosexual/contrasexual	+	++	+/+++	+/+++	−	Tumor	
Adrenal	Contrasexual	+	++	±	+++	−	Congenital adrenal hyperplasia	Dexamethasone suppressible
	Contrasexual/isosexual	+	++	+/+++	+/+++	−	Tumor	
Ectopic	Isosexual	+	++	−/+++	−	−	Aromatase excess	
Exogenous	Contrasexual/isosexual	±	±	−	−	−	Sex steroid exposure	
End organ	Isosexual/contrasexual	−	−	−	−	−	Vaginal foreign body, abuse, tumor	

*HA (height for age) and BA (bone age); − normal; + advanced; ++ markedly advanced.
†Hormone levels; − normal prepubertal; + pubertal level; ++ adult level; +++ abnormally high.
FSH, follicle-stimulating hormone; hCG, human chorionic gonadotropin; LH, luteinizing hormone.

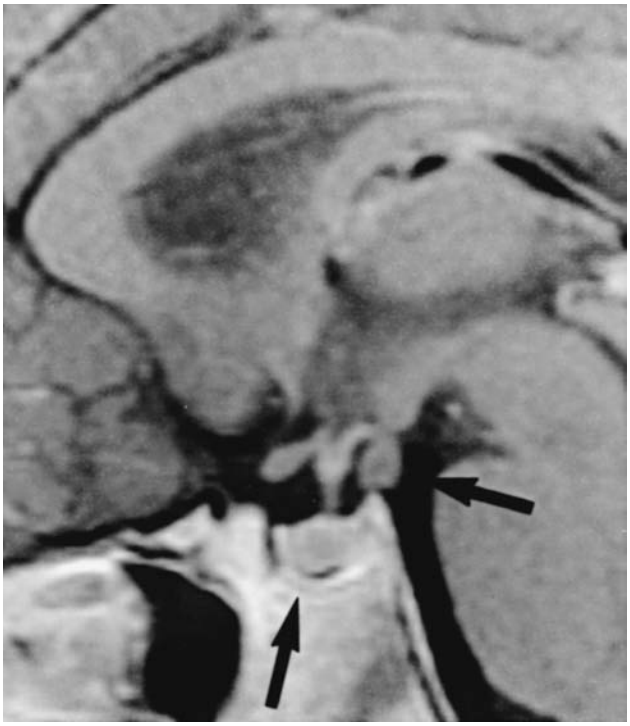


FIGURE 15-35 ■ Magnetic resonance image showing a hypothalamic hamartoma (right-hand arrow) as the cause of true sexual precocity in a 2.5-year-old girl. The hamartoma is hanging from the floor of the hypothalamus just posterior to the pituitary infundibulum. The sella turcica (bottom arrow) contains a normal pituitary gland with pituitary stalk hanging from the infundibulum.

Pineal or hypothalamic hCG-secreting germ cell tumors occasionally cause true sexual precocity.[604,605] Because hCG is a LH receptor agonist, possible explanations for this unusual situation are disinhibition of hypothalamic GnRH release due to a mass effect of the neoplasm, the weak FSH effect of massive elevation of hCG, or the capacity of some dysgerminomas to secrete E2 as well as hCG.[606]

Advancement of somatic maturation due to peripheral endocrine disorders that advance the bone age to a pubertal level sometimes causes true sexual precocity. Thus, true puberty may begin after correction of virilizing or feminizing disorders that have advanced the bone age to 10 to 12 years.[232,233]

The hypergonadotropism of premature ovarian insufficiency has been reported to cause sexual precocity or rapid progression of puberty prior to premature menopause.[607,608] Although polycystic ovary syndrome has occasionally been reported to follow true sexual precocity,[609] there is currently no evidence of a significant association.[593]

Rare causes of true sex precocity include mutations that enhance kisspeptin signaling,[610,611] inactivating mutation in a GnRH release-inhibiting signaling molecule MKRN3,[223] maternal uniparental disomy of chromosome 14, which causes the combination of intrauterine growth retardation and sex precocity,[612] and hyperglycinemia.[613] A genetic variant of a hepatic mixed function oxidase has been associated with female sexual precocity.[379]

Incomplete Precocity. The most common causes of incomplete sexual precocity in girls are the extreme variants of normal mentioned previously, premature thelarche and premature pubarche. These are incomplete forms of sexual precocity in which *either* breast development (thelarche) *or* sexual hair development (pubarche) is of a degree appropriate for an early stage of puberty and isosexual. Isolated prepubertal menses is a rare disorder that has been attributed to transient ovarian activity.[614]

LH- or hCG-producing tumors have not been reported to virilize girls, perhaps because of their limited capacity for thecal androgen production over short periods of time. However, familial isolated elevation of LH has been reported to cause mild virilization of siblings[615]: one was a girl who developed premature pubarche and clitoral hypertrophy at 4 years of age, with slight to moderate advances in height and bone age in association with an adrenarchal level of DHEAS and a moderately elevated testosterone level (91 ng/dL).

The *Van Wyk-Grumbach syndrome* is one of the most puzzling pediatric complexes.[616] This is an unusual syndrome of sexual precocity associated with juvenile hypothyroidism. A case is illustrated in Figure 15-36. This syndrome is often characterized by galactorrhea, which is often not spontaneous; a few drops of milky fluid may become apparent only upon "milking" the subareolar ductal tissue. Multicystic ovaries are often demonstrable by ultrasonography.[617] Modern assays show that levels of LH at baseline and post-GnRH are suppressed and those of FSH are early pubertal.[618] There is little, if any, sexual hair development. There is another clinically unique feature about the sexual precocity of hypothyroidism: it is the only form of sexual precocity in which growth is arrested rather than stimulated and is an exception to the general rule, indeed followed by most chronically hypothyroid children, that a delayed growth pattern is associated with delayed puberty.

Van Wyk and Grumbach postulated that this syndrome resulted from hormonal "overlap" in the negative feedback regulation of pituitary hormone secretion, with overproduction of gonadotropins as well as TSH in response to the thyroid deficiency. The specific nature of hormonal overlap has been considerably clarified, but the pathogenesis remains unclear. The increases in serum TSH and prolactin that characterize the syndrome could well be accounted for by common neurohumoral control systems, TRH stimulating and dopamine inhibiting both hormones. It has been suggested instead that the ovarian FSH receptor is activated by the weak intrinsic FSH activity of extreme TSH elevation,[619] analogous to the rare ovarian hyperstimulation syndrome in which pregnancy levels of hCG activate the FSH receptor.[620]

Prolactin excess has been postulated to underlie the FSH-predominant gonadotropin pattern by slowing GnRH pulsations, and it is this FSH stimulation of the TSH-sensitized ovary that seems to be the proximate cause of the sexual precocity.[621] Hyperprolactinemia alone does not correspond with pubertal development in normal

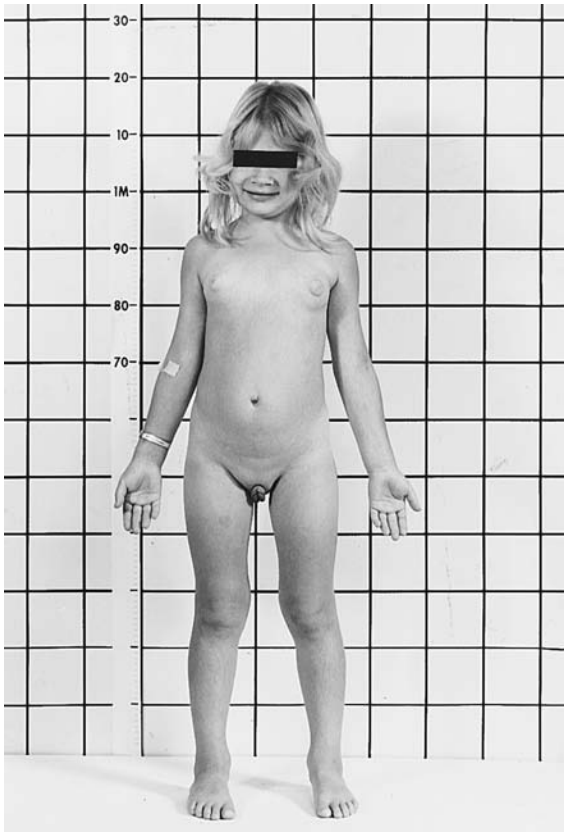


FIGURE 15-36 ■ Sexual precocity caused by hypothyroidism in a 9.1-year-old with breast development since age 7 years and menarche at 9 years. Growth failure had occurred, and her height age was 6 years. In addition to breast enlargement and galactorrhea, the labia minora were noted to be enlarged and pigmented. There was no sexual hair or clitoromegaly. Rectal examination revealed an enlarged and palpable cervix without adnexal masses. There were typical physical findings of hypothyroidism. Bone age was 6.2 years. Thyroxine was less than 1 μg/dL. Thyrotropin-stimulating hormone was 438 μU/mL. Prolactin was 66 ng/mL. Serum estrogens were 72 to 182 pg/mL. Vaginal smear showed 45% superficial cells and 55% large intermediate cells. Immunoreactive luteinizing hormone (LH) and follicle-stimulating hormone were 300 and 174 ng LER-907/mL, respectively (see Figure 15-5 for reference ranges). However, bioactive LH was undetectable. Immunoreactive gonadotropins failed to suppress upon estrogen administration. Their response to a 100-μg gonadotropin-stimulating hormone bolus was minimal, and they seemed responsive to thyrotropin-releasing hormone. All of these hormonal findings were not obviously different from those of hypothyroid girls without sexual precocity except for the higher estrogens. She had withdrawal bleeding and evidence of regression of breast development within the first 3 months of thyroid hormone replacement treatment. After 6 months of treatment, normal puberty began. Menarche occurred at 12.5 years of age.

or hypothyroid children. However, hyperprolactinemia may itself also sensitize the ovaries to gonadotropins. Induced hyperprolactinemia causes sexual precocity in female rats.[312] Ovarian estrogen and progesterone responsiveness to hCG is increased by prolactin, possibly by its induction of ovarian LH receptors. Conversely, suppression of hyperprolactinemia in experimental hypothyroidism blocks the ovarian cyst formation characteristic of hypothyroidism.[622]

McCune-Albright syndrome is another intriguing disorder causing incomplete isosexual feminization.[623,624] This is a syndrome of precocious puberty, cafe-au-lait pigmentation occurring in nevi that have an irregular ("coast of Maine") border, and polyostotic fibrous dysplasia. The disorder is caused by a somatic activating mutation of the G_s-alpha subunit protein that couples transmembrane receptors to adenylate cyclase. The syndrome has been recognized predominantly in females and occurs in incomplete as well as expanded forms. Precocious puberty or mono-ostotic bone lesions may occur in the absence of cutaneous pigmentation; not all patients have sexual precocity. The sexual precocity is of the gonadotropin-independent type. Luteinized follicular cysts within the ovaries function autonomously. Pituitary adenomas capable of secreting excess LH, FSH, GH, or prolactin have been reported. Patients may have Cushing syndrome and hyperthyroidism due to autonomous multinodular hyperplasia. These girls may be at increased risk of breast carcinoma.[625] Nonendocrine abnormalities include cardiopulmonary disease, hypertension, and hepatobiliary disease. Molecular studies have shown an R201H mutation in over 90% of cases where an affected tissue could be studied, but in only 50% of blood samples.[626] Because of the variation in the number and degree of tissue involvement in individual patients, due in large part to the extent of mosaicism present, precocious puberty may be the only feature present in an individual who is mosaic for the activating mutation of G_s-alpha. Thus, these mutations have been found in blood samples from 25% to 33% of subjects with isolated gonadotropin-independent precocity or exaggerated thelarche.[567,626]

Congenital adrenal hyperplasia (CAH) is a well-known cause of premature pubarche. Either nonclassic CAH, a form of the disorder that is so mild that there is no genital defect in girls, or poor control of classic CAH may be responsible. Each form on occasion has been reported to mimic true sex precocity.[627,628]

Tumor may cause isosexual or contrasexual development. The most common tumor is the feminizing benign ovarian follicular cyst.[629,630] Most are isolated and large (> 1 cm diameter). The cells lining these cysts are often luteinized. Estrogen levels may be markedly elevated. Testosterone levels tend to be in the adult female range (about 40 ng/dL). Many function intermittently. They may be gonadotropin dependent and respond to GnRH agonist or progestin therapy. A case is illustrated in Figure 15-37. The second most common hormonally active ovarian neoplasm in girls is the juvenile granulosa cell tumor.[631,632] These have variable degrees of ovarian sex cord–stromal elements and are usually localized and benign in spite of having a malignant histologic appearance. They are more commonly feminizing than masculinizing in young children. They may produce hCG, AMH, and inhibin. Elevation of hCG is found in many ovarian dysgerminomas (a primitive germ cell tumor) and hypercalcemia in some, though less frequently than in small cell carcinoma.[631,633] A granulosa-theca cell tumor is occasionally associated with mesodermal dysplasia syndromes. It has been reported in the adrenal gland, presumably arising in an ovarian rest.[634] FOXL2 mutations are typical of adult-type

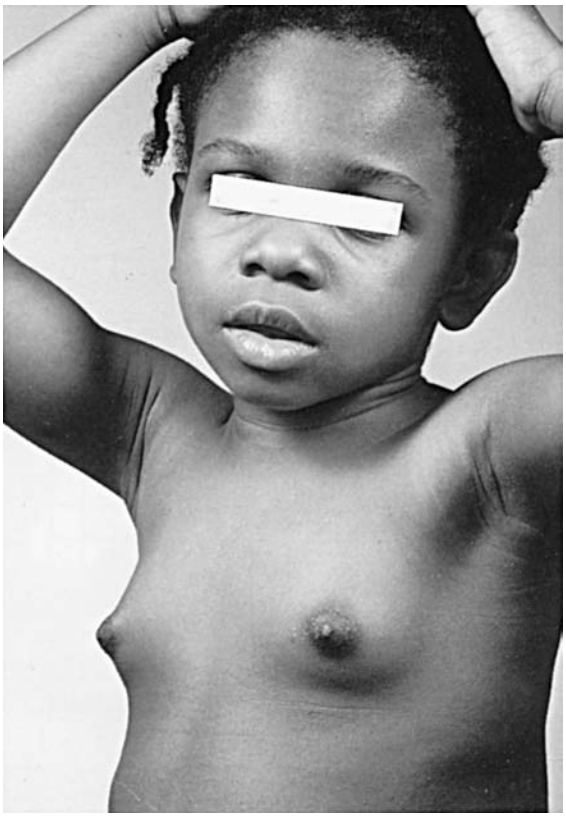


FIGURE 15-37 ■ The appearance of a 5.2-year-old child with complete isosexual precocity caused by a luteinized follicular cyst. Her breast development is no different from that of girls with idiopathic premature thelarche. She presented at 4.7 years with a 2-week history of breast development. Height and bone ages were 5 years. Over a 5-month follow-up period, breast development progressed, she developed sexual pubic hair and presexual axillary hair, and menstrual flow commenced at 3- to 5-week intervals. Four weekly determinations of plasma unconjugated estrogens (estradiol and estrone) showed them to consistently range between 158 and 215 pg/mL. Luteinizing hormone was pubertal and follicle-stimulating hormone was suppressed (averaging 50 and 13 ng LER-907/mL, respectively; see Figure 15-5 for reference ranges). Testosterone was 37 ng/dL, and dehydroepiandrosterone sulfate (DHEAS) was 82 μg/dL. Exploratory laparotomy was performed when she was 5.2 years old. Her height age was 5.8 years, and her bone age was 7.5. The laparotomy revealed a right ovarian cyst about 5 cm in diameter, which was removed. Subsequently, there was a rapid fall in plasma estrogens and testosterone to prepubertal levels. However, DHEAS was unchanged. Menses ceased, but intermittent vaginal cornification (maturation index 90/10/0 to 0/90/10) was found repeatedly. Breast enlargement and sexual hair development resumed at 8.5 years, with normal pubertal sex hormone levels. Menarche occurred at 9.5 years.

granulosa cell tumors but are found in only 10% of the juvenile type. A related feminizing ovarian sex cord–stromal tumor may be caused by loss of a tumor-suppressor gene, as in Peutz-Jeghers syndrome.[635] Adult-type ovarian carcinoma of epithelial cell origin is rare. Ovarian masculinizing tumors are discussed in the section "Hyperandrogenism in Adolescence."

Adrenocortical tumors, as discussed in Chapter 6, typically cause rapid virilization characterized by very high DHEAS production; however, androstenedione is the predominant androgen in many cases. A case discussion is given with Figure 15-38. Many are accompanied by Cushingoid changes. On occasion they cause feminization. When adrenocortical tumors secrete both androgen and estrogen, the clinical picture may resemble complete isosexual precocity.[636] Structural abnormalities of chromosome 11p15 and mutations of the tumor suppressor p53 are fairly common in pediatric adrenocortical tumors.[637]

Aromatase excess syndrome is a rare cause of ectopic feminization.[638] This is an autosomal dominant disorder with variable penetrance that results from constitutive aromatase gene overexpression.

Exogenous steroids can cause sexual precocity. Estrogen-containing contraceptive pills and creams are widely available. Some cases of precocious thelarche may be caused by ingesting food contaminated with artificial estrogens.[639] Soy formulas are potential sources of phytoestrogens, as are many commonly consumed foods, herbs, and topicals.[640-642] It has been proposed that childhood exposure to estrogenic chemical contaminants in underdeveloped countries predisposes to sexual precocity when children emigrate to developed countries.[185] Premature pubarche or acne can result from anabolic steroid use. Topical nonprescription androgen use by a parent, such as for sexual dysfunction or anabolic effects, can cause premature pubarche without necessarily being detectable by standard tests.[643]

Vaginal bleeding in the absence of breast development suggests foreign body, sexual abuse, or tumors of the

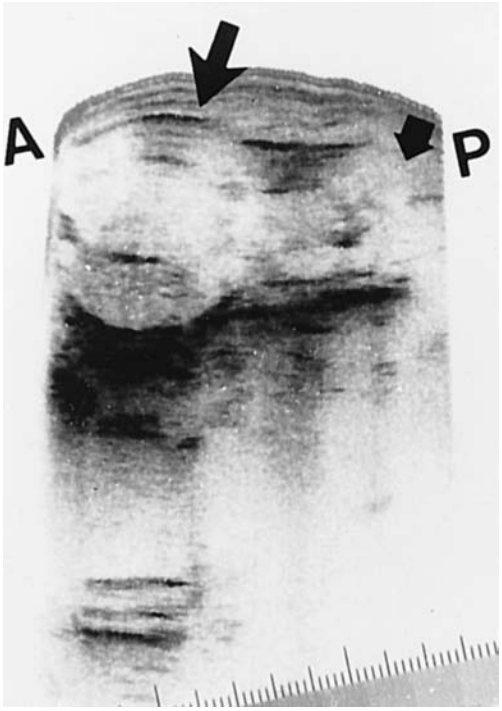


FIGURE 15-38 ■ Abdominal ultrasound (decubitus view) showing a pedunculated encapsulated 5-cm adrenal adenoma (large arrow) near upper pole of kidney (small arrow). This 1.3-year-old girl was virilized. Pubic hair had appeared 2 months previously, height had changed from the 10th to the 30th percentile, and clitoromegaly had occurred. Bone age was 2.3 years. Dehydroepiandrosterone sulfate was 3000 to 4271 μg/dL. Testosterone was 121 ng/dL. A, anterior; P, posterior.

genital tract. A rare cause is "premature menarche," which may result from an extreme variation in the normal intermittent ovarian activation of young girls.[83] Malodorous discharge is highly suggestive of foreign body. A hymenal opening of greater than 5 mm or posterior notches is compatible with sexual abuse.[454,455] Neurofibromas have been reported to simulate breast development and clitoral hypertrophy.

Differential Diagnosis

A physician need not be experienced in endocrinology to diagnose and manage most girls presenting with early breast or pubic hair development. For the most part, the isolated appearance of one of these signs is due to the benign processes of either premature thelarche or premature pubarche, respectively. An otherwise normal history and physical examination, together with a normal bone age constitutes sufficient workup.

In the history, the physician should inquire about the possibility of exposure or access to exogenous steroids in the form of unusual creams, pills, or diet. The possibility of sexual abuse, vaginal infection, or foreign body must be kept in mind when evaluating the child with isolated vaginal bleeding. During the examination the physician should search for nevi, acanthosis nigricans, and signs that might suggest intracranial or abdominal-pelvic disease, and should inspect the external genitalia. The child's height and weight should be carefully recorded, the growth curve examined, and the body mass index (BMI) percentile plotted.

If the history and examination are unremarkable, only a bone age determination is indicated to screen for whether the symptom is indeed an isolated phenomenon or whether appreciable hormone excess exists. If the skeletal age is not abnormally advanced relative to height age, it is likely that the presenting symptom is an isolated and benign extreme variant of normal, which requires no treatment.[540,644] To confirm the diagnosis of premature thelarche or premature pubarche, the child must be similarly reevaluated after 3 to 6 months. If the results are still negative, the family can be reassured with a high degree of confidence that true puberty including menses will not occur until the usual age.

If more than one sign of precocious puberty is present or develops or if the growth is accelerated, a more extensive workup is indicated. For example, if a young girl with early breast development begins to grow pubic hair, or vice versa, then something more than premature thelarche or premature adrenarche is involved. The same is true if she develops a growth spurt or if she begins menstruating. These additional signs indicate the need for more extensive studies. Isolated vaginal bleeding (i.e., bleeding in the absence of secondary sex characteristics) suggests sexual abuse, foreign body, or genital tract tumor, rarely isolated menarche. Cytology, anaerobic culture, pelvic ultrasound, and serum E2 examinations are indicated.

Bone age advancement that is or becomes disproportionate to height (as indicated by compromised height potential or bone age ≥ 20% greater than height age) suggests a sustained excess of sex hormone and is an indication for a more extensive investigation to determine the cause of the precocity. The importance of the recheck is illustrated by the case presented in Figure 15-37.

One must be particularly aware that girls with neurogenic precocity, especially those who have had cranial irradiation, are at risk of paradoxically having concomitant GH deficiency.[566] Coexistent GH deficiency masks the seriousness of the precocity: the growth rate is normal (not accelerated) and breast development is attenuated. However, the disparity between bone age and height age is extreme.

The laboratory investigation of premature pubertal development requires determinations of sex steroids, LH, and FSH by assays of high sensitivity: at least 10 pg/mL for E2, 10 ng/dL for testosterone, 5 μg/dL for DHEAS, and 0.2 U/L for LH and FSH.[593] The modern multichannel platform assays that are widely available generally meet these specifications for the assays of DHEAS, LH, and FSH, but they are totally inadequate for testosterone and E2. These require assays of high sensitivity and specificity, such as are provided by postchromatographic radioimmunoassay or tandem mass spectrometry, in laboratories with well-established normal ranges for children.[645] Prepubertal E2 levels are normally less than 10 pg/mL, and prepubertal testosterone is normally less than 20 ng/dL. Measurement of serum thyroxine and prolactin is indicated if the sexual precocity is accompanied by growth arrest or galactorrhea.

In central precocity, daytime serum E2 concentration is usually pubertal, 10 pg/mL (37 pmol/l) or more.[646] An E2 level in the upper premenarcheal normal range (≥ 75 pg/mL) is atypical and necessitates a prompt workup to distinguish ovarian or adrenal tumor from true isosexual precocity.[647] If the estradiol level is atypically high, weekly determinations of E2 may help to determine whether the level is fluctuating in the normal cyclic fashion of true precocious puberty. Because of the episodic and cyclic nature of sex hormone secretion, examination of the vaginal mucosa (see Figure 15-11) for estrogen effect is a more sensitive indicator of the presence of early puberty than is an E2 blood level because it represents the integrated effect of estrogen over the preceding 2 to 3 weeks. Uterine size (e.g., uterine length > 3.8 cm and endometrial thickness of ≥ 2 mm) has been used as an objective indicator of overall estrogenization.[75,76] Androgen levels are appropriate for the stage of pubarche in true precocity.

Third generation, monoclonal-antibody-based, "pediatric" immunoassays for gonadotropins are necessary for early detection and monitoring of therapy.[593] Early morning basal LH > 0.6 has been reported to be 62% to 95% sensitive and 92% to 100% specific for the diagnosis of central precocious puberty in girls.[541,648] A post-GnRH peak LH > 6.9 U/L has been reported to be 92% sensitive and 100% specific,[541] whereas a post-GnRH agonist peak LH > 4 to 5 U/L has been reported to be ≥ 90% accurate for the diagnosis of central precocious puberty.[544,649,650] The GnRH agonist test also permits assessment of the ovarian gonadotropin-responsiveness: an E2 peak ≥ 34 pg/mL is approximately 90% sensitive and

≥ 60 pg/mL 95% specific for puberty (see Table 15-1).[83] FSH levels are not as helpful diagnostically because prepubertal values overlap considerably with pubertal ones and they may be elevated in premature thelarche. In response to GnRH or GnRH agonist testing, children with unsustained pseudopuberty as variants of normal will have a minimal gonadotropin response, whereas children with gonadotropin-independent precocity will have suppressed responses.[651,652] Demonstration of a sleep-related rise of serum LH is an alternate diagnostic procedure. Box 15-2 summarizes laboratory criteria for the diagnosis of complete sexual precocity.[653]

Premature pubarche must be distinguished from hypertrichosis, the generalized excessive growth of vellus body hair that is prominent in nonsexual areas. The most common cause, by far, of premature pubarche is premature adrenarche. Premature adrenarche must be distinguished from virilizing disorders, the most common of which is virilizing nonclassic congenital adrenal hyperplasia and the most serious of which, though rare, is virilizing tumor.

Determination of the early morning baseline serum androgen pattern is useful for discriminating among the causes of premature pubarche and virilization. Premature adrenarche is characterized by a pubertal level of DHEAS, whereas serum testosterone and androstenedione are, at most, marginally elevated above the prepubertal range. A greatly elevated level of DHEAS suggests either adrenal tumor or the 3β-hydroxysteroid dehydrogenase deficiency form of congenital adrenal hyperplasia. Androstenedione and 17-hydroxyprogesterone levels are disproportionately elevated compared to testosterone or DHEAS levels in other forms of virilizing congenital adrenal hyperplasia and many ovarian tumors.

An ACTH stimulation test is the definitive test to exclude congenital adrenal hyperplasia. We advise performing an ACTH test in children with premature pubarche who have an early morning serum 17-hydroxyprogesterone concentration above 200 ng/dL,[654] though levels above 1500 ng/dL do not require a confirmatory ACTH stimulation test. The diagnosis of congenital adrenal hyperplasia is discussed in detail in Chapter 5 The differential diagnosis of hyperandrogenic disorders is discussed further in the "Hyperandrogenism in Adolescence" section.

Ultrasonography is indicated to screen for abdominal or pelvic masses when feminizing or virilizing disorders are suspected.[655,656] The ovaries of girls with true sexual precocity resemble those of normal pubertal girls.[72,74,657] A "cyst" of 10 mm or more in diameter usually is due to a transient preovulatory follicle. However, the differential diagnosis of a persistent cyst or multicystic ovaries includes McCune-Albright syndrome,[624] tumor,[658] and premature ovarian failure.[659]

The ability of ultrasonography to detect small adrenal neoplasms is highly dependent on the expertise of the ultrasonographer. On rare occasions, ultrasonography has been insensitive in detecting an ovarian tumor in adults.[660] Computed tomography and magnetic resonance imaging (MRI) permit better visualization and a more detailed assessment of tumors.

MRI of the hypothalamic-pituitary area is indicated in progressive true sexual precocity, especially those less than 6 years old or those at risk of organic causes by virtue of their underling condition or neurologic symptoms and signs.[646,661]

Management

The goals in management are to rule out an organic disorder that requires treatment in and of itself and to ascertain whether sexual precocity is either compromising height potential or resulting in important secondary emotional disturbances in the child.

The situation of a girl between 6 and 8 years of age presenting with the onset of breast development or pubic hair warrants special consideration. Breast development between 7 and 8 years of age is normal in blacks and Hispanics. Although presexual pubic hair (stage 2) may be seen in 6- to 8-year-old black and Hispanic girls, sexual pubic hair (stage 3) is abnormal if present before 8 years of age (see Table 15-3). However, pubertal development in girls in the 6- to 8-year age range may be associated with pathology, with excessive adiposity being the major consideration in most [243]

Many 6- to 8-year-old girls with central precocious puberty, including whites, have slowly progressive precocity, with a normal timing of menarche, and are at low risk of short adult stature. Most such girls do not require GnRH agonist therapy to preserve adult stature.[593,594,662,663] Thus, a less comprehensive investigation may be warranted in selected girls between 6 and 8 years of age who present with thelarche or stage 2 pubic hair. For most such girls, a complete history and physical exam, including an obesity evaluation and a bone age determination, may be all that is needed, along with careful longitudinal follow up to rule-out a disorder that requires therapy.[594,663] However, 6- to 8-year-old girls with a suggestion of rapidly progressive or excessive androgenization or feminization, neurologic symptoms, linear growth acceleration, or significant bone age

BOX 15-2 Laboratory Criteria for Diagnosis of Complete Precocious Puberty in Girls

Bone Age Advancement

- Bone age > height age > chronologic age
- Compromised predicted adult height

LH Level Pubertal*

- Sleep-associated LH peak > 1 U/L
- LH (early morning) ≥ 0.6 U/L†
- Post-GnRH (1 hour) agonist LH ≥ 3.2-5.5 U/L†
- Suppressible by chronic GnRH agonist administration

Sex Hormone Level Pubertal (Diurnal Early)

- Estradiol (early morning, cyclically): > 9 pg/mL†
- DHEAS normal for age or early puberty

*Essential criterion
†Typical values for sensitive assays. Exact values vary among laboratories.

advancement should be more completely evaluated, as outlined earlier.

Intracranial lesions must be treated by appropriate measures, such as neurosurgery or irradiation. Shunting for hydrocephalus may stop the precocity. Granulosa cell tumors confined to the ovary have a good prognosis for cure by unilateral oophorectomy. Recurrence of tumor may occur up to 20 years after the initial operation, however. Biopsy of the opposite ovary is indicated in unilateral ovarian neoplasms. Compensatory ovarian hypertrophy can be expected at any age after removal of a single ovary.[664]

The only permanent physical complication of true isosexual precocity, all else being normal, is short adult height. Excessive sex hormone production in the first decade of life causes early maturation of the epiphyses, resulting in their premature closure. About half the girls with this disorder reach an adult height of 53 to 59 inches and the remainder are over 60 inches tall.[594,663] The mismatch between physical, hormonal, and psychological development may cause behavior changes ranging from social withdrawal to aggression or sexuality. However, frank behavioral problems are unusual in girls and so are by themselves seldom indications for treatment.

When central precocity is accompanied by documented progression of pubertal development that accelerates growth and compromises normal height potential, GnRH agonist treatment is indicated.[593] Documentation typically requires 3 to 6 months, but it may be unnecessary if puberty is substantially advanced clinically and hormonally on presentation. The down-regulating effect of GnRH agonists on pituitary gonadotropin release inhibits gonadotropin secretion within 1 month. Suggested criteria for the use of these drugs are presented in Box 15-3.[593,653] The commonly used agents in the United States are leuprolide acetate (ordinarily given as Lupron Depot-Ped 7.5 to 15 mg/mo or 22.5 mg/3month IM),[665,666] nafarelin acetate (Synarel 800 μg bid IN), and a histrelin implant (Supprelin LA 50 mg implant yearly).[667] These starting doses are larger than the usual adult dosage in order to avoid worsening the status by an agonist effect; dosage can be adjusted later as necessary.

Treatment is adequate if the E2 and baseline LH levels become prepubertal[668] or LH is below 4 (1 hour) to 6.6 U/L (2 hours) after GnRH agonist[666,669] one month after institution of therapy. Withdrawal menses may occur at that time, but none should be expected thereafter. Arrest of breast development and the pubertal growth spurt become apparent by 3 to 6 months. Concomitantly, epiphyseal closure is delayed and adult height potential improves, because a type of catch-up growth occurs, in which height age catches up to bone age. Adult height is greatest when treatment is started soon after onset at an early age, yielding an average height gain above pretreatment height prediction of about 1.4 cm for each year of therapy.[593] Adult height prediction at the end of treatment tends to be overestimated from bone age. Therefore, prolonging treatment beyond a chronologic or bone age of 12 to 12.5 years of age generally leads to little further increase in adult height potential, regardless of the prediction of residual height potential.

Coincident GH-deficiency must be treated for optimal growth.[670] GH-sufficient patients with central precocity who are started on treatment relatively late and whose height velocity falls below 4 cm/year after 2 to 3 years appear to gain an average of 2 cm/year when GH therapy is added.[671,672]

Use of the depot form of GnRH agonist is complicated by sterile abscesses at injection sites in about 5% of cases. Anaphylaxis is a rare complication.[673] No other serious side effects have come to light. Long-term safety data remain incomplete, but current studies following subjects into young adulthood are reassuring, including no difference in menstrual cycle characteristics of treated compared to untreated subjects.[674] GnRH agonist treatment does not seem to cause or aggravate obesity, as judged from BMI.[593] Bone mineral density dips with the onset of treatment, but it normalizes after discontinuation of GnRH agonist therapy of precocious puberty.[593]

Girls with idiopathic slowly progressive puberty of onset between 6 and 8 years of age or with early fast puberty between 8 and 9 years of age tend to be tall at the onset of puberty, follow an advanced growth pattern, and reach their target height without GnRH agonist therapy.[594,663,675-677] Therefore, this treatment is only indicated if height potential is compromised or there are other compelling reasons to slow the pace of puberty.

Medroxyprogesterone acetate (Depo-Provera) is useful for stopping menses and as a contraceptive in mentally retarded girls in whom preservation of height potential is not important. It is begun in a dosage commencing at 50 mg/month intramuscularly. Doses as high as 400 mg/month have been used, although cushingoid side effects may be observed at this level.[678] Although this treatment reverses some of the physical changes of premature puberty, it does not reverse the inordinately rapid maturation of the skeleton, possibly because of its inherently weak androgenicity. In addition, use of medroxyprogesterone acetate is associated with a loss of bone mineral density, which must be considered if long-term use is being considered.[679]

A variety of drugs have been used off-label to treat gonadotropin-independent precocity. Both antiestrogen and aromatase inhibitor treatments have demonstrated partial efficacy for McCune-Albright syndrome.[666,680,681] Ketoconazole, an antifungal agent that inhibits 17,20-lyase activity and other steroidogenic enzymes, may be useful.[682] GnRH agonist treatment may be necessary for

BOX 15-3 Indications for Gonadotropin-Releasing Hormone Agonist Therapy of Precocious Puberty

1. Documentation of central precocious puberty
2. Documentation of pubertal progression
3. Plus presence of one of the following:
   - Progressive compromise of predicted adult height *or*
   - Emotional or behavioral disturbance *or*
   - Menses in the emotionally immature or disabled

Modified from Rosenfield, R. L. (1994). Selection of children with precocious puberty for treatment with gonadotropin releasing hormone analogs. J Pediatr, 124, 989.

those in whom true puberty becomes superimposed because the bone age has reached a pubertal level.[232,233,683] Bisphosphonates are often effective at relieving the bone pain of fibrous dysplasia in McCune-Albright syndrome, although they do not appear to have an effect on the course of the lesions and are not a suitable long-term treatment.[684]

Patients with premature thelarche or pubarche as variations of normal are counseled as follows. The child's early development seems to be a matter of a normal stage of puberty occurring early. It is due either to an incomplete, slow kind of puberty or to increased sensitivity to the trace levels of hormones that are normally present in childhood. Feminization with breast development and eventual menstruation can be expected to occur at an appropriate age. No treatment is indicated. To exclude subtle sex hormone excess or eventual anovulatory syndromes, long-term follow-up is advisable.

Besides dealing with the physical consequences of true isosexual precocity, the physician must be ready to help the family and child cope with the psychological problems that come with early physical maturation. The doctor can help the family by explaining that even though their child looks older and more mature than other children of the same age, the child will not behave more maturely. The libido of young children with precocity is not increased. The family should be advised to take some precautions to downplay their child's development—for example, in the choice of clothing and swimsuits. Friendships with children a bit older will help shorten the time affected children spend in social limbo. This may be more easily said than done, however, because the intellectual and social maturity of these patients is not advanced. Early on, any children with these disorders tend to be withdrawn because they feel that they are different from their peers. Later on, they tend to enter into romantic relationships early. It is important to remind the family and child that in a few years the child will not be unique from the standpoint of sexual development.[566] The following books may be helpful in explaining precocious puberty: for children, *What's Happening to Me?* by Peter Mayle (Lyle Stuart, Secaucus, New Jersey, 1973); for parents, Sex *Errors of the Body*, by John Money (Paul H Brookes Publishing, Baltimore, Maryland, ed. 2, 1994).

Hypogonadism

Causes

If hypogonadism is complete and present prepubertally, it causes sexual infantilism. In genetic males, congenital primary hypogonadism may also cause a completely female or ambiguous phenotype (see Chapter 17). If hypogonadism in girls is partial or of onset in the early teenage years, feminization will be of too limited a degree to permit the onset of menses at a normal age (primary amenorrhea). Milder, partial, or incomplete forms of hypogonadism may cause secondary amenorrhea or oligomenorrhea. At its mildest, hypogonadism may present in adolescence with the anovulatory symptoms of dysfunctional uterine bleeding or with excessively frequent periods due to short luteal phase. Consequently, disorders causing hypogonadism appear in the differential diagnosis of disorders of sexual differentiation, sexual infantilism, failure of pubertal progression, and menstrual irregularity. The causes of hypogonadism are listed in the differential diagnosis of amenorrhea in Box 15-4.

Primary Ovarian Failure. Primary ovarian failure is characterized by high levels of gonadotropins, particularly FSH. Two exceptions exist to this rule. First, the gonadotropins may not be elevated until CNS maturation has reached a pubertal stage, as indicated by a bone age of approximately 10 to 11 years (Table 15-5).[685] Second, patients with *partial* ovarian failure (primary ovarian insufficiency), as occurs during the menopausal transition, do not have high baseline gonadotropin levels.[686-688] FSH may hyperrespond to GnRH and estrogen hyporespond to GnRH agonist challenge. It seems as if relatively few ovarian follicles—too few to permit the cyclic emergence of preovulatory follicles—suffice to prevent the characteristic rise in basal FSH levels. Serum AMH levels are a less sensitive indicator of ovarian failure than FSH, but may be useful in prognosticating the potential for fertility.[689,690]

Primary ovarian failure may occur before or during puberty, causing primary amenorrhea, or after puberty has occurred, causing secondary amenorrhea. The latter is termed *premature* ovarian failure and clinically resembles premature menopause, except that about 25% of the cases sometimes resume ovarian function.[691] Because of this fluctuating ovarian function in some women, *premature ovarian insufficiency* may be a better descriptor.

Gonadal dysgenesis due to deficiency of genes on the X chromosome is the most common cause of primary ovarian failure. It is usually due to a relatively large-scale deletion of X-chromosomal material, which is associated with a characteristic, but variable, phenotype and is termed Turner syndrome (see Chapter 16). Fetuses with a 45,X karyotype have a normal number of oocytes in the ovary at midgestation, but a drastic reduction in the number of follicles,[32] which appears to cause gonadal streaks via an accelerated rate of apoptosis. However, the gonadal dysgenesis, like other features of the syndrome, is often incompletely expressed.[692,693] Thus, Turner syndrome should be considered in all girls with primary hypogonadism or secondary amenorrhea whether or not they have the typical stigmata of Turner syndrome.

Specific loci on the X chromosome are associated with primary ovarian failure. Xp11.2 harbors BMP15, a specific ovarian differentiation factor, heterozygous mutation of which is a rare cause of gonadal dysgenesis. Xq harbors two independent loci, in addition to the fragile X premutation, that are associated with about 5% of sporadic and 14% of familial primary ovarian failure.[694] The premutation is an expansion of CGG repeats that is insufficiently long to cause fragile X syndrome. Women with the premutation allele have a substantially increased risk of primary ovarian insufficiency, possibly because raised intracellular mRNA concentrations might sequester CGG binding proteins that are important for RNA processing.

BOX 15-4 Differential Diagnosis of Amenorrhea

Abnormal Genital Structure

- Ambiguous genitalia
  - Disorders of sex development
  - Pseudointersex
- Aplasia*
  - Hymenal
  - Vaginal
  - Müllerian
  - Disorders of sex development
- Endometrial adhesions

Anovulatory Disorders

Hypoestrogenism, FSH Elevated

Primary Ovarian Failure

- Congenital
  - Gonadal dysgenesis
    - —Chromosomal
    - —Genetic
    - Other genetic disorders
- Acquired
  - Oophorectomy
  - Radiotherapy or chemotherapy
  - Oophoritis
  - Idiopathic

Hypoestrogenism, FSH Not Elevated

- Primary ovarian insufficiency
  - Complete if bone age < 11 years*
  - Incomplete if bone age > 11 years
- Delayed puberty
  - Constitutional delay*
  - Growth-retarding disease
- Gonadotropin deficiency
  - Congenital
  - Acquired
  - —Organic
  - —Functional
  - Virilization

Estrogenized

- Hypothalamic anovulation
  - Hypothalamic amenorrhea
  - Athletic amenorrhea
  - Psychogenic amenorrhea
  - Epilepsy
- Nonhypothalamic nonovarian disorders
  - Pregnancy
  - Obesity or undernutrition
  - Chronic disease
  - Cushing syndrome
  - Hypothyroidism
  - Drug abuse
  - Hyperprolactinemia
  - Post-pill amenorrhea
  - Gonadotropin-producing tumor
- Hyperestrogenism
- Hyperandrogenism

*Cause only primary amenorrhea.
FSH, follicle-stimulating hormone.

TABLE-15-5 Bone Age in Workup of Sexually Infantile Girls with a Normal Follicle-Stimulating Hormone Level

	Bone Age (Years)		
	< 11	11-13	> 13
Primary hypogonadism	Yes		
Delayed puberty	Yes	Yes	
Gonadotropin deficiency	Yes	Yes	Yes

Based on Rosenfield, R. L., & Barnes, R. B. (1993). Menstrual disorders in adolescence. Endocrinol Metab Clin North Am, *22, 491.*

Gonadal dysgenesis also results from 46,XY complete gonadal dysgenesis and certain forms of autosomal aneuploidy.[486,694,695] A variable degree of ovarian dysgenesis occurs in trisomy 21; delayed menarche, anovulatory cycles, and primary gonadal failure are occasionally seen.[696] However, pregnancy has been reported; trisomic offspring are common.[697] Oocytes are virtually absent in trisomies 13 and 18. Ovarian dysgenesis also occasionally occurs as part of the Denys-Drash syndrome due to a *WT-1* mutation.[698] Gonadal dysgenesis also occurs in ataxia telangiectasia and related DNA-repair disorders.[699] Other autosomal genetic disorders causing premature ovarian failure include inactivating mutations of FOXL2, which are found in sporadic cases as well in the autosomal dominant type 1 blepharophimosis syndrome.[700] Premature ovarian failure also occurs in galactosemia,[701] leukodystrophies, and myotonia dystrophica.[702,703] Mutation of the inhibin alpha subunit predisposes to premature ovarian failure, an effect that appears to vary depending on ethnicity; this is postulated to be due to deficient paracrine interactions with TGFβ family receptors.[704] Mutations incriminated in the ovarian failure of mouse models[705] or in genes involved in follicle development[24] can be anticipated to be incriminated as causes of human primary ovarian failure.

Injury to the ovary is a common cause of primary ovarian failure. *Mumps* oophoritis is a classic but rare cause of ovarian failure. Very high-dose estrogen treatment in adolescence increases the risk of hypergonadotropic subfertility.[690]

Irradiation and chemotherapy for childhood neoplasia are frequent causes of primary ovarian failure now that life is effectively prolonged.[694,706-709] Ionizing radiation and alkylating agents damage DNA whether or not a cell is replicating.[710] The damage is dose-related and additive. A radiation dose of ≥ 20 Gy causes acute ovarian failure in > 70% of girls. A cumulative cyclophosphamide dose of about 100 mg/kg causes equivalent damage. Prepubertal girls may be about half as sensitive to these therapies as postpubertal females: among girls who receive 1 to 10 Gy, acute ovarian failure develops in about 10% of girls under 13 years but in 25% of those ≥ 13. Some with

early hypergonadotropism will experience ovarian recovery with normal pituitary-ovarian function after several years.[711] After prepubertal cancer treatment, 94% of girls can be anticipated to enter puberty and menstruate regularly, but 8% of these will develop nonsurgical premature menopause because of the reduced number of oocytes.[708] The risk for premature menopause is 30% in those who have received both radiation and chemotherapy and 5% to 13% for those receiving either alone. Fertility is reduced by about 50% in those receiving 5 to 10 Gy abdominal-pelvic radiation and 75% in those receiving more.[712] Fertility is reduced by about 25% in those receiving high cumulative doses of chemotherapy (e.g., cyclophosphamide > 10 gm/m^2).[712] Several nonalkylating chemotherapies are also gonadotoxic.[713,714] However, data are scarce, and interactions among the various classes of chemotherapeutic agents are poorly understood. Methotrexate and vinca alkaloids pose low risk for ovarian failure.[694] As a consequence of gonadotropin elevation when gonadal failure begins, puberty may progress rapidly.[608]

Sterilization by irradiation can be obviated by transposing the ovaries out of the irradiation field if possible. Administration of a GnRH agonist prior to cyclophosphamide administration does not clearly decrease ovarian injury.[710] Imatinib (Gleevec) is a potential oocyte-protective treatment, as it blocks an apoptotic pathway activated by radiation and cisplatin in mouse oocytes.[715]

Gonadotropin resistance (Savage syndrome) can arise from an autosomal recessive loss of function mutation of the LH or FSH receptor.[716-718] The reported cases have had some degree of pubertal development followed by primary or secondary amenorrhea or oligomenorrhea. The ovaries of LH receptor mutants contain follicles in all stages of development, whereas those of FSH receptor mutants vary from hypoplastic to normal size, with antral follicle development varying from nil to 5 mm. Partial gonadotropin resistance is common in the Albright osteodystrophy form of pseudohypoparathyroidism, as part of the generalized defect in G-protein signal transduction.[719]

Autoimmune oophoritis is the basis of approximately half of the cases of spontaneous premature ovarian failure, though estimates vary from 5% to 85% in various series.[720] It is diagnosed by its association with any of a variety of autoimmune endocrine or nonendocrine disorders, manifest or subclinical, that have in common defects in T-cell suppressor function. Autoimmunity may be directed against the granulosa cell, oocyte, or theca cell. The clinical picture may resemble relatively selective resistance to FSH or, less frequently, to LH. The latter results from lymphocytic destruction of theca cells with sparing of granulosa cells from small antral follicles, which lack substrate to form E2 and can only respond to the compensatory increase of FSH by producing inhibin-B.[721] These patients have autoantibodies to steroidogenic cells and are at risk for adrenal failure. Usually these antibodies are directed against 21-hydroxylase, less frequently to side chain cleavage enzyme or 17-hydroxylase, seldom to 3β-hydroxysteroid dehydrogenase. Replacement glucocorticoid therapy may temporarily ameliorate the immune oophoritis in such cases.[722] A case with autoantibodies to testosterone has been reported.[723] Autoimmune gene regulator (AIRE) gene mutations have been identified as causative of type 1 polyendocrine failure. Ultrasonographic and histologic findings are variable in premature ovarian failure and include large or small ovaries, inactive or polyfollicular ovaries, loss or preservation of primordial follicles, and infiltration by lymphocytes or plasma cells[659]

Functional ovarian failure can also result from specific autosomal recessive defects in the biosynthesis of sex steroids. Both androgen and estrogen deficiency occur in lipoid congenital adrenal hyperplasia (StAR and side chain cleavage mutations; see Figure 15-23), 17α-hydroxylase deficiency, P450 oxidoreductase deficiency, 17,20-lyase deficiency, and 17β-HSD3 deficiency.[724,725] Thus, affected genetic males may present with a female phenotype. Hypoestrogenism is associated with virilization in aromatase and 3β-HSD deficiency. Aromatase and 17β-HSD3 deficiency are unique in not being associated with congenital adrenal hyperplasia. Congenital lipoid adrenal hyperplasia is unique in that underlying StAR deficiency has too little direct impact on ovarian function to interfere with the early phases of puberty, but the gradual buildup of intraovarian lipid deposits resulting from enzyme deficiency—a "second hit"—causes ovarian damage with anovulation and late ovarian failure. SF-1 deficiency can cause primary ovarian failure in the absence of adrenal insufficiency.[726]

Estrogen resistance due to inactivating mutation of ER-alpha was recently reported in an 18-year old girl.[726a] Hypoestrogenism and hyperestrogenemia were profound, the ovaries were enlarged and multicystic, and gonadotropin and testosterone levels were marginally elevated.

Gonadotropin Deficiency (Hypogonadotropic Hypogonadism). *Congenital* gonadotropin deficiency can occur in association with cerebral, hypothalamic, or pituitary dysfunction or as an isolated defect.[727] Congenital defects in hypothalamic-hypophyseal formation may be associated with midline facial defects. Congenital hypothalamic dysfunction may be associated with other neurologic or endocrine dysfunction, such as in the Prader-Willi syndrome (congenital hypotonia and neonatal failure to thrive followed by hypothalamic obesity, sometimes with hypopituitarism)[728] or the Laurence-Moon-Biedl syndrome (retinitis pigmentosa, obesity, mental deficiency).

Congenital hypogonadotropic hypogonadism often results from mutations (Table 15-6).[200-202,208,211,213,221,572,729-739] The autosomal recessive forms of congenital combined pituitary hormone deficiency due to PROP1, HESX1, LHX3, and OTX-2 mutations are associated with gonadotropin deficiency. Leptin and leptin receptor inactivating mutations cause gonadotropin deficiency in combination with moderate or extreme obesity.[738,740] The report of a natural pregnancy in a woman with a homozygous mutation in the leptin receptor lends controversy to the current concept that leptin function is essential for reproduction.[741]

Gonadotropin deficiency may be associated with anosmia (olfactory-genital dysplasia or Kallmann syndrome).[202] This syndrome is one tenth as frequent in females as in males. Mutations in the KAL-1 gene on the pseudoautosomal region of the X chromosome,

TABLE 15-6 **Mutations Associated with Congenital Hypogonadotropic Hypogonadism**

Gene	Function	Phenotype	Inheritance	Reference
FGF8	GnRH neuronal migration	Kallmann syndrome or normosmic IHH	Autosomal dominant	202, 571, 730, 731
GnRHR	GnRH signaling	Normosmic IHH	Autosomal recessive	732-734
Hesx1	Somatotroph, thyrotroph, gonadotroph, development, and gene expression	ACTH, GH, gonadotropin, and TSH deficiency	Autosomal dominant, autosomal recessive	735-737
KAL1	GnRH neuronal migration	Kallmann syndrome	X-linked	200, 202
KISSR	GnRH secretion	Normosmic IHH	Autosomal recessive	201, 211, 213, 221
LEPR	GnRH regulation	Obesity and normosmic IHH	Autosomal recessive	738
Lhx3	Somatotroph, thyrotroph, gonadotroph, development, and gene expression	Rigid cervical spine causing limited neck rotation, GH, gonadotropin, and TSH deficiency	Autosomal recessive	735-737, 739
NELF	GnRH neuronal migration	Kallmann syndrome	Autosomal dominant?	202, 571, 730, 731
PROK2, PROKR2	GnRH neuronal migration	Kallmann syndrome or normosmic IHH	Autosomal recessive	730
Prop1	Somatotroph, thyrotroph, gonadotroph, development, and gene expression	GH, gonadotropin, PRL, and TSH deficiency	Autosomal recessive	735-737
Otx2	Somatotroph, thyrotroph, gonadotroph, development, and gene expression	ACTH, GH, gonadotropin, PRL, and TSH deficiency	Autosomal dominant Autosomal recessive	736, 737, 739
TAC3, TACR3	Synchronizing kisspeptin neuron pulsatility?	Normosmic IHH	Autosomal recessive	221

which encodes anosmin, a key protein for GnRH neuronal migration, cause the highly penetrant X-linked form and rarely affect females. Inactivating mutations of other genes in the anosmin signaling pathway (FGF8/FGFR1, PROK2/R2, NELF, and CHD7) account for the majority of female cases; these are inherited as autosomal dominant or recessive (heterozygous, compound heterozygous, or digenic) traits with variable penetrance.[202,572,730,731,742] Neurologic and somatic abnormalities, such as synkinesia, cerebellar ataxia, sensorineural deafness, mental retardation, unilateral renal agenesis, and cleft palate, are variably associated genetic features of Kallmann syndrome. Rare affected individuals have only delayed puberty. These same mutations sometimes are responsible for normosmic idiopathic GnRH deficiency; CHD7 mutations typically have features of CHARGE syndrome. Research is increasingly revealing new elements in the GnRH developmental and signaling pathway. A single-nucleotide polymorphism in the EAP1 gene was associated with amenorrhea/oligomenorrhea in primates.[743]

GnRH receptor mutations account for about half of autosomal recessively inherited cases of isolated, normosmic gonadotropin deficiency.[732] The degree of hypogonadism is variable, even within a family, with delayed puberty and delayed menarche as presentations.[733,734,744]

Loss-of-function mutations of GnRH[745] and in signaling systems that modulate GnRH release (kisspeptin/GPR54, neurokinin B/TAC3R) are rare causes.[211,221] The hypogonadism of most subjects with neurokinin B/TAC3R mutations is reversed by sex steroid therapy, which suggests that this signaling system is important for the startup of puberty, but not its maintenance. Isolated hypogonadotropic hypogonadism also has been reported in a woman homozygous for a nonsense mutation of the X-linked *DAX1* gene, which was associated with adrenal insufficiency in her brothers.[746]

Isolated FSH deficiency due to mutation in the β-subunit has been reported to cause primary amenorrhea in association with a unique test panel—low FSH, elevated LH, and low testosterone levels.[136,718] Carbohydrate-deficient glycoprotein syndrome (phosphomannomutase deficiency) is characterized by high levels of immunoreactive, but bioinactive gonadotropins, mimicking primary ovarian insufficiency.[747] Isolated LH deficiency due to LHβ gene inactivating point mutations has been reported to lead to secondary amenorrhea following normal pubertal development, but undetectable LH, high FSH, low E2, and macrocystic ovaries.[748] However, some inactivating LH mutations in men have high immunoreactive LH levels.[718]

Acquired gonadotropin deficiency can be a consequence of tumors, trauma, autoimmune hypophysitis,[749,750] degenerative disorders involving the hypothalamus and pituitary,[751] irradiation,[752] chemotherapy,[753] or chronic illness.[754] Hypogonadotropic hypogonadism will develop in about one third of those receiving 20 to 30 Gy cranial irradiation, whereas it is typical in those receiving > 50 Gy.[596,755] Pituitary adenoma, craniopharyngioma, and dysgerminoma are the most common neuroendocrine neoplasms responsible for hypopituitarism in children. Most "nonfunctioning" pituitary adenomas are gonadotroph adenomas, which secrete gonadotropin subunits in response to thyrotropin-releasing hormone.[756] A case of

hypothalamic tumor is presented with Figure 15-39. Pinealomas most commonly cause sexual infantilism. They may act by secreting an inhibitory substance, rather than by compressing key areas of the hypothalamus.[603]

Anorexia nervosa is the prototypic form of eating disorder, a common cause of hypogonadotropism in teenagers. It is a syndrome of undernutrition due to voluntary starvation with a particular psychological dysfunction that results in amenorrhea.[757-759] These patients uniformly consider themselves too fat in the face of objective evidence that they are underweight. The psychiatric criteria that distinguish this disorder from food faddism and fear of obesity consist of refusal to gain or maintain body weight to a minimally normal level for height and age (< 85th percentile of expected body weight based on average BMI for age)[760] with all the following being present: (1) intense fear of gaining weight or becoming fat, even though underweight, (2) an inaccurate perception of body weight, such that they have a disturbance in the way that body weight, size, or shape is experienced; undue influence of body shape and weight on self-evaluation; or denial of the seriousness of current low body weight, and (3) amenorrhea for 3 or more months in postmenarcheal females. Bulimia nervosa, the binge-eating/purging variant eating disorder, is similar in the overevaluation of body shape and weight and the use of extreme weight control behaviors. Physical activity tends to be high. These disorders may be manifest at an early stage as atypical eating disorders, before weight or amenorrhea criteria are met or when the binge is subjective. The cognitive defect that weight can serve as the predominant value in judging self-worth is central to anorexia nervosa. In contrast to other depressive individuals, these patients are generally content with themselves in the areas of intellectual and vocational achievement.

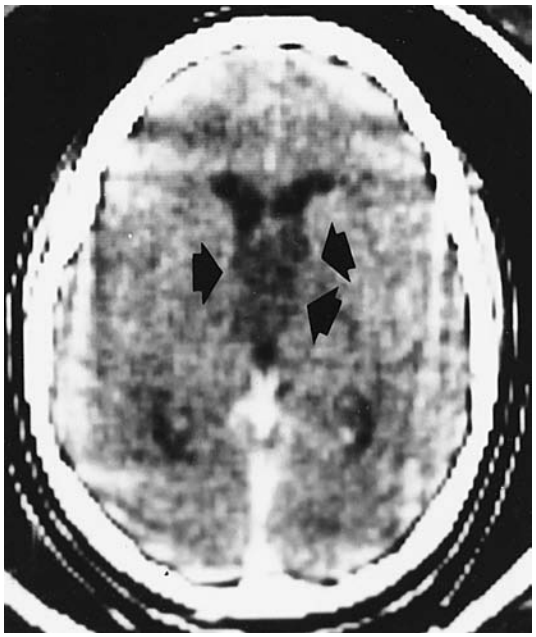


FIGURE 15-39 ■ Computed tomography of the brain of a 16-year-old girl with hypothalamic astrocytoma. The low-density tumor mass (arrows) extends superiorly from the hypothalamus, obliterates the third ventricle, and partially compresses the frontal horns of the lateral ventricles (particularly the right). This patient presented with secondary amenorrhea. Menarche had occurred at age 13, and menses was normal until 15.3 years. The patient then became amenorrheic in association with lethargy, episodic headaches, polyuria, and weight gain—despite little change in appetite. Physical examination was negative. The skull radiograph, electroencephalogram, visual fields, and serum prolactin and thyroxine levels were normal—and urine-specific gravity was 1.016. After biopsy of the cyst wall, studies revealed her to have gonadotropin, growth hormone, and partial antidiuretic hormone deficiencies.

The cause is multifactorial. It involves a genetic predisposition. Concordance rates for the anorexic type are about 50% for monozygous twins, compared with about 5% for dizygotic twins. Many other risk factors have been implicated. Familial factors also include eating disorders of any type, depression, substance abuse, and adverse family interactions. Premorbid experiences, such as sexual abuse or social pressures, or premorbid characteristics, such as low self-esteem, compulsiveness, and perfectionism, are also important. Dieting meets a need for approval in our culture with its emphasis on dietary restriction and thinness as goals for women. Anorexia is often precipitated in vulnerable children by a new experience, such as puberty, leaving home or beginning college, or by adverse life events. The disorder is perpetuated by the complications of starvation, such as depression and reduced gastric emptying.

The onset tends to be at 12 years of age or later. Earlier onset is associated with growth arrest, delay of puberty, and primary amenorrhea.[761] GH deficiency is common.[762,763]

The medical complications of anorexia nervosa are serious. The risk of death is increased approximately 10-fold: electrolyte imbalance, hypoglycemia, cardiovascular instability, bone marrow hypocellularity predisposing to silent infection, and renal failure account for about half of the mortality, and suicide accounts for the rest.

The weight changes leading to cessation or restoration of menstrual cycles are in the range of 10% to 15% of body weight. Recovery is associated with achieving a critical level of body fat stores above the 10th percentile (over approximately 20% body fat) (Figure 15-40), at a BMI of approximately 20 (i.e., midnormal).[764,765] There is an inverse relationship between body weight and the maturity of gonadotropin release in these patients. The 24-hour pattern of gonadotropin release tends to be immature (prepubertal or pubertal), and the diurnal LH pattern becomes mature upon recovery from undernutrition.[766] Luteinizing hormone pulsatility is low and may be restored by opiate antagonists.[89] The gonadotropin response to GnRH and ovulatory response to clomiphene citrate are blunted in the malnourished state and become normal with weight gain to about 80% of ideal.[767,768] Leptin levels are significantly decreased and are a major contributor to both the gonadotropin deficiency and to changes in the thyroid and GH axes.[769]

Mild hypercortisolism is frequent and may contribute to the anovulation by mechanisms discussed further under "Hypothalamic Anovulation."[770] Afternoon ACTH and cortisol levels are significantly higher and the response to CRH is significantly lower than normal.

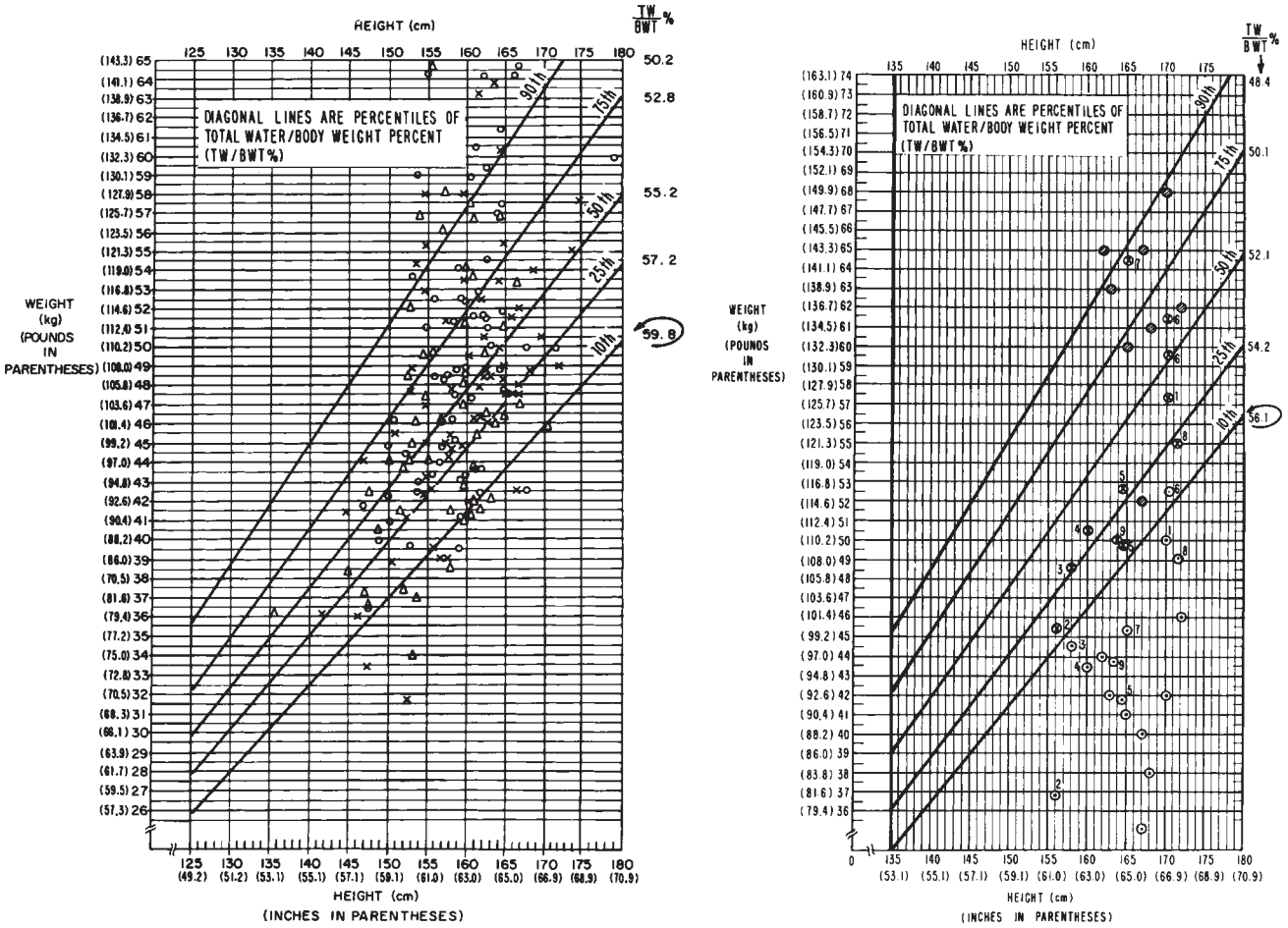


FIGURE 15-40 ■ Percentiles of fatness (diagonal lines) for white girls at menarche (left) and after menarche (right) equated with computed percentiles of total water as a percentage of total body weight. The minimal weight necessary at a particular height for the onset or maintenance of menses is very close to the 10th percentile of fatness on these respective charts. Data for anorexia nervosa cases are shown on the right-hand chart: • at presentation; x at resumption of menses. (From Frisch RE, McArthur JW [1974]. Menstrual cycles: fatness as a determinant of minimum weight for height necessary for their maintenance or onset. Science 185:949. Copyright © by the American Association for the Advancement of Science.)

In contrast to Cushing syndrome, DHEAS levels tend to be blunted as a consequence of undernutrition.[771]

A fundamental neuropsychological flaw or hypothalamic disturbance[772] seems necessary to explain the high incidence of GH deficiency, why some patients become amenorrheic before losing weight, and why about half of the cases remain amenorrheic after treatment. The serotonergic systems implicated in the regulation of feeding and mood seem to remain altered even after weight restoration. The authors favor the concept that these psychological problems lead to amenorrhea only in women who are predisposed to it by a unique preexisting hypothalamic dysfunction. Evidence has been obtained for marked individual differences in reactivity of the neuroendocrine system to stress.[773]

A number of features attributed to hypothalamic dysfunction, such as cold intolerance, may be due to the subtle hypothyroid state that is secondary to the malnutrition.[770] Serum triiodothyronine levels are consistently low, serum thyroxine levels tend to be lower than average (although usually within normal limits), the pattern of TSH release indicates TRH deficiency, and the state of deep tendon reflexes and metabolism is consistent with hypothyroidism. Hypothyroidism may in part complicate malnutrition as a consequence of the interference with IGF-1 generation: low IGF-1 initiates GH excess, compensatory somatostatin release, and subsequent inhibition of the thyrotropin response to thyrotropin-releasing hormone. Undernutrition also diverts the generation of thyroxine metabolites away from triiodothyronine and toward reverse triiodothyronine.

Hyperprolactinemia is a potentially reversible cause of gonadotropin deficiency.[774] Galactorrhea is present in about half of the patients, particularly those with residual estrogen production. The causes of hyperprolactinemia are diverse, including hypothalamic or pituitary disorders, drugs, hypothyroidism, renal or liver failure, peripheral neuropathy, stress, autoimmune disorders, macroprolactinemia, genetic, and idiopathic origins.[775-777a] Elevated serum prolactin levels occur with a variety of tumors that cause functional or anatomic pituitary stalk section, thereby preventing inhibitory pituitary control. About one third of hyperprolactinemic women have an identifiable pituitary adenoma. Prolactinomas smaller than 1 cm

in diameter (microadenomas) cause no problems due to local extension. Prolactinoma may be associated with multiple endocrine neoplasia type 1.[778] In about a quarter of adult hyperprolactinemia cases, the malfunction is due to the ingestion of drugs such as phenothiazines, estrogen, or cocaine.[779] Considerable hyperprolactinemia is idiopathic: decreased sensitivity to dopaminergic inhibition may underlie such cases.[780]

Macroprolactinemia is due to a variant molecule or autoantibody formation. In this situation, direct immunoassay indicates elevated levels of prolactin. However, the biologically available or active prolactin level is normal; thus there is no physiologic consequence to the macroprolactinemia.

Hyperprolactinemia results in LH pulses that tend to be infrequent and LH secretion that is variable in response to GnRH.[781] Selective prolactin excess causes variable degrees of gonadotropin deficiency, ranging from severe to partial (hypothalamic amenorrhea). Adrenal hyperandrogenism, hirsutism, and seborrhea are common.[314]

Frank virilization as a result of very high androgen levels suppresses gonadotropin levels and so causes defeminization. However, the moderately hyperandrogenic disorders discussed later, which are more common, are associated with normal estrogenization.

Differential Diagnosis

The differential diagnosis of hypogonadism is included in Box 15-4. Investigation should begin for hypogonadism when puberty is delayed or does not progress normally. Delayed puberty is indicated by a lack of thelarche by the chronologic or bone age of 13 years. Abnormal progression of puberty is suggested by failure of menses to occur within 4.5 years of the onset of puberty or if secondary amenorrhea or oligomenorrhea has persisted for 1 year. A family history of delayed puberty is compatible with the delay being constitutional rather than having an organic basis. The history should include a thorough past medical history and systems review, including intracranial, visual, olfactory, emotional, abdominal, or pelvic symptoms and systemic symptoms that might indicate chronic endocrine, metabolic, or systemic disorders that delay puberty. Upon examining the patient, the height and weight should be carefully measured and growth rate and appropriateness of weight for height determined (see Figure 15-40). Careful categorization of the stage of breast and sexual hair development are essential. Inspection of the external genitalia is indicated, but an internal pelvic examination seldom is necessary for diagnosis.[782] Examination of the mature breast should include an attempt to express milk from the ducts to the nipple. The finding of a structural genital abnormality may indicate that amenorrhea is due to abnormal genital tract development, whereas clitoromegaly[783] is a clue to a virilizing disorder. Neurologic examination should include evaluation of eye movements, visual fields, and optic fundi, as well as a search for anosmia and midline defects.

An algorithmic approach to the workup of patients with menstrual disorders is shown in Figures 15-41 through 15-43.[784] The laboratory workup depends on the degree of estrogenization, as initially assessed from the stage of breast development: it includes a bone age radiograph in adolescents who are not sexually mature and generally begins with a chronic disease panel, and determination of gonadotropins, E2 and testosterone level. A pregnancy test is indicated in a sexually mature adolescent. The diagnostic considerations differ in the anovulatory girl without FSH elevation depending on whether she is hypoestrogenic or estrogenized (see Box 15-4; also see Figures 15-41 and 15-42).

FSH elevation indicates primary ovarian failure (see Figures 15-41 and 15-42). Chromosome abnormalities are ordinarily the first consideration, as the most common cause is Turner syndrome and its variants. Individuals with primary ovarian failure that is not due to Turner syndrome and its variants should be investigated for the fragile X premutation.

Lack of FSH elevation in a prepubertal patient does not rule out primary ovarian failure if bone age is below 11 years, because neuroendocrine puberty may not have occurred; in this situation primary ovarian failure is not hypergonadotropic (see Table 15-5).[685] If FSH is not elevated and bone age has reached 11 years, in a prepubertal girl without a growth-attenuating or retarding disorder, one is dealing either with constitutional delay of puberty or isolated gonadotropin deficiency (see Figure 15-41). "Constitutional" delay of puberty is the most likely diagnosis until the bone age reaches 11 to 13 years (see Table 15-5).[685] Its distinction from isolated gonadotropin deficiency may be difficult. The features that help to distinguish it from isolated gonadotropin deficiency are listed in Box 15-5 and discussed in the footnotes to Figure 15-41. The single most useful test is the LH level in response to GnRH testing because random LH levels in hypogonadotropic patients often overlap those of pre- and midpubertal normal children.[95] GnRH agonist testing may discriminate between these disorders better because the LH response at 3 to 4 hours is the best indicator of gonadotropin secretory reserve and because this test permits assessing the gonadal secretory response to the secreted gonadotropins at 24 hours.[651]

The assessment of an adolescent's degree of estrogenization is often difficult. Breast development indicates that there has been estrogen exposure, but it does not mean that the exposure is current. Determination of serum E2 is the simplest test, but diurnal and cyclic variations must be taken into account. Determination of the hormonal effects on vaginal cytology is the most indicative of overall estrogen exposure (see Figure 15-29), but patients do not accept it as well. A progestin withdrawal test is often helpful. A female who does not experience progestin withdrawal bleeding (see Figure 15-42) probably has an ambient E2 level of less than about 40 pg/mL.[785] If bleeding does not occur in response to this maneuver, the integrity of the uterus can be demonstrated by eliciting withdrawal bleeding after a 3-week course of estrogen-progestin, most conveniently administered in the form of birth control pills.

A prolactin level is indicated in the initial workup of normogonadotropic patients, regardless of their

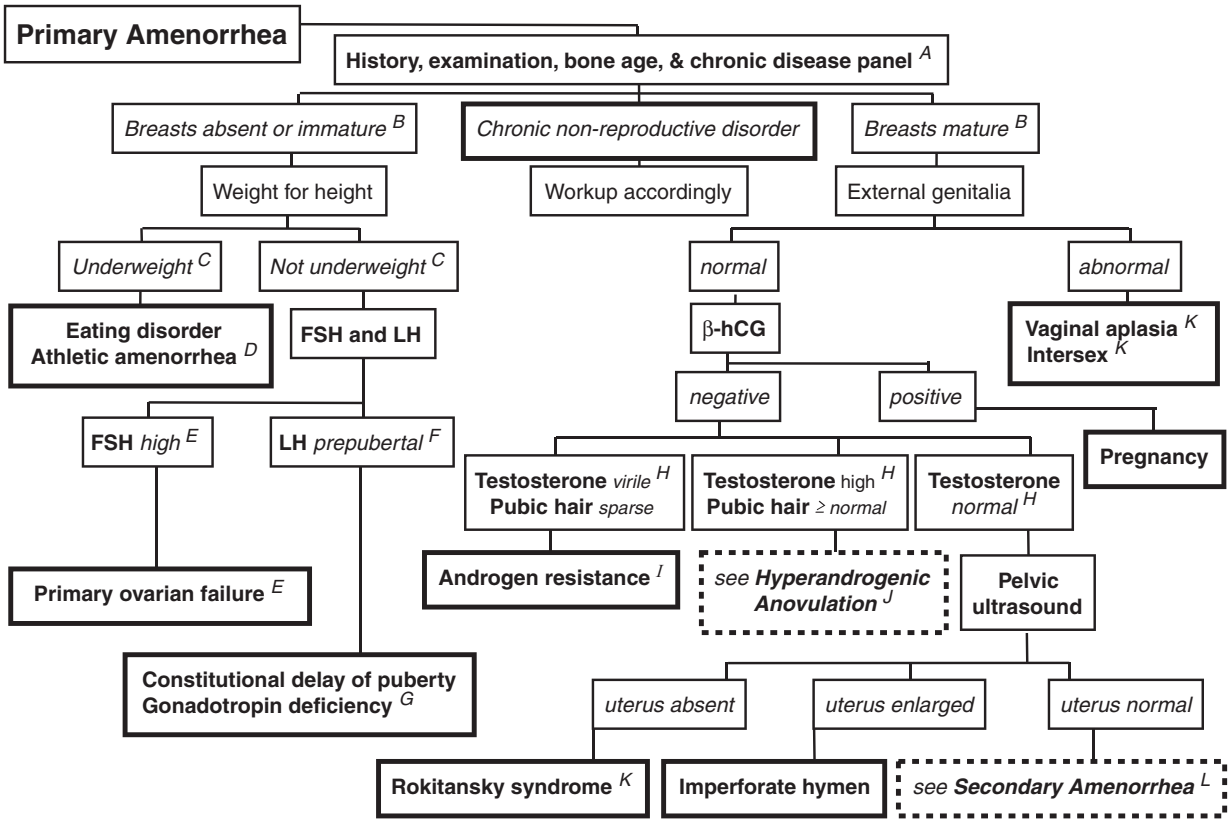


FIGURE 15-41 ■ Differential diagnosis of primary amenorrhea. (Modified with permission from Rosenfield, R. L. (2003). Menstrual disorders and hyperandrogenism in adolescence. In S. Radovick, & M. H. MacGillivray (Eds.), *Pediatric endocrinology: a practical clinical guide* (pp. 451–478). Totowa, NJ: Humana Press.)

A. Prime among the causes of primary amenorrhea are growth-retarding or attenuating disorders. In the absence of specific symptoms or signs to direct the workup, laboratory assessment for chronic disease typically includes a bone age radiograph if the adolescent is not sexually mature and a chronic disease panel (complete blood count and differential, sedimentation rate, comprehensive metabolic panel, celiac panel, thyroid panel, cortisol and insulin-like growth factor-I levels, and urinalysis).

B. Breast development ordinarily signifies the onset of pubertal feminization. However, mature breast development does not ensure ongoing pubertal estrogen secretion (see Figures 15-5 and 15-6).

C. BMI < 10th percentile generally corresponds to a body composition that is < 20% body fat, which is the critical factor.

D. BMI may not accurately reflect body fat in serious athletes (who have a disproportionately greater muscle mass) or bulimia nervosa.

E. FSH is preferentially elevated over LH in primary ovarian failure. The most common cause of primary amenorrhea due to primary ovarian failure is gonadal dysgenesis due to Turner syndrome, but acquired causes must be considered (such as cytotoxic therapy). The workup of primary ovarian failure is considered in detail in the next algorithm (Figure 15-5, secondary amenorrhea and oligomenorrhea). Lack of FSH elevation virtually rules out primary ovarian failure only when the bone age is appropriate for puberty (11 years or more).

F. "Pediatric" gonadotropin assays sensitive to ≤ 0.15 U/L are critical to the accurate diagnosis of gonadotropin deficiency and delayed puberty. A low LH level is more characteristic of these disorders than a low FSH level. Congenital gonadotropin deficiency is closely mimicked by the more common extreme variation of normal, constitutional delay of puberty.

G. History and examination may yield clues to the cause of hypogonadotropic hypogonadism, such as evidence of hypopituitarism (midline facial defect, extreme short stature, visual field defect), anosmia (Kallmann syndrome), or functional hypothalamic disturbance (bulimia, excessive exercise). Random LH levels in hypogonadotropic patients are usually below 0.15 IU/L, but they often overlap those of normal pre- and midpubertal children. The GnRH test, measuring the gonadotropin response to a 50- to 100-mg bolus, in the premenarcheal teenager strongly suggests gonadotropin deficiency if the LH peak is less than 4.2 IU/L by monoclonal assay. However, the GnRH test has limitations because of overlap between hypogonadotropic and normal teenager responses. GnRH agonist testing (e.g., leuprolide acetate injection 10 mg/kg SC) may discriminate better. It may not be possible to definitively establish the diagnosis of gonadotropin deficiency until puberty fails to begin by 16 years of age or progress at a normal tempo.

H. Plasma total testosterone is normally 20 to 60 ng/dL (0.7 to 2.1 nM) in women, 300 to 1200 ng/dL in men, but varies somewhat among laboratories. Plasma free (or bioavailable) testosterone is about 50% more sensitive than total testosterone in detecting hyperandrogenemia. However, there are many pitfalls in testosterone assays at the low levels common among women, reliable testosterone assays are not available to many physicians, and assaying the free testosterone introduces other potential sources of error. Therefore, it is reasonable to begin the evaluation with a total testosterone determination if a free testosterone test in a reliable specialty laboratory is not available to the practitioner.

I. Androgen resistance is characterized by a frankly male plasma testosterone level when sexual maturation is complete, male karyotype (46,XY), and absent uterus. External genitalia may be ambiguous (partial form) or normal female (complete form).

J. The differential diagnosis of hyperandrogenism is shown in a later algorithm (see Figure 15-7).

K. Vaginal aplasia in a girl with normal ovaries may be associated with uterine aplasia (Rokitansky-Kustner-Hauser syndrome). When the vagina is blind and the uterus aplastic, this disorder must be distinguished from androgen resistance. If the external genitalia are ambiguous, it must be distinguished from other disorders of sex development (intersex).

L. Secondary amenorrhea differential diagnosis is presented in Figure 15-42.

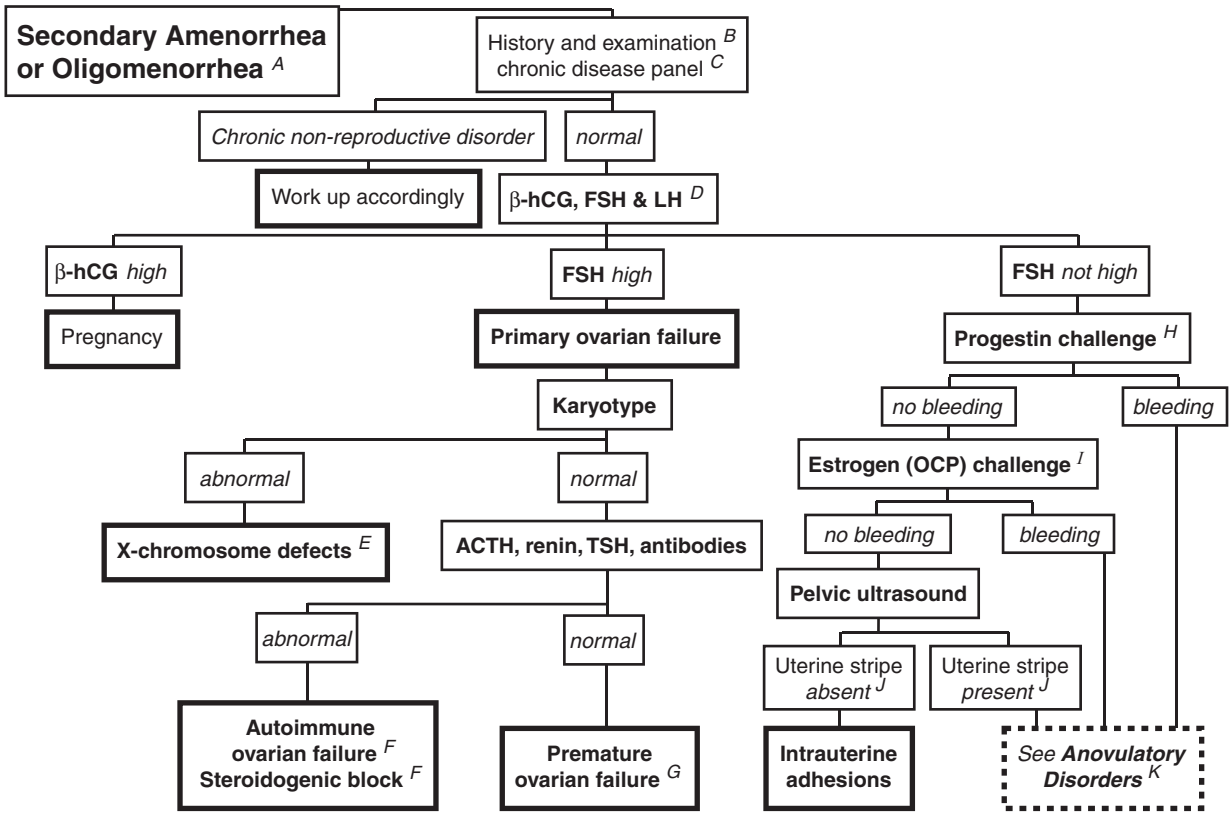


FIGURE 15-42 ■ Differential diagnosis of secondary amenorrhea or oligomenorrhea. (Modified with permission from Rosenfield, R. L. (2003). Menstrual disorders and hyperandrogenism in adolescence. In S. Radovick, & M. H. MacGillivray (Eds.), *Pediatric endocrinology: a practical clinical guide* (pp. 451–478). Totowa, NJ: Humana Press.)

A. Mature secondary sex characteristics are characteristic because the occurrence of menarche indicates a substantial degree of development of the reproductive system.

B. Diverse disorders of many systems cause anovulation. The history may reveal excessive exercise, symptoms of depression, gastrointestinal symptoms, radiotherapy to the brain or pelvis, or rapid virilization. Physical findings may include hypertension (forms of congenital adrenal hyperplasia, chronic renal failure), short stature (hypopituitarism, Turner syndrome, pseudohypoparathyroidism), abnormal weight for height (anorexia nervosa, obesity), decreased sense of smell (Kallmann syndrome), optic disk or visual field abnormality (pituitary tumor), cutaneous abnormalities (neurofibromatosis, lupus), goiter, galactorrhea, hirsutism, or abdominal mass.

C. In the absence of specific symptoms or signs to direct the workup, evaluation for chronic disease in a sexually mature adolescent typically includes complete blood count and differential, sedimentation rate, comprehensive metabolic panel, celiac panel, thyroid panel, cortisol and insulin-like growth factor 1 levels, and urinalysis.

D. "Pediatric" gonadotropin assays sensitive to ≤ 0.2 U/L are critical to the early diagnosis of many anovulatory disorders.

E. Patients missing only a small portion of an X chromosome may not have the Turner syndrome phenotype. Indeed, among 45,X patients, the classic Turner syndrome phenotype is found in less than one third (with the exception of short stature in 99%). Ovarian function is sufficient for about 10% to undergo some spontaneous pubertal development and for 5% to experience menarche. If chromosomal studies are normal and there is no obvious explanation for the hypogonadism, special studies for fragile X premutation and autoimmune oophoritis should be considered.

F. Autoimmune ovarian failure may be associated with tissue-specific antibodies and autoimmune endocrinopathies such as chronic autoimmune thyroiditis, diabetes, adrenal insufficiency, and hypoparathyroidism. Nonendocrine autoimmune disorders may occur, such as mucocutaneous candidiasis, celiac disease, and chronic hepatitis. Rare gene mutations causing ovarian insufficiency include steroidogenic defects that affect mineralocorticoid status (17-hydroxylase deficiency is associated with mineralocorticoid excess and lipoid adrenal hyperplasia with mineralocorticoid deficiency) and mutations of the gonadotropins or their receptors. Ovarian biopsy is of no prognostic or therapeutic significance. LH is disproportionately high in steroidogenic defects or autoimmune disease specifically affecting theca cells.

G. The history may provide a diagnosis (e.g., cancer chemotherapy or radiotherapy). Other acquired causes include surgery and autoimmunity. Chromosomal causes of premature ovarian failure include X-chromosome fragile site and point mutations. Other genetic causes include gonadotropin-resistance syndromes such as LH or FSH receptor mutation and pseudohypoparathyroidism. A pelvic ultrasound that shows the preservation of ovarian follicles carries some hope for fertility.

H. Withdrawal bleeding in response to a 5- to 10-day course of progestin (e.g., medroxyprogesterone acetate 10 mg HS) suggests an overall estradiol level greater than 40 pg/mL. However, this is not entirely reliable, thus in the interest of making a timely diagnosis it is often worthwhile to proceed to further studies.

I. A single cycle of an OCP containing 30 to 35 mg ethinyl estradiol generally suffices to induce withdrawal bleeding if the endometrial lining is intact.

J. A thin uterine stripe suggests hypoestrogenism. A thick one suggests endometrial hyperplasia, as may occur in polycystic ovary syndrome.

K. The differential diagnosis of other anovulatory disorders continues in Figure 15-43.

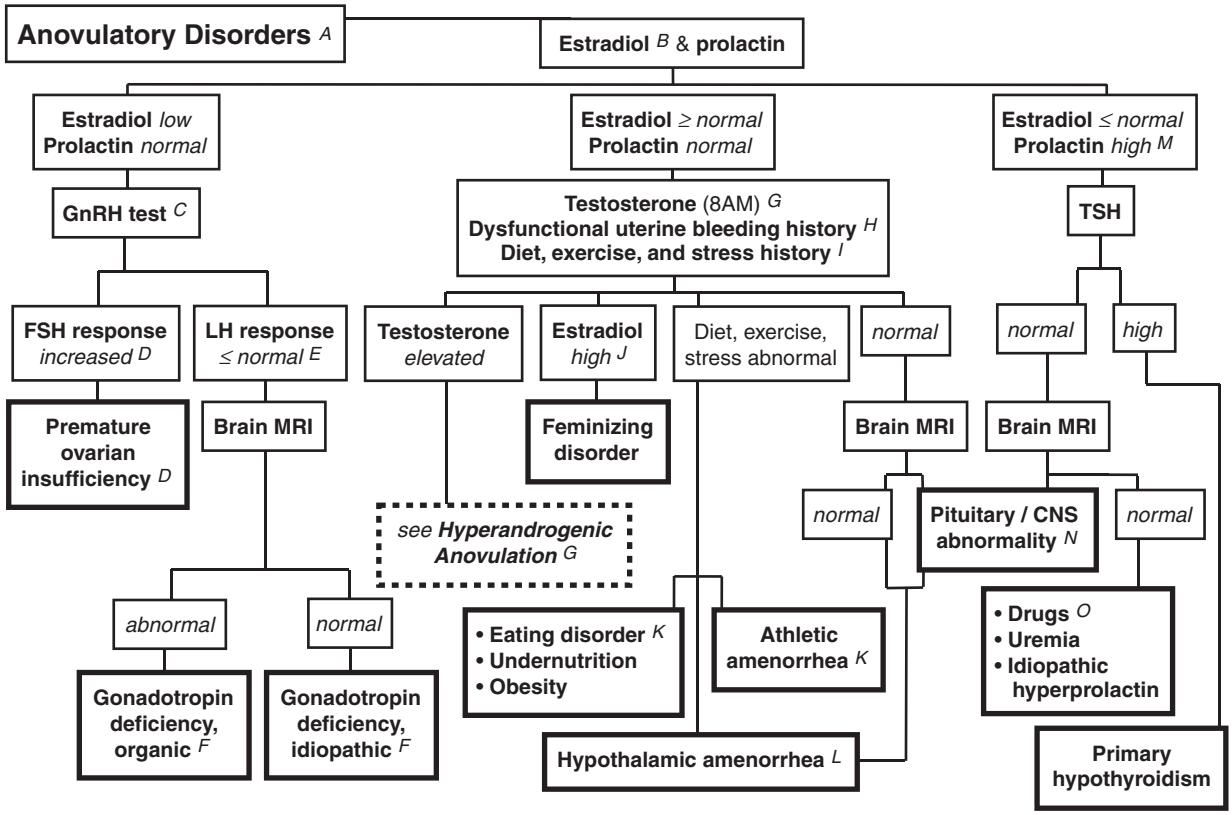


FIGURE 15-43 ■ **Differential diagnosis of anovulatory disorders.** (Modified with permission from Rosenfield, R. L. (2003). Menstrual disorders and hyperandrogenism in adolescence. In S. Radovick, & M. H. MacGillivray (Eds.), *Pediatric endocrinology: a practical clinical guide* (pp. 451–478). Totowa, NJ: Humana Press.)

A. Anovulatory disorders should be considered in any girl with unexplained amenorrhea or oligomenorrhea, irregular menstrual bleeding, short cycles, or excessive menstrual bleeding. The workup in this algorithm progresses from negative studies in the Figure 15-5 algorithm.

B. Once breast development has matured, the breast contour does not substantially regress when hypoestrogenism develops. Hypoestrogenism is suggested if plasma estradiol is persistently < 40 pg/mL in a "pediatric" assay sensitive to < 10 pg/mL. However, a single estradiol level may be misleading because of cyclic or episodic variations.

C. Gonadotropin-releasing hormone (GnRH) testing is usually performed by assaying LH and FSH before and 0.5 hour after the administration of 1 μg/kg GnRH intravenously. GnRH agonist testing may alternatively be performed by administering 10 μg/kg leuprolide acetate subcutaneously and assaying LH and FSH at 3 to 4 hours to assess gonadotropin reserve and at 18 to 24 hours to assess the ovarian steroid response to endogenous gonadotropin release.

D. Baseline gonadotropin levels may be normal as the ovary begins to fail, as in early menopause, but an exaggerated FSH response to GnRH and subnormal E2 response to the gonadotropin elevation induced by acute GnRH agonist challenge are characteristic. The further workup is shown in Figure 15-5.

E. LH responses to GnRH may vary from nil to normal in gonadotropin deficiency: normal LH and FSH responses in the presence of hypoestrogenism indicate inadequate compensatory hypothalamic GnRH secretion.

F. Gonadotropin deficiency may be congenital or acquired, organic or functional. Congenital causes include midline brain malformations or specific genetic disorders such as Prader-Willi syndrome, Laurence-Moon-Biedl syndrome, or Kallmann syndrome. Kallmann syndrome, the association of anosmia with gonadotropin deficiency, occurs in both the X-linked and autosomal-recessive forms. Special MRI views often demonstrate the absence of the olfactory tracts. Acquired gonadotropin deficiency may be secondary to a variety of organic CNS disorders, varying from hypothalamic-pituitary tumor to radiation damage to empty sella syndrome. Autoimmune hypophysitis is a rare disorder, sometimes accompanying a polyendocrine deficiency syndrome. The prototypic form of functional gonadotropin deficiency is anorexia nervosa. Idiopathic hypogonadotropic deficiency may sometimes occur in families with anosmia, suggesting a relationship to Kallmann syndrome.

G. Plasma free (or bioavailable) testosterone is about 50% more sensitive than total testosterone in detecting hyperandrogenemia. However, there are many pitfalls in testosterone assays at the low levels observed in women, reliable testosterone assays are not available to many physicians, and assaying the free testosterone introduces other potential sources of error. Therefore, it is reasonable to begin the evaluation with a total testosterone determination if a free testosterone test in a reliable specialty laboratory is not available to the practitioner. Simultaneous assay of 17-hydroxyprogesterone is indicated in subjects at high-risk for congenital adrenal hyperplasia, such as Ashkenazi Jews. A differential diagnosis of hyperandrogenic evaluation is outlined in Figure 15-46.

H. Dysfunctional uterine bleeding or menorrhagia not controlled by progestin or OCP therapy additionally requires a pelvic ultrasound examination (for genital tract tumor or feminizing tumor), coagulation workup (which includes platelet count, prothrombin time, thromboplastin generation test, bleeding time, and von Willebrand factor), and consideration of the possibility of sexual abuse.

I. The equivalent of 4 miles per day or more is generally required before body fat stores fall to the point where amenorrhea occurs. Physical or psychosocial stress may cause amenorrhea.

J. The normal range for estradiol over the menstrual cycle is wide: values > 95 pg/mL usually indicate the preovulatory or luteal phase but are compatible with a feminizing disorder.

K. Mild forms of stress disorders associated with low body fat (anorexia nervosa, bulimia nervosa, and athletic amenorrhea) may cause acquired hypothalamic amenorrhea rather than frank gonadotropin deficiency. The low body fat content of athletic amenorrhea may not be reflected by weight for height because of high muscularity. A dual-photon absorptiometry scan may be useful for documenting body fat below 20%.

Patients with anorexia nervosa may become amenorrheic before or when weight loss begins, indicating an important psychological component to the etiology. Obesity is also associated with anovulatory cycles and raises the possibility of Cushing syndrome.

L. Hypothalamic amenorrhea is a diagnosis of exclusion. It is a form of partial gonadotropin deficiency in which baseline estrogen secretion is normal but a preovulatory LH surge cannot be generated. It may result from organic CNS disorders or be functional, due to stress, undernutrition or obesity, diverse types of endocrine dysfunction, chronic disease, or idiopathic causes. It may be difficult to distinguish from hyperandrogenemia.

M. Hyperprolactinemia is heterogeneous in its presentation. Some have normoestrogenic anovulation, which may be manifest as hypothalamic anovulation, hyperandrogenism, dysfunctional uterine bleeding, or short luteal phase. On the other hand, some are hypoestrogenic; these do not have galactorrhea.

N. Large hypothalamic-pituitary tumors or other types of CNS injury cause variable pituitary dysfunction, which may include complete or partial gonadotropin deficiency and various manifestations of hypopituitarism (including secondary hypothyroidism). If they interrupt the pituitary stalk, hyperprolactinemia ensues. Hyperprolactinemia may also be caused by prolactinomas.

O. Drugs, particularly neuroleptics of the phenothiazine or tricyclic type, may induce hyperprolactinemia.

BOX 15-5 Features That Distinguish Gonadotropin Deficiency from Constitutional Delay of Puberty

In a healthy delayed prepubertal girl with BA >11 years and prepubertal FSH, gonadotropin deficiency is:

- Possible if:
  - Weight loss greater than 5% to 8% (BMI < 10th to 15th percentile for height age)
  - Midline facial defect
  - CNS dysfunction
  - CT or MRI brain scan abnormal
- Probable if:
  - BA > 13 years and LH < 0.15 U/L in early daytime
  - Anosmia or panhypopituitarism
- Diagnostic if:
  - Sleep-associated increase in LH lacking
  - GnRH agonist test subnormal response
  - Chronologic age >16 years

Modified with permission from Rosenfield, R. L., Barnes, R. B. (1993). Menstrual disorders in adolescence. Endocrinol Metab Clin N Am, 22, 491–505.

estrogen status. The prolactin level correlates with the size of prolactinomas, and a level over 200 ng/mL is typical of a macroprolactinoma. A prolactin level that does not correlate with the size of a large pituitary tumor suggests that either the tumor is not a prolactinoma and is causing a functional pituitary stalk section or it is a macroprolactinoma elaborating such high levels of prolactin as to artifactually lower the immunoassayable prolactin level by a "hook effect."[786] Very high blood or cerebrospinal fluid prolactin levels suggest invasiveness. The workup for this should include formal testing of visual fields (Goldman perimetry or evoked response). Pituitary microadenomas may be "incidentalomas" of no clinical significance, judging from an approximate 10% incidence in autopsy material.[787] However, they require careful assessment of pituitary function and follow-up.[788] Macroprolactinemia should be considered in the absence of clearly related symptoms and when the MRI is negative or in the setting of autoimmune disease.[776,777] Macroprolactinemia is confirmed when the prolactin level measured after precipitation of serum using polyethylene glycol is normal (or substantially reduced compared to the level measured in untreated serum.)

Imaging studies are important ancillary measures. Pelvic ultrasound may demonstrate hypoplastic ovaries, endometrial hypoplasia or disorders, or polycystic ovaries. Magnetic resonance imaging of the hypothalamic-pituitary area is important in the workup of gonadotropin deficiency, hyperprolactinemia, and hypothalamic anovulation.

Anorexic patients require psychiatric evaluation and consideration of brain tumor and partial bowel obstruction. Diet faddism and athletic addiction may be difficult to distinguish from anorexia nervosa. Constitutionally thinness is a variant of normal with normal menses and a distinctive hormonal profile.[789] It is unclear whether the superior mesenteric artery syndrome is a primary disorder that mimics anorexia nervosa or is a complication of it.[790]

Management

Underlying disorders must be treated appropriately. For example, tumors require surgery or radiotherapy. For prolactinoma, dopaminergic treatment is the initial treatment of choice unless the patient's condition or eyesight is critical.[778,791] Hyperprolactinemia will be maximally suppressed within 1 month and the menstrual cycle normalized within 3 months by an effective dopaminergic agonist regimen. Cabergoline 0.5 to 1 mg once or twice weekly will usually control galactorrhea and shrink prolactinomas.[775,792] To minimize nausea it is best to start with a low dose at bedtime. Data implicate cabergoline as increasing the risk of cardiac valve regurgitation about 5-fold in the elderly, albeit at generally 10-fold or higher doses than used to treat hyperprolactinemia. Bromocriptine does not activate the serotonin 5-HT(2B) receptor, the proposed mechanism through which cabergoline is thought to stimulate valve hypertrophy, and so bromocriptine does not seem to be associated with an increased risk of cardiac valve regurgitation and may be considered as an alternative to cabergoline treatment, albeit a less-effective one. The usual bromocriptine maintenance dose is 0.25 to 0.5 mg twice daily. After 2 years of treatment, if the prolactin level is normal and there is no tumor evident on MRI,

dopamine agonist may be tapered and possibly discontinued. Prolactin levels are then measured to monitor for recurrance.[793]

Anorexia nervosa is best managed by an experienced multidisciplinary team. Refeeding is the first priority, and once steady weight gain is evident, the psychodynamic issues can be addressed.[794] Family therapy of medically uncomplicated cases of anorexia nervosa on an outpatient basis generally yields the best results, with good improvement in over half of patients. Inpatient intervention for rehydration and metabolic stabilization or failure of weight gain with ongoing cachexia may be required at any time. Menses resumes when psychotherapy is effective and body fat is restored to normal (see Figure 15-40). The induction of menses by estrogen-progestin replacement is usually injudicious because it provides a false sense of recovery and does not yield the recovery of bone loss that occurs with weight gain.[795] Although the acute episode can usually be successfully treated, there is a high rate of ongoing psychiatric disability and medical complications. Anorexia nervosa "by proxy" has been described in the offspring of former patients.[796]

There are two aspects of therapy that are uniformly involved in managing hypogonadism: psychological support and hormone administration. Patients with delayed development that is a variation of normal should be reassured that there is nothing wrong, only a delay in timing of the onset of puberty. The wide normal variation in the pattern and time of the pubertal growth spurt should be explained in detail and the girl should be informed of her predicted eventual height. Most children with delayed puberty do not have overt psychological symptoms. Complex compensations and sublimations obviously occur. However, peer group pressures may make adjustment to sexual infantilism especially difficult when the child approaches age 13,[797] and a poor self-image may lead to social withdrawal and feelings of hopelessness. Physical immaturity may prolong psychological immaturity. A 6- to 12-month course of physiologic sex hormone therapy at this time may help alleviate these anxieties. The physician should discuss the fact, when the evidence favors it, that the odds are overwhelmingly in favor of the "timer in the subconscious area of the brain" eventually turning on. When this will happen can be approximated from the skeletal age. One should not hesitate to advise more intensive psychological counseling if it becomes apparent that the concern about puberty is but one aspect of a more general maladjustment. Ultimately the decision as to whether to undertake treatment for delayed puberty is up to the patient and her family.

It is important to assure the teenager with an organic basis for hypoestrogenism that feminization will occur, although in response to appropriate hormone treatment. Some genetic forms of gonadotropin deficiency are actually reversed by sex steroid therapy.[221] However, most will benefit from lifelong hormone replacement therapy. It should be kept in mind that attainment of normal breast development in the girl with panhypopituitarism requires replacement of GH and cortisol deficits. It is difficult, however, to induce secondary sex characteristics in some patients with systemic chronic inflammatory disease such as lupus erythematosus.

In patients in whom short stature is an important concern, as in Turner syndrome, growth potential must be considered before undertaking estrogen replacement. GH therapy improves the adult height potential of patients with Turner syndrome, especially when started as soon as growth failure becomes apparent.[798] GH therapy in the United States is generally initiated at a dose of 0.375 mg/kg per week as approved by the U.S. Food and Drug Administration (FDA). In older girls, or those with extremely short stature, therapy with oxandrolone (2-oxo-17α-methyldihydrotestosterone [Anavar]) 0.05 mg/kg/day, which augments GH action, can be considered.[799] Clitoromegaly is ordinarily negligible on this dosage; liver function should be monitored.

Two controlled studies have shown that pubertal estrogen replacement therapy is safe and efficacious when started as young as 11 to 12 years of age using very low estrogen doses in conjunction with growth hormone therapy in Turner syndrome.[800,801] Both therapeutic regimens, one utilizing monthly depot E2 and the other using daily oral ethinyl E2, maximized growth potential and age-appropriate feminization. However, the doses used were far below those available by prescription. The estrogen content of oral contraceptive pills is supraphysiologic for the induction of breast development or for linear growth.

We favor the use of one of the following hormone replacement regimens. Whichever form of estrogen is used, pubertal development and growth should be monitored every 6 months, with bone age determinations at 6- to 12-month intervals to avoid unanticipated loss of growth potential.

Intramuscular depot E2 in a starting dose of 0.2 mg/month will usually induce breast budding; the dose should be increased by 0.2 mg every 6 months.[800] A midpubertal dose of 1 to 1.5 mg monthly, which is half the adult replacement dose, typically induces menarche within 1 year. An alternative oral regimen begins with 5 μg/kg micronized E2 (Estrace, 0.25 mg for a 50 kg girl) daily; the adult replacement dose is 1 to 2 mg/day.[802]

Transdermal E2 is a convenient, physiologic form of therapy[803] that appears to have long-term health advantages over commonly used oral estrogens,[524,525] but there are little data on its use for inducing puberty. We recommend starting transdermal feminization with 25 μg daily for 1 week per month, a marginally feminizing dose that is in accord with current guidelines,[798] and escalating at 6-month intervals to an adult dose at 3 years. A suggested protocol for female pubertal induction for hypogonadal patients is provided in Table 15-7.[802]

For girls with hypogonadism and an intact uterus, cyclic progestin should be added after 2 years of estrogen therapy or when bleeding begins to occur at unpredictable times. A simple regimen is to use 100 mg of micronized progesterone (Prometrium) at bedtime for 7 to 14 days during the second to third week of estrogen therapy or equivalent doses of medroxyprogesterone acetate (5 to 10 mg/day) or norethindrone acetate (5 mg/day). This will bring about normal menstruation during the week

TABLE 15-7 **Suggested Pubertal Transdermal Estradiol Replacement Regimen Beginning at 11 years of Age***

Age	Estradiol Dose
0-6 months	25 μg, day 1-7 each month
6-12 months	25 μg, day 1-14 each month
12-18 months	25 μg, day 1-21 each month
18-24 months	37.5 μg, day 1-21 each month
2-2.5 years	28-day cycle: 50 μg day 1-21 and Prometrium 100 mg† day 12-21 every 28 days
	OR
	Continuous: 50 μg day 1-14, then CombiPatch (50 μg estradiol/0.14 mg norethindrone) day 15-28 every 28 days
2.5-3 years	28-day cycle: 75 μg day 1-21 and Prometrium 100 mg day 12-21 every 28 days
	OR
	Continuous: 75 μg day 1-14 then CombiPatch (50 μg estradiol/norethindrone) day 15-28 every 28 days
3-3.5 years	28-day cycle: 100 μg day 1-21 and Prometrium 100 mg day 12-21 every 28 days
	OR
	100 μg day 1-14 then CombiPatch (50 μg estradiol/norethindrone) day 15-28 every 28 days
> 3.5 years	Continue regimen or offer oral contraceptive pill

*In children ≥ 13 years old, consider starting with 25 μg for 2 to 3 weeks monthly and increasing the dose at shorter intervals (e.g., 3 months). †If inadequate bleeding, increase Prometrium to 200 mg day 12-21 or change to CombiPatch 50 μg E2/0.25 mg norethindrone.

Modified from Rosenfield, R., Kiess, W., & Keizer-Schrama, S. (2006). Physiologic induction of puberty in Turner syndrome with very low-dose estradiol. In C. Gravholt, & C. Bondy (Eds.), Wellness for girls and women with Turner syndrome *(pp. 71–79). Amsterdam: Elsevier Science.*

preceding the resumption of estrogen therapy. The addition of progestin will decrease the risk of endometrial hyperplasia and endometrial carcinoma, but premenstrual symptoms should be anticipated.

Once optimal height is achieved, most patients prefer to switch to birth control pills as a convenient form of estrogen-progestin therapy. The pills containing the lowest dose of estrogen that will result in normal menstrual cycles are advisable. The potential risks of oral contraceptives must be kept in mind when counseling adolescents.[804] The lowest estrogen dosages currently available in combination contraceptive pills in the United States contain 20 μg (Mircette) to 30 μg (Yasmin) ethinyl E2. Low-dose androgen replacement has been controversial but may confer benefits to body composition, cognition, bone mineralization, and libido.[805]

Hypogonadotropic patients can achieve ovulation with gonadotropin therapy. Hypothalamic GnRH deficiency can be successfully treated by pulsatile GnRH.[89,806] Because several genes in the GnRH signaling system are expressed in the gonads, some hypogonadotropic patients have primary defects in gonadal function.[728,807] Induction of ovulation is best carried out by a gynecologist specializing in reproductive endocrinology.

Patients with primary ovarian failure usually require oocyte donation and in vitro fertilization to successfully achieve pregnancy.[691,694,808] Activation of residual follicle growth in autografts has recently been accomplished, with successful pregnancy after embryo transfer.[808a] Turner syndrome patients are, however, at high risk for obstetrical complications in the areas of uterine anomalies, carbohydrate intolerance, and cardiovascular complications. Oocyte cryopreservation and ovarian tissue cryopreservation and transplantation have been explored to preserve fertility in patients with gonadal dysgenesis and disorders requiring cytotoxic chemotherapy or gonadectomy.[809-811] There has been some success with these experimental procedures, but outcomes data are sparse. Feasibility is a limitation in children because of time constraints and the need for ovarian stimulation.[709] Female germline stem cell research holds promise as a future fertility treatment.[38] Up-to-date information for physicians and patients can be found through the Oncofertility Consortium (myoncofertility.org).

Nonhypoestrogenic Menstrual Disturbances

Hypothalamic Anovulation

Hypothalamic anovulation causes menstrual disturbances in sexually mature women through a deficiency in GnRH secretion that is too subtle to cause frank hypoestrogenism. The neuroendocrine system stimulates ovarian estrogen secretion to a level normal for an early- or midfollicular phase female, but follicular development is inadequate for a normal dominant follicle to emerge. Amenorrhea or oligomenorrhea may result. However, in some patients sufficient estrogenization occurs to cause dysfunctional uterine bleeding, which is discussed in the next section.

Reduced LH pulsatility[812] and failure to generate a midcycle LH surge[813,814] are characteristic. The pathophysiology seems to be mediated primarily by undernutrition or CRH excess. Negative energy balance may be present even in patients of normal, but less than average, weight and fat stores.[418,769] Leptin deficiency is an important determinant of the decreased LH pulsatility. Ghrelin may play a role in LH inhibition.[815] The anovulation of psychic or physical stress seems to involve CRH excess.[770] In the brain, CRH releases β-endorphin from proopiomelanocortin, and the endorphin in turn inhibits GnRH release. Naloxone blockade of opioid action normalizes gonadotropin secretion.[89] In the pituitary, CRH increases the set point for ACTH release. This brings about a new steady state of increased cortisol secretion. Further ACTH response to CRH is blunted by the negative feedback of this cortisol excess. The result is a mildly Cushingoid cortisol rhythm. Cortisol excess itself can contribute to the amenorrhea by inhibiting the response to GnRH,[816] as well as antagonizing some sex hormone actions. Adrenal androgens are elevated in competitive athletes who maintain body fat stores.[817]

Causes. *Functional hypothalamic amenorrhea* is the term commonly used to describe hypothalamic anovulation that is unexplained by organic CNS disorders or chronic disease. The endocrine features of these patients resemble those of patients with the athletic or psychogenic types of hypothalamic anovulation. Twenty-four percent of women in one series had a history of delayed menarche.[573] A primate model indicates that hypothalamic anovulation develops in stress-sensitive individuals from an innocuous combination of mild stress and mild caloric restriction.[818] Heterozygosity for genes associated with Kallmann syndrome has been identified as a predisposing factor in 13% of cases; more than half of those in this subgroup had a family history of hypothalamic amenorrhea or delayed puberty.[573]

Athletic amenorrhea is the term given to hypothalamic anovulation associated with excessive exercise related to low body fat stores. The female athletic triad consists of menstrual disturbance, eating disorder, and osteoporosis.[819] Primary or secondary amenorrhea, oligomenorrhea, or short luteal phase are common in athletes.[820] Ovarian function decreases approximately in proportion to the amount of physical activity and dietary restriction. Weight-bearing exercise only partially protects against the effects of hypoestrogenism on weight-bearing bone. There is concern that amenorrheic athletes may be left with a permanent deficit in bone mass.[821] Weight loss to 10% below ideal body weight and body fat less than 12% are risk factors for amenorrhea. Body mass index does not accurately reflect body fat stores in athletes.[822] Energy balance seems to be more critical than low body fat stores in mediating the anovulation.[820,823] Menarche may occur or menses may resume when the athlete's activity level suddenly decreases and before weight gain occurs. Other factors also contribute to amenorrhea. Nutritional deficiencies may coexist. Chronic undernutrition may suppress thyroid function as in anorexia nervosa.[770] Athletic amenorrhea resembles anorexia nervosa in patients' obsession with weight control.[820,824]

Psychogenic amenorrhea from severe psychic stress has long been known (e.g., "boarding-school amenorrhea").[825] The onset of psychogenic amenorrhea may be identified as being associated with a discrete event, but the ovarian dysfunction tends to be long lasting. Subtle nutritional deficits contribute.[418]

Epilepsy causes menstrual disturbances that seem to result from abnormal neuroendocrine regulation independently of drug treatment.[826]

Pseudocyesis is an extremely rare form of psychogenic amenorrhea that is due to the persistence of the corpus luteum. This syndrome tends to occur in infertile women with an overwhelming desire for pregnancy and conversion hysteria. Prolactin and LH excess appear to mediate this rare syndrome.[827]

Differential Diagnosis. *Disorders outside the neuroendocrine-gonadal axis* may cause or mimic hypothalamic anovulation. These include pregnancy, nutritional disturbance, glucocorticoid excess, disturbed thyroid function, drug abuse, chronic illness, hyperprolactinemia, and ectopic gonadotropin secretion.

Pregnancy must be excluded in all sexually mature adolescents with amenorrhea. An elevation of the serum β-hCG level is the earliest laboratory sign.[828] Placental hCG initially drives constant overproduction of estrogens and progestins by the maternal corpus luteum, then production of estrogen and other sex steroids shifts to the fetoplacental unit and suppresses maternal pituitary gonadotropin release.

Optimal fat mass is necessary for normal gonadotropin levels in sexually mature women, and both *obesity* and *undernutrition* suppress gonadotropins: thus, the gonadotropin response to relative adipose mass seems biphasic.[266] Obesity is associated with blunted LH pulse amplitude that is partially attributable to the increased LH clearance rate. Overproduction of estrogen from plasma precursors in adipose tissue[378] may play a role in suppressing LH pulsatility.[829] The extent to which sleep disruption may contribute to LH suppression is unclear.[830] The effect of undernutrition seems to be mediated by factors related to energy balance, as discussed under "Hypothalamic Anovulation."

Cushing syndrome (glucocorticoid excess) of any etiology causes anovulation by inhibiting the gonadotropin response to GnRH.[816] *Thyroid hormone deficiency* interferes with gonadotropin action on the ovary[831] and may interfere with endometrial function by influences on steroid metabolism[832] and action.[833] *Drug abuse* with tetrahydrocannabinol, ethanol, or opiates causes hypothalamic anovulation.[834,835] Cocaine causes menstrual irregularity by suppressing gonadotropin secretion through mechanisms that include depletion of dopaminergic stores, resulting in hyperprolactinemia, and stimulation of CRH release.[779,836] *Inflammatory illness* acutely disrupts the E2-induced LH surge,[837] and chronic illness causes gonadotropin deficiency, which may be mediated partly by undernutrition and partly by cytokines.[188,754] Disorders as diverse as *diabetes mellitus* and *iron overload* all affect GnRH secretion.[838,839] *Chronic renal failure* causes complex dysfunction of the reproductive system, including poor clearance of

gonadotropins and prolactin in the presence of an inhibition of gonadotropins by a nondialyzable factor.[840]

Hyperprolactinemia occasionally causes secondary amenorrhea without frank hypoestrogenism.[841] This situation probably results from a mild diminution in FSH secretion that only inhibits the emergence of a dominant follicle and, therefore, ovulation.

Post-pill amenorrhea has been a term applied to the amenorrhea that sometimes follows the long-term use of hormonal contraceptives. In the past this was attributed to oversuppression, but oversuppression should not be expected to be the case with the current generation of oral contraceptives.[292] About one third of patients with secondary amenorrhea after discontinuation of estrogen and progestin-containing pills have a history of previous menstrual disturbance and ongoing menstrual problems.[842] Another third can expect spontaneous remission of the amenorrhea. In about half of the remaining cases, the patient's menstrual disturbance will resolve after induced pregnancy. The most common cause of post-pill amenorrhea is probably hyperprolactinemia, because in over 20% of such cases the patients have galactorrhea. How often this antedates ingestion of the contraceptive pill is unknown. Menses may be restored in normoprolactinemic cases by dopaminergic treatment, which suggests that in such cases there is excessive pituitary prolactin secretion that is too subtle to be detected by measurement of serum levels.[843] The anovulation resulting from depot-medroxyprogesterone acetate contraception is related to the extremely slow rate of absorption and metabolism of this steroid; menses returns when the blood levels of this progestin fall below the threshold for suppression of the LH surge,[844] and only rarely has it been associated with disturbed prolactin secretion.[845]

Gonadotropin or hCG secretion by a *tumor* can cause normoestrogenic or hyperestrogenic anovulation.[846,847] In one LH-producing tumor, sex steroid levels were normal; the lack of virilization was attributed to ovarian desensitization to LH,[846] whereas in another case, virilization occurred, which was attributed to preexisting polycystic ovary syndrome-hyperthecosis and extreme LH elevation.[848] Other hyperestrogenic disorders that cause anovulatory bleeding are discussed under "Precocious Puberty."

Hypothalamic anovulation is ordinarily a diagnosis of exclusion. The medical evaluation should be performed as discussed in the preceding section, with particular attention paid to the possibilities of emotional stressors, excessive exercise, the use of birth control pills or other drugs, and state of health. The physical examination should be particularly directed to the state of nutrition, the possibilities of intracranial or systemic disease, galactorrhea, thyroid dysfunction, glucocorticoid excess, hirsutism, and obesity. If this workup is negative, an MRI of the hypothalamic-pituitary area is indicated. Hypothalamic anovulation may be documented by demonstrating subnormal LH pulse frequency, but this is not generally practical. Leptin levels tend to be low but nondiagnostic.[769] The response to a GnRH agonist test is normal but seems to lack the normal priming response to repeat testing.[849] Dysfunctional uterine bleeding from hypothalamic anovulation must be distinguished from that due to other causes (see next section).

Management. Many patients with hypothalamic anovulation will benefit from nutritional counseling. Diet faddists and athletes should be advised about the necessity of optimal body energy reserves for the maintenance of normal menstrual cycles (see Figure 15-40). The teleologic significance of this factor may be pointed out, namely, that inherent in the evolutionary process is the inhibition of pregnancy in times of inadequate food supplies. Ongoing psychological counseling is advisable for patients who cannot change their dietary or exercise patterns because of an abnormal body image. Estrogen replacement only partially corrects osteoporosis unless nutrition is optimized.[795] Obese girls should be advised that there is a substantial possibility that reduction to a normal weight will restore menses and improve the probability of fertility.

Mature teenagers whose amenorrhea is unexplained should be assured that they have a high likelihood of fertility with appropriate endocrinologic treatment. However, such treatment is unlikely to be of any benefit to them until such time as they desire to become pregnant. In the meanwhile, the main objective of therapy is to normalize the endometrial cycle by periodic progestin administration. For this purpose, progestin (micronized progesterone 100 to 200 mg orally at bedtime for 14 consecutive days) usually is effective in inducing withdrawal periods. During the first few years after menarche, it is reasonable to administer this treatment on alternate months to allow detection of late maturation of a regular menstrual cycle.

Induction of an ovulatory cycle has been reported to occasionally result in the resumption of spontaneous normal menses.[842] An ovulatory cycle can normally be induced by the administration of clomiphene citrate once nightly for five doses. If treatment is successful, bleeding generally occurs about 1 month from commencement of the treatment. One should start with the 50-mg dose, because larger doses may cause hyperstimulation of the ovaries with the development of ovarian cysts. For this reason, an ultrasound examination should be performed to rule out cystic ovaries before the patient progresses successively to a 100 to 150 mg dosage. This treatment is not generally recommended in the teenage years, however. Dopaminergic therapy has been reported to be successful in causing the resumption of ovulation in post-pill amenorrhea, modest undernutrition, and other unexplained cases of secondary amenorrhea. Otherwise, induction of ovulation is best left to the endocrinologic gynecologist to supervise at such time as the woman wishes to conceive. The majority of patients with no obvious cause for their secondary amenorrhea will become pregnant after appropriate treatment with estrogen, clomiphene, dopaminergic agonist, human menopausal gonadotropins, or pulsatile GnRH therapy.

Dysfunctional Uterine Bleeding

Causes. Heavy uterine bleeding is usually due to ovulatory dysfunction. It may be abnormally frequent or

intermenstrual ("polymenorrhea"), as indicated by intervals less than 21 days, or excessively prolonged or profuse ("menometrorrhagia"), as indicated by a menstrual flow that lasts more than 7 days or soaks more than one pad or tampon every 1 to 2 hours.[578] Dysfunctional bleeding occurs from an uncycled, hyperplastic endometrium.[685] It is most often a manifestation of physiologic adolescent anovulation. Hyperandrogenism, particularly polycystic ovary syndrome and its variants, is a common cause of dysfunctional bleeding. In some cases it arises from hypothalamic anovulation. Less common are estrogen-producing cysts or tumors, hypothyroidism, hyperprolactinemia, and incipient premature ovarian failure. The workup should, therefore, include measurement of serum androgen, prolactin, thyroid, and gonadotropin levels.

Corpus luteum insufficiency may present as short (less than 21 days) or infertile *ovulatory* cycles. The immediate cause of an inadequate luteal phase is insufficient progesterone production to sustain sufficient endometrial development to support implantation.[850,851] This in turn may arise from subtle deficiency of LH or FSH during the early follicular phase or an inadequate preovulatory LH surge, resultant incomplete emergence of a dominant follicle, and the subsequent formation of an inadequate corpus luteum. Alternatively, the corpus luteum may not be responsive to LH.[852] Luteal insufficiency is common during early post menarcheal cycles[579] and may otherwise be the result of hyperprolactinemia,[853] obesity,[829] hypothalamic amenorrhea, or hyperandrogenism.

Differential Diagnosis. Dysfunctional uterine bleeding must be distinguished from the other causes of abnormal genital bleeding listed in Box 15-6.[685,854] The possibility that it is pregnancy related must be considered and a pregnancy test performed. Sexual abuse is a prime consideration in recurrent vaginal bleeding. Genital tract or feminizing tumors characteristically cause bleeding that cannot be controlled with cyclic progestin or estrogen-progestin therapy. Menstrual bleeding can be considered to be excessive if it is associated with iron deficiency anemia. Often, abnormally heavy menstrual flow in adolescents is idiopathic ("essential menorrhagia"); it is theorized to result from an imbalance of vasodilating and vasoconstricting prostanoid action on the endometrium.[855] However pathologic causes must be considered because bleeding disorders are present in about 20% of adolescents with menorrhagia requiring hospitalization and in 50% of those presenting at menarche. Patients requiring hospitalization for abnormal bleeding should have a platelet count, prothrombin time, partial thromboplastin time, von Willebrand panel, and bleeding time performed. Transvaginal ultrasound, which is not often feasible in the virginal adolescent, is as reliable as hysteroscopy in determining whether or not the endometrial cavity is normal.

Failure of serum progesterone to rise over 500 ng/dL during the luteal phase is diagnostic of corpus luteum insufficiency with 71% accuracy.[851] However, a higher progesterone level than this must be sustained to transform the endometrium sufficiently to support implantation.

BOX 15-6 Differential Diagnosis of Abnormal Genital Bleeding

- Ovulatory dysfunction ("dysfunctional") uterine bleeding
  - Physiologic anovulation (perimenarcheal)
  - Hyperandrogenism
    —Polycystic ovary syndrome
  - Hyperestrogenism
  - Hypothyroidism
  - Hypothalamic anovulation
    —Hyperprolactinemia
  - Chronic disease
  - Incipient premature ovarian failure
  - Luteal phase defects
- Pregnancy-related uterine bleeding
  - Threatened, missed, or incomplete abortion
  - Molar pregnancy
  - Ectopic pregnancy
- Uterine tumor, polyp, adenomyosis
- Coagulopathy
- Endometrial
  - Idiopathic ("essential menorrhagia")
- Iatrogenic
  - Breakthrough bleeding (intrauterine device or contraceptive pills)
- Vaginal bleeding
  - Trauma
  - Tumor
  - Foreign body
  - Infection

Based on Rosenfield, R. L., & Barnes, R. B. (1993). Menstrual disorders in adolescence. Endocrinol Metab Clin North Am, 22, 491.

Management. Nonsteroidal anti-inflammatory agents may lessen the flow. An estrogen-progestin oral contraceptive with 35 μg ethinyl E2 is first-line treatment to stop dysfunctional bleeding that is acute or associated with anemia. For active bleeding, the dosage is advanced rapidly until bleeding stops, up to four times daily, and then sustained for 7 days. Treatment is then stopped for 5 days and the patient warned that heavy withdrawal bleeding with cramps may occur. Therapy with a low-dose pill, given as for contraception, is then initiated to prevent the recurrence of dysfunctional bleeding and is continued for about three cycles. Hemoglobin should be monitored and supplemental iron prescribed.

Cyclic progestin can be used as an alternative to the oral contraceptive pill to prevent recurrent dysfunctional bleeding in a patient who is not sexually active. Micronized progesterone 100 to 200 mg/day for 1 week is given at 3- to 4-week intervals. After the third month, therapy is stopped and the patient is observed for 1 to 2 months for spontaneous bleeding. If none occurs, the progestin can be given every other month in a dosage of 5 to 10 mg for 7 to 14 days to prevent recurrent dysfunctional bleeding. If in the progestin-free month a normal spontaneous menstrual period occurs, progestins are withheld in the subsequent month to determine if the patient has developed regular ovulatory cycles.

Hemodynamic instability is an indication for hospitalization and treatment with intravenous fluids and blood products as necessary. Premarin can be administered in a

dose of 25 mg intravenously every 3 to 4 hours for three to four doses.

When medical management fails, a bleeding diathesis or uterine structural abnormality should be considered. If heavy bleeding persists, a gynecologist should perform uterine curettage.

Unexplained ("essential") menorrhagia is treated much the same way as dysmenorrhea. The oral contraceptive pill will decrease menstrual blood loss by about 50% in these women. Antiprostaglandins, such as naproxen 500 mg twice a day, decrease blood loss nearly as effectively.

Perimenstrual Symptoms

Dysmenorrhea. Uterine cramping is characteristic of normal ovulatory cycles, apparently as the result of prostaglandin release within the endometrium upon the withdrawal of progesterone. Pain with menses becomes a source of morbidity in 14% of adolescents.[856] When pain is acute and qualitatively different from the usual menstrual pain, *ectopic pregnancy* must be considered.[828,857] An ectopic pregnancy often causes vaginal bleeding that occurs 2½ weeks later than the time of the expected next menstrual period and is typically light. However, the bleeding may be heavy and so resemble an episode of dysfunctional uterine bleeding. Ectopic pregnancy is usually diagnosable by a combination of ultrasonography, a serum β-hCG level over 1000 IU/L, and a progesterone level of < 2500 ng/dL.

In patients with chronic pelvic pain unresponsive to antiprostaglandins or the oral contraceptive pill, *psychological overlay* is possible, but attention should be directed to the possibility of *endometriosis*, *uterine outlet obstruction*, *gynecologic tract masses*, or the poorly defined entity of *vulvodynia*.[858] Ultrasonography and laparoscopy may be indicated to further evaluate these patients. Endometriosis is an estrogen-dependent disorder that accounts for approximately half of the cases of chronic pelvic pain in teenagers.[859] Genetic factors and congenital obstruction of the genital tract predispose to endometriosis, and aberrant E2 formation in endometrial stroma has been incriminated in the pathogenesis.[860] GnRH agonist therapy is approved to provide symptomatic relief, but adolescents are at particular risk for bone loss, and progestin therapy may be an effective alternative.

Dysmenorrhea may be ameliorated by antiprostaglandin therapy. Naproxen (275 mg qid after a 550 mg loading dose) has been shown to be superior to aspirin (650 mg qid) or placebo when begun 2 days before the anticipated onset of menses.[861] The oral contraceptive pill is an alternative that will relieve dysmenorrhea in about 90% of cases by reducing endometrial mass.[685] Because smoking, alcohol intake, and excessive weight are risk factors, lifestyle counseling is advisable.

Premenstrual Syndrome. This is the term applied when cyclic mood changes confined to the second half of the menstrual cycle become debilitating.[862] It is often disruptive to women's personal, social, and occupational function. If symptoms of marked mood swings, depressed mood, anxiety, and irritability occur, it is classified as premenstrual dysphoric disorder.[863] Neuropsychiatric symptoms may include epilepsy[864] and bizarre behavior.[865] These seem to represent aberrant responses to normal cyclic hormonal changes.[866] Subnormal activation of the hypothalamic-pituitary-adrenal axis in response to progesterone has been found.[867] Some evidence indicates that variation in the degree of progesterone metabolism to neuroactive steroids affects the severity of symptomatology.[868] Oral contraceptive therapy with the antimineralocorticoid progestin drospirenone is indicated if psychotropic therapy is unsuccessful. Down-regulation of pituitary gonadotropin secretion by GnRH agonist therapy is efficacious, but its usefulness is limited by the side effects of estrogen deficiency.

The relationship of premenstrual syndrome to rare other luteal phase symptomatology, such as recurrent fever and autoimmune symptoms, may be related to the hyperresponsiveness of cytokines to progesterone.[869,870]

Hyperandrogenism in Adolescence

Hyperandrogenism of a mild to moderate degree is the most common cause of normoestrogenic menstrual disturbances. It is usually due to polycystic ovary syndrome, but the differential diagnosis includes other ovarian or adrenal disorders, abnormal peripheral formation of androgen, and drugs (Box 15-7).[871,872]

BOX 15-7 Causes of Adolescent Hyperandrogenism

Functional Gonadal Hyperandrogenism

- Primary functional ovarian hyperandrogenism (common form of polycystic ovary syndrome)
- Secondary polycystic ovary syndrome
  - Virilizing congenital adrenal hyperplasia
  - Ovarian steroidogenic blocks
  - Syndromes of severe insulin resistance
  - Acromegaly
  - Portohepatic shunting
  - Epilepsy ± valproic acid therapy
  - Disorders of sex development
  - Chorionic gonadotropin excess

Functional Adrenal Hyperandrogenism

- Primary functional adrenal hyperandrogenism (uncommon form of polycystic ovary syndrome)
- Congenital adrenal hyperplasia and related adrenal steroid metabolism disorders
- Prolactin excess
- Dexamethasone-resistant functional adrenal hyperandrogenism
  - Cushing syndrome
  - Glucocorticoid resistance

Peripheral Androgen Overproduction

- Idiopathic hyperandrogenism
- Obesity

Tumoral Hyperandrogenism

Androgenic Drugs

Modified with permission from Buggs, C., & Rosenfield, R. L. (2005). Polycystic ovary syndrome in adolescence. Endocrinol Metab Clin North Am, 34, 677–705.

Causes

Polycystic Ovary Syndrome. Polycystic ovary syndrome (PCOS) is the most common cause of hyperandrogenism presenting at or after the onset of puberty. Ever since its description by Stein and Leventhal as a syndrome of amenorrhea and polycystic ovaries, with hirsutism (57%) or acne (14%) and/or obesity (57%),[873] the definition of PCOS has evolved.[874,875] Most recently, three international conferences developed somewhat different, but overlapping, diagnostic criteria for adult women: National Institutes of Health conference criteria (1992),[876] Rotterdam consensus criteria (2004),[877] and Androgen Excess-PCOS Society consensus criteria (AES, 2006).[878]

Rotterdam criteria are the broadest and include features of all the others, namely all combinations of otherwise unexplained hyperandrogenism, anovulation, and a polycystic ovary. This generates four phenotypes, which are here listed in order of decreasing specificity (Box 15-8).[879] Insulin resistance, obesity, and LH excess, though not diagnostic features of the syndrome, are common and contribute to its pathogenesis; like hyperandrogenism, their severity has generally proven to correlate with specificity.[880,881] Phenotypes 1 to 3 have hyperandrogenic ovarian dysfunction of successively lesser degree. Phenotype 3, which allows diagnosis in the presence of cutaneous or metabolic symptoms in the absence of anovulatory symptoms ("ovulatory PCOS"), is controversial because it permits a PCOS diagnosis in apparently normal females with a polycystic ovary and subclinical hyperandrogenemia.[534] Phenotype 4, which lacks hyperandrogenemia, is the most controversial[878]; the extent to which it is due to undetected ovarian hyperandrogenism is unclear.[880,881] Furthermore, it is too nonspecific for application to adolescents.

Criteria for PCOS diagnosis have been additionally controversial in adolescents because menstrual cycles, hyperandrogenic features, polycystic ovary morphology, and insulin resistance have special characteristics during this developmental stage that have led to the following questions.[579] How can one be certain that adolescent hyperandrogenemia is not a normal phase of pubertal development (i.e., the consequence of prolonged anovulatory cycles) rather than PCOS? Is the correlation between adolescent and adult androgen levels and menstrual patterns sufficiently high that adolescent hyperandrogenic anovulation accurately predicts adult PCOS? Is the polycystic ovary, as currently defined in adults, normal during adolescence?

Endocrine Society guidelines recently suggested that the diagnosis in adolescents be confined to those who have persistent hyperandrogenic oligomenorrhea (Box 15-8).[882] Persistence of symptoms for two years has been suggested by reproductive endocrinology consensus to avoid overdiagnosis.[883]

However, the actuarial risk of 1 year of persistent menstrual abnormality persisting is substantial, and the combination of PCOS risk factors and an abnormal menstrual pattern may facilitate the documentation of hyperandrogenemia and permit PCOS diagnosis by NIH criteria within 1 year of the last menstrual period. Waiting ≥ 2 years to diagnose and treat PCOS may unnecessarily delay treatment and recognition of comorbidities and increase the possibility of lost follow-up.[579] The syndrome in adolescents resembles that in adults, with similar clinical and endocrinologic heterogeneity. The cardinal symptoms typically begin in the perimenarcheal stage, and PCOS has been documented in children as young as 10 years of age.

Clinical Manifestations (Figure 15-44). Cutaneous signs of hyperandrogenism are variably expressed. They are present in about two thirds of cases. Hirsutism is the most common manifestation, but the hirsutism equivalents, seborrhea, acne vulgaris, or androgenetic alopecia, sometimes occur instead. On the other hand, hyperandrogenemia may be entirely cryptic, manifesting neither cutaneous signs nor anovulatory symptoms.

Menstrual irregularity indicative of anovulation is present in approximately two-thirds of cases with the PCOS type of ovarian dysfunction.[141] The distinction between PCOS and physiologic anovulation is often delayed in adolescents because patients, families, and physicians alike often are unsure about the normal range of menstrual cycle variation. Abnormal degrees of adolescent menstrual irregularity are discussed in the "Physiologic Adolescent Anovulation" section. PCOS is a state of relative, not absolute, infertility in which oligo-amenorrheic patients typically ovulate unpredictable. Menstrual regularity does not exclude the presence of ovulatory dysfunction. On the other hand, many eumenorrheic cases have virtually normal ovulatory cycles ("ovulatory PCOS") or have a subtle

BOX 15-8 Diagnostic Criteria for Polycystic Ovary Syndrome*

Adult Criteria

Hyperandrogenic Rotterdam Criteria Phenotypes** (2004)

1. Phenotype 1 (Classic)
   - Clinical or biochemical evidence of hyperandrogenism
   - Evidence of oligo-anovulation
   - Ultrasonographic evidence of a polycystic ovary
2. Phenotype 2 (National Institutes of Health Criteria, 1992)
   - Clinical and/or biochemical evidence of hyperandrogenism
   - Evidence of oligo-anovulation
3. Phenotype 3 ("Ovulatory PCOS")
   - Clinical and/or biochemical evidence of hyperandrogenism
   - Ultrasonographic evidence of a polycystic ovary

Nonhyperandrogenic "Rotterdam criteria" Phenotype (2004)

4. Phenotype 4 (Nonhyperandrogenic PCOS)
   - Evidence of oligo-anovulation
   - Ultrasonographic evidence of a polycystic ovary

Adolescent Criteria

The Endocrine Society Clinical Practice Guideline (2013)

- Clinical or biochemical evidence of hyperandrogenism
- Persistent oligomenorrhea

*All criteria involve exclusion of other causes of hyperandrogenism and anovulation.
**AES-endorsed criteria

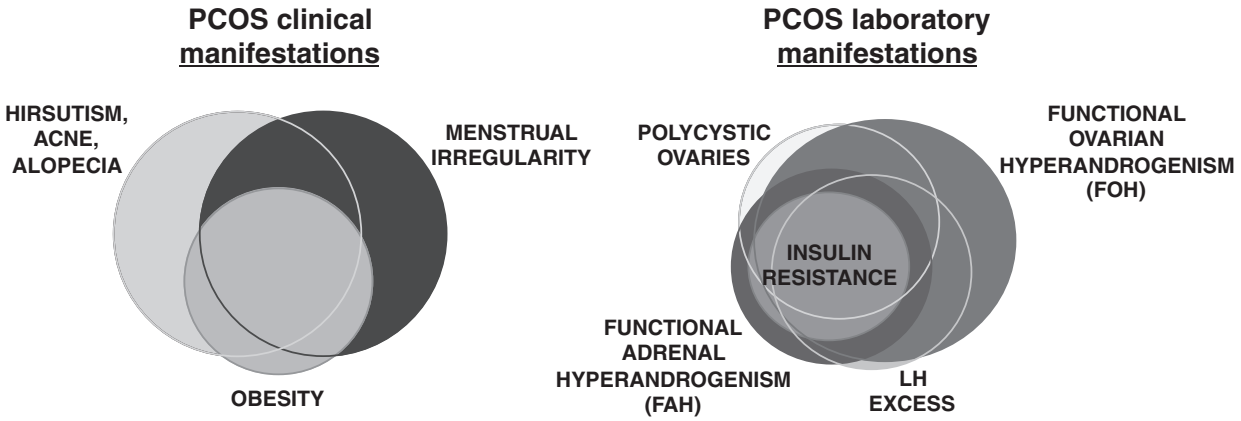


FIGURE 15-44 ■ The major clinical and laboratory manifestations of polycystic ovary syndrome (PCOS) are shown in approximate proportion to their relative incidence and coincidence. (Modified with permission from Buggs, C., & Rosenfield, R. L. (2005). Polycystic ovary syndrome in adolescence. *Endocrinol Metab Clin North Am, 34,* 677–705.)

ovulatory abnormality that escapes notice until presentation in adulthood with unexplained infertility[884] or recurrent miscarriages.[885]

Obesity, present in approximately half of patients with PCOS, often is the initial complaint. PCOS is the single most common endocrine obesity syndrome in females. The obesity occasionally begins in mid-childhood. Even normal-weight young women with PCOS are reported to have a body fat content that is 50% greater than normal.[886] The concept that central obesity or visceral fat is more fundamentally related than global adiposity to the insulin resistance of PCOS has been challenged.[887] Acanthosis nigricans, a manifestation of insulin resistance, may be the presenting complaint of patients with PCOS.

Laboratory Manifestations (see Figure 15-44). Hyperandrogenism may be defined on the basis of cutaneous signs (see Box 15-8) but is best established biochemically if a reliable testosterone assay is available because hirsutism, particularly mild hirsutism, is an unreliable surrogate for hyperandrogenemia. The problematic nature of many androgen assays is discussed in the "Differential Diagnosis" section.

Functional ovarian hyperandrogenism (FOH) can be documented in 85% of PCOS cases by specific tests of ovarian androgenic function.[141,888] The GnRH agonist test and hCG tests evaluate the gonadal response to, respectively, endogenous gonadotropin release or exogenous administration of the LH analog hCG. In two thirds of PCOS patients these tests show a distinctive pattern of ovarian steroidogenic hyperresponsiveness in which 17-hydroxyprogesterone (17OHP) responses are increased compared to normal women without PCOS; there is no evidence of a steroidogenic block, and, indeed, E2 is significantly hyperresponsive. The dexamethasone androgen-suppression test (DAST) is predicated on the principle that residual concentrations of androgens after suppression of adrenal function by glucocorticoid administration ordinarily arise from the ovary. It shows elevated testosterone postdexamethasone in 80% of PCOS patients. Both ovarian function tests are normal in 15% of PCOS patients: the androgen excess of this "nonovarian" PCOS appears to be secondary to isolated functional adrenal hyperandrogenism (FAH) in about one third of cases or to obesity in about two thirds of cases.

A polycystic ovary is found in about two thirds of adolescents with PCOS, which is slightly less frequently than in adult PCOS.[534] An adult polycystic ovary is defined as one with ≥12 small antral follicles (2-9 mm) or >10 cc in volume (in the absence of a follicle >10mm);[877] these follicle number criteria are likely to change with improved ultrasound technology since recent studies indicate that these parameters fall during the adult reproductive years and about half of normal young adults have a polycystic ovary by follicle count criteria.[579] The normal adolescent ovary often exceeds these adult criteria [73,116-119] (see the section of this chapter Maturation of the Neuroendocrine-Ovarian Axis, Adolescent).

A polycystic ovary occurs in 10% of regularly menstruating schoolgirls,[73,117] and up to half of adolescent research volunteers.[116,119,534] Polycystic ovary morphology is not highly stable during adolescence: it develops transiently in about one third of adolescents, yet it may not develop until 2 or more years after menarche.[116,121]

Polycystic ovaries are functionally heterogeneous.[534] Most asymptomatic young women with a polycystic ovary have normal serum androgen concentrations. Such women typically ovulate normally and seldom develop PCOS. Serum levels of anti-Müllerian hormone (AMH) are mildly, but significantly, increased in such females.[889] This seems to indicate an enlarged follicle pool ("increased ovarian reserve") and prognosticate a lengthened reproductive life span.[889]

On the other hand, many asymptomatic volunteers with a polycystic ovary have subclinical abnormalities of ovarian androgenic function. This group is itself heterogeneous. Some of them have baseline hyperandrogenemia and thus meet Rotterdam/AES criteria for PCOS. Those without baseline hyperandrogenemia have been postulated to be carriers for PCOS.[534,890] These considerations call into question the clinical significance of making a diagnosis of PCOS by polycystic ovary criteria in the absence of hyperandrogenic symptoms.

AMH levels are independently associated with polycystic ovaries and hyperandrogenism.[889] Thus, although a mildly increased AMH level is common in asymptomatic females with a polycystic ovary, AMH elevation of twofold or more suggests PCOS with high specificity. AMH elevation occurs in PCOS because of an increased number of small growing follicles. The underlying altered granulosa cell function of PCOS seems likely to result from excessive androgen production by intrinsically defective theca cells,[891] although it may in part be intrinsic.[889]

The increased LH of PCOS was initially considered the cause of the androgen excess and was long considered diagnostic.[892] However, accumulating evidence suggests that LH levels in PCOS are determined by the severity of hyperandrogenemia, moderate degrees of which stimulate LH production, and the extent of obesity, which suppresses LH levels.[266]

Insulin resistance has been estimated from homeostatic model assessment data to occur in 50% to 75% of adults with PCOS.[893] It is excessive for the degree of adiposity. About half of obese adolescents with PCOS have greater insulin resistance than do age-, stage- and BMI-matched adolescents according to the euglycemic clamp method.[894] Adolescents with PCOS are also at increased risk for glucose intolerance,[895,896] consistent with an independent relationship of PCOS to pancreatic beta-cell dysfunction.

Metabolic syndrome results from the interaction of insulin resistance with obesity and age. It is a cluster of critical levels of abdominal obesity, blood pressure, serum triglycerides, HDL cholesterol, and glucose. It is found in approximately 25% of adolescents with PCOS, which is two- threefold the prevalence expected for BMI of matched populations.[897] It poses a twofold increased risk for sleep-disordered breathing in adolescence, as well as long-tem risks for diabetes and cardiovascular disease.[898]

Pathogenesis. Excessive LH secretion, found in 50% to 75% of cases, was once thought to be central to the pathogenesis of PCOS. Although PCOS is gonadotropin dependent, gonadotropins being necessary for the expression of gonadal steroidogenic enzymes, LH excess does not seem to ordinarily be the fundamental cause of the hyperandrogenism. The variable LH excess seems to depend on obesity-androgen balance.[266] When LH is elevated, homologous desensitization normally limits the androgenic response to it: thus, LH excess alone seems unlikely to cause the hyperandrogenism of PCOS, although it may aggravate it in PCOS, where intrinsic ovarian dysfunction causes "escape" from desensitization. Evidence is accumulating that LH excess results from hyperandrogenemia interfering with the progesterone negative feedback effect on LH secretion. Nevertheless, the possibility of a primary role for LH excess remains, particularly in the PCOS that is secondary to congenital virilizing disorders.

PCOS ordinarily seems to be due to intraovarian androgen excess (Figure 15-45). The disproportionately high intraovarian androgen concentration arising from FOH seems to cause polycystic ovaries by recruiting excessive growth of small follicles while hindering the emergence of a dominant follicle, as well as causing thecal and stromal hyperplasia. The hyperandrogenemia results in the pilosebaceous manifestations.

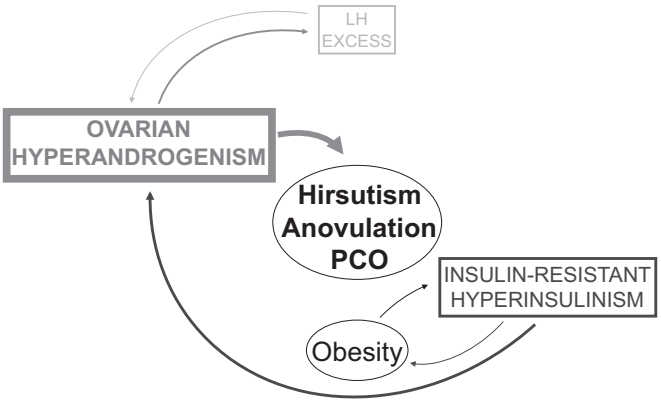


FIGURE 15-45 ■ A model for the pathogenesis of PCOS. Ovarian hyperandrogenism is nearly universal and causes the cardinal clinical features of the syndrome, including the polycystic ovary (PCO). About half of patients have insulin-resistant hyperinsulinism, which aggravates ovarian hyperandrogenism and contributes to adiposity. Androgen excess may also cause LH excess, which aggravates ovarian hyperandrogenism in the presence of hyperinsulinism. Obesity increases insulin-resistance, and the resultant increased hyperinsulinism further aggravates hyperandrogenism. The cause of the ovarian hyperandrogenism and insulin resistance is usually intrinsic and may have common genetic or environmental determinants. This model does not exclude the possibility that the unknown intrinsic ovarian defects that underpin the ovarian steroidogenic dysfunction involve granulosa cell folliculogenesis as well, and it does not depict other associated defects, such as the adrenal hyperandrogenism that parallels the ovarian hyperandrogenism. Excess adipose tissue can produce excess androgen as well as estrogen. However, as with most other extrinsic causes of androgen excess, the hyperandrogenemia of simple obesity is mild and polycystic ovaries unusual. In contrast to PCOS, the anovulation of simple obesity is the consequence of suppression, rather than augmentation, of LH pulse amplitude.

The FOH of PCOS, in turn, seems to arise from a unique ("primary") dysregulation of steroidogenesis that is ordinarily due to intrinsic theca cell dysfunction.[141,299,300] The dysregulation is thought to result from imbalance among the various intrinsic and extrinsic factors involved in the modulation of trophic hormone action. Within the ovary, there appear to be flaws in the processes that normally coordinate androgen and estrogen secretion (see Figure 15-20). The intrinsic theca cell defect causes constitutive overexpression of most steroidogenic enzymes, most prominently at the level of 17-hydroxylase and 17,20-lyase activites, both properties of P450c17, which are the rate-limiting steps in the biosynthesis of testosterone precursors. As in the ovary, dysregulation of local steroidogenic regulatory processes within the adrenal cortex appears to cause a characteristic type of primary FAH in which excessive dehydroepiandrosterone is formed as a by-product of cortisol secretion.

As a consequence of dysregulated steroidogenesis, PCOS theca cells do not undergo the normal down-regulation of steroidogenesis in response to excess LH stimulation, and so are hypersensitive to LH stimulation. "Escape" from desensitization to LH excess appears to be

the basis for the distinctive pattern of steroidogenic hyperresponse to GnRH agonist or hCG testing, which is characterized by disproportionate hyperresponsiveness of 17-hydroxyprogesterone relative to other ovarian steroids without evidence of a steroidogenic block.

Granulosa cell functions are also defective in PCOS. Folliculogenesis of small follicles is excessive, which contributes to the tendency of PCOS women to develop the dangerous ovarian hyperstimulation syndrome during fertility treatment. Inhibin-B hyperresponsiveness to FSH also appears to aggravate thecal androgen secretion via a paracrine action.[899]

Insulin-resistant hyperinsulinism appears to be an important extrinsic factor in dysregulation. Insulin resistance results in compensatory hyperinsulinemia. Insulin, like IGFs, synergizes with trophic hormones to cause ovarian or adrenal androgen excess. The ovaries and adrenal glands function as if responding to the hyperinsulinemic state in spite of the resistance to the effects of insulin on skeletal glucose metabolism. This paradox seems to result from differential insulin sensitivity of insulin target tissues, some of which appear to be result from intrinsic signaling defects and others from the endocrine environment.[900,901] Notably, a common transcription factor, KLF15, mediates insulin stimulation of both adipogenesis and testosterone formation.[902] This has led to the proposal that increased KLF15 expression in response to the compensatory hyperinsulinemia of insulin resistance may mediate the obesity of PCOS, while aggravating hyperandrogenism. These observations suggest that PCOS commonly results from intrinsic ovarian dysfunction that seems to be but one manifestation of a generalized disorder in which there is dysregulation of transcription factors common to the function of a wide variety of tissues.

Etiology. Increasing evidence suggests that PCOS arises as a complex trait with contributions from both heritable and nonheritable factors.[15] Twin studies indicate that genetic factors explain about 70% of the variance in pathogenesis. Hyperandrogenemia and polycystic ovaries each appear to be inherited as an independent autosomal dominant trait. Nearly half of sisters of women with PCOS have an elevated serum testosterone level, although only half of them are symptomatic. Seventy percent of sisters of PCOS probands, diagnosed by either NIH (80%) or Rotterdam criteria, had a polycystic ovary; 25% of these met NIH criteria and 42% met other Rotterdam criteria for PCOS.[903] Central obesity and insulin resistance not only seem to play important roles in PCOS by accentuating steroidogenic dysregulation, but polycystic ovaries in PCOS probands that were unrelated to a maternal polycystic ovary were reported to be associated with paternal metabolic syndrome. Gestational factors have also been incriminated: the syndrome has been associated with both high and low birthweight, and it can develop secondary to fetal virilization. Thus, the syndrome seems to become manifest when an intrinsic genetic trait interacts at puberty with other congenital or environmental factors, excessive adiposity being a common precipitant.

Other Causes of Functional Ovarian Hyperandrogenism. Secondary PCOS can result from several disorders (see Box 15-7).[904] Virilizing congenital adrenal hyperplasia frequently causes ovarian hyperandrogenism. Three mechanisms are involved.[905] For one, poor control of adrenal hyperandrogenism causes polycystic ovaries and amenorrhea by direct effects on the ovary. Adrenal rests of the ovaries may cause polycystic ovaries and hyperandrogenism. Finally, patients with CAH, particularly those with classic CAH, are at high risk for the emergence of PCOS at puberty due to developmental programming (see "Disorders of Sex Development," presented earlier).

Steroidogenic blocks in ovarian steroid synthetic pathways, such as caused by 3β-HSD[906] or aromatase deficiency,[907] can cause hyperandrogenism in association with grossly polycystic ovaries and elevated LH levels. Ovarian 17-ketosteroid reductase deficiency has been reported to be responsible for a PCOS-like picture in two families, but there has been no molecular confirmation of an underlying mutation.[908]

All known forms of extreme insulin resistance, including hereditary cases of insulin receptor mutations and acquired lipodystrophy, are accompanied by PCOS, possibly by acting through the IGF-1 signal transduction pathway to cause escape from desensitization to LH. Acromegaly itself is associated with PCOS. PCOS has also been reported as a complication of portasystemic shunting; impaired steroid metabolism has been postulated as the mechanism. The antiepileptic drug valproic acid causes hyperandrogenism and polycystic ovaries, and an association of epilepsy itself with PCOS is possible.[383,826] Functional ovarian hyperandrogenism may also result from an ovotesticular disorder of sex development.

Excessive hCG stimulation mediates the hyperandrogenism of hyperreactio luteinalis and luteoma of pregnancy,[909] and excessive LH appeared to mediate hilus cell hyperplasia in a case of FSH-resistant ovarian follicles.[910] An extremely high level of hCG due to tumor has been reported to virilize a nonpregnant woman with preexisting PCOS.[848]

Other Causes of Functional Adrenal Hyperandrogenism. The PCOS type of primary FAH that appears to arise from dysregulation of adrenal steroidogenesis occurs as an isolated entity, not associated with FOH, in 15% to 25% of hyperandrogenic women.[888] This may sometimes be an outcome of premature adrenarche. This type of adrenal dysfunction was previously mistaken for nonclassical 3β-hydroxysteroid dehydrogenase deficiency, which is now known to be a rare disorder.[911] Less than 10% of adrenal hyperandrogenism can be attributed to the more well-understood disorders listed in Box 15-7. The most common of these are nonclassic congenital adrenal hyperplasia, which accounts for under 5% of hyperandrogenism in the general U.S. population, and the more rare various hyperandrogenic forms of Cushing syndrome. Prolactin excess causes adrenal hyperandrogenism, sometimes in association with polycystic ovaries. Adrenal hyperandrogenism can on rare occasions arise from other rare congenital disorders of adrenal steroid action or metabolism, such as glucocorticoid resistance, apparent cortisone reductase deficiency, and apparent sulfotransferase deficiency.[326,912,913]

Peripheral Androgen Overproduction. In about 10% of hyperandrogenemic patients, an ovarian or adrenal source cannot be ascertained by thorough testing. This is discovered in two settings. When hirsutism is not accompanied by evidence of ovarian dysfunction, it is termed *idiopathic hyperandrogenism* (as distinct from *idiopathic hirsutism*, discussed later).[383] Quirks in steroid peripheral metabolism have been suspected to be the cause. When the source of androgen cannot be located in subjects with hyperandrogenic anovulation, it has been termed functionally atypical PCOS.[888] Obesity may explain such cases, because adipose tissue has the capacity to both cause anovulation and to form testosterone from androstenedione.

Tumoral Hyperandrogenism. Virilizing tumors are rare, accounting for about 0.2% of hyperandrogenism; about half are malignant.[383] About half are ovarian, half adrenal. Masculinizing sex cord-stromal tumors of the ovary are unusual before the teenage years. Leydig-Sertoli cell tumor (androblastoma, arrhenoblastoma) is the most common type. Virilizing granulosa-theca cell tumor (thecoma) is unusual before menopause.[848,914] Dysgerminomas virilize only if they have interstitial cell elements. Lipid cell tumors tend to respond to ACTH as well as LH and produce 17-hydroxyprogesterone; thus, they must be considered in the differential diagnosis of late-onset congenital adrenal hyperplasia.[915] The abnormal differentiation that underlies tumor formation typically leads to an abnormal pattern of steroid secretion with androstenedione predominating over testosterone secretion.[916] However, some thecomas have been reported to predominantly secrete testosterone.[917,918] Gonadoblastomas are virilizing tumors virtually confined to individuals with dysgenetic gonads with Y-chromosomal material in their genome. Some masculinizing ovarian sex cord-stromal tumors may be caused by activating mutations of stimulatory G proteins.[919] Leydig cell and adrenal rest tumors of the ovary are extremely rare causes of masculinization in childhood.[20,358,920] Adrenal virilizing tumors are rare in adolescence; their peak incidence is in early childhood and adulthood[637] (see the "Incomplete Precocity" section).

Androgenic Drugs. Drug-induced masculinization in adolescence is encountered most often in athletes. The medical history is important in detection, as standard clinical laboratory tests for androgens are not helpful in the detection of either natural or artificial androgens.[921] This is also the case for masculinization that results from unintended contact with topical androgens used by a parent or by a sexual partner.[643] Valproic acid use in epileptics raises testosterone levels and may mimic PCOS.[383]

Differential Diagnosis.

Hyperandrogenism should be considered in any girl who presents with hirsutism or cutaneous hirsutism equivalents, menstrual disturbance, or central obesity during puberty.

Hirsutism and hirsutism equivalents are variably expressed manifestations of excess androgens that are present in approximately two thirds of hyperandrogenic females. Hirsutism may be absent in young adolescents whose hyperandrogenism has not evolved fully.

Hirsutism is defined clinically as excessive sexual hair that appears in a male pattern.[383] It is commonly graded according to the hormonal Ferriman-Gallwey system, which quantitates the extent of hair growth in the most androgen-sensitive areas (Figure 15-46). Focal hirsutism (score < 8) is a common normal variant, whereas generalized hirsutism (score of 8 or more) is abnormal in the general U.S. population. The normal score is lower in Asian populations and higher in Mediterranean populations.[922]

Hirsutism must be distinguished from hypertrichosis, the generalized excess growth of vellus hair that sometimes occurs on a hereditary basis or in patients taking glucocorticoids, phenytoins, diazoxide, or cyclosporine. Hypertrichosis is distributed in a nonsexual pattern (e.g., generalized distribution or more prominent distribution on the forehead or shoulders) and is not caused by excess androgen, although it may be aggravated by excess androgen.

The absence of hirsutism in approximately one third of hyperandrogenic adults appears to be because of relatively low sensitivity of their pilosebaceous unit to androgens. Conversely, hirsutism without elevated circulating levels of androgen—"idiopathic hirsutism"—accounts for approximately half of mild hirsutism and one sixth of moderate-severe hirsutism.

Acne vulgaris, androgenetic (pattern) alopecia, seborrhea, hyperhidrosis, and hidradenitis suppurativa are cutaneous hirsutism equivalents. Hyperandrogenism should be considered in adolescent females who have inflammatory acne that is unusual in severity (≥ moderate) or poorly responsive to topical dermatologic therapy.[583] Balding may be either male pattern (affecting the fronto-temporo-occipital scalp) or pattern (affecting the crown, typically manifest early as a midline part widened in a "Christmas tree" pattern).

Symptomatic menstrual abnormality (primary or secondary amenorrhea, oligo-amenorrhea, or dysfunctional uterine bleeding) in a normally feminized girl is reason to consider hyperandrogenism. Failure to establish normal adult menstrual cyclicity by 2 years after menarche is a strong indication for investigation even in the absence of hirsutism or obesity (see the "Physiologic Adolescent Anovulation" section, presented earlier).

Intractable obesity, high waist circumference (> 88 cm), or acanthosis nigricans should raise concern for PCOS. The possibility of PCOS is heightened if the preceding symptoms are associated with a history of prepubertal risk factors for PCOS: congenital virilizing disorders; premature adrenarche; intractable prepubertal obesity, particularly if associated with pseudo-Cushing syndrome or pseudo-acromegaly; or a family history of PCOS or metabolic syndrome.

Diagnostic Approach

The goals of the laboratory evaluation for hyperandrogenism are to attempt to obtain evidence of hyperandrogenism and anovulation, to determine the specific etiology, and to provide a baseline in case it becomes necessary to reassess because of progression of the disorder. The diagnosis of hyperandrogenism is on the firmest

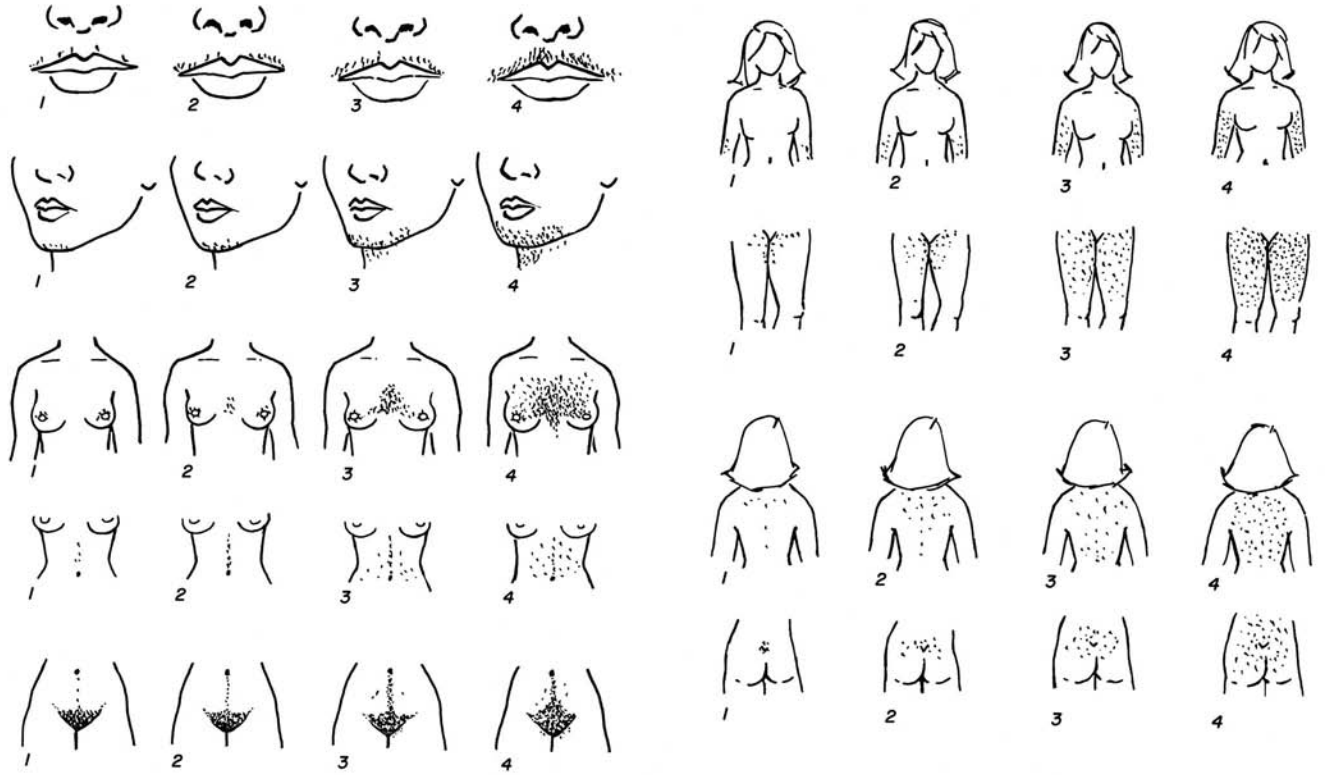


FIGURE 15-46 ■ Ferriman-Gallwey hirsutism scoring system. Each of the nine body areas most sensitive to androgen is assigned a score from 0 (no hair) to 4 (frankly virile), and these are summed to provide a hormonal hirsutism score. (Copyright © 2008, The Endocrine Society. Reproduced with permission from Martin, K. A., Chang, R. J., Ehrmann, D. A., et al. (2008). Evaluation and treatment of hirsutism in premenopausal women: an Endocrine Society Clinical Practice Guideline. *J Clin Endocrin Metab, 93*, 1105–1120.)

grounds if hyperandrogenemia is demonstrated biochemically, rather than relying on hirsutism as a clinical surrogate for it, although documentation of hyperandrogenemia can be problematic. Menstrual abnormality of most sorts provides evidence of anovulation.

Most hyperandrogenic girls present when fully feminized. In girls with menstrual irregularity who lack cutaneous manifestations of hyperandrogenism, the evaluation should address the possibility of hypoestrogenism by including bone age, E2, and gonadotropin levels, as outlined earlier (see Figure 15-43).

An approach to the workup that depends on assessing the degree of hirsutism, identifying anovulatory symptoms, and elucidating risk factors for virilizing disorders, androgenic medications, and other endocrinopathies (see Box 15-7) is suggested (Figure 15-47).[383] A rapid pace of development or progression of hirsutism, evidence of virilization (such as clitoromegaly, genital ambiguity, or increasing muscularity), or an abdominal mass raise concern for an androgen-secreting neoplasm. However, tumors producing moderately excessive androgen can have indolent presentations. Inspection of the external genitalia is indicated, but an internal pelvic examination seldom is necessary.[782] If hirsutism is sufficiently mild or focal that it can be managed by dermatologic means and menses is regular with no evidence of risk factors that would suggest an underlying cause, it is reasonable *not* to pursue laboratory evaluation, given the high likelihood of idiopathic hirsutism (a cutaneous rather than an endocrine disorder), unless the patient is of Asian ethnicity. If hirsutism is sufficiently symptomatic that hormonal therapy is contemplated[922] or there are features to suggest an underlying disorder, excess androgen production should be ruled out. Risk factor assessment includes follow-up to evaluate response to therapy.

Serum testosterone is the single most important androgen to evaluate (see Figure 15-47). Serum free testosterone is about 50% more sensitive in detecting excessive androgen production because hyperandrogenic women have a relatively low level of SHBG. There are many pitfalls in testosterone assays at the low levels found in women and children, and reliable testosterone assays are not available to many physicians. Assays of high sensitivity and specificity, such as those provided by postchromatographic radioimmunoassay or tandem mass spectrometry, are required; furthermore, they are best performed by a specialty laboratory that has extensively validated them. Assaying the free testosterone level introduces other potential sources of error. Direct assays of the free testosterone concentration are inaccurate and should be avoided. The best methods calculate free testosterone as the product of the total testosterone and a function of SHBG: free testosterone = total testosterone × percentage of free testosterone, where the percentage of free testosterone is most commonly determined by dialysis or calculated from the SHBG concentration. The combination of a

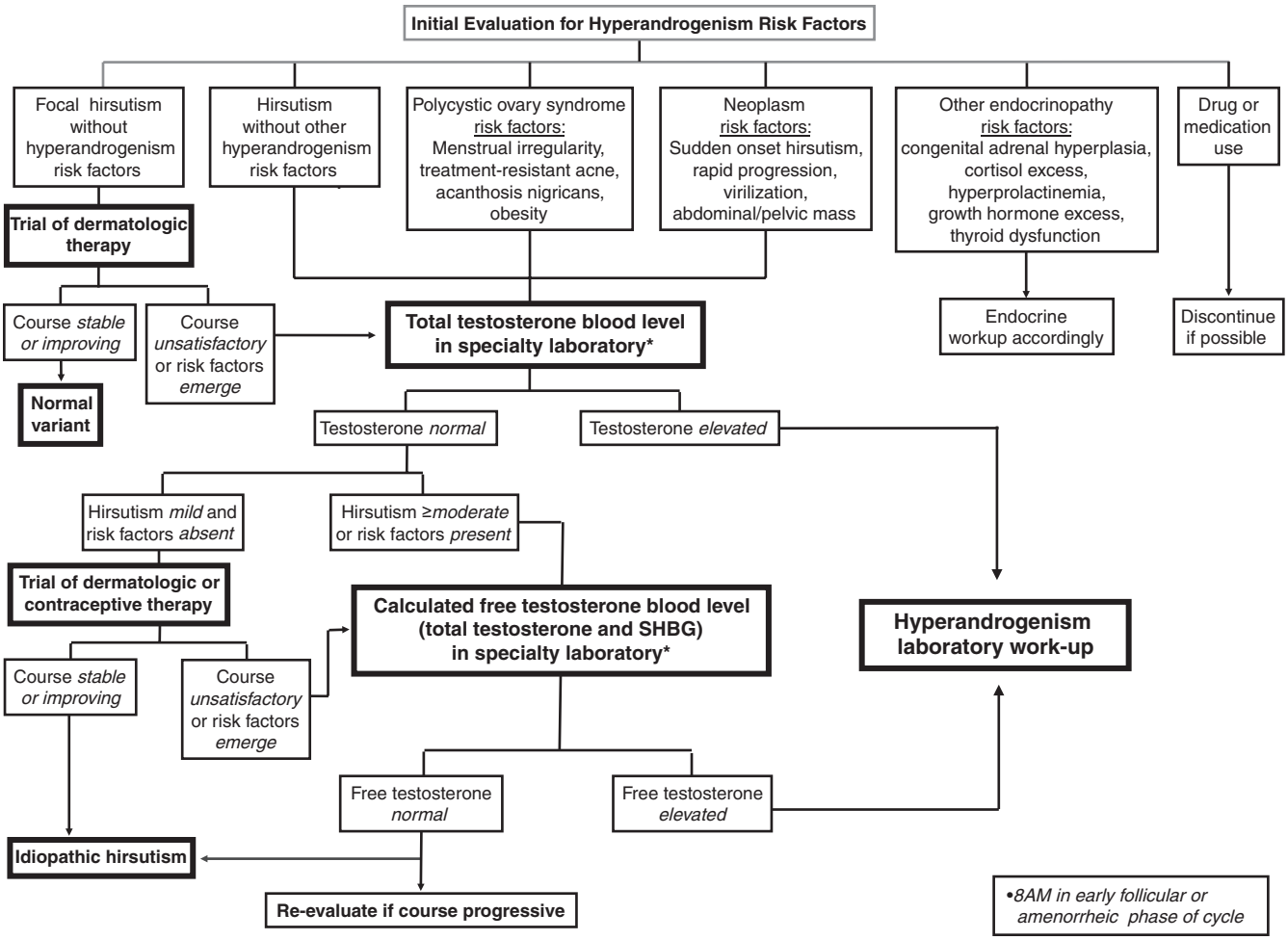


FIGURE 15-47 ■ Initial evaluation of women for hyperandrogenism. Risk assessment includes more than the degree of hirsutism. Drugs that cause hirsutism include anabolic or androgenic steroids (which should be considered in athletes and patients with sexual dysfunction) and valproic acid (which should be considered for neurologic disorders). Localized areas of hair growth that do not meet the criteria for hirsutism ("focal hirsutism") and are not accompanied by other risk factors for hyperandrogenism do not require an endocrine workup if hormonal treatment is not contemplated. In women with symptomatic hirsutism who will have hormonal treatment, androgen levels should be assessed. PCOS is the most common cause to be considered, but androgen-secreting tumors, congenital adrenal hyperplasia, and diverse androgenic disorders, as shown, should be excluded. Serum testosterone is best assessed in the early morning, on days 4 to 10 of the menstrual cycle in eumenorrheic women and on random days in amenorrheic women. Females with mild hirsutism (a score of 8 to 15), normal total testosterone level, and no risk factors probably have idiopathic hirsutism, which may be responsive to oral contraceptive therapy. Serum free testosterone should be measured (which involves measuring total testosterone and either SHBG or the percentage of free testosterone by dialysis) in a specialty reference laboratory if the serum total testosterone is normal in the presence of risk factors or progression of hirsutism on therapy. A simultaneous assay of 17-hydroxyprogesterone may be indicated in subjects at high risk for congenital adrenal hyperplasia. Some women diagnosed with idiopathic hirsutism by this algorithm will have polycystic ovaries on ultrasound, but the significance is unclear in the absence of ovulatory dysfunction. Progression of hyperandrogenism in the presence of a normal serum free testosterone is very unusual; such patients should be thoroughly reevaluated. (Modified with permission from Martin, K. A., Chang, R. J., Ehrmann, D. A., et al. (2008). Evaluation and treatment of hirsutism in premenopausal women: an endocrine society clinical practice guideline. *J Clin Endocrin Metab, 93,* 1105–1120. © The Endocrine Society).

high-normal total testosterone and a low-normal SHBG yields a high free testosterone concentration.

Although other androgens are present in blood, their assessment ordinarily makes little difference in diagnosis and management if serum free testosterone is normal. However, the variation in androgen levels may miss an occasional case of nonclassic congenital adrenal hyperplasia, so further studies are indicated in patients at high risk by virtue of family history or ethnicity.

If otherwise unexplained hyperandrogenism is confirmed, the next step in the workup is ordinarily to obtain an ultrasound examination of the pelvis (Figure 15-48). These findings are not completely specific for PCOS, and require excluding disorders that may secondarily cause PCOS. On the other hand, a normal ultrasound examination does not exclude PCOS. However, it is useful for ruling out tumor and disorders of sexual differentiation as a cause of the androgen excess. Simultaneous ultrasound imaging of the abdomen can be a cost-effective screening test for adrenal neoplasm in the hands of an experienced ultrasonographer.

Endocrine screening is indicated to rule out the most common mimes of PCOS. These include exclusion of pregnancy and hyperprolactinemia. It may also include measurement of DHEAS and early morning 17-hydroxyprogesterone to screen for nonclassic congenital adrenal

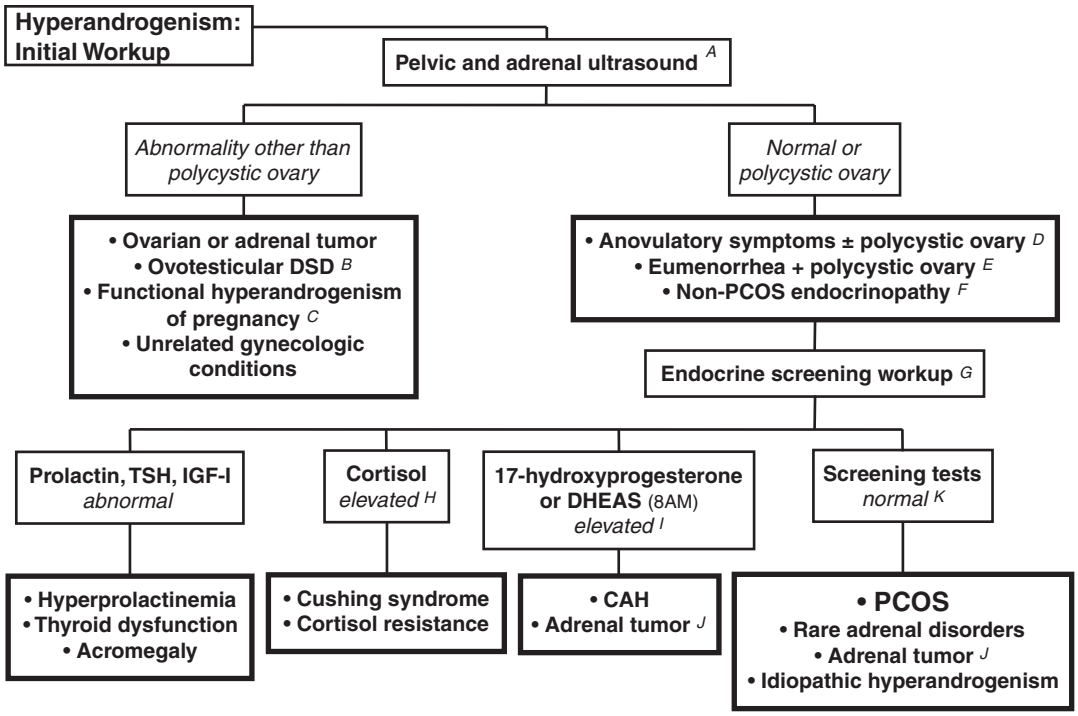


FIGURE 15-48 ■ Initial workup for causes of hyperandrogenism. This algorithm identifies the common causes of hyperandrogenism, which is most often due to PCOS. (Modified with permission from Rosenfield, R. L. (2012). Polycystic ovary syndrome in adolescence: clinical features and diagnosis of polycystic ovary syndrome in adolescents. UpToDate. Retrieved from http://www.uptodate.com)

A. Ultrasonography is the initial study that detects polycystic ovaries and excludes ovarian pathology other than polycystic ovaries. The abdominal ultrasound that is indicated for pelvic ultrasonographic imaging in virginal adolescents can be used to screen for adrenal enlargement/mass. A polycystic ovary has been defined by international consensus in adults as an ovary with a volume > 10 cc or containing ≥ 12 follicles 2 to 9 mm in diameter in the absence of a dominant follicle (≥ 10 mm diameter) or corpus luteum. In adolescents ovaries are normally are up to 10-8-11.8 cc in volume with 10-17 follicles in the maximum plane. Unless the ultrasound reveals an abnormality other than a polycystic ovary, further workup for hyperandrogenism is indicated.
B. Ovotesticular disorder of sex development (DSD) was formerly termed *true hermaphroditism*.
C. Virilization during pregnancy may be due to androgen hypersecretion by a luteoma or hyperreactio luteinalis.
D. The presence of a polycystic ovary supports the diagnosis of PCOS in hyperandrogenic patients. The presence of a polycystic ovary is not necessary—nor does it suffice—for the diagnosis of PCOS in a patient with hyperandrogenic anovulation.
E. In a eumenorrheic symptomatically hyperandrogenic adolescent with normal menses, the presence of a polycystic ovary (which meets Rotterdam-AES diagnostic criteria in adults) is provisional evidence for the diagnosis of PCOS.
F. A polycystic ovary is not specific for PCOS; it has been reported in several specific endocrinopathies (e.g., hypothyroidism and Cushing disease) and is also common in asymptomatic individuals.
G. Further evaluation should include levels of serum prolactin, thyroid-stimulating hormone (TSH), insulin-like growth factor 1 (IGF-1), cortisol, 17-hydroxyprogesterone, and dehydroepiandrosterone sulfate (DHEAS). An abnormality of any of these endocrine tests is suggestive of one of the hyperandrogenic disorders that most commonly mimics PCOS.
H. Plasma cortisol < 10 μg/dL essentially rules out endogenous Cushing syndrome unless the clinical index of suspicion is high.
I. 8AM 17-hydroxyprogesterone > 170 to 200 ng/dL is approximately 95% sensitive and 90% specific for detecting common type (21-hydroxylase deficient) nonclassic congenital adrenal hyperplasia (CAH) in anovulatory or follicular phase women; it is often found in virilizing neoplasms. DHEAS > 700 μg/dL suggests an adrenal virilizing tumor or a rare type of CAH (3β-hydroxysteroid dehydrogenase deficiency).
J. Computed tomographic scanning of the adrenal gland is a more definitive study for identifying adrenal tumor than is ultrasound.
K. Exclusion of the preceding disorders in a hyperandrogenic patient with menstrual dysfunction meets standard diagnostic criteria for PCOS with approximately 95% reliability. However, this workup does not identify rare adrenal disorders (e.g., some types of CAH and related types of congenital adrenal steroid metabolic disorders), the rare testosterone-secreting adrenal tumor, or, most commonly, idiopathic hyperandrogenism (hyperandrogenism of unknown origin, which can arise from obesity or possibly metabolic abnormalities).

hyperplasia, and assessment for Cushing syndrome, thyroid dysfunction, or acromegaly, if clinically indicated. If this evaluation for the most common disorders that mimic polycystic ovary syndrome is negative, the combination of testosterone elevation with anovulatory symptoms fulfills the most widely accepted diagnostic criteria (criteria established by the National Institutes of Health [NIH]) for PCOS. The combination of testosterone elevation with a polycystic ovary in the absence of menstrual irregularity permits a provisional diagnosis of PCOS by Rotterdam/AES criteria (see Box 15-8). However, this evaluation does not exclude some fairly rare hyperandrogenic disorders that mimic PCOS. The approach to further studies to determine the source of hyperandrogenemia varies among subspecialists and with the needs of the individual patient.

Our preference is to use a dexamethasone androgen-suppression test (DAST) to attempt to make a positive diagnosis of the ovarian dysfunction of PCOS versus determining whether further workup is necessary for rare forms of CAH or other rare adrenal disorders (Figure 15-49).[888] Suppression of serum androgens and cortisol in response to a low-dose dexamethasone suppression test segregates patients diagnostically. Subnormal testosterone suppression

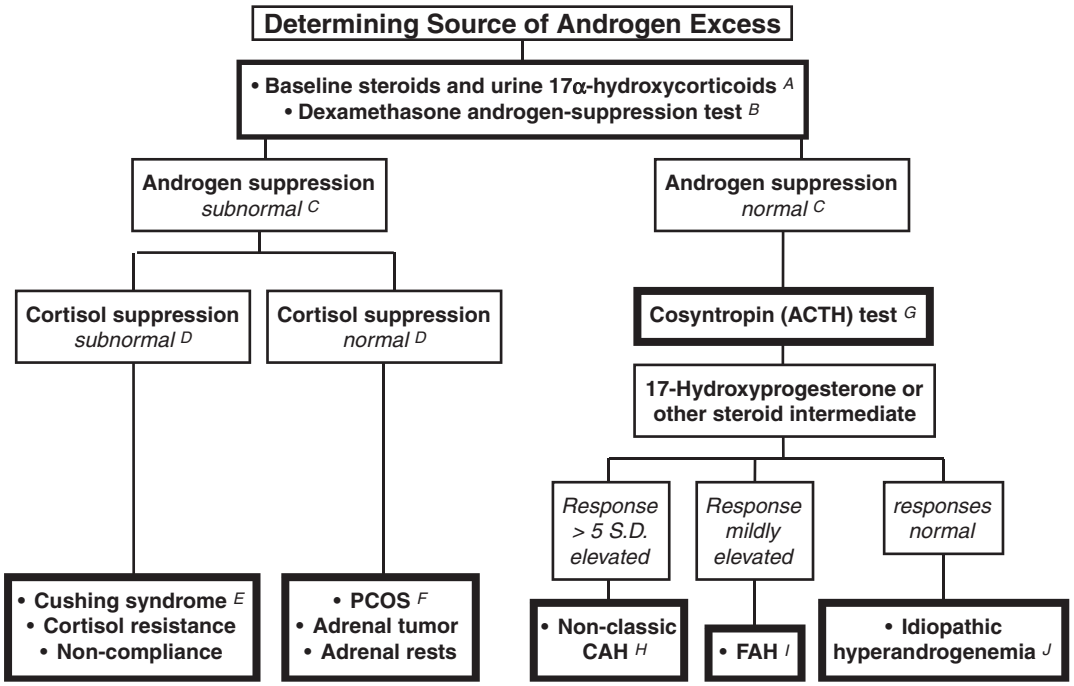


FIGURE 15-49 ■ An approach to determining the source of androgen excess. The association of testosterone elevation with otherwise unexplained anovulatory symptoms (see Figures 15-42 and 15-43) or a polycystic ovary fulfills standard diagnostic criteria for PCOS, which accounts for over 80% of adolescent hyperandrogenism. Determination of the source of excess androgen often permits a positive diagnosis of the characteristic ovarian and adrenal dysfunction of PCOS and will elucidate rare disorders that mimic PCOS. (Modified with permission from Rosenfield, R. L. (2012). Polycystic ovary syndrome in adolescence: clinical features and diagnosis of polycystic ovary syndrome in adolescents. UpToDate. Retrieved from http://www.uptodate.com)

A. After obtaining an early morning blood sample for baseline steroid intermediates (e.g., 17-hydroxypregnenolone, 17-hydroxyprogesterone, 11-deoxycortisol, dehydroepiandrosterone, androstenedione) and a 24-hour urine for corticoids (i.e., free cortisol and 17α-hydroxycorticoids, as well as creatinine to control for completeness of collection), a dexamethasone androgen-suppression test (DAST) is performed.
B. A short DAST (sampling blood 4 hours after a single noontime 0.5 mg dexamethasone dose) maximally suppresses total and free testosterone and 17-hydroxyprogesterone, but DHEAS and cortisol are not maximally suppressed in comparison to the 4-day DAST. A long (4-day) DAST is a definitive test: this consists of a 4-day course of dexamethasone 0.5 mg four times daily prior to an early morning posttest blood sample on day 5.
C. Normal androgen suppression in response to the 4-day DAST is indicated by total testosterone < 28 ng/dL (1 nmol/L), free testosterone < 8 pg/mL (28 pmol/L), DHEAS < 40 mg/dL (1 micromol/L) (> 75% fall), and 17-hydroxyprogesterone < 26 ng/dL (0.8 nmol/L).
D. Normal corticoid suppression is indicated by serum cortisol < 1.5 mg/dL (40 nmol/L) and urinary cortisol < 10 mg (27 nmol) per 24 hours by the second day of dexamethasone administration.
E. If androgen and cortisol are both not normally suppressed, Cushing syndrome and cortisol resistance should be considered. Poor suppression can also result from noncompliance with the dexamethasone regimen.
F. Subnormal suppression of testosterone (as well as androstenedione and 17-hydroxyprogesterone) and a normal suppression of cortisol and DHEAS are characteristic of PCOS, but the rare virilizing adrenal tumor or adrenal rests should be considered on the basis of clinical factors.
G. A cosyntropin (ACTH) stimulation test is appropriate if androgen suppression by dexamethasone is normal.
H. The diagnosis of congenital adrenal hyperplasia (CAH) is suggested if the response to ACTH is > 5 SD above the average: for 17-hydroxyprogesterone this is > 1000 ng/dL (30 nmol/L) and for 17-hydroxypregnenolone it is > 5000 ng/dL (158 nmol/L).
I. Primary functional adrenal hyperandrogenism (FAH) (suggested by a modest rise in 17-hydroxypregnenolone or 17-hydroxyprogesterone that does not meet the criteria for the diagnosis of CAH) is sometimes the only demonstrable source of androgen excess in PCOS. A rare mime of FAH and idiopathic hyperandrogenemia is (apparent) cortisone reductase deficiency: baseline urinary corticoids consist primarily of cortisone metabolites rather than cortisol metabolites, so 17α-hydroxycorticoid excretion is elevated, but cortisol excretion is normal.
J. Idiopathic hyperandrogenism (distinct from idiopathic hirsutism) is the diagnosis when the source of hyperandrogenemia remains unexplained after intensive investigation (occurs in approximately 10% of cases). It often appears to be due to obesity.

with normal adrenocortical suppression indicates a source of androgen other than an ACTH-dependent adrenal one and is found in 80% of PCOS. However, tumor or other ovarian pathology must be excluded by ultrasound examination. If both cortisol and androgen suppression are subnormal, then the androgen excess may be secondary to noncompliance with taking dexamethasone, Cushing syndrome, or glucocorticoid resistance. If testosterone suppression is normal, then ACTH (cosyntropin) stimulation testing to rule out nonclassic congenital adrenal hyperplasia is recommended. If both dexamethasone and ACTH testing are normal, the most likely diagnosis is idiopathic hyperandrogenism, of which obesity seems to be the most likely cause.

A short (4-hour) DAST, as described in the legend for Figure 15-49, suffices in the absence of high suspicion for virilizing disorder. It is helpful in distinguishing the potentially reversible pseudo-PCOS of simple obesity, in which case testosterone suppresses normally, from the persistent ovarian dysfunction of ordinary PCOS.[888] However, a more prolonged course of low-dose dexamethasone (long DAST, 4-days) is required to

suppress the hyperandrogenism of congenital adrenal hyperplasia.

Further extensive diagnostic studies are seldom indicated unless there is reason to suspect a virilizing tumor or a disorder of sexual differentiation. On rare occasions, ultrasonography has been insensitive in detecting a virilizing ovarian tumor in adults.[660] Computed tomography and magnetic resonance imaging permit the best visualization and more detailed assessment of tumors. Further or alternative workup may include acute gonadotropin releasing hormone agonist testing or assessment of the response to ovarian suppression treatment to determine the source of androgen.

Management

Management is individualized according to symptoms and patient goals—hirsutism, acne, and alopecia; menstrual irregularity; obesity and insulin resistance—and the source of androgen excess.[871,872,923] Because PCOS is associated with the early development of type 2 diabetes mellitus and metabolic syndrome, a fasting lipid panel and oral glucose tolerance test are recommended in patients with obesity or risk factors for these metabolic dysfunctions.[897] Obesity and insulin-resistant patients should be screened for sleep-disordered breathing. Because PCOS is closely related to parental metabolic syndrome, we recommend a similar evaluation of primary relatives. Women with PCOS are at increased risk of mood and anxiety disorders and depression and should be screened for these disorders.[924]

Cosmetic measures are the cornerstone of care for hirsutism.[383] Bleaching and shaving suffice for many. Depilating agents and waxing treatments are useful but prone to cause skin irritation. Eflornithine hydrochloride cream brings about marked improvement of hirsutism in 32%, with the maximal effect by 2 to 6 months. The FDA has permitted marketing of many laser devices, and equivalents such as diode and flashlamp, as effective for permanent hair reduction, for which the criterion is persistent reduction in hair density by 30% or more after three to four treatments of a site. Wavelengths between 694 and 1064 nm damage hair follicles by combining relatively selective absorption of heat by dark hairs with penetration into the dermis. Light-skinned individuals are the best candidates, requiring the lower energy pulses. Those with heavily tanned or darker skin require the use of cooling procedures and adjustment of energy levels to minimize the risk of skin side effects. Laser treatment is preferred to electrolysis, but both types of treatment require trained personnel; are repetitious, expensive, and painful; are practical only for treating limited areas; and may result in local reactions, including burns, dyspigmentation, and scarring. Endocrine therapy is directed at interrupting androgen production or action. This causes the pilosebaceous unit to revert toward the prepubertal vellus type.

Endocrinologic treatment of cutaneous symptoms is indicated before undertaking treatment with laser treatment, Accutane, or Rogaine if standard cosmetic or topical dermatologic measures are inadequate. The maximal effect on the sebaceous gland occurs within 3 months, but that on sexual hairs requires 9 to 12 months of treatment, because of the long duration of the hair growth cycle. All are effective only as long as the patient wishes to maintain her improvement in hirsutism.

Combination oral contraceptive pills (OCPs) are the first-line endocrine treatment for women with the dermatologic or menstrual abnormalities of PCOS. They act by suppressing serum androgens, particularly free testosterone, mainly by inhibiting ovarian function. They also raise SHBG and modestly lower DHEA sulfate levels. They normalize androgen levels by 3 weeks of therapy.

All estrogen-progestin combinations generally suffice for women with acne or mild hirsutism, in combination with cosmetic measures. Those with nonandrogenic progestins, such as norgestimate or ethynodiol diacetate combined with 35-μg ethinyl E2, have generally favorable risk-benefit ratios and optimize lipid profiles. Those with antiandrogenic progestins in low doses may confer an additional benefit: drospirenone is available in the United States with 30-μg ethinyl E2 and cyproterone acetate 2 mg with 35-μg ethinyl E2 in Canada, Mexico, and elsewhere. The larger estrogen doses may be necessary in larger girls to provide menstrual regularity. Overall, combination pills carry about a 4-fold increased risk of venous thromboembolism in first-time users; this risk decreases with duration of use and decreasing estrogen dose but is less than that of pregnancy.[528,529,579]

OCPs are also effective in the management of menstrual irregularity, which requires treatment because chronic anovulation is associated with increased risk of developing endometrial hyperplasia and carcinoma. There are, however, several potential disadvantages to the use of OCPs in the management of PCOS in adolescents. They will bring growth to end in perimenarcheal girls. OCPs may be contraindicated in patients who are at risk for venous thrombosis, and they should be used with caution and in the lowest estrogen dose possible in patients with migraine headaches. Patients may use OCPs as an excuse for not losing weight. The patient may believe that the treatment is curative and defer a definitive diagnostic workup. OCPs do not permit conception if and when it is desired. The long-term consequences of these agents on fertility are unknown; although there is the theoretic possibility of post-pill amenorrhea, very high-dose estrogen initiated in early adolescence increases the risk of primary ovarian insufficiency rather than hypogonadotropinism.[690]

It is advisable to recheck patients after 3 months of therapy to assess the efficacy of treatment and normalization of androgen levels. As a general rule, OCP treatment should be continued until the patient is gynecologically mature (5 years postmenarcheal) or has lost a substantial amount of excess weight. At that point, withholding treatment for a few months to allow recovery of suppression of pituitary-gonadal function and to ascertain whether the menstrual abnormality persists is usually advisable. In doing so, however, one must keep in mind that the anovulatory cycles of PCOS lead to relative infertility, not absolute infertility. The need for continued use of OCP for contraceptive purposes must be considered.

Progestin monotherapy is an alternative to OCPs for the control of menstrual irregularities. Micronized progesterone (Prometrium) 100 to 200 mg daily at bedtime

for 7 to 10 days induces withdrawal bleeding in the majority of patients, but some do not respond, apparently because of an antiestrogenic effect of androgen excess on the endometrium, and breakthrough bleeding is more likely than with OCPs. Progestin therapy has the appeal of permitting the detection of the emergence of normal menstrual cyclicity. However, it does not normalize androgen levels and is not an adequate treatment if hirsutism or hirsutism equivalents are a problem. The perimenarcheal girl who responds well to progestin therapy can be maintained at approximately 6-week cycles to permit the detection of spontaneous menses. Side effects of progestin include mood symptoms (depression), bloating, and breast soreness. Patients must be informed that oral progestin dosed in this way is not a means of contraception.

Antiandrogens generally yield improvement in hirsutism beyond that attainable with OCPs. They can be expected to reduce the Ferriman-Gallwey score by 15% to 40%, although there is considerable variation among individuals. Antiandrogen use for this purpose is off-label because all carry the risk of causing pseudohermaphroditism of the male fetus. Therefore, all antiandrogens should be prescribed with a contraceptive, preferably an OCP. They may have a modest effect on the metabolic abnormalities associated with PCOS.[925]

Spironolactone in high dosage is the safest potent antiandrogen in the United States. We recommend starting with 100 mg twice a day until the maximal effect has been achieved and then attempting to reduce the dose to 50 mg twice a day for maintenance therapy. Spironolactone usually is well tolerated, but it is contraindicated in patients with adrenal, hepatic, or renal insufficiency. Women are at risk of hyperkalemia if on potassium-sparing diuretics, potassium supplements, daily nonsteroidal anti-inflammatory drugs, angiotensin converting enzyme inhibitors, heparin, or such drugs. Therefore, electrolytes should be monitored. Alone, it tends to cause irregular bleeding.

Other antiandrogens used to treat hirsutism and hirsutism equivalents include cyproterone acetate, flutamide, and finasteride. Cyproterone acetate is a potent progestational antiandrogen that is used with estrogen in a reverse sequential regimen: 50 to 100 mg is given during days 1 to 10 of cycles in which estrogen is given from days 1 to 21. Flutamide is a more specific antiandrogen with efficacy similar to that of cyproterone and spironolactone, but it is more expensive and carries a seemingly idiosyncratic risk of fatal hepatocellular toxicity. Finasteride, a type 1 5-alpha-reductase inhibitor, may be as effective as other antiandrogens in the treatment of hirsutism, but it is less effective in pattern hair loss in females than in males. Although topical minoxidil is the only medication approved for alopecia treatment, antiandrogen-OCP therapy may be superior in those with PCOS.

Insulin-lowering treatments, from weight loss to drug treatment, uniformly improve hyperandrogenism. They have about a 50% probability of improving menstrual cyclicity and ovulatory status, which seems greater than explicable by the modest reduction in androgen levels that they bring about. The effect on hirsutism is negligible. Although weight reduction is indicated in obese PCOS patients, it is typically difficult to achieve. Bariatric surgery has led to improvement in androgen levels and menses in the majority, but improvements in hirsutism and ovulation have been inconsistent in adults. [926,927] This option is currently limited in adolescents to select patients with extremely high BMI and access to highly specialized centers.[926]

Metformin appears to have more utility than thiazolidines in management because it suppresses appetite and enhances weight loss. However, well-controlled studies in adults indicate that metformin therapy offers no advantage over lifestyle modification in regard to weight, menstrual frequency, or ovulation,[883,928,929] though it may have additive biochemical effects.[930] Abnormal glucose tolerance is the only clear indication for metformin. Thus, metformin is most effective in combination with a behavioral weight-reduction program.[925,926] Therapy should start with 500 mg daily of the extended release form before the evening meal, with an increase in the dose by 500 mg per week to a maximal dose of 2000 mg daily as tolerated. The larger doses often are better tolerated when divided into two daily doses. It is advisable to obtain a comprehensive metabolic panel at baseline to confirm normal hepatic and renal function. Although extremely rare, lactic acidosis is a potential complication of metformin use; patients should be made aware of this risk.

Other hormonal manipulations may be useful in specific unusual situations. Prednisone therapy has little utility in the management of the hirsutism or menstrual irregularity of PCOS. Gonadotropin releasing hormone agonists are an alternative when OCPs are contraindicated; they should be used with E2 replacement therapy.

FUTURE DIRECTIONS

Tremendous advances continue to occur in our understanding of puberty. The identification of genes involved in ovarian differentiation, the discovery of new hormones and hormone receptors, new insights into the regulation of gene transcription and signal transduction, further identification of the role of genetic factors and prenatal imprinting on pubertal disorders, and advances in the application of mass spectrometry to steroid assays can also be anticipated to occur in the coming years. We are in the midst of an explosion of information in the biologic sciences. It is becoming clear that the body puts a wide but finite repertoire of hormones and growth factors to myriad and unexpected uses. Many concepts that we hold dear at this moment are, at best, likely to be shown to be oversimplifications or, at worst, likely to be wrong. New information comes to light faster than we can assimilate it. The understanding of the interactions of the human genome with environmental factors can be expected to yield new insights into our understanding of puberty and its disorders.

REFERENCES

1. Donovan, B., & van der Werff ten Bosch, J. (1965). *Physiology of puberty*. London: Edward Arnold.
2. Wilkins, L. (1965). The dagnosis and treatment of endocrine disorders in childhood and adolescence. In C. Thomas (Ed.), Springfield.
3. Ramirez, D., & McCann, S. (1963). Comparison of the regulation of luteinizing hormone (LH) secretion in immature and adult rats. *Endocrinology*, *72*, 452.
4. Mills, I., Brooks, R., & Prunty, F. (1962). The relationship between the production of cortisol and androgen by the human adrenal. In A. Currie, T. Symington, & J. Grant (Eds.), *The human adrenal cortex*. Baltimore: Williams & Wilkins.
5. Krsmanovic, L. Z., Hu, L., Leung, P. K., et al. (2009). The hypothalamic GnRH pulse generator: multiple regulatory mechanisms. *Trends Endocrinol Metab*, *20*, 402–408.
6. Grumbach, M., Roth, J., Kaplan, S., & Kelch, R. (1974). Hypothalamic-pituitary regulation of puberty in man: evidence and concepts derived from clinical research. In M. Grumbach, C. Grave, & F. Mayer (Eds.), *The control of the onset of puberty* (pp. 115–207). New York: John Wiley & Sons.
7. Winter, J. S., Faiman, C., Hobson, W. C., et al. (1975). Pituitary-gonadal relations in infancy. I. Patterns of serum gonadotropin concentrations from birth to four years of age in man and chimpanzee. *J Clin Endocrinol Metab*, *40*, 545–551.
8. Arey, L. (1974). Development of the female reproductive system. *Developmental anatomy*. Philadelphia: WB Saunders.
9. Schwanzel-Fukuda, M., Jorgenson, K., Bergen, H., et al. (1992). Biology of normal luteinizing hormone-releasing hormone neurons during and after their migration from olfactory placode. *Endocr Rev*, *13*, 623.
10. Clements, J., Reyes, F., Winter, J., et al. (1980). Ontogenesis of gonadotropin-releasing hormone in the human fetal hypothalamus. *Proc Soc Exp Biol Med*, *163*, 437.
11. Kaplan, S., Grumbach, M., & Aubert, M. (1976). The ontogenesis of pituitary hormones and hypothalamic factors in the human fetus. *Recent Prog Horm Res*, *32*, 161.
12. King, J., & Gerall, A. (1976). Localization of luteinizing hormone-releasing hormone. *J Histochem Cytochem*, *24*, 829.
13. Raisman, G., & Field, P. (1973). Sexual dimorphism in the neuropil of the preoptic area of the rat and its dependence on neonatal androgen. *Brain Res*, *54*, 1.
14. Gorski, R. (1985). Sexual differentiation of the brain: possible mechanisms and implications. *Can J Physiol Pharmacol*, *63*, 577–594.
15. Rosenfield, R. L. (2007). Identifying children at risk of polycystic ovary syndrome. *J Clin Endocrinol Metab*, *92*, 787–796.
16. Foecking, E. M., Szabo, M., Schwartz, N. B., & Levine, J. E. (2005). Neuroendocrine consequences of prenatal androgen exposure in the female rat: absence of luteinizing hormone surges, suppression of progesterone receptor gene expression, and acceleration of the gonadotropin-releasing hormone pulse generator. *Biol Reprod*, *72*, 1475–1483.
17. Tapanainen, J., Koivisto, M., Vihko, R., & Huhtaniemi, I. (1981). Enhanced activity of the pituitary-gonadal axis in premature human infants . J Clin Endocrinol Metab, *52*, 235–238.
18. Kuiri-Hanninen, T., Kallio, S., Seuri, R., et al. (2011). Postnatal developmental changes in the pituitary-ovarian axis in preterm and term infant girls. *J Clin Endocrinol Metab*, *96*, 3432–3439.
19. Gulyas, B. J., Hodgen, G. D., Tullner, W. W., & Ross, G. T. (1977). Effects of fetal or maternal hypophysectomy on endocrine organs and body weight in infant rhesus monkeys *(Macaca mulatto)*: with particular emphasis on oogenesis. *Biol Reprod*, *16*, 216.
20. Merrill, J. (1959). Ovarian hilus cells. *Am J Obstet Gynecol*, *78*, 1258.
21. Carney, J. A. (1987). Unusual tumefactive spindle-cell lesions in the adrenal gland. *Hum Pathol*, *18*, 980–985.
22. Rabinovici, J., & Jaffe, R. (1990). Development and regulation of growth and differentiated function in human and subhuman primate fetal gonads. *Endocr Rev*, *11*, 532.
23. Cox, J. J., Willatt, L., Homfray, T., & Woods, C. G. (2011). A SOX9 duplication and familial 46,XX developmental testicular disorder. *N Engl J Med*, *364*, 91–93.
24. Richards, J. S., & Pangas, S. A. (2010). The ovary: basic biology and clinical implications. *J Clin Invest*, *120*, 963–972.
25. Lim, H. N., & Hawkins, J. R. (1998). Genetic control of gonadal differentiation. *Baillieres Clin Endocrinol Metab*, *12*, 1–16.
26. Peters, H. (1970). Migration of gonocytes into the mammalian gonad and their differentiation. *Philos Trans R Soc Lond* [Biol], *259*, 291.
27. Baker, T. (1963). A quantitative and cytological study of germ cells in human ovaries. *Proc R Soc Lond B Biol Sci*, *158*, 417–433.
28. Peters, H., Byskov, A., & Grinsted, J. (1978). Follicular growth in fetal and prepubertal ovaries of humans and other primates. *Clin Endocrinol Metab*, *7*, 469.
29. Ross, G. T., & Schreiber, J. R. (1978). The ovary. In S. S. C. Yen, & R. Jaffe (Eds.), *Reproductive endocrinology* (pp. 1978). Philadelphia: WB Saunders Company.
30. Peters, H. (1979). The human ovary in childhood and early maturity. *Eur J Obstet Gynecol Reprod Biol*, *9*, 137.
31. Gougeon, A. (1996). Regulation of ovarian follicular development in primates: facts and hypotheses. *Endocr Rev*, *17*, 121–155.
32. Carr, D., Naggar, R., & Hart, A. (1968). Germ cells in the ovaries of XO female infants. *Am J Clin Pathol*, *19*, 521.
33. Block, E. (1953). A quantitative morphological investigation of the follicular system in newborn female infants. *Acta Anat*, *17*, 201–206.
34. Richardson, S., Senikas, V., & Nelson, J. (1987). Follicular depletion during the menopausal transition: evidence for accelerated loss and ultimate exhaustion. *J Clin Endocrinol Metab*, *65*, 1231.
35. Gartler, S., Andina, R., & Cant, N. (1975). Ontogeny of X chromosome inactivation in the female germ line. *Exp Cell Res*, *91*, 454.
36. Zinn, A. R. (2001). The X chromosome and the ovary. *J Soc Gynecol Investig*, *8*, S34–S36.
37. Schmidt, D., Ovitt, C. E., Anlag, K., et al. (2004). The murine winged-helix transcription factor Foxl2 is required for granulosa cell differentiation and ovary maintenance. *Development*, *131*, 933–942.
38. Zou, K., Yuan, Z., Yang, Z., et al. (2009). Production of offspring from a germline stem cell line derived from neonatal ovaries. *Nat Cell Biol*, *11*, 631–636.
39. Pru, J. K., & Tilly, J. L. (2001). Programmed cell death in the ovary: insights and future prospects using genetic technologies. *Mol Endocrinol*, *15*, 845–853.
40. Robles, R., Morita, Y., Mann, K. K., et al. (2000). The aryl hydrocarbon receptor, a basic helix-loop-helix transcription factor of the PAS gene family, is required for normal ovarian germ cell dynamics in the mouse. *Endocrinology*, *141*, 450–453.
41. Besedovsky, H., & Sorkin, E. (1974). Thymus involvement in female sexual maturation. *Nature*, *249*, 356.
42. de Bruin, J. P., Dorland, M., Bruinse, H. W., et al. (1998). Fetal growth retardation as a cause of impaired ovarian development. *Early Hum Dev*, *51*, 39–46.
43. Sir-Petermann, T., Hitchsfeld, C., Codner, E., et al. (2007). Gonadal function in low birth weight infants: a pilot study. *J Pediatr Endocrinol Metab*, *20*, 405–414.
44. Ibañez, L., Valls, C., Cols, M., et al. (2002). Hypersecretion of FSH in infant boys and girls born small for gestational age. *J Clin Endocrinol Metab*, *87*, 1986–1988.
45. Chernausek, S. D. (2012). Update: consequences of abnormal fetal growth. *J Clin Endocrinol Metab*, *97*, 689–695.
46. Cole, B., Hensinger, K., Maciel, G. A., et al. (2006). Human fetal ovary development involves the spatiotemporal expression of p450c17 protein. *J Clin Endocrinol Metab*, *91*, 3654–3661.
47. Gagnon, R. (1998). Fetal endocrine adaptation to placental insufficiency. *Endocrinologist*, *8*, 436–442.
48. Winter, J., & Faiman, C. (1973). Pituitary-gonadal relations in female children and adolescents. *Pediatr Res*, *7*, 948–953.
49. Winter, J. S. D., Hughes, I. A., Reyes F. L., & Faiman, C. (1976). Pituitary-gonadal relations in infancy: 2. Patterns of serum gonadal steroid concentrations in man from birth to two years of age. *J Clin Endocrinol Metab*, *42*, 679.
50. Danon, M., Velez, O., Ostrea, T., et al. (1988). Dynamics of bioactive luteinizing hormone-human chorionic gonadotropin during the first 7 days of life. *Pediatr Res*, *23*, 530–533.
51. Bangham, D. R., Berryman, I., Burger, H., et al. (1973). An international collaborative study of 69–104, a reference preparation of human pituitary FSH and LH. *J Clin Endocrinol Metab*, *36*, 647–660.

52. Greaves, R. F., Hunt, R. W., Chiriano, A. S., & Zacharin, M. R. (2008). Luteinizing hormone and follicle-stimulating hormone levels in extreme prematurity: development of reference intervals. *Pediatrics*, *121*, e574–e580.
53. Perlman, M., Schenker, J., Glassman, M., & Ben-david, M. (1978). Prolonged hyperprolactinemia in preterm infants. *J Clin Endocrinol Metab*, *47*, 894–897.
54. Bouvattier, C., Carel, J. C., Lecointre, C., et al. (2003). Postnatal changes of T, LH, and FSH in 46,XY infants with mutations in the AR gene. *J Clin Endocrinol Metab*, *87*, 29–32.
55. Sullivan, S. D., & Moenter, S. M. (2004). Prenatal androgens alter GABAergic drive to gonadotropin-releasing hormone neurons: implications for a common fertility disorder. *Proc Natl Acad Sci USA*, *101*, 7129–7134.
56. Bidlingmaier, F., & Kiiorr, D. (1978). Oestrogens: physiological and clinical aspects. *Pediatr Adolesc Endocrinol*, *4*, 43.
57. Betend, B., Claustrat, B., Bizollon, C., et al. (1975). Etude de la fonction gonadotrope hypophysaire par le test a la LH-RH pendant la premiere année de la vie. *Ann Endocrinol* (Paris), *36*, 325.
58. Rosenfield, R. L., Burstein, S., Cuttler, L., et al. (1989). Use of nafarelin for testing pituitary-ovarian function. *J Reprod Med*, *34*, 1044–1050.
59. Chellakooty, M., Schmidt, I. M., Haavisto, A. M., et al. (2003). Inhibin A, inhibin B, follicle-stimulating hormone, luteinizing hormone, estradiol, and sex hormone-binding globulin levels in 473 healthy infant girls. *J Clin Endocrinol Metab*, *88*, 3515–3520.
60. Conte, F. A., Grumbach, M. M., Kaplan, S. L., & Reiter, E. O. (1980). Correlation of luteinizing hormone-releasing factor-induced luteinizing hormone and follicle-stimulating hormone release from infancy to 19 years with the changing pattern of gonadotropin secretion in agonadal patients: relation to the restraint of puberty. *J Clin Endocrinol Metab*, *50*, 163–168.
61. Penny, R., Olambiwonnu, N., & Frasier S (1974). Serum gonadotropin concentrations during the first four years of life. *J Clin Endocrinol Metab*, *38*, 320.
62. Elsholz, D. D., Padmanabhan, V., Rosenfield, R. L., et al. (2004). GnRH agonist stimulation of the pituitary-gonadal axis in children: age and sex differences in circulating inhibin-B and activin-A. *Hum Reprod*, *19*, 2748–2758.
63. Bourguignon, J. P., Hoyoux, C., Reuter, A., & Franchimont, P. (1979). Urinary excretion of immunoreactive luteinizing hormone-releasing hormone-like material and gonadotropins at different stages of life. *J Clin Endocrinol Metab*, *48*, 78–84.
64. Rosenfield, R. L. (1977). Hormonal events and disorders of puberty. In J. Givens (Ed.), *Gynecologic endocrinology* (pp. 1–19). Chicago: Year Book Medical Publishers.
65. Attardi, B., & Ohno, S. (1976). Androgen and estrogen receptors in the developing mouse brain. *Endocrinology*, *99*, 1279.
66. Rifkind, A. B., Kulin, H. E., & Ross, G. T. (1967). Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in the urine of prepubertal children. *J Clin Invest*, *46*, 1925–1931.
67. Apter, D., Butzow, T. L., Laughlin, G. A., & Yen, S. S. (1993). Gonadotropin-releasing hormone pulse generator activity during pubertal transition in girls: pulsatile and diurnal patterns of circulating gonadotropins. *J Clin Endocrinol Metab*, *76*, 940–949.
68. Wilson, M. E., Fisher, J., & Chikazawa, K. (2004). Estradiol negative feedback regulates nocturnal luteinizing hormone and follicle-stimulating hormone secretion in prepubertal female rhesus monkeys. *J Clin Endocrinol Metab*, *89*, 3973–3978.
69. Mitamura, R., Yano, K., Suzuki, N., et al. (2000). Diurnal rhythms of luteinizing hormone, follicle-stimulating hormone, testosterone, and estradiol secretion before the onset of female puberty in short children. *J Clin Endocrinol Metab*, *85*, 1074–1080.
70. Merrill, J. (1963). The morphology of the prepubertal ovary: relationship to the polycystic ovary syndrome. *South Med J*, *56*, 225–231.
71. Kraus, F., & Neubecker, R. (1962). Luteinization of the ovarian theca in infants and children. *Am J Clin Pathol*, *37*, 389–397.
72. Stanhope, R., Adams, J., Jacobs, H., & Brook, C. (1985). Ovarian ultrasound assessment in normal children, idiopathic precocious puberty, and during low dose pulsatile gonadotrophin releasing hormone treatment of hypogonadotrophic hypogonadism. *Arch Dis Child*, *60*, 116.
73. Bridges, N. A., Cooke, A., Healy, M. J. R., et al. (1993). Standards for ovarian volume in childhood and puberty. *Fertil Steril*, *60*, 456–460.
74. Buzi, F., Pilotta, A., Dordoni, D., et al. (1998). Pelvic ultrasonography in normal girls and in girls with pubertal precocity. *Acta Paediatr*, *87*, 1138–1145.
75. de Vries, L., Horev, G., Schwartz, M., & Phillip, M. (2006). Ultrasonographic and clinical parameters for early differentiation between precocious puberty and premature thelarche. *Eur J Endocrinol*, *154*, 891–898.
76. Badouraki, M., Christoforidis, A., Economou, I., et al. (2008). Evaluation of pelvic ultrasonography in the diagnosis and differentiation of various forms of sexual precocity in girls. *Ultrasound Obstet Gynecol*, *32*, 819–827.
77. Hagen, C. P., Aksglaede, L., Sorensen, K., et al. (2010). Serum levels of anti-Mullerian hormone as a marker of ovarian function in 926 healthy females from birth to adulthood and in 172 Turner syndrome patients. *J Clin Endocrinol Metab*, *95*, 5003–5010.
78. Sedin, G., Bergquist, C., & Lindgren, P. G. (1985). Ovarian hyperstimulation syndrome in preterm infants. *Pediatr Res*, *19*, 548–552.
79. McKiernan, J. F., & Hull, D. (1981). Breast development in the newborn. *Arch Dis Child*, *56*, 525–529.
80. Feuillan, P. P., Jones, J. V., Barnes, K., et al. (1999). Reproductive axis after discontinuation of gonadotropin-releasing hormone analog treatment of girls with precocious puberty: long term follow-up comparing girls with hypothalamic hamartoma to those with idiopathic precocious puberty. *J Clin Endocrinol Metab*, *84*, 44–49.
81. Collett-Solberg, P., & Grumbach, M. (1965). A simplified procedure for evaluating estrogenic effects and the sex chromatin pattern in exfoliated cells in urine: studies in premature thelarche and gynecomastia of adolescence. *J Pediatr*, *66*, 883–890.
82. Potau, N., Ibanez, L., Sentis, M., & Carrascosa, A. (1999). Sexual dimorphism in the maturation of the pituitary-gonadal axis, assessed by GnRH agonist challenge. *Eur J Endocrinol*, *141*, 27–34.
83. Rosenfield, R. L., Bordini, B., & Yu, C. (2013). Comparison of detection of normal puberty in girls by a hormonal sleep test and a gonadotropin-releasing hormone agonist test. *J Clin Endocrinol Metab*, *98*, 1591–1601.
84. Norjavaara, E., Ankargerg, C., & Albertsson-Wikland, K. (1996). Diurnal rhythm of 17β-estradiol secretion throughout pubertal development in healthy girls: evaluation by a sensitive radioimmunoassay. *J Clin Endocrinol Metab*, *81*, 4095–4102.
85. Dickerman, Z., Prager-Lewin, R., & Laron, Z. (1976). Response of plasma LH and FSH to synthetic LHRH in children at various pubertal stages. *Am J Dis Child*, *130*, 634–638.
86. Rettig, K., Duckett, C. E., Sweetland, M., et al. (1981). Urinary excretion of immunoreactive luteinizing hormone-releasing hormone-like material in children: correlation with pubertal development. *J Clin Endocrinol Metab*, *52*, 1150.
87. Barnea, A., Cho, C., & Porter, J. (1979). A role for the ovaries in maturational processes of hypothalamic neurons containing luteinizing hormone-releasing hormone. *Endocrinology*, *105*, 1303.
88. Knobil, E. (1980). The neuroendocrine control of the menstrual cycle. *Recent Prog Horm Res*, *36*, 53–88.
89. Marshall, J., & Kelch, R. (1986). Gonadotropin-releasing hormone: role of pulsatile secretion in the regulation of reproduction. *N Engl J Med*, *315*, 1459–1468.
90. Crowley, W. F., Jr., Filicori, M., & Santoro, N. F. (1987). GnRH secretion across the normal menstrual cycle. In W. J. Crowley, J. Hofler (Eds.), *The episodic secretion of hormones* (pp. 219–231). New York: John Wiley/Churchill Livingstone.
91. Hansen, J. W., Hoffman, H. J., & Ross, G. T. (1975). Monthly gonadotropin cycles in premenarcheal girls. *Science*, *190*, 161–163.
92. Kapen, S., Boyar, R., Finkelstein, J., et al. (1974). Effect of sleep-wake cycle reversal on luteinizing hormone secretory pattern in puberty. *J Clin Endocrinol Metab*, *39*, 293–299.
93. Boyar, R. M., Wu, R. H.K, Roffwarg, H., et al. (1976). Human puberty: 24-hour estradiol patterns in pubertal girls. *J Clin Endocrinol Metab*, *43*, 1418–1421.
94. Lucky, A. W., Rich, B. H., Rosenfield, R. L., et al. (1980). LH bioactivity increases more than immunoreactivity during puberty. *J Pediatr*, *97*, 205.
95. Rosenfield, R. L., & Helke, J. (1992). Is an immunoassay available for the measurement of bioactive LH in serum? *J Androl*, *13*, 1–10.
96. Beitins, I., & Padmanabhan, V. (1991). Bioactivity of gonadotropins. *Endocrinol Metab Clin North Am*, *20*, 85–120.

97. Legro, R. S., Lin, H. M., Demers, L. M., & Lloyd, T. (2000). Rapid maturation of the reproductive axis during perimenarche independent of body composition. *J Clin Endocrinol Metab*, *85*, 1021–1025.
98. Elkind-Hirsch, K., Ravnikar, V., Schiff, I., et al. (1982). Determinations of endogenous immunoreactive luteinizing hormone-releasing hormone in human plasma. *J Clin Endocrinol Metab*, *54*, 602–607.
99. Xia, L., Van Vugt, D., Alston, E., et al. (1992). A surge of gonadotropin-releasing hormone accompanies the estradiol-induced gonadotropin surge in the Rhesus monkey. *Endocrinology*, *131*, 2812–2820.
100. Gasc, J. M., & Baulieu, E. E. (1988). Regulation by estradiol of the progesterone receptor in the hypothalamus and pituitary: an immunohistochemical study in the chicken. *Endocrinology*, *122*, 1357–1365.
101. Lin, W., & Ramirez, V. (1988). Effect of pulsatile infusion of progesterone on the in vivo activity of the luteinizing hormone-releasing hormone neural apparatus of awake unrestrained female and male rabbits. *Endocrinology*, *122*, 868.
102. Moll, G. W., Jr., & Rosenfield, R. L. (1984). Direct inhibitory effect of estradiol on pituitary luteinizing hormone responsiveness to luteinizing hormone releasing hormone is specific and of rapid onset. *Biol Reprod*, *30*, 59.
103. Young, J., & Jaffe, R. (1976). Strength-duration characteristics of estrogen effects on gonadotropin response to gonadotropin-releasing hormone in women. II. *J Clin Endocrinol Metab*, *42*, 432–442.
104. March, C. M., Goebelsmann, U., Nakamura, R. M., & Mishell, D. R., Jr. (1979). Roles of estradiol and progesterone in eliciting the midcycle luteinizing hormone and follicle-stimulating hormone surges. *J Clin Endocrinol Metab*, *49*, 507–513.
105. Chang, R., & Jaffe, R. (1978). Progesterone effects on gonadotropin release in women pretreated with estradiol. *J Clin Endocrinol Metab*, *47*, 1191–1125.
106. Advis, J., Krause, J., & McKelvy, J. (1983). Evidence that endopeptidase-catalyzed luteinizing hormone releasing hormone cleavage contributes to the regulation of median eminence LHRH levels during positive steroid feedback. *Endocrinology*, *112*, 1147–1149.
107. Harlow, C., Shaw, N., Hillier, S., & Hodges, J. (1988). Factors influencing follicle-stimulating hormone-responsive steroidogenesis in marmoset granulosa cells: effects of androgens and the stage of follicular maturity. *Endocrinology*, *122*, 2780.
108. Richards, J., & Bogovich, K. (1982). Effects of human chorionic gonadotropin and progesterone on follicular development in the immature rat. *Endocrinology*, *111*, 1429–1438.
109. Jia, X.-C., Kessel, B., Welsh, T. N. J., & Hsueh, A. J. (1985). Androgen inhibition of follicle-stimulating hormone-stimulated luteinizing hormone receptor formation in cultured rat granulosa cells. *Endocrinology*, *117*, 13–22.
110. Hsueh, A. J. W., Adashi, E. Y., Jones, P. B. C., & Welsh, T. N. J. (1984). Hormonal regulation of the differentiation of cultured ovarian granulosa cells. *Endocrinol Rev*, *5*, 76–127.
111. Findlay, J. (1994). Peripheral and local regulators of folliculogenesis. *Reprod Fertil Dev*, *6*, 1–13.
112. White, S. S., & Ojeda, S. R. (1981). Changes in ovarian LHRH receptor content during the onset of puberty in the female rat. *Endocrinology*, *108*, 347.
113. Eckstein, B., Shani, J., Ravid, R., & Goldhaber, G. (1981). Effect of androstanediol sulfates on luteinizing hormone release in ovariectomized rats. *Endocrinology*, *108*, 500.
114. Polhemus, D. (1953). Ovarian maturation and cyst formation in children. *Pediatr*, *11*, 588–593.
115. Adams, J., Franks, S., Polson, D. W., et al. (1985). Multifollicular ovaries: clinical and endocrine features and response to pulsatile gonadotrophin releasing hormone. *Lancet*, *326*, 1375–1379.
116. Venturoli, S., Porcu, E., Fabbri, R., et al. (1995). Longitudinal change of sonographic ovarian aspects and endocrine parameters in irregular cycles of adolescence. *Pediatr Res*, *38*, 974–980.
117. van Hooff, M. H., Voorhorst, F. J., Kaptein, M. B., et al. (2000). Polycystic ovaries in adolescents and the relationship with menstrual cycle patterns, luteinizing hormone, androgens, and insulin. *Fertil Steril*, *74*, 49–58.
118. Mortensen, M., Rosenfield, R. L., & Littlejohn, E. (2006). Functional significance of polycystic-size ovaries in healthy adolescents. *J Clin Endocrinol Metab*, *91*, 3786–3790.
119. Villarroel, C., Merino, P. M., Lopez, P., et al. (2011). Polycystic ovarian morphology in adolescents with regular menstrual cycles is associated with elevated anti-Mullerian hormone. *Hum Reprod*, *26*, 2861–2868.
120. Balen, A. H., Laven, J. S., Tan, S. L., & Dewailly, D. (2003). Ultrasound assessment of the polycystic ovary: international consensus definitions. *Hum Reprod Update*, *9*, 505–514.
121. Codner, E., Villarroel, C., Eyzaguirre, F. C., et al. (2011). Polycystic ovarian morphology in postmenarchal adolescents. *Fertil Steril*, *95*, 702–706.
122. Abraham, G. (1974). Ovarian and adrenal contributions to peripheral androgens during the menstrual cycle. *J Clin Endocrinol Metab*, *39*, 340.
123. Ross, G. T., Cargille, C. M., Lipsett, M. B., et al. (1970). Pituitary and gonadal hormones in women during spontaneous and induced ovulatory cycles. *Recent Prog Horm Res*, *26*, 1–62.
124. McCartney, C. R., Gingrich, M. B., Hu, Y., et al. (2002). Hypothalamic regulation of cyclic ovulation: evidence that the increase in gonadotropin-releasing hormone pulse frequency during the follicular phase reflects the gradual loss of the restraining effects of progesterone. *J Clin Endocrinol Metab*, *87*, 2194–2200.
125. Shaw, N. D., Klingman, K. M., Srouji, S. S., et al. (2012). Gonadotropin responses to estrogen-positive and -negative feedback are identical in African-American and Caucasian women. *J Clin Endocrinol Metab*, *97*, E106–E109.
126. McCartney, C. R., Blank, S. K., & Marshall, J. C. (2007). Progesterone acutely increases LH pulse amplitude but does not acutely influence nocturnal LH pulse frequency slowing during the late follicular phase in women. *Am J Physiol Endocrinol Metab*, *292*, E900–E906.
127. Johnson, D., & Naqvi, R. (1969). A positive feedback action of androgen on pituitary follicle stimulating hormone: induction of a cyclic phenomenon. *Endocrinology*, *85*, 881.
128. Melrose, P., & Gross, R. (1987). Steroid effects on the secretory modalities of gonadotropin-releasing hormone release. *Endocrinology*, *121*, 190.
129. Soules, M., Steiner, R., Clifton, D., et al. (1984). Progesterone modulation of pulsatile luteinizing hormone secretion in normal women. *J Clin Endocrinol Metab*, *58*, 378–383.
130. McGee, E., & Hsueh, A. (2000). Initial and cyclic recruitment of ovarian follicles. *Endocr Rev*, *21*, 200–214.
131. Erickson, G. F., Magoffin, D. A., Dyer, C. A., & Hofeditz, C. (1985). The ovarian androgen producing cells: a review of structure/function relationships. *Endocr Rev*, *6*, 371–399.
132. Richards, J. (1994). Hormonal control of gene expression in the ovary. *Endocr Rev*, *15*, 725.
133. Li, J., Kawamura, K., Cheng, Y., et al. (2010). Activation of dormant ovarian follicles to generate mature eggs. *Proc Natl Acad Sci USA*, *107*, 10280–10284.
134. Ross, C. (1974). Gonadotropins and preantral follicular maturation in women. *Fertil Steril*, *25*, 52.
135. Kumar, T. R., Wang, Y., Lu, N., & Matzuk, M. M. (1997). Follicle stimulating hormone is required for ovarian follicle maturation but not male fertility. *Nat Genet*, *15*, 201–204.
136. Barnes, R. B., Namnoum, A. B., Rosenfield, R. L., & Layman, L. C. (2002). The role of LH and FSH in ovarian androgen secretion and ovarian follicular development: clinical studies in a patient with isolated FSH deficiency and multicystic ovaries: case report. *Hum Reprod*, *17*, 88–91.
137. Meduri, G., Touraine, P., Beau, I., et al. (2003). Delayed puberty and primary amenorrhea associated with a novel mutation of the human follicle-stimulating hormone receptor: clinical, histological, and molecular studies. *J Clin Endocrinol Metab*, *88*, 3491–498.
138. Weil, S., Vendola, K., Zhou, J., & Bondy, C. A. (1999). Androgen and follicle-stimulating hormone interactions in primate ovarian follicle development. *J Clin Endocrinol Metab*, *84*, 2951–2956.
139. Vendola, K. A., Zhou, J., Adesanya, O. O., Weil, S. J., & Bondy, C. A. (1998). Androgens stimulate early stages of follicular growth in the primate ovary. *J Clin Invest*, *15*, 2622–2629.

140. Shiina, H., Matsumoto, T., Sato, T., et al. (2006). Premature ovarian failure in androgen receptor-deficient mice. Proc Natl Acad Sci USA, *103*, 224–229.
141. Ehrmann, D. A., Barnes, R. B., & Rosenfield, R. L. (1995). Polycystic ovary syndrome as a form of functional ovarian hyperandrogenism due to dysregulation of androgen secretion. *Endocr Rev*, *16*, 322–353.
142. Inkster, S., & Brodie, A. (1991). Expression of aromatase cytochrome P-450 in premenopausal and postmenopausal human ovaries: an immunocytochemical study. *J Clin Endocrinol Metab*, *73*, 717–726.
143. McNatty, K. P., Makris, A., Reinhold, V. N., et al. (1979). Metabolism of androstenedione by human ovarian tissues in vitro with particular reference to reductase and aromatase activity. *Steroids*, *34*, 429–443.
144. McNatty, K. P., Smith, D. M., Makris, A., et al. (1979). The microenvironment of the human antral follicle: interrelationships among the steroid levels in antral fluid, the population of granulosa cells, and the status of the oocyte in vivo and in vitro. *J Clin Endocrinol Metab*, *49*, 851–860.
145. Erickson, G. F., Hsueh, A. J., Quigley, M. E., et al. (1979). Functional studies of aromatase activity in human granulosa cells from normal and polycystic ovaries. *J Clin Endocrinol Metab*, *49*, 514–519.
146. Tsang, B., Armstrong, D., & Whitfield, J. (1980). Steroid biosynthesis by isolated human ovarian follicular cells in vitro. *J Clin Endocrinol Metab*, *51*, 1407–1411.
147. Mason, H. D., Willis, D. S., Beard, R. W., et al. (1994). Estradiol production by granulosa cells of normal and polycystic ovaries: relationship to menstrual cycle history and concentrations of gonadotropins and sex steroids in follicular fluid. *J Clin Endocrinol Metab*, *79*, 1355–1360.
148. Haning, R. V., Jr., Hackett, R. J., Flood, C. A., et al. (1993). Testosterone, a follicular regulator: key to anovulation. *J Clin Endocrinol Metab*, *77*, 710–715.
149. Karnitis, V., Townson, D., Friedman, C., & Danforth, D. (1994). Recombinant human follicle-stimulating hormone stimulates multiple follicular growth, but minimal estrogen production in gonadotropin-releasing hormone antagonist-treated monkeys: examining the role of luteinizing hormone in follicular development and steroidogenesis. *J Clin Endocrinol Metab*, *79*, 91–97.
150. Zhou, J., Kumar, R., Matzuk, M. M., & Bondy, C. (1997). Insulin-like growth factor I regulates gonadotropin responsiveness in the murine ovary. *Molec Endocrinol*, *11*, 1924–1933.
151. McNatty, K. P., Hunter, W. M., MacNeilly, A. S., & Sawers, R. S. (1975). Changes in the concentration of pituitary and steroid hormones in the follicular fluid of human graafian follicles throughout the menstrual cycle. *J Endocrinol*, *64*, 555.
152. McNatty, K. P., Makris, A., De Grazia, C., et al. (1980). Steroidogenesis by recombined follicular cells from the human ovary in vitro. *J Clin Endocrinol Metab*, *51*, 1286.
153. Couse, J. F., Hewitt, S. C., Bunch, D. O., et al. (1999). Postnatal sex reversal of the ovaries in mice lacking estrogen receptors alpha and beta. *Science*, *286*, 2328–2331.
154. Lofrano-Porto, A., Barra, G. B., Giacomini, L. A., et al. (2007). Luteinizing hormone beta mutation and hypogonadism in men and women. *N Engl J Med*, *357*, 897–904.
155. Shoham, Z., Balen, A., Patel, A., & Jacobs, H. S. (1991). Results of ovulation induction using human menopausal gonadotropin or purified follicle-stimulating hormone in hypogonadotropic hypogonadism patients. *Fertil Steril*, *56*, 1048–1053.
156. Schoot, D., Bennink, H., Mannaerts, B., et al. (1992). Human recombinant follicle-stimulating hormone induces growth of preovulatory follicles without concomitant increase in androgen and estrogen biosynthesis in a woman with isolated gonadotropin deficiency. *J Clin Endocrinol Metab*, *74*, 1471–1473.
157. Richards, J. S., Russell, D. L., Ochsner, S., et al. (2002). Novel signaling pathways that control ovarian follicular development, ovulation, and luteinization. *Recent Prog Horm Res*, *57*, 195–220.
158. Radu, A., Pichon, C., Camparo, P., et al. (2010). Expression of follicle-stimulating hormone receptor in tumor blood vessels. *N Engl J Med*, *363*, 1621–1630.
159. Jonassen, J., Bose, K., & Richards, J. (1982). Enhancement and desensitization of hormone-responsive adenylate cyclase in granulosa cells of preantral and antral ovarian follicles: effects of estradiol and follicle-stimulating hormone. *Endocrinology*, *111*, 74.
160. Masciarelli, S., Horner, K., Liu, C., et al. (2004). Cyclic nucleotide phosphodiesterase 3A-deficient mice as a model of female infertility. *J Clin Invest*, *114*, 196–205.
161. Lindner, H. R., Tsafriri, A., Lieberman, M. E., et al. (1974). Gonadotropin action on cultured graafian follicles: induction of maturation division of the mammalian oocyte and differentiation of the luteal cell. *Recent Prog Horm Res*, *30*, 79.
162. Moor, R. M., Dai, Y., Lee, C., & Fulka, J., Jr. (1998). Oocyte maturation and embryonic failure. *Hum Reprod Update*, *4*, 223–236.
163. Tsafriri, A., Cao, X., Ashkenazi, H., et al. (2005). Resumption of oocyte meiosis in mammals: on models, meiosis activating sterols, steroids and EGF-like factors. *Mol Cell Endocrinol*, *234*, 37–45.
164. Hillier, S., & Ross, C. (1979). Effects of exogenous testosterone on ovarian weight, follicular morphology and intraovarian progesterone concentration in estrogen-primed hypophysectomized immature female rats. *Biol Reprod*, *20*, 261.
165. Poland, M., Seu, D., & Tarlatzis, B. (1986). Human chorionic gonadotropin stimulation of estradiol production and androgen antagonism of gonadotropin-stimulated responses in cultured human granulosa luteal cells. *J Clin Endocrinol Metab*, *62*, 628.
166. Chaffkin, L., Luciano, A., & Peluso, J. (1993). The role of progesterone in regulating human granulosa cell proliferation and differentiation in vitro. *J Clin Endocrinol Metab*, *76*, 696–700.
167. Schreiber, J., Nakamura, K., Truscella, A., & Erickson, G. (1982). Progestins inhibit FSH-induced functional LH receptors in cultured rat granulosa cells. Molec Cell Endocrinol 25, 113.
168. Fauser, B. C., & Van Heusden, A. M. (1997). Manipulation of human ovarian function: physiological concepts and clinical consequences. *Endocr Rev*, *18*, 71–106.
169. Couse, J. F., Bunch, D. O., Lindzey, J., et al. (1999). Prevention of the polycystic ovarian phenotype and characterization of ovulatory capacity in the estrogen receptor-alpha knockout mouse. *Endocrinology*, *140*, 5855–5865.
170. Couse, J. F., & Korach, K. S. (1999). Estrogen receptor null mice: what have we learned and where will they lead us? *Endocr Rev*, *20*, 358–417. Erratum in *Endocr Rev*, *20*, 459.
171. Cheng, G., Weihua, Z., Makinen, S., et al. (2002). A role for the androgen receptor in follicular atresia of estrogen receptor beta knockout mouse ovary. *Biol Reprod*, *66*, 77–84.
172. La Marca, A., Broekmans, F. J., Volpe, A., et al. (2009). Anti-Mullerian hormone (AMH): what do we still need to know? *Hum Reprod*, *24*, 2264–2275.
173. Grossman, M. P., Nakajima, S. T., Fallat, M. E., & Siow, Y. (2008). Mullerian-inhibiting substance inhibits cytochrome P450 aromatase activity in human granulosa lutein cell culture. *Fertil Steril*, *89*, 1364–1370.
174. Hehenkamp, W. J., Looman, C. W., Themmen, A. P., et al. (2006). Anti-Mullerian hormone levels in the spontaneous menstrual cycle do not show substantial fluctuation. *J Clin Endocrinol Metab*, *91*, 4057–4063.
175. Grynberg M, Pierre A, Rey R, et al. (2012) Differential regulation of ovarian anti-mullerian hormone (AMH) by estradiol through alpha- and beta-estrogen receptors. *J Clin Endocrinol Metab*, *97*: E1649–57.
176. Freeman, E. W., Sammel, M. D., Lin, H., & Gracia, C. R. (2012). Anti-mullerian hormone as a predictor of time to menopause in late reproductive age women. *J Clin Endocrinol Metab*, *97*, 1673–1680.
177. Burger, H. G., Groome, N. P., & Robertson, D. M. (1998). Both inhibin A and B respond to exogenous follicle-stimulating hormone in the follicular phase of the human menstrual cycle. *J Clin Endocrinol Metab*, *83*, 4167–4169.
178. Welt, C. K., Smith, Z. A., Pauler, D. K., & Hall, J. E. (2001). Differential regulation of inhibin A and inhibin B by luteinizing hormone, follicle-stimulating hormone, and stage of follicle development. *J Clin Endocrinol Metab*, *86*, 2531–2537.
179. Auletta, F., & Flint, A. (1988). Mechanisms controlling corpus luteum function in sheep, cows, nonhuman primates, and women especially in relation to the time of luteolysis. *Endocr Rev*, *9*, 88.
180. Vande Wiele, R. L., Bogumil, J., Dyrenfurth, I., et al. (1970). Mechanisms regulating the menstrual cycle in women. *Recent Prog Horm Res*, *26*, 63.

181. Patel, S. S., Beshay, V. E., Escobar, J. C., et al. (2009). Molecular mechanism for repression of 17alpha-hydroxylase expression and androstenedione production in granulosa cells. *J Clin Endocrinol Metab*, *94*, 5163–5168.
182. Richards, J., Hegin, L., & Caston, L. (1986). Differentiation of rat ovarian thecal cells: evidence for functional luteinization. *Endocrinology*, *118*, 1660–1668.
183. Moghissi, K. S., Syner, F. N., & Evans, T. N. (1972). A composite picture of the menstrual cycle. *Am J Obstet Gynecol*, *114*, 405–418.
184. Stouffer, R., Hodgen, C., Ottobre, A., & Christina, C. (1984). Follicular fluid treatment during the follicular versus luteal phase of the menstrual cycle: effects on corpus luteum function. *J Clin Endocrinol Metab*, *58*, 1027.
185. Parent, A. S., Teilmann, G., Juul, A., et al. (2003). The timing of normal puberty and the age limits of sexual precocity: variations around the world, secular trends, and changes after migration. *Endocr Rev*, *24*, 668–693.
186. Massart, F., & Saggese, G. (2010). Oestrogenic mycotoxin exposures and precocious pubertal development. *Int J Androl*, *33*, 369–376.
187. Jacobson-Dickman, E., & Lee, M. M. (2009). The influence of endocrine disruptors on pubertal timing. *Curr Opin Endocrinol Diabetes Obes*, *16*, 25–30.
188. Deboer, M. D., Steinman, J., & Li, Y. (2012). Partial normalization of pubertal timing in female mice with DSS colitis treated with anti-TNF-alpha antibody. *J Gastroenterol*, *47*, 647–654.
189. Fischbein, S. (1977). Intra-pair similarity in physical growth of monozygotic and of dizygotic twins during puberty. *Ann Hum Biol*, *4*, 417–430.
190. Garn, S. M., & Bailey, S. M. (1978). Genetics and maturational processes. In F. Falkner, J. M. Tanner (Eds.), *Human growth 1: principles and prenatal growth* (pp. 307–330). New York: Plenum.
191. Sharma, J. C. (1983). The genetic contribution to pubertal growth and development studied by longitudinal growth data on twins. *Ann Hum Biol*, *10*, 163–171.
192. Kaprio, J., Rimpela, A., Winter, T., et al. (1995). Common genetic influences on BMI and age at menarche. *Hum Biol*, *67*, 739–753.
193. Sun, S. S., Schubert, C. M., Chumlea, W. C., et al. (2002). National estimates of the timing of sexual maturation and racial differences among US children. *Pediatrics*, *110*, 911–919.
194. Chumlea, W. C., Schubert, C. M., Roche, A. F., et al. (2003). Age at menarche and racial comparisons in US girls. *Pediatrics*, *111*, 110–113.
195. Sedlmeyer, I. L., Hirschhorn, J. N., & Palmert, M. R. (2002). Pedigree analysis of constitutional delay of growth and maturation: determination of familial aggregation and inheritance patterns. *J Clin Endocrinol Metab*, *87*, 5581–5586.
196. Sedlmeyer, I. L., & Palmert, M. R. (2002). Delayed puberty: analysis of a large case series from an academic center. *J Clin Endocrinol Metab*, *87*, 1613–1620.
197. Eaves, L., Silberg, J., Foley, D., et al. (2004). Genetic and environmental influences on the relative timing of pubertal change. *Twin Res*, *7*, 471–481.
198. Herman-Giddens, M. E., Kaplowitz, P. B., & Wasserman, R. (2004). Navigating the recent articles on girls' puberty in *Pediatrics*: what do we know and where do we go from here? *Pediatrics*, *113*, 911–917.
199. Nathan, B. M., Hodges, C. A., & Palmert, M. R. (2006). The use of mouse chromosome substitution strains to investigate the genetic regulation of pubertal timing. *Mol Cell Endocrinol*, *254–255*, 103–108.
200. Franco, B., Guioli, S., Pragliola, A., et al. (1991). A gene deleted in Kallmann's syndrome shares homology with neural cell adhesion and axonal path-finding molecules. *Nature*, *353*, 529–536.
201. Seminara, S. B. (2006). Mechanisms of disease: the first kiss-a crucial role for kisspeptin-1 and its receptor, G-protein-coupled receptor 54, in puberty and reproduction. *Nat Clin Pract Endocrinol Metab*, *2*, 328–334.
202. Pitteloud, N., Meysing, A., Quinton, R., et al. (2006). Mutations in fibroblast growth factor receptor 1 cause Kallmann syndrome with a wide spectrum of reproductive phenotypes. *Mol Cell Endocrinol*, *254–255*, 60–69.
203. Pfaeffle, R. W., Savage, J. J., Hunter, C. S., et al. (2007). Four novel mutations of the LHX3 gene cause combined pituitary hormone deficiencies with or without limited neck rotation. *J Clin Endocrinol Metab*, *92*, 1909–1919.
204. Elks, C. E., Perry, J. R., Sulem, P., et al. (2010). Thirty new loci for age at menarche identified by a meta-analysis of genome-wide association studies. *Nat Genet*, *42*, 1077–1085.
205. Sedlmeyer, I. L., Pearce, C. L., Trueman, J. A., et al. (2005). Determination of sequence variation and haplotype structure for the gonadotropin-releasing hormone (GnRH) and GnRH receptor genes: investigation of role in pubertal timing. *J Clin Endocrinol Metab*, *90*, 1091–1099.
206. Terasawa, E., & Fernandez, D. L. (2001). Neurobiological mechanisms of the onset of puberty in primates. *Endocr Rev*, *22*, 111–151.
207. Plant, T. M., & Barker-Gibb, M. L. (2004). Neurobiological mechanisms of puberty in higher primates. *Hum Reprod Update*, *10*, 67–77.
208. Ojeda, S. R., & Urbanski, H. F. (1994). Puberty in the rat. In E. Knobil, & J. D. Neill (Eds.), *The physiology of reproduction* (pp. 363–407). New York: Raven Press.
209. Plant, T. M., & Witchel, S. F. (2006). Puberty in non-human primates and humans. In J. R. G. Challis, D. M. de Kretser, J. D. Neill, et al. (Eds.), *Knobil and Neill's physiology of reproduction* (pp. 2177–2230). New York: Elsevier.
210. Morelli, A., Marini, M., Mancina, R., et al. (2008). Sex steroids and leptin regulate the "first Kiss" (KiSS 1/G-protein-coupled receptor 54 system) in human gonadotropin-releasing-hormone-secreting neuroblasts. *J Sex Med*, *5*, 1097–1113.
211. Seminara, S. B., Messager, S., Chatzidaki, E. E., et al. (2003). The GPR54 gene as a regulator of puberty. *N Engl J Med*, *349*, 1614–1627.
212. Semple, R. K., Achermann, J. C., Ellery, J., et al. (2005). Two novel missense mutations in g protein-coupled receptor 54 in a patient with hypogonadotropic hypogonadism. *J Clin Endocrinol Metab*, *90*, 1849–1855.
213. Lanfranco, F., Gromoll, J., von Eckardstein, S., et al. (2005). Role of sequence variations of the GnRH receptor and G protein-coupled receptor 54 gene in male idiopathic hypogonadotropic hypogonadism. *Eur J Endocrinol*, *153*, 845–852.
214. Navarro, V. M., Fernandez-Fernandez, R., Castellano, J. M., et al. (2004). Advanced vaginal opening and precocious activation of the reproductive axis by KiSS-1 peptide, the endogenous ligand of GPR54. *J Physiol*, *561*, 379–386.
215. Shahab, M., Mastronardi, C., Seminara, S. B., et al. (2005). Increased hypothalamic GPR54 signaling: a potential mechanism for initiation of puberty in primates. *Proc Natl Acad Sci USA*, *102*, 2129–2134.
216. Castellano, J. M., Navarro, V. M., Fernandez-Fernandez, R., et al. (2005). Changes in hypothalamic KiSS-1 system and restoration of pubertal activation of the reproductive axis by kisspeptin in undernutrition. *Endocrinology*, *146*, 3917–3925.
217. Navarro, V. M., & Tena-Sempere, M. (2011). Neuroendocrine control by kisspeptins: role in metabolic regulation of fertility. *Nat Rev Endocrinol*, *8*, 40–53.
218. Han, S. K., Gottsch, M. L., Lee, K. J., et al. (2005). Activation of gonadotropin-releasing hormone neurons by kisspeptin as a neuroendocrine switch for the onset of puberty. *J Neurosci*, *25*, 11349–11356.
219. Chan, Y. M., Butler, J. P., Sidhoum, V. F., et al. (2012). Kisspeptin administration to women: a window into endogenous kisspeptin secretion and GnRH responsiveness across the menstrual cycle. *J Clin Endocrinol Metab*, *97*, E1458–E1467.
220. Chan, Y. M., Broder-Fingert, S., Wong, K. M., & Seminara, S. B. (2009). Kisspeptin/Gpr54-independent gonadotrophin-releasing hormone activity in Kiss1 and Gpr54 mutant mice. *J Neuroendocrinol*, *21*, 1015–1023.
221. Gianetti, E., Tusset, C., Noel, S. D., et al. (2010). TAC3/TACR3 mutations reveal preferential activation of gonadotropin-releasing hormone release by neurokinin B in neonatal life followed by reversal in adulthood. *J Clin Endocrinol Metab*, *95*, 2857–2867.
222. d'Anglemont de Tassigny, X., & Colledge, W. H. (2010). The role of kisspeptin signaling in reproduction. *Physiology* (Bethesda), *25*, 207–217.

223. Abreu, A. P., Dauber, A., Macedo, D. B., et al. (2013). Central precocious puberty caused by mutations in the imprinted gene MKRN3. *N Engl J Med*, *368*, 2467–2475.
224. Ojeda, S. R., Dubay, C., Lomniczi, A., et al. (2010). Gene networks and the neuroendocrine regulation of puberty. *Mol Cell Endocrinol*, *324*, 3–11.
225. Clarkson, J., & Herbison, A. E. (2006). Development of GABA and glutamate signaling at the GnRH neuron in relation to puberty. Mol Cell Endocrinol, 254–255, 32–38.
226. Ojeda, S. R., & Terasawa, E. (2002). Neuroendocrine regulation of puberty. In Pfaff D, et al. (eds.), *Hormones, brain and behavior* (Vol. 4) (pp. 589–659). New York: Elsevier.
227. DeFazio, R. A., Heger, S., Ojeda, S. R., Fernandez-Fernandez, R., & Moenter, S. M. (2002). Activation of A-type gamma-aminobutyric acid receptors excites gonadotropin-releasing hormone neurons. *Mol Endocrinol*, *16*, 2872–2891.
228. Mueller, J. K., Koch, I., Lomniczi, A., et al. (2012). Transcription of the human EAP1 gene is regulated by upstream components of a puberty-controlling Tumor Suppressor Gene network. *Mol Cell Endocrinol*, *351*, 184–198.
229. Partsch, C. J., Dreyer, G., Gosch, A., et al. (1999). Longitudinal evaluation of growth, puberty, and bone maturation in children with Williams syndrome. *J Pediatr*, *134*, 82–89.
230. Terasawa, E., Noonan, J., Nass, T., & Loose, M. (1984). Posterior hypothalamic lesions advance the onset of puberty in the female rhesus monkey. *Endocrinology*, *115*, 2241.
231. Mayer, C., Acosta-Martinez, M., Dubois, S. L., et al. (2010). Timing and completion of puberty in female mice depend on estrogen receptor alpha-signaling in kisspeptin neurons. *Proc Natl Acad Sci USA*, *107*, 22693–22698.
232. Pescovitz, O. H., Comite, F., Cassorla, F., et al. (1984). True precocious puberty complicating congenital adrenal hyperplasia: treatment with a luteinizing hormone-releasing hormone analog. *J Clin Endocrinol Metab*, *58*, 857.
233. Foster, C., Comite, F., Pescovitz, O., et al. (1984). Variable response to a long-acting agonist of luteinizing hormone-releasing hormone in girls with McCune-Albright syndrome. *J Clin Endocrinol Metab*, *59*, 801.
234. Doherty, L. F., Bromer, J. G., Zhou, Y., et al. (2010). In utero exposure to diethylstilbestrol (DES) or bisphenol-A (BPA) increases EZH2 expression in the mammary gland: an epigenetic mechanism linking endocrine disruptors to breast cancer. *Horm Cancer*, *1*, 146–155.
235. Wilson, M. E., & Tanner, J. M. (1994). Somatostatin analog treatment slows growth and the tempo of reproductive maturation in female rhesus monkeys. *J Clin Endocrinol Metab*, *79*, 495–501.
236. Hiney, J. K., Srivastava, V., Nyberg, C. L., et al. (1996). Insulin-like growth factor I of peripheral origin acts centrally to accelerate the initiation of female puberty. *Endocrinology*, *137*, 3717–3728.
237. Childs, G. V. (2000). Growth hormone cells as co-gonadotropes: partners in the regulation of the reproductive system. *Trends Endocrinol Metab*, *11*, 168–175.
238. Marshall, W. (1974). Interrelationships of skeletal maturation, sexual development and somatic growth in man. *Ann Human Biol*, *1*, 29.
239. Simmons, K., & Greulich, W. (1943). Menarcheal age and the height, weight, and skeletal age of girls age 7 to 17 years. *J Pediatr*, *22*, 518.
240. Boyar, R. M., Finkelstein, J. W., David, R., et al. (1973). Twenty-four hour patterns of plasma luteinizing hormone and follicle-stimulating hormone in sexual precocity. *N Engl J Med*, *289*, 282–286.
241. Tanner, J., & Whitehouse, R. (1975). A note on the bone age at which patients with true isolated growth hormone deficiency enter puberty. *J Clin Endocrinol Metab*, *41*, 788.
242. Frisch, R. (1984). Body fat, puberty, and fertility. *Biol Rev Camb Philos Soc*, *59*, 161–188.
243. Rosenfield, R. L., Lipton, R. B., & Drum, M. L. (2009). Thelarche, pubarche, and menarche attainment in children with normal and elevated body mass index. *Pediatrics*, *123*, 84–88. Erratum in *Pediatrics*, *123*, 1255.
244. Thankamony, A., Ong, K. K., Ahmed, M. L., et al. (2012). Higher levels of IGF-I and adrenal androgens at age 8 years are associated with earlier age at menarche in girls. *J Clin Endocrinol Metab*, *97*, E786–E790.
245. Rosenbaum, M., & Leibel, R. L. (1999). The role of leptin in human physiology [Editorial and comment]. *N Engl J Med*, *341*, 913–915.
246. Ahima, R. S., Kelly, J., Elmquist, J. K., & Flier, J. S. (1999). Distinct physiologic and neuronal responses to decreased leptin and mild hyperleptinemia. *Endocrinology*, *140*, 4923–4931.
247. Tortoriello, D. V., McMinn, J. E., & Chua, S. C. (2007). Increased expression of hypothalamic leptin receptor and adiponectin accompany resistance to dietary-induced obesity and infertility in female C57BL/6J mice. *Int J Obes* (Lond), *31*, 395–402.
248. Mann, D. R., Johnson, A. O., Gimpel, T., & Castracane, V. D. (2003). Changes in circulating leptin, leptin receptor, and gonadal hormones from infancy until advanced age in humans. *J Clin Endocrinol Metab*, *88*, 3339–3345.
249. Donato, J., Jr., Cravo, R. M., Frazao, R., et al. (2011). Leptin's effect on puberty in mice is relayed by the ventral premammillary nucleus and does not require signaling in Kiss1 neurons. *J Clin Invest*, *121*, 355–368.
250. Schwartz, J., & Cherny, R. (1992). Intercellular communication within the anterior pituitary influencing the secretion of hypophysial hormones. *Endocr Rev*, *13*, 453.
251. Evans, J. J. (1999). Modulation of gonadotropin levels by peptides acting at the anterior pituitary gland. *Endocr Rev*, *20*, 46–67.
252. Campbell, R. E., French-Mullen, J. M., Cowley, M. A., et al. (2001). Hypothalamic circuitry of neuropeptide Y regulation of neuroendocrine function and food intake via the Y5 receptor subtype. *Neuroendocrinology*, *74*, 106–119.
253. Lloyd, B., Ravi, P., Mendes, N., et al. (2010). Peptide YY levels across pubertal stages and associations with growth hormone. *J Clin Endocrinol Metab*, *95*, 2957–2962.
254. Loucks, A. B., & Thuma, J. R. (2003). Luteinizing hormone pulsatility is disrupted at a threshold of energy availability in regularly menstruating women. *J Clin Endocrinol Metab*, *88*, 297–311.
255. Kluge, M., Schussler, P., Schmidt, D., et al. (2012). Ghrelin suppresses secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in women. *J Clin Endocrinol Metab*, *97*, E448–E451.
256. Bruning, J. C., Gautam, D., Burks, D. J., et al. (2000). Role of brain insulin receptor in control of body weight and reproduction. *Science*, *289*, 2122–2125.
257. Reiter, R. (1980). The pineal and its hormones in the control of reproduction in mammals. *Endocrinology*, *1*, 109.
258. Brzezinski, A. (1997). Melatonin in humans. *N Engl J Med*, *336*, 186–195.
259. Reiter, E., Kulin, H., & Hamwood, S. (1974). The absence of positive feedback between estrogen and luteinizing hormone in sexually immature girls. *Pediatr Res*, *8*, 740.
260. Rosenfield, R. L., & Fang, V. S. (1974). The effects of prolonged physiologic estradiol therapy on the maturation of hypogonadal teenagers. *J Pediatr*, *85*, 830–837.
261. Wray, S., & Hoffman, G. (1986). Postnatal morphological changes in rat LHRH neurons correlated with sexual maturation. *Neuroendocrinology*, *43*, 93–97.
262. Cottrell, E. C., Campbell, R. E., Han, S. K., & Herbison, A. E. (2006). Postnatal remodeling of dendritic structure and spine density in gonadotropin-releasing hormone neurons. *Endocrinology*, *147*, 3652–3661.
263. Plant, T. M. (2007). Gonadotropin-releasing hormone neuron remodeling: causal for puberty onset? *Trends Endocrinol Metab*, *18*, 50–51.
264. Terasawa, E. (2006). Postnatal remodeling of gonadotropin-releasing hormone I neurons: toward understanding the mechanism of the onset of puberty. Endocrinology, 147, 3650–3651.
265. Meriggiola, M., Dahl, K., Mather, J., & Bremner, W. (1994). Follistatin decreases activin-stimulated FSH secretion with no effect on GnRH-stimulated FSH secretion in prepubertal male monkeys. *Endocrinology*, *134*, 1967.
266. Rosenfield, R. L., & Bordini, B. (2010). Evidence that obesity and androgens have independent and opposing effects on gonadotropin production from puberty to maturity. *Brain Res*, *1364*, 186–197.
267. Shaw, N. D., Histed, S. N., Srouji, S. S., et al. (2010). Estrogen negative feedback on gonadotropin secretion: evidence for a

direct pituitary effect in women. *J Clin Endocrinol Metab*, *95*, 1955–1961.
268. Levine, J. E. (1999). Editorial: pulsatility in primates—a perspective from the placode. *Endocrinology*, *140*, 1033–1035.
269. Wuttke, W., Leonhardt, S., Jarry, H., et al. (1992). Involvement of catecholamines and amino acid neurotransmitters in the generation of GnRH pulses. In W. Rosmanith, & W. Scherbaum (Eds.), *New developments in biosciences 6: neuroendocrinology of sex steroids, basic knowledge and clinical implications* (pp. 109–123). Berlin: de Gruyter.
270. Favit, A., Wetsel, W., & Negro-Vilar, A. (1993). Differential expression of g-aminobutyric acid receptors in immortalized luteinizing hormone-releasing hormone neurons. *Endocrinology*, *133*, 1983–1989.
271. Dissen, G. A., Lomniczi, A., Heger, S., et al. (2012). Hypothalamic EAP1 (enhanced at puberty 1) is required for menstrual cyclicity in nonhuman primates. *Endocrinology*, *153*, 350–361.
272. Chappell, P. E., Lee, J., & Levine, J. E. (2000). Stimulation of gonadotropin-releasing hormone surges by estrogen. II. Role of cyclic adenosine 3′,5′-monophosphate. *Endocrinology*, *141*, 1486–1492.
273. Radovick, S., Ticknor, C. M., Nakayama, Y., et al. (1991). Evidence for direct estrogen regulation of the human gonadotropin-releasing hormone gene. *J Clin Invest*, *88*, 1649–1655.
274. Roy, D., Angelini, N. L., & Belsham, D. D. (1999). Estrogen directly represses gonadotropin-releasing hormone (GnRH) gene expression in estrogen receptor-a (ERa)- and ERβ-expressing GT1–7 GnRH neurons. *Endocrinology*, *140*, 5045–5053.
275. Kepa, J. K., Jacobsen, B. M., Boen, E. A., et al. (1996). Direct binding of progesterone receptor to nonconsensus DNA sequences represses rat GnRH. *Mol Cell Endocrinol*, *117*, 27–39.
276. Couzinet, B., Young, J., Kujas, M., et al. (1999). The antigonadotropic activity of a 19-nor-progesterone derivative is exerted both at the hypothalamic and pituitary levels in women. *J Clin Endocrinol Metab*, *84*, 4191–4196.
277. Wildt, L., Hutchison, J. S., Marshall, G., et al. (1981). On the site of action of progesterone in the blockade of the estradiol-induced gonadotropin discharge in the rhesus monkey. *Endocrinology*, *109*, 1293–1294.
278. Garcia, A., Herbon, L., Barkan, A., et al. (1985). Hyperprolactinemia inhibits gonadotropin-releasing hormone (GnRH) stimulation of the number of pituitary GnRH receptors. *Endocrinology*, *117*, 954.
279. Milenkovic, L., D'Angelo, G., Kelly, P. A., & Weiner, R. I. (1994). Inhibition of gonadotropin hormone-releasing hormone release by prolactin from GT1 neuronal cell lines through prolactin receptors. *Proc Natl Acad Sci USA*, *91*, 1244–1247.
280. Shaw, N. D., Gill, S., Lavoie, H. B., et al. (2011). Persistence of sleep-associated decrease in GnRH pulse frequency in the absence of gonadal steroids. *J Clin Endocrinol Metab*, *96*, 2590–2595.
281. Petraglia, F., Sutton, S., Vale, W., et al. (1987). Corticotropin-releasing factor decreases plasma luteinizing hormone levels in female rats by inhibiting gonadotropin-releasing hormone release into hypophysial-portal circulation. *Endocrinology*, *120*, 1083–1088.
282. Rivest, S., Lee, S., Attardi, B., & Rivier, C. (1993). The chronic intracerebroventricular infusion of interleukin-1β alters the activity of the hypothalamic-pituitary-gonadal axis of cycling rats. I. Effect on LHRH and gonadotropin biosynthesis and secretion. *Endocrinology*, *133*, 2424–2430.
283. Vitale, M., & Chiocchio, S. (1993). Serotonin, a neurotransmitter involved in the regulation of luteinizing hormone release. *Endocr Rev*, *14*, 480.
284. Conn, P., & Crowley, W. J. (1991). Gonadotropin-releasing hormone and its analogues. *New Engl J Med*, *324*, 93–103.
285. Lahlou, N., Carel, J. C., Chaussain, J. L., & Roger, M. (2000). Pharmacokinetics and pharmacodynamics of GnRH agonists: clinical implications in pediatrics. *J Pediatr Endocrinol Metab*, *13*(Suppl. 1), 723–737.
286. Hirono, M., Igarashi, M., & Matsumoto, S. (1971). Short- and auto-feedback mechanism of LH. *Endocrinology*, *18*, 175.
287. Patritti-Laborde, N., Wolfsen, A., & Odell, W. (1981). Short loop feedback system for the control of follicle-stimulating hormone in the rabbit. *Endocrinology*, *108*, 172.
288. Kanasaki, H., Bedecarrats, G. Y., Kam, K. Y., et al. (2005). Gonadotropin-releasing hormone pulse frequency-dependent activation of extracellular signal-regulated kinase pathways in perifused LbetaT2 cells. *Endocrinology*, *146*, 5503–5513.
289. McArdle, C. (1994). Pituitary adenylate cyclase-activating polypeptide: a key player in reproduction? *Endocrinol*, *135*, 815–816.
290. Drouin, J., & Labrie, F. (1981). Interactions between 17β-estradiol and progesterone in the control of luteinizing hormone and follicle-stimulating hormone release in rat anterior pituitary cells in culture. *Endocrinology*, *108*, 52–57.
291. Turgeon, J. L., & Waring, D. W. (1999). Androgen modulation of luteinizing hormone secretion by female rat gonadotropes. *Endocrinology*, *140*, 1767–1774.
292. Hemrika, D. J., Slaats, E. H., Kennedy, J. C., et al. (1993). Pulsatile luteinizing hormone patterns in long term oral contraceptive users. *J Clin Endocrinol Metab*, 77, 420–426.
293. Couse, J. F., Yates, M. M., Walker, V. R., & Korach, K. S. (2003). Characterization of the hypothalamic-pituitary-gonadal axis in estrogen receptor (ER) Null mice reveals hypergonadism and endocrine sex reversal in females lacking ERalpha but not ERbeta. *Mol Endocrinol*, *17*, 1039–1053.
294. Griffin, L. D., & Mellon, S. H. (2001). Biosynthesis of the neurosteroid 3alpha-hydroxy-4-pregnen-20-one (3alphahp), a specific inhibitor of FSH release. *Endocrinology*, *142*, 4617–4622.
295. Groome, N., Illingworth, P., O'Brien, M., et al. (1996). Measurement of dimeric inhibin B throughout the human menstrual cycle. *J Clin Endocrinol Metab*, *81*, 1401–1405.
296. Hayes, F. J., Hall, J. E., Boepple, P. A., & Crowley, W. F., Jr. (1998). Clinical review 96: differential control of gonadotropin secretion in the human: endocrine role of inhibin. *J Clin Endocrinol Metab*, *83*, 1835–1841.
297. Lumpkin, M., Negro-Vilar, A., Franchimont, P., et al. (1981). Evidence for a hypothalamic site of action of inhibin to suppress FSH release. *Endocrinology*, *108*, 1101.
298. Bilezikjian, L. M., Blount, A. L., Donaldson, C. J., & Vale, W. W. (2006). Pituitary actions of ligands of the TGF-beta family: activins and inhibins. *Reproduction*, *132*, 207–215.
299. Nelson, V. L., Legro, R. S., Strauss, J. F., 3rd, & McAllister, J. M. (1999). Augmented androgen production is a stable steroidogenic phenotype of propagated theca cells from polycystic ovaries. *Mol Endocrinol*, *13*, 946–957.
300. Jakimiuk, A. J., Weitsman, S. R., Navab, A., & Magoffin, D. A. (2001). Luteinizing hormone receptor, steroidogenesis acute regulatory protein, and steroidogenic enzyme messenger ribonucleic acids are overexpressed in thecal and granulosa cells from polycystic ovaries. *J Clin Endocrinol Metab*, *86*, 1318–1323.
301. Rosenfield, R. L. (1999). Ovarian and adrenal function in polycystic ovary syndrome. *Endocrinol Metab Clin N Am*, *28*, 265–293.
302. Taniguchi, F., Couse, J. F., Rodriguez, K. F., et al. (2007). Estrogen receptor-alpha mediates an intraovarian negative feedback loop on thecal cell steroidogenesis via modulation of Cyp17a1 (cytochrome P450, steroid 17alpha-hydroxylase/17,20 lyase) expression. *FASEB J*, *21*, 586–595.
303. Udoff, L. C., & Adashi, E. Y. (1999). Autocrine/paracrine regulation of the ovarian follicle. *Endocrinologist*, *9*, 99–106.
304. Yoshimura, Y., Washita, M., Karube, M., et al. (1994). Growth hormone stimulates follicular development by stimulating ovarian production of insulin-like growth factor-I. *Endocrinology*, *135*, 887–894.
305. Mason, H. D., Martikainen, H., Beard, R. W., et al. (1990). Direct gonadotrophic effect of growth hormone on oestradiol production by human granulosa cells in vitro. *J Endocrinol*, *126*, R1–R4.
306. Mayerhofer, A., Hemmings, H. C., Jr., Snyder, G. L., et al. (1999). Functional dopamine-1 receptors and DARPP-32 are expressed in human ovary and granulosa luteal cells in vitro. *J Clin Endocrinol Metab*, *84*, 257–264.
307. Yamamoto, N., Christenson, L. K., McAllister, J. M., & Strauss, J. F., 3rd (2002). Growth differentiation factor-9 inhibits 3′5′-adenosine monophosphate-stimulated steroidogenesis in human granulosa and theca cells. *J Clin Endocrinol Metab*, *87*, 2849–2856.
308. Agarwal, S. K., Vogel, K., Weitsman, S. R., & Magoffin, D. A. (1999). Leptin antagonizes the insulin-like growth factor-I augmentation of steroidogenesis in granulosa and theca cells of the human ovary. *J Clin Endocrinol Metab*, *84*, 1072–1076.

309. Feng, P., Catt, K., & Knecht, M. (1988). Transforming growth factor-β stimulates meiotic maturation of the rat oocyte. *Endocrinology*, *122*, 181.
310. Aten, R., Poland, M., Bayless, R., & Behrman, H. (1987). A gonadotropin-releasing hormone (GnRH)-like protein in human ovaries: similarity to the GnRH-like ovarian protein of the rat. *J Clin Endocrinol Metab*, *64*, 1288.
311. Barnes, R. B., Scommegna, A., & Schreiber, J. R. (1987). Decreased ovarian response to human menopausal gonadotropin caused by subcutaneously administered gonadotropin-releasing hormone agonist. *Fertil Steril*, *47*, 512.
312. Advis, J., Richards, J., & Ojeda, S. (1981). Hyperprolactinemia-induced precocious puberty: studies on the intraovarian mechanism(s) by which PRL enhances ovarian responsiveness to gonadotropins in prepubertal rats. *J Clin Endocrinol Metab*, *108*, 1333.
313. Demura, R., Ono, M., Demura, H., et al. (1982). Prolactin directly inhibits basal as well as gonadotropin-stimulated secretion of progesterone and 17β-estradiol in the human ovary. *J Clin Endocrinol Metab*, *54*, 1246.
314. Glickman, S. P., Rosenfield, R. L., Bergenstal, R. M., & Helke, J. (1982). Multiple androgenic abnormalities, including elevated free testosterone, in hyperprolactinemic women. *J Clin Endocrinol Metab*, *55*, 251–257.
315. Rosenfield, R. L. (2012). Normal adrenarche. In B. D. Rose (Ed.), UpToDate. Retrieved from http://www.uptodate.com/contents/normal-adrenarche
316. Rainey, W. E., Carr, B. R., Sasano, H., et al. (2002). Dissecting human adrenal androgen production. *Trends Endocrinol Metab*, *13*, 234–239.
317. Rittmaster, R., & Givner, M. (1988). Effect of daily and alternate day low dose prednisone on serum cortisol and adrenal androgens in hirsute women. *J Clin Endocrinol Metab*, *67*, 400–403.
318. Cutler, G. J., Davis, S., Johnsonbaugh, R., & Loriaux, L. (1979). Dissociation of cortisol and adrenal androgen secretion in patients with secondary adrenal insufficiency. *J Clin Endocrinol Metab*, *49*, 604.
319. Auchus, R. J., & Rainey, W. E. (2004). Adrenarche: physiology, biochemistry and human disease. *Clin Endocrinol* (Oxf), *60*, 288–296.
320. Taha, D., Mullis, P. E., Ibanez, L., & de Zegher, F. (2005). Absent or delayed adrenarche in Pit-1/POU1F1 deficiency. *Horm Res*, *64*, 175–179.
321. Biason-Lauber, A., Zachmann, M., & Schoenle, E. J. (2000). Effect of leptin on CYP17 enzymatic activities in human adrenal cells: new insight in the onset of adrenarche. *Endocrinology*, *141*, 1446–1354.
322. Topor, L. S., Asai, M., Dunn, J., & Majzoub, J. A. (2011). Cortisol stimulates secretion of dehydroepiandrosterone in human adrenocortical cells through inhibition of 3betaHSD2. *J Clin Endocrinol Metab*, *96*, E31–E39.
323. Ehrhart-Bornstein, M., Hinson, J., Bornstein, S., et al. (1998). Intraadrenal interactions in the regulation of adrenocortical steroidogenesis. *Endocr Rev*, *19*, 101–143.
324. Martin, D. D., Schweizer, R., Schwarze, C. P., et al. (2004). The early dehydroepiandrosterone sulfate rise of adrenarche and the delay of pubarche indicate primary ovarian failure in Turner syndrome. *J Clin Endocrinol Metab*, *89*, 1164–1168.
325. Cumming, D., Rebar, R., Hopper, B., & Yen, S. (1982). Evidence for an influence of the ovary on circulating dehydroepiandrosterone sulfate levels. *J Clin Endocrinol Metab*, *54*, 1069–1071.
326. Noordam, C., Dhir, V., McNelis, J. C., et al. (2009). Inactivating PAPSS2 mutations in a patient with premature pubarche. *N Engl J Med*, *360*, 2310–2318.
327. Paul, S., & Purdy, R. (1992). Neuroactive steroids. *FASEB J*, *6*, 2311–2322.
328. Apaja, P. M., Aatsinki, J. T., Rajaniemi, H. J., & Petaja-Repo, U. E. (2005). Expression of the mature luteinizing hormone receptor in rodent urogenital and adrenal tissues is developmentally regulated at a posttranslational level. *Endocrinology*, *146*, 3224–3232.
329. Krsmanovic, L. Z., Stojilkovic, S. S., & Catt, K. J. (1996). Pulsatile gonadotropin-releasing hormone release and its regulation. *Trends Endocrinol Metab*, *7*, 56–59.
330. Spiegel, A., Shenker, A., & Weinstein, L. (1992). Receptor-effector coupling by G proteins: implications for normal and abnormal signal transduction. *Endocr Rev*, *13*, 536.
331. Carvalho, C. R., Carvalheira, J. B., Lima, M. H., et al. (2003). Novel signal transduction pathway for luteinizing hormone and its interaction with insulin: activation of Janus kinase/signal transducer and activator of transcription and phosphoinositol 3-kinase/Akt pathways. *Endocrinology*, *144*, 638–647.
332. Adelman, J., Mason, A., Hayflick, J., & Seeburg, P. (1986). Isolation of the gene and hypothalamic cDNA for the common precursor of gonadotropin-releasing hormone and prolactin release-inhibiting factor in human and rat. *Proc Natl Acad Sci USA*, *83*, 179.
333. Redding, T. W., Schally, A. V., Arimura, A., & Matsuo, H. (1972). Stimulation of release and synthesis of luteinizing hormone (LH) and follicle stimulating hormone (FSH) in tissue cultures of rat pituitaries in response to natural and synthetic LH and FSH releasing hormone. *Endocrinology*, *90*, 764–770.
334. Kim, H. H., Mui, K. L., Nikrodhanond, A. A., & Tamayo, N. C. (2007). Regulation of gonadotropin-releasing hormone in non-hypothalamic tissues. *Semin Reprod Med*, *25*, 326–336.
335. Kauffman, A. S., & Rissman, E. F. (2004). A critical role for the evolutionarily conserved gonadotropin-releasing hormone II: mediation of energy status and female sexual behavior. *Endocrinology*, *145*, 3639–3646.
336. Millar, R., Lowe, S., Conklin, D., et al. (2001). A novel mammalian receptor for the evolutionarily conserved type II GnRH. *Proc Natl Acad Sci USA*, *98*, 9636–9641.
337. Phifer, R., Midgley, A., & Spicer, S. (1973). Immunohistologic and histologic evidence that follicle-stimulating hormone and luteinizing hormone are present in the same cell type in the human pars distalis. *J Clin Endocrinol Metab*, *36*, 125.
338. Hammond, E., Griffin, J., & Odell, W. (1991). A chorionic gonadotropin-secreting human pituitary cell. *J Clin Endocrinol Metab*, *72*, 747–754.
339. Combarnous, Y. (1992). Moecular basis of the specificity of binding of glycoprotein hormones to their receptors. *Endocr Rev*, *13*, 670–685.
340. Dahl, K., & Stone, M. (1992). FSH isoforms, radioimmunoassays, bioassays, and their significance. *J Andrology*, *13*, 11–12.
341. Arey, B. J., Stevis, P. E., Deecher, D. C., et al. (1997). Induction of promiscuous G protein coupling of the follicle-stimulating hormone (FSH) receptor: a novel mechanism for transducing pleiotropic actions of FSH isoforms. *Mol Endocrinol*, *11*, 517–526.
342. West, C. R., Carlson, N. E., Lee, J. S., et al. (2002). Acidic mix of FSH isoforms are better facilitators of ovarian follicular maturation and E2 production than the less acidic. *Endocrinology*, *143*, 107–116.
343. Mi, Y., Fiete, D., & Baenziger, J. U. (2008). Ablation of GalNAc-4-sulfotransferase-1 enhances reproduction by altering the carbohydrate structures of luteinizing hormone in mice. *J Clin Invest*, *118*, 1815–1824.
344. Weiss, J., Axelrod, L., Whitcomb, R., et al. (1992). Hypogonadism caused by a single amino acid substitution in the β subunit of luteinizing hormone. *New Engl J Med*, *326*, 179–184.
345. Wide, L., Eriksson, K., Sluss, P. M., & Hall, J. E. (2009). Serum half-life of pituitary gonadotropins is decreased by sulfonation and increased by sialylation in women. *J Clin Endocrinol Metab*, *94*, 958–964.
346. Solano, A. R., Garcia-Vela, A., Catt, K. J., & Dufau, M. L. (1980). Modulation of serum and pituitary luteinizing hormone bioactivity by androgen in the rat. *Endocrinology*, *106*, 1941–1948.
347. Lucky, A. W., Rebar, R. W., Rosenfield, R. L., et al. (1979). Reduction of the potency of luteinizing hormone by estrogen. *N Engl J Med*, *300*, 1034–1036.
348. Dahl, K., Bicxak, T., & Hsueh, A. (1988). Naturally occurring antihormones: secretion of FSH antagonists by women treated with a GnRH analog. *Science*, *239*, 72.
349. Tilly, J., Aihara, T., Nishimori, K., et al. (1992). Expression of recombinant human follicle-stimulating hormone receptor: species-specific ligand binding, signal transduction, and identification of multiple ovarian messenger ribonucleic acid transcripts. *Endocrinology*, *131*, 799–806.
350. Taylor, A., Khoury, R., & Crowley, W. J. (1994). A comparison of 13 different immunometric assay kits for gonadotropins: implications for clinical investigation. *J Clin Endocrinol Metab*, *79*, 240–247.

351. Raiti, S., Foley, T. P., Jr., Penny, R., & Blizzard, R. M. (1975). Measurement of the production rate of human luteinizing hormone using the urinary excretion technique. *Metabolism*, *24*, 937–941.
352. Prentice, L., & Ryan, R. (1975). LH and its subunits in human pituitary, serum and urine. *J Clin Endocrinol Metab*, *40*, 303.
353. Yen, S., Llerena, O., Little, B., & Pearson, O. H. (1968). Disappearance rates of endogenous luteinizing hormone and chorionic gonadotropin in man. *J Clin Endocrinol Metab*, *28*, 1763–1767.
354. Yen, S., Vela, P., & Rankin, J. (1970). Inappropriate secretion of follicle-stimulating hormone and luteinizing hormone in polycystic ovarian disease. *J Clin Endocrinol Metab*, *30*, 435–442.
355. Veldhuis, J. D., Fraioli, F., Rogol, A. D., & Dufau, M. L. (1986). Metabolic clearance of biologically active luteinizing hormone in man. *J Clin Invest*, *77*, 1122–1128.
356. Kohler, P., Ross, C., & Odell, W. (1968). Metabolic clearance and production rates of human luteinizing hormone in pre- and postmenopausal women. *J Clin Invest*, *47*, 38.
357. Coble, Y. D., Jr., Kohler, P. O., Cargille, C. M., & Ross, G. T. (1969). Production rates and metabolic clearance rates of human follicle-stimulating hormone in premenopausal and postmenopausal women. *J Clin Invest*, *48*, 359.
358. Rosenfield, R. L. (1972). Role of androgens in growth and development of the fetus, child, and adolescent. *Adv Pediatr*, *19*, 171–213.
359. Baulieu, E. E., Corp'Echot, C., Dray, F., et al. (1965). An adrenal-secreted "androgen": dehydroisoandrosterone sulfate. Its metabolism and a tentative generalization on the metabolism of other steroid conjugates in man. *Recent Prog Horm Res*, *21*, 411–500.
360. Sandberg, E., Gurpide, E., & Lieberman, S. (1964). Quantitative studies on the metabolism of dehydroisoandrosterone sulfate. *Biochemistry*, *3*, 1256–1267.
361. Longcope, C., & Pratt, J. (1977). Blood production rates of estrogens in women with differing ratios of urinary estrogen conjugates. *Steroids*, *29*, 483–492.
362. Strott, C., Hoshimi, T., & Lipsett, M. (1989). Plasma progesterone and 17-hydroxyprogesterone in normal men and children with congenital adrenal hyperplasia. *J Clin Invest*, *48*, 930–939.
363. Sinha, Y. (1992). Prolactin variants. *Trends Endocrinol Metab*, *3*, 100–106.
364. Frawley, L. (1994). Role of the hypophyseal neurointermediate lobe in the dynamic release of prolactin. *Trends Endocrinol Metab*, *5*, 107–112.
365. Pangas, S. A., Rademaker, A. W., Fishman, D. A., & Woodruff, T. K. (2002). Localization of the activin signal transduction components in normal human ovarian follicles: implications for autocrine and paracrine signaling in the ovary. *J Clin Endocrinol Metab*, *87*, 2644–2657.
366. Rosenfield, R. L., Lucky, A. W., & Allen, T. D. (1980). The diagnosis and management of intersex. *Curr Probl Pediatr*, *10*, 1–66.
367. Miller, W. L., & Auchus, R. J. (2011). The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. *Endocr Rev*, *32*, 81–151.
368. Qin, K., & Rosenfield, R. L. (1998). Role of cytochrome P450c17 in polycystic ovary syndrome. *Molec Cell Endocrinol*, *145*, 111–121.
369. Suzuki, T., Miki, Y., Nakata, T., et al. (2003). Steroid sulfatase and estrogen sulfotransferase in normal human tissue and breast carcinoma. *J Steroid Biochem Mol Biol*, *86*, 449–454.
370. Nelson, V. L., Qin Kn, K., Rosenfield, R. L., et al. (2001). The biochemical basis for increased testosterone production in theca cells propagated from patients with polycystic ovary syndrome. *J Clin Endocrinol Metab*, *86*, 5925–5933.
371. Rosenfield, R. L. (1973). Relationship of androgens to female hirsutism and infertility. *J Reprod Med*, *11*, 87–95.
372. Alonso, L. C., & Rosenfield, R. L. (2002). Oestrogens and puberty. *Best Pract Res Clin Endocrinol Metab*, *16*, 13–30.
373. Lin, T. J., Billiar, R. B., & Little, B. (1972). Metabolic clearance of progesterone in the menstrual cycle. *J Clin Endocrinol Metab*, *35*, 879–886.
374. Siiteri, P., & MacDonald, P. (1973). Role of extraglandular estrogen in human endocrinology. In R. Greep, & E. Astwood (Eds.), *Handbook of physiology* (p. 615). Washington, DC: American Physiology Society.
375. Morimoto, I., Edmiston, A., Hawks, D., & Horton, R. (1981). Studies of the origin of androstanediol and androstanediol glucuronide in young and elderly men. *J Clin Endocrinol Metab*, *52*, 772.
376. Heinrichs, W. L., Tabei, T., Kuwabara, Y., et al. (1979). Differentiation and regulation of peripheral androgen metabolism in rats and rhesus monkeys. *Am J Obstet Gyn*, *135*, 974.
377. Mode, A., Norstedt, G., Eneroth, H., & Gustafsson, J.-A. (1983). Purification of liver feminizing factor from rat pituitaries and demonstration of its identity with growth hormone. *Endocrinology*, *113*, 1250–1260.
378. Edman, C. D., & MacDonald, P. C. (1979). Effect of obesity on conversion of plasma androstenedione to estrone in ovulatory and anovulatory young women. *Am J Obstet Gynecol*, *130*, 456–461.
379. Kadlubar, F. F., Berkowitz, G. S., Delongchamp, R. R., et al. (2003). The CYP3A4*1B variant is related to the onset of puberty, a known risk factor for the development of breast cancer. *Cancer Epidemiol Biomarkers Prev*, *12*, 327–331.
380. Schroth, W., Goetz, M. P., Hamann, U., et al. (2009). Association between CYP2D6 polymorphisms and outcomes among women with early stage breast cancer treated with tamoxifen. *JAMA*, *302*, 1429–1436.
381. Harris, R. M., Wood, D. M., Bottomley, L., et al. (2004). Phytoestrogens are potent inhibitors of estrogen sulfation: implications for breast cancer risk and treatment. *J Clin Endocrinol Metab*, *89*, 1779–1787.
382. Giorgi, E., & Stein, W. (1981). The transport of steroids into animal cells in culture. *Endocrinology*, *108*, 688.
383. Martin, K. A., Chang, R. J., Ehrmann, D. A., et al. (2008). Evaluation and treatment of hirsutism in premenopausal women: an Endocrine Society Clinical Practice Guideline. *J Clin Endocrin Metab*, *93*, 1105–1120.
384. Rosenfield, R. L. (1975). Studies of the relation of plasma androgen levels to androgen action in women. *J Steroid Biochem*, *6*, 5695–5702.
385. Kahn, S. M., Hryb, D. J., Nakhla, A. M., et al. (2002). Sex hormone-binding globulin is synthesized in target cells. *J Endocrinol*, *175*, 113–120.
386. Hammes, A., Andreassen, T. K., Spoelgen, R., et al. (2005). Role of endocytosis in cellular uptake of sex steroids. *Cell*, *122*, 751–762. Comments in *Cell*, *122*, 647–649 and *Cell*, *124*, 455–456; author reply in *Cell*, *124*, 456–457.
387. Rosenfield, R. L., & Moll, G. W. (1983). The role of proteins in the distribution of plasma androgens and estradiol. In G. Molinatti, L. Martini, & V. James (Eds.), *Androgenization in women* (pp. 25–45). New York: Raven Press.
388. Pugeat M, Nader N, Hogeveen K, et al. (2010) Sex hormone-binding globulin gene expression in the liver: drugs and the metabolic syndrome. *Mol Cell Endocrinol*, *316*, 53–9.
389. Simo, R., Barbosa-Desongles, A., Lecube, A., et al. (2012). Potential role of tumor necrosis factor-alpha in downregulating sex hormone-binding globulin. *Diabetes*, *61*, 372–382.
390. Nimrod, A., Rosenfield, R. L., & Otto, P. (1980). Relationship of androgen action to androgen metabolism in isolated rat granulosa cells. *J Steroid Biochem*, *13*, 1015–1019.
391. Wilson, J. D., Griffin, J. E., & Russell, D. W. (1993). Steroid 5 alpha-reductase 2 deficiency. *Endocr Rev*, *14*, 577–593.
392. Yang, S., Fang, Z., Gurates, B., et al. (2001). Stromal PRs mediate induction of 17beta-hydroxysteroid dehydrogenase type 2 expression in human endometrial epithelium: a paracrine mechanism for inactivation of E2. *Mol Endocrinol*, *15*, 2093–2105.
393. Hochberg, R. B. (1998). Biological esterification of steroids. *Endocr Rev*, *19*, 331–348.
394. Baracat, E., Haidar, M., Lopez, F. J., et al. (1999). Estrogen activity and novel tissue selectivity of delta8,9-dehydroestrone sulfate in postmenopausal women. *J Clin Endocrinol Metab*, *84*, 2020–2027.
395. Katzenellenbogen, B. S., Montano, M. M., Ediger, T. R., et al. (2000). Estrogen receptors: selective ligands, partners, and distinctive pharmacology. *Recent Prog Horm Res*, *55*, 163–193. (Discussion 194–195.)

396. O'Malley, B. W. (2005). A life-long search for the molecular pathways of steroid hormone action. *Mol Endocrinol*, *19*, 1402–1411.
397. McDevitt, M. A., Glidewell-Kenney, C., Jimenez, M. A., et al. (2008). New insights into the classical and non-classical actions of estrogen: evidence from estrogen receptor knock-out and knock-in mice. *Mol Cell Endocrinol*, *290*, 24–30.
398. Chen, H., Hu, B., Huang, G. H., et al. (2003). Purification and characterization of a novel intracellular 17 beta-estradiol binding protein in estrogen-resistant New World primate cells. *J Clin Endocrinol Metab*, *88*, 501–504.
399. Cheung, J., & Smith, D. F. (2000). Molecular chaperone interactions with steroid receptors: an update. *Molec Endocrinol*, *14*, 939–946.
400. Alarid, E. T., Bakopoulos, N., & Solodin, N. (1999). Proteasome-mediated proteolysis of estrogen receptor: a novel component in autologous down-regulation. *Mol Endocrinol*, *13*, 1522–1534.
401. Korenman, S. (1968). Radio-ligand binding assay of specific estrogens using a soluble uterine macromolecule. *J Clin Endocrinol Metab*, *28*, 127.
402. French, F. S., Lubahn, D. B., Brown, T. R., et al. (1990). Molecular basis of androgen insensitivity. *Rec Prog Horm Res*, *46*, 1.
403. Connor, C. E., Norris, J. D., Broadwater, G., et al. (2001). Circumventing tamoxifen resistance in breast cancers using antiestrogens that induce unique conformational changes in the estrogen receptor. *Cancer Res*, *61*, 2917–2922.
404. Melamed, M., Castano, E., Notides, A. C., & Sasson, S. (1997). Molecular and kinetic basis for the mixed agonist-antagonist activity of estriol. *Molec Endocrinol*, *11*, 1868–1878.
405. Kemppainen, J. A., Langley, E., Wong, C. I., et al. (1999). Distinguishing androgen receptor agonists and antagonists: distinct mechanisms of activation by medroxyprogesterone acetate and dihydrotestosterone. *Mol Endocrinol*, *13*, 440–454.
406. McDonnell, D. P., & Wardell, S. E. (2010). The molecular mechanisms underlying the pharmacological actions of ER modulators: implications for new drug discovery in breast cancer. *Curr Opin Pharmacol*, *10*, 620–628.
407. Schmidt, A., Kimmel, D. B., Bai, C., et al. (2010). Discovery of the selective androgen receptor modulator MK-0773 using a rational development strategy based on differential transcriptional requirements for androgenic anabolism versus reproductive physiology. *J Biol Chem*, *285*, 17054–17064.
408. Yang, N. N., Venugopalan, M., Hardikar, S., & Glasebrook, A. (1996). Identification of an estrogen response element activated by metabolites of 17beta-estradiol and raloxifene. Science, 273, 1222–1225. Erratum in *Science*, *275*, 1249.
409. Dowsett, M., & Ashworth, A. (2003). New biology of the oestrogen receptor. *Lancet*, *362*, 260–262.
410. Smid-Koopman, E., Blok, L. J., Kuhne, L. C., et al. (2003). Distinct functional differences of human progesterone receptors A and B on gene expression and growth regulation in two endometrial carcinoma cell lines. *J Soc Gynecol Investig*, *10*, 49–57.
411. McPhaul, M. J., & Young, M. (2001). Complexities of androgen action. *J Am Acad Dermatol*, *45*, S87–S94.
412. Lindberg, M. K., Moverare, S., Skrtic, S., et al. (2003). Estrogen receptor (ER)-beta reduces ERalpha-regulated gene transcription, supporting a "ying yang"relationship between ERalpha and ERbeta in mice. *Mol Endocrinol*, *17*, 203–208.
413. Smith, E. P., Boyd, J., Frank, G. R., et al. (1994). Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. *N Engl J Med*, *331*, 1056–1061.
414. Nilsson, S., Kuiper, G., & Gustafsson, J.-A. (1998). ERβ: a novel estrogen receptor offers the potential for new drug development. *Trends Endocrinol Metab*, *9*, 387–395.
415. Conneely, O. M., Lydon, J. P., De Mayo, F., & O'Malley, B. W. (2000). Reproductive functions of the progesterone receptor. *J Soc Gynecol Investig*, 7, S25–S32.
416. Barbulescu, K., Geserick, C., Schuttke, I., et al. (2001). New androgen response elements in the murine pem promoter mediate selective transactivation. *Mol Endocrinol*, *15*, 1803–1816.
417. McKenna, N. J., Lanz, R. B., & O'Malley, B. W. (1999). Nuclear receptor coregulators: cellular and molecular biology. *Endocr Rev*, *20*, 321–344.
418. Laughlin, G. A., Dominguez, C. E., & Yen, S. S. (1998). Nutritional and endocrine-metabolic aberrations in women with functional hypothalamic amenorrhea. *J Clin Endocrinol Metab*, *83*, 25–32.
419. Lu, S., Jenster, G., & Epner, D. E. (2000). Androgen induction of cyclin-dependent kinase inhibitor p21 gene: role of androgen receptor and transcription factor Sp1 complex. *Mol Endocrinol*, *14*, 753–760.
420. Kousteni, S., Bellido, T., Plotkin, L. I., et al. (2001). Nongenotropic, sex-nonspecific signaling through the estrogen or androgen receptors: dissociation from transcriptional activity. *Cell*, *104*, 719–730.
421. Nilsson, O., Falk, J., Ritzen, E. M., et al. (2003). Raloxifene acts as an estrogen agonist on the rabbit growth plate. *Endocrinology*, *144*, 1481–1485.
422. Cranney, A., & Adachi, J. D. (2005). Benefit-risk assessment of raloxifene in postmenopausal osteoporosis. *Drug Saf*, *28*, 721–730.
423. Kelly, M. J., Ronnekleiv, O. K. (2008). Membrane-initiated estrogen signaling in hypothalamic neurons. *Mol Cell Endocrinol*, *290*, 14–23.
424. Zhao, Z., Park, C., McDevitt, M. A., et al. (2009). p21-Activated kinase mediates rapid estradiol-negative feedback actions in the reproductive axis. *Proc Natl Acad Sci USA*, *106*, 7221–7226.
425. Hammes, S. R., & Levin, E. R. (2007). Extranuclear steroid receptors: nature and actions. *Endocr Rev*, *28*, 726–741.
426. Park, C. J., Zhao, Z., Glidewell-Kenney, C., et al. (2011). Genetic rescue of nonclassical ERα signaling normalizes energy balance in obese Erα-null mutant mice. *J Clin Invest*, *121*, 604–612.
427. Apostolakis, E. M., Garai, J., Lohmann, J. E., et al. (2000). Epidermal growth factor activates reproductive behavior independent of ovarian steroids in female rodents. *Molec Endocrinol*, *14*, 1086–1098.
428. Mellon, S. (1994). Neurosteroids: biochemistry, modes of action. and clinical relevance. *J Clin Endocrinol Metab*, *78*, 1003–1008.
429. Baulieu, E. E. (1999). Neuroactive neurosteroids: dehydroepiandrosterone (DHEA) and DHEA sulphate. *Acta Paediatr Suppl*, *88*, 78–80.
430. Zwain, I. H., & Yen, S. S. (1999). Neurosteroidogenesis in astrocytes, oligodendrocytes, and neurons of cerebral cortex of rat brain. *Endocrinology*, *140*, 3843–3852.
431. Rhodes, M. E., Harney, J. P., & Frye, C. A. (2004). Gonadal, adrenal, and neuroactive steroids' role in ictal activity. *Brain Res*, *1000*, 8–18.
432. Ramirez, V. D., & Zheng, J. (1996). Membrane sex-steroid receptors in the brain. *Front Neuroendocrinol*, *17*, 402–439.
433. Wetzel, C. H., Hermann, B., Behl, C., et al. (1998). Functional antagonism of gonadal steroids at the 5-hydroxytryptamine type 3 receptor. *Mol Endocrinol*, *12*, 1441–1451.
434. McEwen, B. S. (1999). Clinical review 108: the molecular and neuroanatomical basis for estrogen effects in the central nervous system. *J Clin Endocrinol Metab*, *84*, 1790–1797.
435. MacLusky, N., & McEwen, B. (1980). Progestin receptors in rat brain: distribution and properties of cytoplasmic progestin-binding sites. *Endocrinology*, *106*, 192–202.
436. Attardi, B. (1981). Facilitation and inhibition of the estrogen-induced luteinizing hormone surge in the rat by progesterone: effects on cytoplasmic and nuclear estrogen receptors in the hypothalamic-preoptic area, pituitary, and uterus. *Endocrinology*, *108*, 1487–1496.
437. Pacifici, R. (19988). Cytokines, estrogen, and postmenopausal osteoporosis—the second decade. [Editorial and comment]. *Endocrinology*, *139*, 2659–2661.
438. Gupta, C., & Goldman, A. (1986). The arachidonic acid cascade is involved in the masculinizing action of testosterone on embryonic external genitalia in mice. *Develop Biol*, *83*, 4346–4349.
439. Rosenfield, R. L., Kentsis, A., Deplewski, D., & Ciletti, N. (1999). Rat preputial sebocyte differentiation involves peroxisome proliferator-activated receptors. *J Invest Dermatol*, *112*, 226–232.
440. Maor, G., Segev, Y., & Phillip, M. (1999). Testosterone stimulates insulin-like growth factor-I and insulin-like growth factor-I-receptor gene expression in the mandibular condyle—a model of endochondral ossification. *Endocrinology*, *140*, 1901–1910.
441. Ammini, A., Vijyaraghavan, M., & Sabherwal, U. (1994). Human female phenotypic development: role of fetal ovaries. *J Clin Endocrinol Metab*, *79*, 604–608.

442. Kalloo, N., Gearhart, J., & Barrack, E. (1993). Sexually dimorphic expression of estrogen receptors, but not of androgen receptors in human fetal external genitalia. *J Clin Endocrinol Metab*, *77*, 692–698.
443. Tewari, K., Bonebrake, R. G., Asrat, T., & Shanberg, A. M. (1997). Ambiguous genitalia in infant exposed to tamoxifen in utero [Letter]. *Lancet*, *350*, 183.
444. Greco, T., Duello, T., & Gorski, J. (1993). Estrogen receptors, estradiol, and diethylstilbestrol in early development: the mouse as a model for the study of estrogen receptors and estrogen sensitivity in embryonic development of male and female reproductive tracts. *Endocr Rev*, *14*, 59.
445. Woodruff, J., & Pickar, J. (1994). Incidence of endometrial hyperplasia in postmenopausal women taking conjugated estrogens (Premarin) with medroxyprogesterone acetate or conjugated estrogens alone. *Am J Obstet Gynecol*, *170*, 1213–1223.
446. Futterweit, W., & Deligdisch, L. (1986). Histopathological effects of exogenously administered testosterone in 19 female to male transsexuals. *J Clin Endocrinol Metab*, *62*, 16–21.
447. Westhoff, C. (2003). Clinical practice. Emergency contraception. *N Engl J Med*, *349*, 1830–1835.
448. Rochwerger, L., & Buchwald, M. (1993). Stimulation of the cystic fibrosis transmembrane regulator expression by estrogen in vivo. *Endocrinology*, *133*, 921–930.
449. Paek, S. C., Merritt, D. F., & Mallory, S. B. (2001). Pruritus vulvae in prepubertal children. *J Am Acad Dermatol*, *44*, 795–802.
450. Wied, C., & Bibbo, M. (1975). Evaluation of endocrinologic condition by exfoliative cytology. In Cold J (ed.), *Gynecologic endocrinology*. New York: Harper & Row, 1975.
451. Rosenfield, R. L., Fang, V. S., Dupon, C., et al. (1973). The effects of low doses of depot estradiol and testosterone in teenagers with ovarian failure and Turner's syndrome. *J Clin Endocrinol Metab*, *37*, 574–580.
452. Kuon, R. J., Shi, S. Q., Maul, H., et al. (2010). Pharmacologic actions of progestins to inhibit cervical ripening and prevent delivery depend on their properties, the route of administration, and the vehicle. *Am J Obstet Gynecol*, *202*, 455 e1–e9.
453. Armstrong, J. (2011). Unintended consequences—the cost of preventing preterm births after FDA approval of a branded version of 17OHP. *N Engl J Med*, *364*, 1689–1691.
454. Berenson, A., Heger, A., Hayes, J., et al. (1992). Appearance of the hymen in prepubertal girls. *Pediatr*, *89*, 387–394.
455. Gardner, J. (1992). Descriptive study of genital variation in healthy, nonabused premenarcheal girls. *J Pediatr*, *120*, 251–257.
456. Robbins, S., & Cotran, R. (1979). The breast. In V. Kumar (Ed.), *Pathologic basis of disease* (pp. 1165–1191). Philadelphia: WB Saunders Company.
457. Rilemma, J. (1994). Development of the mammary gland and lactation. *Trends Endocrinol Metab*, *5*, 149–154.
458. Lyons, W. (1958). Hormonal synergism in mammary growth. *Proc Roy Soc Lond* [Biol], *149*, 303.
459. Bole-Feysot, C., Goffin, V., Edery, M., et al. (1998). Prolactin (PRL) and its receptor: actions, signal transduction pathways and phenotypes observed in PRL receptor knockout mice. *Endocr Rev*, *19*, 225–268.
460. Brisken, C., & O'Malley, B. (2010). Hormone action in the mammary gland. *Cold Spring Harb Perspect Biol*, *2*, a003178.
461. Butler, L. M., Potischman, N. A., Newman, B., et al. (2000). Menstrual risk factors and early-onset breast cancer. *Cancer Causes Control*, *11*, 451–458.
462. Poynter, J. N., Langholz, B., Largent, J., et al. (2010). Reproductive factors and risk of contralateral breast cancer by BRCA1 and BRCA2 mutation status: results from the WECARE study. Cancer Causes Control, 21, 839–846.
463. Ma, Y., Katiyar, P., Jones, L. P., et al. (2006). The breast cancer susceptibility gene BRCA1 regulates progesterone receptor signaling in mammary epithelial cells. *Mol Endocrinol*, *20*, 14–34.
464. Deplewski, D., & Rosenfield, R. L. (2000). Role of hormones in pilosebaceous unit development. *Endocr Rev*, *21*, 363–392.
465. Jave-Suarez, L. F., Langbein, L., Winter, H., et al. (2004). Androgen regulation of the human hair follicle: the type I hair keratin hHa7 is a direct target gene in trichocytes. *J Invest Dermatol*, *122*, 555–564.
466. Walker, W. H. (2010). Is the "comb over" dying? A mouse model for male pattern baldness (androgenic alopecia). *Endocrinology*, *151*, 1981–1983.
467. Ohnemus, U., Uenalan, M., Inzunza, J., et al. (2006). The hair follicle as an estrogen target and source. *Endocr Rev*, *27*, 677–706.
468. Daughaday, W. H., & Rotwein, P. (1989). Insulin-like growth factors I and II. Peptide, messenger ribonucleic acid and gene structures, serum, and tissue concentrations. *Endocr Rev*, *10*, 68–91.
469. Callewaert, F., Sinnesael, M., Gielen, E., et al. (2010). Skeletal sexual dimorphism: relative contribution of sex steroids, GH-IGF1, and mechanical loading. *J Endocrinol*, *207*, 127–134.
470. Smith, E. P., Specker, B., & Korach, K. S. (2010). Recent experimental and clinical findings in the skeleton associated with loss of estrogen hormone or estrogen receptor activity. *J Steroid Biochem Mol Biol*, *118*, 264–272.
471. Seeman, E. (2003). The structural and biomechanical basis of the gain and loss of bone strength in women and men. *Endocrinol Metab Clin North Am*, *32*, 25–38.
472. Mora, S., & Gilsanz V (2003). Establishment of peak bone mass. Endocrinol Metab Clin North Am, 32, 39–63.
473. Bachrach, B. E., & Smith, E. P. (1996). The role of sex steroids in bone growth and development: evolving new concepts. *Endocrinologist*, *6*, 362–368.
474. Migliaccio, S., Newbold, R. R., Bullock, B. C., et al. (1996). Alterations of maternal estrogen levels during gestation affect the skeleton of female offspring. *Endocrinology*, *137*, 2118–2125.
475. Eastell, R. (2005). Role of oestrogen in the regulation of bone turnover at the menarche. *J Endocrinol*, *185*, 223–234.
476. Cooke, P. S., & Naaz, A. (2004). Role of estrogens in adipocyte development and function. *Exp Biol Med* (Maywood), *229*, 1127–1135.
477. Woo, J. G., Dolan, L. M., Daniels, S. R., et al. (2005). Adolescent sex differences in adiponectin are conditional on pubertal development and adiposity. *Obes Res*, *13*, 2095–2101.
478. Singh, R., Artaza, J. N., Taylor, W. E., et al. (2003). Androgens stimulate myogenic differentiation and inhibit adipogenesis in C3H 10T1/2 pluripotent cells through an androgen receptor-mediated pathway. *Endocrinology*, *144*, 5081–5088.
479. Gupta, V., Bhasin, S., Guo, W., et al. (2008). Effects of dihydrotestosterone on differentiation and proliferation of human mesenchymal stem cells and preadipocytes. *Mol Cell Endocrinol*, *296*, 32–40.
480. Perseghin, G., Scifo, P., Pagliato, E., et al. (2001). Gender factors affect fatty acids-induced insulin resistance in nonobese humans: effects of oral steroidal contraception. *J Clin Endocrinol Metab*, *86*, 3188–3196.
481. Carruth, L. L., Reisert, I., & Arnold, A. P. (2002). Sex chromosome genes directly affect brain sexual differentiation. *Nat Neurosci*, *5*, 933–934.
482. Gregg, C., Zhang, J., Butler, J. E., et al. (2010). Sex-specific parent-of-origin allelic expression in the mouse brain. *Science*, *329*, 682–685.
483. Vige, A., Gallou-Kabani, C., & Junien, C. (2008). Sexual dimorphism in non-mendelian inheritance. *Pediatr Res*, *63*, 340–347.
484. Sherwin, B. B. (2003). Estrogen and cognitive functioning in women. *Endocr Rev*, *24*, 133–151.
485. Swaab, D. F., & Hofman, M. A. (1995). Sexual differentiation of the human hypothalamus in relation to gender and sexual orientation. *Trends Neurosci*, *18*, 264–270.
486. Hughes, I. A., Houk, C., Ahmed, S. F., & Lee, P. A. (2006). Consensus statement on management of intersex disorders. *Arch Dis Child*, *91*, 554–1563.
487. Cosgrove, K. P., Mazure, C. M., & Staley, J. K. (2007). Evolving knowledge of sex differences in brain structure, function, and chemistry. *Biol Psychiatry*, *62*, 847–855.
488. Davis, E. C., Popper, P., & Gorski, R. A. (1996). The role of apoptosis in sexual differentiation of the rat sexually dimorphic nucleus of the preoptic area. *Brain Res*, *734*, 10–18.
489. Abdelgadir, S., Resko, J., Ojeda, S., et al. (1994). Androgens regulate aromatase cytochrome P450 messenger ribonucleic acid in rat brain. *Endocrinology*, *135*, 395–401.
490. Beyer, C., Green, S., & Hutchison, J. (1994). Androgens influence sexual differentiation of embryonic mouse hypothalamic aromatase neurons in vitro. *Endocrinology*, *135*, 1220–1226.

491. Ogawa, S., Eng, V., Taylor, J., et al. (1998). Roles of estrogen receptor-alpha gene expression in reproduction-related behaviors in female mice. *Endocrinology*, *139*, 5070–5081.
492. Gladue, B., & Clemens, L. (1980). Flutamide inhibits testosterone-induced masculine sexual behavior in male and female rats. *Endocrinology*, *106*, 1917–1922.
493. Poletti, A., Negri-Cesi, P., Rabuffetti, M., et al. (1998). Transient expression of the 5alpha-reductase type 2 isozyme in the rat brain in late fetal and early postnatal life. *Endocrinology*, *139*, 2171–2178.
494. Roselli, C. E., Klosterman, S. A. (1998). Sexual differentiation of aromatase activity in the rat brain: effects of perinatal steroid exposure. *Endocrinology*, *139*, 3193–3201.
495. Shughrue, P., Stumpf, W., Elger, W., et al. (1991). Progestin receptor cells in the 8-day-old male and female mouse cerebral cortex: autoradiographic evidence for a sexual dimorphism in target cell number. *Endocrinology*, *128*, 87–95.
496. Mani, S., Blaustein, J., Allen, J., et al. (1994). Inhibition of rat sexual behavior by antisense oligonucleotides to the progesterone receptor. *Endocrinology*, *135*, 1409–1414.
497. Cooke, B. M., & Woolley, C. S. (2005). Gonadal hormone modulation of dendrites in the mammalian CNS. *J Neurobiol*, *64*, 34–46.
498. Naftolin, F., Garcia-Segura, L. M., Horvath, T. L., et al. (2007). Estrogen-induced hypothalamic synaptic plasticity and pituitary sensitization in the control of the estrogen-induced gonadotrophin surge. *Reprod Sci*, *14*, 101–116.
499. Lu, S. F., McKenna, S. E., Cologer-Clifford, A., et al. (1998). Androgen receptor in mouse brain: sex differences and similarities in autoregulation. *Endocrinology*, *139*, 1594–1601.
500. Cooke, B. M., Tabibnia, G., & Breedlove, S. M. (1999). A brain sexual dimorphism controlled by adult circulating androgens. *Proc Natl Acad Sci USA*, *96*, 7538–7540.
501. Franssen, R. A., Rzucidlo, A. M., Franssen, C. L., et al. (2012). Reproductive experience facilitates recovery from kainic acid-induced neural insult in female Long-Evans rats. *Brain Res*, *1454*, 80–89.
502. Tsuji, M., Taguchi, A., Ohshima, M., et al. (2012). Progesterone and allopregnanolone exacerbate hypoxic-ischemic brain injury in immature rats. *Exp Neurol*, *233*, 214–220.
503. Levy, J., & Heller, W. (1992). Gender differences in human neuropsychological function. In A. A. Gerall, H. Moltz, & I. Ward (Eds.), *Sexual differentiation: a lifespan approach*, *handbook of behavioral neurobiology* (Vol. 11) (pp. 245–274). New York: Plenum Press.
504. Levine, S. C., Huttenlocher, J., Taylor, A., & Langrock, A. (1999). Early sex differences in spatial skill. *Dev Psychol*, *35*, 940–949.
505. Hyde, J. S., & Mertz, J. E. (2009). Gender, culture, and mathematics performance. *Proc Natl Acad Sci USA*, *106*, 8801–8807.
506. Schoentjes, E., Deboutte, D., & Friedrich, W. (1999). Child sexual behavior inventory: a Dutch-speaking normative sample. *Pediatrics*, *104*, 885–893.
507. Herdt, G., & McClintock, M. (2000). The magical age of 10. *Arch Sex Behav*, *29*, 587–606.
508. Finkelstein, J. W., Susman, E. J., Chinchilli, V. M., et al. (1997). Estrogen or testosterone increases self-reported aggressive behaviors in hypogonadal adolescents. *J Clin Endocrinol Metab*, *82*, 2433–2438.
509. Finkelstein, J. W., Susman, E. J., Chinchilli, V. M., et al. (1998). Effects of estrogen or testosterone on self-reported sexual responses and behaviors in hypogonadal adolescents . *J Clin Endocrinol Metab*, *83*, 2281–2285.
510. Wilson, J. D. (2001). Androgens, androgen receptors, and male gender role behavior. *Horm Behav*, *40*, 358–366.
511. LeVay, S. (1991). A difference in hypothalamic structure between heterosexual and homosexual men. *Science*, *253*, 1034–1037.
512. Allen, L. S., & Gorski, R. A. (1992). Sexual orientation and the size of the anterior commissure in the human brain. *Proc Natl Acad Sci USA*, *89*, 7199–7202.
513. Zhou J-N, Hofman, M., Gooren, L., & Swaab, D. (1995). A sex difference in the human brain and its relation to transsexuality. *Nature*, *378*, 68–70.
514. Berglund, H., Lindstrom, P., & Savic, I. (2006). Brain response to putative pheromones in lesbian women. *Proc Natl Acad Sci USA*, *103*, 8269–8274.
515. Keller, A., Zhuang, H., Chi, Q., et al. (2007). Genetic variation in a human odorant receptor alters odour perception. *Nature*, *449*, 468–472.
516. Stern, K., & McClintock, M. K. (1998). Regulation of ovulation by human pheromones. *Nature*, *392*, 177–179.
517. Jacob, S., & McClintock, M. K. (2000). Psychological state and mood effects of steroidal chemosignals in women and men. *Horm Behav*, *37*, 57–78.
518. Yoon, H., Enquist, L. W., & Dulac, C. (2005). Olfactory inputs to hypothalamic neurons controlling reproduction and fertility. *Cell*, *123*, 669–682.
519. Cohen, L., Morgan, J., & Bozyk, M. E. (1977). Effects of the simultaneous administration of diethylstilbestrol and prednisolone on serum enzymes in Duchenne's muscular dystrophy. *J Med*, *8*, 123–134.
520. Papanicolaou, D. A., & Vgontzas, A. N. (2000). Interleukin-6: the endocrine cytokine [Editorial and comment]. *J Clin Endocrinol Metab*, *85*, 1331–1333.
521. Chow, R. W., Handelsman, D. J., & Ng, M. K. (2010). Minireview: rapid actions of sex steroids in the endothelium. *Endocrinology*, *151*, 2411–2422.
522. Rosing, J., Middeldorp, S., Curvers, J., et al. (1999). Low-dose oral contraceptives and acquired resistance to activated protein C: a randomised cross-over study. *Lancet*, *354*, 2036–2040.
523. O'Donnell, E., Goodman, J. M., & Harvey, P. J. (2011). Clinical review: cardiovascular consequences of ovarian disruption: a focus on functional hypothalamic amenorrhea in physically active women. *J Clin Endocrinol Metab*, *96*, 3638–3648.
524. Laliberte, F., Dea, K., Duh, M. S., et al. (2011). Does the route of administration for estrogen hormone therapy impact the risk of venous thromboembolism? Estradiol transdermal system versus oral estrogen-only hormone therapy. *Menopause*, *18*, 1052–1059.
525. Renoux, C., Dell'Aniello, S., & Suissa, S. (2010). Hormone replacement therapy and the risk of venous thromboembolism: a population-based study. *J Thromb Haemost*, *8*, 979–986.
526. Santen, R. J., Allred, D. C., Ardoin, S. P., et al. (2010). Postmenopausal hormone therapy: an Endocrine Society scientific statement. *J Clin Endocrinol Metab*, *95*(Suppl. 1), S1–S66.
527. Shufelt, C. L., Johnson, B. D., Berga, S. L., et al. (2011). Timing of hormone therapy, type of menopause, and coronary disease in women: data from the National Heart, Lung, and Blood Institute-sponsored Women's Ischemia Syndrome Evaluation. *Menopause*, *18*, 943–950.
528. Lidegaard, O., Lokkegaard, E., Svendsen, A. L., & Agger, C. (2009). Hormonal contraception and risk of venous thromboembolism: national follow-up study. *BMJ*, *339*, b2890.
529. Jick, S. S., & Hernandez, R. K. (2011). Risk of non-fatal venous thromboembolism in women using oral contraceptives containing drospirenone compared with women using oral contraceptives containing levonorgestrel: case-control study using United States claims data. *BMJ*, *342*, d2151.
530. Lidegaard, O., Lokkegaard, E., Jensen, A., et al. (2012). Thrombotic stroke and myocardial infarction with hormonal contraception. *N Engl J Med*, *366*, 2257–2266.
531. Wang, X., Magkos, F., & Mittendorfer, B. (2011). Sex differences in lipid and lipoprotein metabolism: it's not just about sex hormones. *J Clin Endocrinol Metab*, *96*, 885–893.
532. Forest, M. (1979). Function of the ovary in the neonate and infant. *Eur J Obstet Gynecol Reprod Biol*, *9*, 145–160.
533. de Peretti, E., & Forest, M. G. (1982). Pitfalls in the etiological diagnosis of congenital adrenal hyperplasia in the early neonatal period. *Horm Res*, *16*, 10–22.
534. Mortensen, M., Ehrmann, D. A., Littlejohn, E., & Rosenfield, R. L. (2009). Asymptomatic volunteers with a polycystic ovary are a functionally distinct but heterogeneous population. *J Clin Endocrinol Metab*, *94*, 1579–1586.
535. Hingre, R. V., Gross, S. J., Hingre, K. S., et al. (1994). Adrenal steroidogenesis in very low birth weight preterm infants. *J Clin Endocrinol Metab*, *78*, 266–270.
536. Langlois, D., Li, J. Y., & Saez, J. M. (2002). Development and function of the human fetal adrenal cortex. *J Pediatr Endocrinol Metab*, *15*(Suppl. 5), 1311–1322.
537. Heckmann, M., Hartmann, M. F., Kampschulte, B., et al. (2006). Persistent high activity of the fetal adrenal cortex in preterm

infants: is there a clinical significance? *J Pediatr Endocrinol Metab*, *19*, 1303–1312.
538. Kuiri-Hanninen T, Haanpaa M, Turpeinen U, et al. (2013) Postnatal ovarian activation has effects in estrogen target tissues in infant girls. *J Clin Endocrinol Metab*, *98*, 4709–16.
539. Solomon, L. M., & Esterly, N. B. (1970). Neonatal dermatology. I. The newborn skin. *J Pediatr*, 77, 888–894.
540. Rosenfield, R. L. (1994). Normal and almost normal variants of precocious puberty: premature pubarche and premature thelarche revisited. *Horm Res*, *41*(Suppl. 2), 7–13.
541. Brito, V. N., Batista, M. C., Borges, M. F., et al. (1999). Diagnostic value of fluorometric assays in the evaluation of precocious puberty. *J Clin Endocrinol Metab*, *84*, 3539–3544.
542. Resende, E. A., Lara, B. H., Reis, J. D., et al. (2007). Assessment of basal and gonadotropin-releasing hormone-stimulated gonadotropins by immunochemiluminometric and immunofluorometric assays in normal children. *J Clin Endocrinol Metab*, *92*, 1424–1429.
543. Maesaka, H., Tachibana, K., Adachi, M., Okada T (1996). Monthly urinary gonadotropin and ovarian hormone excretory patterns in normal girls and female patients with idiopathic precocious puberty. *Pediatr Res*, *40*, 853–860.
544. Bordini, B. D., Littlejohn, E. E., & Rosenfield, R. L. (2010). LH dynamics in overweight girls with premature adrenarche and slowly progressive sexual precocity. *Intl J Pediatr Endocrinol*, *2010*, 724696.
545. Goodpasture, J., Ghai, K., Cara, J., & Rosenfield, R. (1993). Potential of gonadotropin-releasing hormone agonists in the diagnosis of pubertal disorders in girls. *Clin Obstet Gynecol*, *36*, 773–785.
546. Ehara, Y., Yen, S., & Siler, T. (1975). Serum prolactin levels during puberty. *Am J Obstet Gynecol*, *121*, 995.
547. Marshall, W., & Tanner, J. (1969). Variations in pattern of pubertal changes in girls. *Arch Dis Child*, *44*, 291.
548. Ross, G. T., & Vande Wiele, R. (1974). The ovary. In R. Williams (Ed.), *Textbook of endocrinology*. Philadelphia: WB Saunders Company.
549. Rosenfield, R. L., Bachrach, L. K., Chernausek, S. D., et al. (2000). Current age of onset of puberty. *Pediatrics*, *106*, 622.
550. Freedman, D. S., Khan, L. K., Serdula, M. K., et al. (2002). Relation of age at menarche to race, time period, and anthropometric dimensions: the Bogalusa Heart Study. *Pediatrics*, *110*, e43.
551. Demerath, E. W., Towne, B., Chumlea, W. C., et al. (2004). Recent decline in age at menarche: the Fels Longitudinal Study. *Am J Hum Biol*, *16*, 453–457.
552. de Ridder, C. M., Thijssen, J. H., Bruning, P. F., et al. (1992). Body fat mass, body fat distribution, and pubertal development: a longitudinal study of physical and hormonal sexual maturation of girls. *J Clin Endocrinol Metab*, *75*, 442–446.
553. Marti-Henneberg, C., & Vizmanos, B. (1997). The duration of puberty in girls is related to the timing of its onset. *J Pediatr*, *131*, 618–621.
554. Biro, F. M., Huang, B., Crawford, P. B., et al. (2006). Pubertal correlates in black and white girls. *J Pediatr*, *148*, 234–240.
555. Lee, J. M., Appugliese, D., Kaciroti, N., et al. (2007). Weight status in young girls and the onset of puberty. *Pediatrics*, *119*, e624–e630.
556. Bordini, B. D., Littlejohn, E. E., & Rosenfeld, R. L. (2009). Blunted sleep-related LH rise in healthy premenarcheal pubertal girls with elevated body mass index. J *Clin Endocrinol Metab*, *94*, 1168–1175. Comment in *J Clin Endocrinol Metab*, *94*, 1094–1096.
557. Ibañez, L., Jimenez, R., & de Zegher, F. (2006). Early puberty-menarche after precocious pubarche: relation to prenatal growth. *Pediatrics*, *117*, 117–121.
558. Tanner, J. M., & Davies, P. S. (1985). Clinical longitudinal standards for height and height velocity for North American children. *J Pediatr*, *107*, 317–329.
559. Bennett, D., Ward, M., & Daniel, W. J. (1976). The relationship of serum alkaline phosphatase concentrations to sex maturity ratings in adolescents. *J Pediatr*, *88*, 633.
560. Treloar, A., Boynton, R., Benn, B., & Brown, B. (1967). Variation of human menstrual cycle through reproductive life. *Int J Fertil*, *12*, 77–84.
561. Rosenfield, R. L. (1979). Plasma free androgen patterns in hirsute women and their diagnostic implications. *Am J Med*, *66*, 417–421.
562. Vekemans, M., Delvoye, P., L'Hermite, M., & Robyn, C. (1977). Serum prolactin levels during the menstrual cycle. *J Clin Endocrinol Metab*, *44*, 989.
563. Kolodny, R., Jacobs, L., & Daughaday, W. (1972). Mammary stimulation causes prolactin secretion in non-lactating women. *Nature*, *238*, 284.
564. Herman-Giddens, M. E., Slora, E. J., Wasserman, R. C., et al. (1997). Secondary sexual characteristics and menses in young girls seen in office practice: a study from the Pediatric Research in Office Settings network. *Pediatrics*, *99*, 505–512.
565. Klein, K. O., Mericq, V., Brown-Dawson, J. M., et al. (1999). Estrogen levels in girls with premature thelarche compared with normal prepubertal girls as determined by an ultrasensitive recombinant cell bioassay. *J Pediatr*, *134*, 190–192.
566. Kreiter, M., Cara, J., & Rosenfield, R. (1993). Modifying the outcome of complete precocious puberty: to treat or not to treat. In G. Grave, & G. Cutler (Eds.), *Sexual precocity: etiology, diagnosis, and management* (pp. 109–120). New York: Raven Press.
567. Roman, R., Johnson, M. C., Codner, E., et al. (2004). Activating GNAS1 gene mutations in patients with premature thelarche. *J Pediatr*, *145*, 218–222.
568. Rosenfield, R. L. (2012). Premature adrenarche. In B. Rose (Ed.), UpToDate. Retrieved from http://www.uptodate.com/contents/premature-adrenarche
569. Witchel, S. F., Lee, P. A., Suda-Hartman, M., & Hoffman, E. P. (1997). Hyperandrogenism and manifesting heterozygotes for 21-hydroxylase deficiency. B *iochem Mol Med*, *62*, 151–158.
570. Nayak, S., Lee, P. A., & Witchel, S. F. (1998). Variants of the type II 3beta-hydroxysteroid dehydrogenase gene in children with premature pubic hair and hyperandrogenic adolescents. *Mol Genet Metab*, *64*, 184–192.
571. Wehkalampi, K., Widen, E., Laine, T., et al. (2008). Patterns of inheritance of constitutional delay of growth and puberty in families of adolescent girls and boys referred to specialist pediatric care. *J Clin Endocrinol Metab*, *93*, 723–728.
572. Sykiotis, G. P., Plummer, L., Hughes, V. A., et al. (2010). Oligogenic basis of isolated gonadotropin-releasing hormone deficiency. *Proc Natl Acad Sci USA*, *107*, 15140–15144.
573. Caronia, L. M., Martin, C., Welt, C. K., et al. (2011). A genetic basis for functional hypothalamic amenorrhea. *N Engl J Med*, *364*, 215–225.
574. Chevalley, T., Bonjour, J. P., Ferrari, S., & Rizzoli, R. (2009). The influence of pubertal timing on bone mass acquisition: a predetermined trajectory detectable five years before menarche. *J Clin Endocrinol Metab*, *94*, 3424–431.
575. Vollman, R. F. (1977). The menstrual cycle. *Major Probl Obstet Gynecol*, 7, 72–123.
576. Apter, D., & Vihko, R. (1977). Serum pregnenolone, progesterone, 17-hydroxyprogesterone, testosterone, and 5alpha-dihydrotestosterone during female puberty. *J Clin Endocrinol Metab*, *45*, 1039–1048.
577. Zhang, K., Pollack, S., Ghods, A., et al. (2008). Onset of ovulation after menarche in girls: a longitudinal study. *J Clin Endocrinol Metab*, *93*, 1186–1194.
578. American Academy of Pediatrics Committee on Adolescence, American College of Obstetricians and Gynecologists Committee on Adolescent Health Care, Diaz, A., Laufer, M. R., & Breech, L. L. (2006). Menstruation in girls and adolescents: using the menstrual cycle as a vital sign. *Pediatrics*, *118*, 2245–2250.
579. Rosenfield, R. L. (2013). Adolescent anovulation: maturational mechanisms and implications. *J Clin Endocrinol Metab*. Advance online publication. doi:10.1210/jc.2013-1770
580. Siegberg, R., Nilsson, C. G., Stenman, U. H., & Widholm, O. (1986). Endocrinologic features of oligomenorrheic adolescent girls. *Fertil Steril*, *46*, 852–857.
581. Venturoli, S., Porcu, E., Fabbri, R., et al. (1986). Menstrual irregularities in adolescents: hormonal pattern and ovarian morphology. *Hormone Res*, *24*, 269–279.
582. Lucky AW, Biro FM, Simbartl LA, et al. (1997) Predictors of severity of acne vulgaris in young adolescent girls: results of a five-year longitudinal study. *J Pediatr*, *130*, 30–9.

583. Eichenfield, L. F., Krakowski, A. C., Piggott, C., et al. (2013). Evidence-based recommendations for the diagnosis and treatment of pediatric acne. *Pediatrics*, *131*(Suppl. 3), S163-S186.
584. McClintock, M. (1971). Menstrual synchrony and suppression. *Nature*, *229*, 244.
585. Steinberg, L., & Morris, A. S. (2001). Adolescent development. *Annu Rev Psychol*, *52*, 83–110.
586. Susman, E. J., Finkelstein, J. W., Chinchilli, V. M., et al. (1998). The effect of sex hormone replacement therapy on behavior problems and moods in adolescents with delayed puberty. *J Pediatr*, *133*, 521–525.
587. Dewald, J. F., Meijer, A. M., Oort, F. J., et al. (2010). The influence of sleep quality, sleep duration and sleepiness on school performance in children and adolescents: a meta-analytic review. *Sleep Med Rev*, *14*, 179–189.
588. Feinberg, I., Higgins, L. M., Khaw, W. Y., & Campbell, I. G. (2006). The adolescent decline of NREM delta, an indicator of brain maturation, is linked to age and sex but not to pubertal stage. *Am J Physiol Regul Integr Comp Physiol*, *291*, R1724–R1729.
589. Nada, S. E., Thompson, R. C., & Padmanabhan, V. (2010). Developmental programming: differential effects of prenatal testosterone excess on insulin target tissues. *Endocrinology*, *151*, 5165–5173.
590. Aono, T., Miyake, A., Kinugasa, T., et al. (1978). Absence of positive feedback effect of oestrogen on LH release in patients with testicular feminization syndrome. *Acta Endocrinol* (Copenh), *87*, 259–267.
591. Golan, A., Langer, R., Bukovsky, I., & Caspi, E. (1989). Congenital anomalies of the müllerian system. *Fertil Steril*, *51*, 747–754.
592. Philibert, P., Biason-Lauber, A., Rouzier, R., et al. (2008). Identification and functional analysis of a new WNT4 gene mutation among 28 adolescent girls with primary amenorrhea and Mullerian duct abnormalities: a French collaborative study. *J Clin Endocrinol Metab*, *93*, 895–900.
593. Carel, J. C., Eugster, E. A., Rogol, A., et al. (2009). Consensus statement on the use of gonadotropin-releasing hormone analogs in children. *Pediatrics*, *123*, e752–e762.
594. Brauner, R., Adan, L., Malandry, F., & Zantleifer, D. (1994). Adult height in girls with idiopathic true precocious puberty. *J Clin Endocrinol Metab*, *79*, 415–420.
595. Junier M-P, Wolff, A., Hoffman, G., et al. (1992). Effect of hypothalamic lesions that induce precocious puberty on the morphological and functional maturation of the luteinizing hormone-releasing hormone neuronal system. *Endocrinology*, *131*, 787–798.
596. Cohen, L. E. (2005). Endocrine late effects of cancer treatment. *Endocrinol Metab Clin North Am*, *34*, 769–789.
597. Cacciari, E., Zucchini, S., Ambrosetto, P., et al. (1994). Empty sella in children and adolescents with possible hypothalamic-pituitary disorders. *J Clin Endocrinol Metab*, *78*, 767–771.
598. Laue, I., Comite, F., Hench, K., et al. (1985). Precocious puberty associated with neurofibromatosis and optic gliomas. *Am J Dis Child*, *139*, 1097.
599. Zacharin, M. (1997). Precocious puberty in two children with neurofibromatosis type I in the absence of optic chiasmal glioma. *J Pediatr*, *130*, 155–157.
600. Mahachoklertwattana, P., Kaplan, S., & Grumbach, M. (1993). The luteinizing hormone-releasing hormone-secreting hypothalamic hamartoma is a congenital malformation: natural history. *J Clin Endocrinol Metab*, *77*, 118–124.
601. Chan YM, Fenoglio-Simeone KA, Paraschos S, et al. (2010) Central precocious puberty due to hypothalamic hamartomas correlates with anatomic features but not with expression of GnRH, TGFalpha, or KISS1. *Horm Res Paediatr*, *73*, 312–9.
602. Kitay, J., & Altschule, M. (1954). *The pineal gland*. Cambridge, MA: Harvard University Press.
603. Cohen, R., Wurtman, R., Axelrod, J., et al. (1964). Some clinical. biochemical, and physiological actions of the pineal gland. *Ann Intern Med*, *61*, 1144.
604. Hibi, I., & Fujiwara, K. (1987). Precocious puberty of cerebral origin: a cooperative study in Japan. *Prog Exp Tumor Res*, *30*, 224–238.
605. Starzyk, J., Starzyk, B., Bartnik-Mikuta, A., et al. (2001). Gonadotropin releasing hormone-independent precocious puberty in a 5 year-old girl with suprasellar germ cell tumor secreting beta-hCG and alpha-fetoprotein. *J Pediatr Endocrinol Metab*, *14*, 789–796.
606. O'Marcaigh, A. S., Ledger, G. A., Roche, P. C., et al. (1995). Aromatase expression in human germinomas with possible biological effects. *J Clin Endocrinol Metab*, *80*, 3763–3766.
607. Baer, K. (1977). Premature ovarian failure and precocious puberty. *Obstet Gynecol*, *49*, 15s.
608. Quigley, C., Cowell, C., Jimenez, M., et al. (1989). Normal or early development of puberty despite gonadal damage in children treated for acute lymphoblastic leukemia. *N Engl J Med*, *321*, 143–151.
609. Root, A. W., & Moshang, T., Jr. (1984). Evolution of the hyperandrogenism-polycystic ovary syndrome from isosexual precocious puberty: report of two cases. *Am J Obstet Gynecol*, *149*, 763–767.
610. Teles, M. G., Silveira, L. F., Tusset, C., & Latronico, A. C. (2011). New genetic factors implicated in human GnRH-dependent precocious puberty: the role of kisspeptin system. *Mol Cell Endocrinol*, *346*, 84–90.
611. Silveira, L. G., Noel, S. D., Silveira-Neto, A. P., et al. (2010). Mutations of the KISS1 gene in disorders of puberty. *J Clin Endocrinol Metab*, *95*, 2276–2280.
612. Fokstuen, S., Ginsburg, C., Zachmann, M., & Schinzel, A. (1999). Maternal uniparental disomy 14 as a cause of intrauterine growth retardation and early onset of puberty. *J Pediatr*, *134*, 689–695.
613. Bourguignon, J. P., Jaeken, J., Gerard, A., & de Zegher, F. (1997). Amino acid neurotransmission and initiation of puberty: evidence from nonketotic hyperglycinemia in a female infant and gonadotropin-releasing hormone secretion by rat hypothalamic explants. *J Clin Endocrinol Metab*, *82*, 1899–1903.
614. Blanco-Garcia, M., Evain-Brion, D., Roger, M., & Job, M. (1985). Isolated menses in prepubertal girls. Pediatrics 76, 43.
615. Rosenfeld, R. G., Reitz, R. E., King, A. B., & Hintz, R. L. (1980). Familial precocious puberty associated with isolated elevation of luteinizing hormone. *N Engl J Med*, *303*, 859–862.
616. Van Wyk, J., & Grumbach, M. (1960). Syndrome of precocious menstruation and galactorrhea in juvenile hypothyroidism: an example of hormonal overlap in pituitary feedback. *J Pediatr*, *57*, 416.
617. Lindsay, A., Voorhess, M., & MacGillivray, M. (1980). Multicystic ovaries detected by sonography in children with hypothyroidism. *Am J Dis Child*, *134*, 588.
618. Baranowski, E., & Hogler, W. (2012). An unusual presentation of acquired hypothyroidism: the Van Wyk-Grumbach syndrome. *Eur J Endocrinol*, *166*, 537–542.
619. Ryan, G. L., Feng, X., d'Alva, C. B., et al. (2007). Evaluating the roles of follicle-stimulating hormone receptor polymorphisms in gonadal hyperstimulation associated with severe juvenile primary hypothyroidism. *J Clin Endocrinol Metab*, *92*, 2312–2317.
620. De Leener, A., Montanelli, L., Van Durme, J., et al. (2006). Presence and absence of follicle-stimulating hormone receptor mutations provide some insights into spontaneous ovarian hyperstimulation syndrome physiopathology. *J Clin Endocrinol Metab*, *91*, 555–562.
621. Cabrera, S. M., Dimeglio, L. A., & Eugster, E. A. (2013). Incidence and characteristics of pseudoprecocious puberty because of severe primary hypothyroidism. *J Pediatr*, *162*, 637–639.
622. Copmann, T., & Adams, W. (1981). Relationship of polycystic ovary induction to prolactin secretion: prevention of cyst formation by bromocriptine in the rat. *Endocrinology*, *108*, 1095.
623. Shenker, A., Weinstein, L., Moran, A., et al. (1993). Severe endocrine and nonendocrine manifestations of the McCune-Albright syndrome associated with activating mutations of stimulatory G protein Gs. *J Pediatr*, *123*, 509–518.
624. Frisch, L., Copeland, K., & Boepple, P. (1992). Recurrent ovarian cysts in childhood: diagnosis of McCune-Albright syndrome by bone scan. *Pediatr*, *90*, 102–104.
625. Scanlon, E., Burkett, F., Sener, S., et al. (1974). Breast carcinoma in an 11-year-old girl with Albright's syndrome. *Breast*, *6*, 5.
626. Lumbroso, S., Paris, F., & Sultan, C. (2004). Activating Gsalpha mutations: analysis of 113 patients with signs of McCune-Albright syndrome—a European Collaborative Study. *J Clin Endocrinol Metab*, *89*, 2107–2113.
627. Kalter-Leibovici, O., Dickerman, Z., Zamir, R., et al. (1989). Late onset 21-hydroxylase deficiency in a girl mimicking true sexual precocity. *J Pediat Endocrinol*, *3*, 121–124.

628. Uli, N., Chin, D., David, R., et al. (1997). Menstrual bleeding in a female infant with congenital adrenal hyperplasia: altered maturation of the hypothalamic-pituitary-ovarian axis. *J Clin Endocrinol Metab*, *82*, 3298–3302.
629. Boepple, P. (1989). Case records of the Massachusetts General Hospital. *N Engl J Med*, *321*, 1463.
630. Brauner, R., Bashamboo, A., Rouget, S., et al. (2010). Clinical, biological and genetic analysis of prepubertal isolated ovarian cyst in 11 girls. *PLoS One*, *5*, e11282.
631. Young, R. H., Goodman, A., Penson, R. T., et al. (2010). Case records of the Massachusetts General Hospital. Case 8–2010. A 22-year-old woman with hypercalcemia and a pelvic mass. *N Engl J Med*, *362*, 1031–1040.
632. Jamieson, S., & Fuller, P. J. (2012). Molecular pathogenesis of granulosa cell tumors of the ovary. *Endocr Rev*, *33*, 109–144.
633. Gallion, H. H., van Nagell, J. R. Jr, Donaldson, E. S., & Powell, D. E. (1988). Ovarian dysgerminoma: report of seven cases and review of the literature. *Am J Obstet Gynecol*, *158*, 591–595.
634. Orselli, R. C., & Bassler, T. J. (1973). Theca granulosa cell tumor arising in adrenal. *Cancer*, *31*, 474–477.
635. Mehenni, H., Blouin, J. L., Radhakrishna, U., et al. (1997). Peutz-Jeghers syndrome: confirmation of linkage to chromosome 19p13.3 and identification of a potential second locus, on 19q13.4. *Am J Hum Genet*, *61*, 1327–1334.
636. Phornphutkul, C., Okubo, T., Wu, K., et al. (2001). Aromatase p450 expression in a feminizing adrenal adenoma presenting as isosexual precocious puberty. *J Clin Endocrinol Metab*, *86*, 649–652.
637. Wilkin, F., Gagne, N., Paquette, J., et al. (2000). Pediatric adrenocortical tumors: molecular events leading to insulin-like growth factor II gene overexpression. *J Clin Endocrinol Metab*, *85*, 2048–2056.
638. Fukami, M., Shozu, M., Soneda, S., et al. (2011). Aromatase excess syndrome: identification of cryptic duplications and deletions leading to gain of function of CYP19A1 and assessment of phenotypic determinants. *J Clin Endocrinol Metab*, *96*, E1035–E1043.
639. Saenz de Rodriguez, C. A., Bongiovanni, A. M., & Conde de Borrego, L. (1985). An epidemic of precocious development in Puerto Rican children. *J Pediatr*, *107*, 393–396.
640. Setchell, K. D., Zimmer-Nechemias, L., Cai, J., & Heubi, J. E. (1997). Exposure of infants to phyto-oestrogens from soy-based infant formula. Lancet 350, 23–27.
641. Zava, D. T., Dollbaum, C. M., & Blen, M. (1998). Estrogen and progestin bioactivity of foods, herbs, and spices. *Proc Soc Exp Biol Med*, *217*, 369–378.
642. Henley, D. V., Lipson, N., Korach, K. S., & Bloch, C. A. (2007). Prepubertal gynecomastia linked to lavender and tea tree oils. *N Engl J Med*, *356*, 479–485. Comment in *N Engl J Med*, *356*, 2541–2544
643. Kunz, G. J., Klein, K. O., Clemons, R. D., et al. (2004). Virilization of young children after topical androgen use by their parents. *Pediatrics*, *114*, 282–284.
644. Balducci, R., Boscherini, B., Mangiantini, A., et al. (1994). Isolated precocious pubarche: an approach. *J Clin Endocrinol Metab*, *79*, 582–589.
645. Wartofsky, L., & Handelsman, D. J. (2010). Standardization of hormonal assays for the 21st century. *J Clin Endocrinol Metab*, *95*, 5141–5143.
646. Trivin, C., Couto-Silva, A. C., Sainte-Rose, C., et al. (2006). Presentation and evolution of organic central precocious puberty according to the type of CNS lesion. *Clin Endocrinol* (Oxf), *65*, 239–245.
647. Bidlingmaier, F., Butenandt, O., & Knorr, D. (1977). Plasma gonadotropins and estrogens in girls with idiopathic precocious puberty. Pediatr Res 11, 91.
648. Houk, C. P., Kunselman, A. R., & Lee, P. A. (2009). Adequacy of a single unstimulated luteinizing hormone level to diagnose central precocious puberty in girls. *Pediatrics*, *123*, e1059–e1063.
649. Houk, C. P., Kunselman, A. R., & Lee, P. A. (2008). The diagnostic value of a brief GnRH analogue stimulation test in girls with central precocious puberty: a single 30-minute post-stimulation LH sample is adequate. *J Pediatr Endocrinol Metab*, *21*, 1113–1118.
650. Sathasivam, A., Garibaldi, L., Shapiro, S., et al. (2010). Leuprolide stimulation testing for the evaluation of early female sexual maturation. *Clin Endocrinol* (Oxf), *73*, 375–381.
651. Ibañez, L., Potau, N., Zampolli, M., et al. (1994). Use of leuprolide acetate response patterns in the early diagnosis of pubertal disorders: comparison with the gonadotropin-releasing hormone test. *J Clin Endocrinol Metab*, *78*, 30–35.
652. Rosenthal, I., Refetoff, S., Rich, B., et al. (1996). Response to challenge with gonadotropin-releasing hormone agonist in a mother and her two sons with a constitutively activating mutation of the luteinizing hormone receptor—a Clinical Research Center study. *J Clin Endocrinol Metab*, *81*, 3802–3806.
653. Rosenfield, R. L. (1994). Selection of children with precocious puberty for treatment with gonadotropin releasing hormone analogs. *J Pediatr*, *124*, 989–991.
654. Armengaud, J. B., Charkaluk, M. L., Trivin, C., et al. (2009). Precocious pubarche: distinguishing late-onset congenital adrenal hyperplasia from premature adrenarche. *J Clin Endocrinol Metab*, *94*, 2835–2840.
655. Shah, R. U., Lawrence, C., Fickenscher, K. A., et al. (2011). Imaging of pediatric pelvic neoplasms. *Radiol Clin North Am*, *49*, 729–748, vi.
656. Balassy, C., Navarro, O. M., & Daneman, A. (2011). Adrenal masses in children. *Radiol Clin North Am*, *49*, 711–727, vi.
657. Salardi, S., Orsini, L., Cacciari, E., et al. (1988). Pelvic ultrasonography in girls with precocious puberty, congenital adrenal hyperplasia, obesity, or hirsutism. *J Pediatr*, *112*, 880.
658. Stratakis, C. A., Papageorgiou, T., Premkumar, A., et al. (2000). Ovarian lesions in Carney complex: clinical genetics and possible predisposition to malignancy. *J Clin Endocrinol Metab*, *85*, 4359–4366.
659. Lonsdale, R. N., Roberts, P. F., & Trowell, J. E. (1991). Autoimmune oophoritis associated with polycystic ovaries. *Histopathology*, *19*, 77–81.
660. Pascale, M. M., Pugeat, M., Roberts, M., et al. (1994). Androgen suppressive effect of GnRH agonist in ovarian hyperthecosis and virilizing tumours. *Clin Endocrinol* (Oxf), *41*, 571–576.
661. Grunt, J. A., Midyett, L. K., Simon, S. D., & Lowe, L. (2004). When should cranial magnetic resonance imaging be used in girls with early sexual development? *J Pediatr Endocrinol Metab*, *17*, 775–780.
662. Lazar, L., Padoa, A., & Phillip, M. (2007). Growth pattern and final height after cessation of gonadotropin-suppressive therapy in girls with central sexual precocity. *J Clin Endocrinol Metab*, *92*, 3483–3489.
663. Palmert, M. R., Malin, H. V., & Boepple, P. A. (1999). Unsustained or slowly progressive puberty in young girls: initial presentation and long-term follow-up of 20 untreated patients. *J Clin Endocrinol Metab*, *84*, 415–423.
664. Alvarez, R., Grizzle, W., Smith, L., & Miller, D. (1989). Compensatory ovarian hypertrophy occurs by a mechanism distinct from compensatory growth in the regenerating liver. *Am J Obstet Gynecol*, *161*, 1653–1657.
665. Fuld, K., Chi, C., & Neely, E. K. (2011). A randomized trial of 1- and 3-month depot leuprolide doses in the treatment of central precocious puberty. *J Pediatr*, *159*, 982–987, e1.
666. Fuqua, J. S. (2013). Treatment and outcomes of precocious puberty: an update. *J Clin Endocrinol Metab*, *98*, 2198–207.
667. Rahhal, S., Clarke, W. L., Kletter, G. B., et al. (2009). Results of a second year of therapy with the 12-month histrelin implant for the treatment of central precocious puberty. *Int J Pediatr Endocrinol*. doi:10.1155/2009/812517
668. Klein, K. O., Baron, J., Barnes, K. M., et al. (1998). Use of an ultrasensitive recombinant cell bioassay to determine estrogen levels in girls with precocious puberty treated with a luteinizing hormone-releasing hormone agonist. *J Clin Endocrinol Metab*, *83*, 2387–2389.
669. Brito, V. N., Latronico, A. C., Arnhold, I. J., & Mendonca, B. B. (2004). A single luteinizing hormone determination 2 hours after depot leuprolide is useful for therapy monitoring of gonadotropin-dependent precocious puberty in girls. *J Clin Endocrinol Metab*, *89*, 4338–4342.
670. Adan, L., Souberbielle, J. C., & Zucker, J. M. (1997). Adult height in 24 patients treated for growth hormone deficiency and early puberty. *J Clin Endocrinol Metab*, *82*, 229–233.

671. Pasquino, A. M., Pucarelli, I., Segni, M., et al. (1999). Adult height in girls with central precocious puberty treated with gonadotropin-releasing hormone analogues and growth hormone. *J Clin Endocrinol Metab*, *84*, 449–452. Erratum in *J Clin Endocrinol Metab*, *84*, 1978.
672. Carel, J. C., Roger, M., Ispas, S., et al. (1999). Final height after long-term treatment with triptorelin slow release for central precocious puberty: importance of statural growth after interruption of treatment. French study group of Decapeptyl in Precocious Puberty. *J Clin Endocrinol Metab*, *84*, 1973–1978.
673. Letterie, G. S., Stevenson, D., & Shah, A. (1991). Recurrent anaphylaxis to a depot form of GnRH analogue. *Obstet Gynecol*, *78*, 943–946.
674. Magiakou, M. A., Manousaki, D., Papadaki, M., et al. (2010). The efficacy and safety of gonadotropin-releasing hormone analog treatment in childhood and adolescence: a single center, long-term follow-up study. *J Clin Endocrinol Metab*, *95*, 109–117.
675. Rosenfield, R. L. (1996). Essentials of growth diagnosis. *Endocrinol Metab Clin N Am*, *25*, 743–758.
676. Papadimitriou, A., Beri, D., Tsialla, A., et al. (2006). Early growth acceleration in girls with idiopathic precocious puberty. *J Pediatr*, *149*, 43–46.
677. Lazar, L., Kauli, R., Pertzelan, A., & Phillip, M. (2002). Gonadotropin-suppressive therapy in girls with early and fast puberty affects the pace of puberty but not total pubertal growth or final height. *J Clin Endocrinol Metab*, *87*, 2090–2094.
678. Richman, R. A., Underwood, L. E., French, F. S., & Van Wyk, J. J. (1971). Adverse effects of large doses of medroxyprogesterone (MPA) in idiopathic isosexual precocity. *J Pediatr*, *79*, 963.
679. Lopez, L. M., Grimes, D. A., Schulz, K. F., & Curtis, K. M. (2006). Steroidal contraceptives: effect on bone fractures in women. *Cochrane Database Syst Rev*, CD006033.
680. Eugster, E. A., Rubin, S. D., Reiter, E. O., et al. (2003). Tamoxifen treatment for precocious puberty in McCune-Albright syndrome: a multicenter trial. *J Pediatr*, *143*, 60–66.
681. Wit, J. M., Hero, M., & Nunez, S. B. (2012). Aromatase inhibitors in pediatrics. *Nat Rev Endocrinol*, *8*, 135–147.
682. Holland, F., Kirsch, S., & Selby, R. (1987). Gonadotropin-independent precocious puberty ("testotoxicosis"): influence of maturational status on response to ketoconazole. *J Clin Endocrinol Metab*, *64*, 328–333.
683. Laven, J. S., Lumbroso, S., Sultan, C., & Fauser, B. C. (2001). Dynamics of ovarian function in an adult woman with McCune—Albright syndrome. *J Clin Endocrinol Metab*, *86*, 2625–2630.
684. Leet, A. I., & Collins, M. T. (2007). Current approach to fibrous dysplasia of bone and McCune-Albright syndrome. *J Child Orthop*, *1*, 3–17.
685. Rosenfield, R. L., & Barnes, R. B. (1993). Menstrual disorders in adolescence. *Endocrinol Metab Clin N Am*, *22*, 491–505.
686. Razdan, A., Rosenfield, R., & Kim, M. (1976). Endocrinologic characteristics of partial ovarian failure. *J Clin Endocrinol Metab*, *43*, 449.
687. Page, L., Beauregard, L., Bode, H., & Beitins, I. (1990). Hypothalamic-pituitary-ovarian function in menstruating women with Turner syndrome (45,X). *Pediatr Res*, *28*, 514–517.
688. Winslow, K. L., Toner, J. P., Brzyski, R. G., et al. (1991). The gonadotropin-releasing hormone agonist stimulation test—a sensitive predictor of performance in the flare-up in vitro fertilization cycle. *Fertil Steril*, *56*, 711–717.
689. Knauff, E. A., Eijkemans, M. J., Lambalk, C. B., et al. (2009). Anti-Mullerian hormone, inhibin B, and antral follicle count in young women with ovarian failure. *J Clin Endocrinol Metab*, *94*, 786–792.
690. Hendriks, A. E., Laven, J. S., Valkenburg, O., et al. (2011). Fertility and ovarian function in high-dose estrogen-treated tall women. *J Clin Endocrinol Metab*, *96*, 1098–1105.
691. Bidet, M., Bachelot, A., Bissauge, E., et al. (2011). Resumption of ovarian function and pregnancies in 358 patients with premature ovarian failure. *J Clin Endocrinol Metab*, *96*, 3864–3872.
692. Mazzanti, L., Cacciari, E., Bergamaschi, R., et al. (1997). Pelvic ultrasonography in patients with Turner syndrome: age-related findings in different karyotypes. *J Pediatr*, *131*, 135–140.
693. Birgit, B., Julius, H., Carsten, R., et al. (2009). Fertility preservation in girls with turner syndrome: prognostic signs of the presence of ovarian follicles. *J Clin Endocrinol Metab*, *94*, 74–80.
694. De Vos, M., Devroey, P., & Fauser, B. C. (2011). Primary ovarian insufficiency. *Lancet*, *376*, 911–921.
695. Cunniff, C., Jones, K., & Benirschke, K. (1990). Ovarian dysgenesis in individuals with chromosomal abnormalities. *Hum Genet*, *86*, 552–556.
696. Hansen, J., Boyar, R., & Shapiro, L. (1980). Gonadal function in trisomy 21. *Horm Res*, *12*, 345.
697. Bovicelli, L., Orsini, L. F., Rizzo, N., et al. (1982). Reproduction in Down syndrome. *Obstet Gynecol*, *59*, 13S–17S.
698. Pelletier, J., Bruening, W., Kashtan, C., et al. (1991). Germline mutations in the Wilms' tumor suppressor gene are associated with abnormal urogenital development in Denys-Drash syndrome. *Cell*, *67*, 437–447.
699. Chrzanowska, K. H., Szarras-Czapnik, M., Gajdulewicz, M., et al. (2010). High prevalence of primary ovarian insufficiency in girls and young women with Nijmegen breakage syndrome: evidence from a longitudinal study. *J Clin Endocrinol Metab*, *95*, 3133–3140.
700. Beysen, D., De Paepe, A., & De Baere, E. (2009). FOXL2 mutations and genomic rearrangements in BPES. *Hum Mutat*, *30*, 158–169.
701. Spencer JB, Badik JR, Ryan EL, et al. (2013) Modifiers of ovarian function in girls and women with classic galactosemia. *J Clin Endocrinol Metab*, *98*, E1257–65.
702. Lieman, H., & Santoro, N. (1997). Premature ovarian failure: a modern approach to diagnosis and treatment. *Endocrinologist*, *7*, 314–321.
703. McDonough, P. G. (2003). Selected enquiries into the causation of premature ovarian failure. *Hum Fertil* (Camb), *6*, 130–136.
704. Chand, A. L., Harrison, C. A., & Shelling, A. N. (2010). Inhibin and premature ovarian failure. *Hum Reprod Update*, *16*, 39–50.
705. Layman, L. C. (2006). Editorial: BMP15—the first true ovarian determinant gene on the X-chromosome? *J Clin Endocrinol Metab*, *91*, 1673–1676.
706. Wallace, W. H. (2011). Oncofertility and preservation of reproductive capacity in children and young adults. *Cancer*, *117*, 2301–2310.
707. Chemaitilly, W., Mertens, A. C., Mitby, P., et al. (2006). Acute ovarian failure in the childhood cancer survivor study. *J Clin Endocrinol Metab*, *91*, 1723–1728.
708. Sklar, C. A., Mertens, A. C., Mitby, P., et al. (2006). Premature menopause in survivors of childhood cancer: a report from the childhood cancer survivor study. *J Natl Cancer Inst*, *98*, 890–896.
709. Ginsberg, J. P. (2011). New advances in fertility preservation for pediatric cancer patients. *Curr Opin Pediatr*, *23*, 9–13.
710. Oktay, K., Sonmezer, M., Oktem, O., et al. (2007). Absence of conclusive evidence for the safety and efficacy of gonadotropin-releasing hormone analogue treatment in protecting against chemotherapy-induced gonadal injury. *Oncologist*, *12*, 1055–1066.
711. Sarafoglou, K., Boulad, F., Gillio, A., & Sklar, C. (1997). Gonadal function after bone marrow transplantation for acute leukemia during childhood. *J Pediatr*, *130*, 210–216.
712. Green, D. M., Kawashima, T., Stovall, M., et al. (2009). Fertility of female survivors of childhood cancer: a report from the childhood cancer survivor study. *J Clin Oncol*, *27*, 2677–2685.
713. Yeung, S. C., Chiu, A. C., Vassilopoulou-Sellin, R., & Gagel, R. F. (1998). The endocrine effects of nonhormonal antineoplastic therapy. *Endocr Rev*, *19*, 144–172.
714. Larsen, E. C., Muller, J., Schmiegelow, K., et al. (2003). Reduced ovarian function in long-term survivors of radiation- and chemotherapy-treated childhood cancer. *J Clin Endocrinol Metab*, *88*, 5307–5314.
715. Woodruff, T. K. (2009). Preserving fertility during cancer treatment. *Nat Med*, *15*, 1124–1125.
716. Toledo, S. P., Brunner, H. G., Kraaij, R., et al. (1996). An inactivating mutation of the luteinizing hormone receptor causes amenorrhea in a 46,XX female. *J Clin Endocrinol Metab*, *81*, 3850–3854.
717. Layman, L. C., & McDonough, P. G. (2000). Mutations of follicle stimulating hormone-beta and its receptor in human and mouse: genotype/phenotype. *Mol Cell Endocrinol*, *161*, 9–17.
718. Salvi, R., & Pralong, F. P. (2010). Molecular characterization and phenotypic expression of mutations in genes for gonadotropins and their receptors in humans. *Front Horm Res*, *39*, 1–12.

719. Namnoum, A. B., Merriam, G. R., Moses, A. M., & Levine, M. A. (1998). Reproductive dysfunction in women with Albright's hereditary osteodystrophy. *J Clin Endocrinol Metab*, *83*, 824–829.
720. Nelson, L. M. (2009). Clinical practice: primary ovarian insufficiency. *N Engl J Med*, *360*, 606–614.
721. Tsigkou, A., Marzotti, S., Borges, L., et al. (2008). High serum inhibin concentration discriminates autoimmune oophoritis from other forms of primary ovarian insufficiency. *J Clin Endocrinol Metab*, *93*, 1263–1269.
722. Lucky, A. W., Rebar, R. W., Blizzard, R. M., & Goren, E. M. (1977). Pubertal progression in the presence of elevated serum gonadotropins in girls with multiple endocrine deficiencies. *J Clin Endocrinol Metab*, *45*, 673–678.
723. Kuwahara, A., Kamada, M., Irahara, M., et al. (1998). Autoantibody against testosterone in a woman with hypergonadotropic hypogonadism. *J Clin Endocrinol Metab*, *83*, 14–16.
724. Sahakitrungruang, T., Huang, N., Tee, M. K., et al. (2009). Clinical, genetic, and enzymatic characterization of P450 oxidoreductase deficiency in four patients. *J Clin Endocrinol Metab*, *94*, 4992–5000.
725. Tanae, A., Katsumata, N., Sato, N., et al. (2000). Genetic and endocrinological evaluations of three 46,XX patients with congenital lipoid adrenal hyperplasia previously reported as having presented spontaneous puberty. *Endocr J*, *47*, 629–634.
726. Lourenco, D., Brauner, R., Lin, L., et al. (2009). Mutations in NR5A1 associated with ovarian insufficiency. *N Engl J Med*, *360*, 1200–1210.
726a. Quaynor SD, Stradtman EW, Jr., Kim HG, et al. (2013) Delayed puberty and estrogen resistance in a woman with estrogen receptor alpha variant. *N Engl J Med*, 369, 164-71.
727. Layman, L. C. (1999). Genetics of human hypogonadotropic hypogonadism. *Am J Med Genet*, *89*, 240–248.
728. Eldar-Geva, T., Hirsch, H. J., Benarroch, F., et al. (2010). Hypogonadism in females with Prader-Willi syndrome from infancy to adulthood: variable combinations of a primary gonadal defect and hypothalamic dysfunction. *Eur J Endocrinol*, *162*, 377–384.
729. Pitteloud, N., Quinton, R., Pearce, S., et al. (2007). Digenic mutations account for variable phenotypes in idiopathic hypogonadotropic hypogonadism. *J Clin Invest*, *117*, 457–463.
730. Cole, L. W., Sidis, Y., Zhang, C., et al. (2008). Mutations in prokineticin 2 and prokineticin receptor 2 genes in human gonadotrophin-releasing hormone deficiency: molecular genetics and clinical spectrum. *J Clin Endocrinol Metab*, *93*, 3551–3559.
731. Jongmans, M. C., van Ravenswaaij-Arts, C. M., Pitteloud, N., et al. (2009). CHD7 mutations in patients initially diagnosed with Kallmann syndrome—the clinical overlap with CHARGE syndrome. *Clin Genet*, *75*, 65–71.
732. Beranova, M., Oliveira, L. M., Bedecarrats, G. Y., et al. (2001). Prevalence, phenotypic spectrum, and modes of inheritance of gonadotropin-releasing hormone receptor mutations in idiopathic hypogonadotropic hypogonadism. *J Clin Endocrinol Metab*, *86*, 1580–1588.
733. de Roux, N., Young, J., Brailley-Tabard, S., et al. (1999). The same molecular defects of the gonadotropin-releasing hormone receptor determine a variable degree of hypogonadism in affected kindred. *J Clin Endocrinol Metab*, *84*, 567–572.
734. Seminara, S. B., Beranova, M., Oliveira, L. M. B., et al. (2000). Successful use of pulsatile gonadotropin-releasing hormone (GnRH) for ovulation induction and pregnancy in a patient with GnRH receptor mutations. *J Clin Endocrinol Metab*, *85*, 556–562.
735. Kelberman, D., Rizzoti, K., Lovell-Badge, R., et al. (2009). Genetic regulation of pituitary gland development in human and mouse. *Endocr Rev*, *30*, 790–829.
736. Prince, K. L., Walvoord, E. C., & Rhodes, S. J. (2011). The role of homeodomain transcription factors in heritable pituitary disease. *Nat Rev Endocrinol*, *7*, 727–737.
737. Romero, C. J., Nesi-Franca, S., & Radovick, S. (2009). The molecular basis of hypopituitarism. *Trends Endocrinol Metab*, *20*, 506–516.
738. Fischer-Posovszky, P., von Schnurbein, J., Moepps, B., et al. (2010). A new missense mutation in the leptin gene causes mild obesity and hypogonadism without affecting T cell responsiveness. *J Clin Endocrinol Metab*, *95*, 2836–2840.
739. Romero, C. J., Pine-Twaddell, E., & Radovick, S. (2011). Novel mutations associated with combined pituitary hormone deficiency. *J Mol Endocrinol*, *46*, R93–R102.
740. Israel, D., & Chua, S., Jr. (2010). Leptin receptor modulation of adiposity and fertility. *Trends Endocrinol Metab*, *21*, 10–16.
741. Nizard, J., Dommergue, M., & Clement, K. (2012). Pregnancy in a woman with a leptin-receptor mutation. *N Engl J Med*, *366*, 1064–1065.
742. Layman, W. S., Hurd, E. A., & Martin, D. M. (2011). Reproductive dysfunction and decreased GnRH neurogenesis in a mouse model of CHARGE syndrome. *Hum Mol Genet*, *20*, 3138–3150.
743. Lomniczi, A., Garcia-Rudaz, C., Ramakrishnan, R., et al. (2012). A single-nucleotide polymorphism in the EAP1 gene is associated with amenorrhea/oligomenorrhea in nonhuman primates. *Endocrinology*, *153*, 339–349.
744. Beate, K., Joseph, N., Nicolas de, R., & Wolfram, K. (2012). Genetics of isolated hypogonadotropic hypogonadism: role of GnRH receptor and other genes. Int J Endocrinol. doi:10.1155/2012/147893
745. Bouligand, J., Ghervan, C., Tello, J. A., et al. (2009). Isolated familial hypogonadotropic hypogonadism and a GNRH1 mutation. *N Engl J Med*, *360*, 2742–2748.
746. Merke, D. P., Tajima, T., Baron, J., & Cutler, G. B., Jr. (1999). Hypogonadotropic hypogonadism in a female caused by an X-linked recessive mutation in the DAX1 gene. *N Engl J Med*, *340*, 1248–1252.
747. de Zegher, F., & Jaeken, J. (1995). Endocrinology of the carbohydrate-deficient glycoprotein syndrome type 1 from birth through adolescence. *Pediatr Res*, *37*, 395.
748. Achard, C., Courtillot, C., Lahuna, O., et al. (2009). Normal spermatogenesis in a man with mutant luteinizing hormone. *N Engl J Med*, *361*, 1856–1863.
749. Barkan, A., Kelch, R., & Marshall, J. (1985). Isolated gonadotrope failure in the polyglandular autoimmune syndrome. *N Engl J Med*, *312*, 1535.
750. Komatsu, M., Kondo, T., Yamauchi, K., et al. (1988). Antipituitary antibodies in patients with the primary empty sella syndrome. *J Clin Endocrinol Metab*, *67*, 633.
751. Hendricks, S. A., Lippe, B. M., Kaplan, S. A., & Bentson, J. R. (1981). Hypothalamic atrophy with progressive hypopituitarism in an adolescent girl. *J Clin Endocrinol Metab*, *52*, 562.
752. Rappaport, R., Brauner, R., Czernichow, P., et al. (1982). Effect of hypothalamic and pituitary irradiation on pubertal development in children with cranial tumors. *J Clin Endocrinol Metab*, *54*, 1164–1168.
753. Rose, S. R., Schreiber, R. E., Kearney, N. S., et al. (2004). Hypothalamic dysfunction after chemotherapy. *J Pediatr Endocrinol Metab*, *17*, 55–66.
754. Van den Berghe, G., de Zegher, F., & Bouillon, R. (1998). Clinical review 95: acute and prolonged critical illness as different neuroendocrine paradigms. *J Clin Endocrinol Metab*, *83*, 1827–1834.
755. Green, D. M., Nolan, V. G., Kawashima, T., et al. (2011). Decreased fertility among female childhood cancer survivors who received 22–27 Gy hypothalamic/pituitary irradiation: a report from the Childhood Cancer Survivor Study. *Fertil Steril*, *95*, 1922–1927, 1927, e1.
756. Molitch, M. (1991). Gonadotroph-cell pituitary adenomas. *N Engl J Med*, *324*, 626–627.
757. Fairburn, C. G., & Harrison, P. J. (2003). Eating disorders. *Lancet*, *361*, 407–416.
758. American Academy of Pediatrics Policy Statement (2003). Identifying and treating eating disorders. *Pediatrics*, *111*, 204–211.
759. Yager, J., & Andersen, A. E. (2005). Clinical practice. Anorexia nervosa. *N Engl J Med*, *353*, 1481–1488.
760. Le Grange, D., Doyle, P. M., Swanson, S. A., et al. (2012). Calculation of expected body weight in adolescents with eating disorders. Pediatrics. doi:10.1542/peds.2011–1676
761. Pugliese, M., Lifshitz, F., Grad, C., et al. (1983). Fear of obesity: a cause of short stature and delayed puberty. *N Engl J Med*, *309*, 513.
762. Golden, N., Kreitzer, P., Jacobson, M., et al. (1994). Disturbances in growth hormone secretion and action in adolescents with anorexia nervosa. *J Pediatr*, *125*, 655–660.

763. Newman, M., & Halmi, K. (1988). The endocrinology of anorexia nervosa and bulimia nervosa. *Endocrinol Metab Clin N Am*, *17*, 195.
764. Frisch, R., & McArthur, J. (1974). Menstrual cycles as a determinant of minimum weight for height necessary for their maintenance or onset. Science, 185, 949.
765. Misra, M., Prabhakaran, R., Miller, K. K., et al. (2006). Role of cortisol in menstrual recovery in adolescent girls with anorexia nervosa. *Pediatr Res*, *59*, 598–603.
766. Boyar, R. M., Katz, J., Finkelstein, J. W., et al. (1974). Anorexia nervosa: immaturity of the 24-hour luteinizing hormone secretory pattern. *N Engl J Med*, *291*, 861.
767. Beumont, P. J., George, G. C., Pimstone, B. L., & Vinik, A. I. (1976). Body weight and the pituitary response to hypothalamic releasing hormones in patients with anorexia nervosa. *J Clin Endocrinol Metab*, *43*, 487.
768. Marshall, J., & Fraser, T. (1971). Amenorrhoea in anorexia nervosa: assessment and treatment with clomiphene citrate. *Br Med J*, *4*, 590.
769. Welt, C. K., Chan, J. L., Bullen, J., et al. (2004). Recombinant human leptin in women with hypothalamic amenorrhea. *N Engl J Med*, *351*, 987–997.
770. Chrousos, G., & Gold, P. (1992). The concepts of stress and stress system disorders: overview of physical and behavioral homeostasis. *JAMA*, *267*, 1244–1252.
771. Winterer, J., Gwirtsman, H. E., George, D. T., et al. (1985). Adrenocorticotropin-stimulated adrenal androgen secretion in anorexia nervosa: impaired secretion at low weight with normalization after long-term weight recovery. *J Clin Endocrinol Metab*, *61*, 693.
772. Mecklenburg, R. S., Loriaux, D. L., Thompson, R. H., et al. (1974). Hypothalamic dysfunction in patients with anorexia nervosa. *Medicine*, *53*, 147.
773. Petrides, J., Mueller, G., Kalogeras, K., et al. (1994). Exercise-induced activation of the hypothalamic-pituitary-adrenal axis: marked differences in the sensitivity to glucocorticoid suppression. *J Clin Endocrinol Metab*, *79*, 377–383.
774. Molitch, M. (1992). Pathologic hyperprolactinemia. *Endocrinol Metab Clin N Am*, *21*, 877–901.
775. Colao, A., Loche, S., Cappa, M., et al. (1998). Prolactinomas in children and adolescents: clinical presentation and long-term follow-up. *J Clin Endocrinol Metab*, *83*, 2777–2780.
776. Blanco-Favela, F., Quintal Ma, G., Chavez-Rueda, A. K., et al. (2001). Anti-prolactin autoantibodies in paediatric systemic lupus erythematosus patients. *Lupus*, *10*, 803–808.
777. Schlechte, J. A. (2002). The macroprolactin problem. *J Clin Endocrinol Metab*, *87*, 5408–5409.
777a. Newey PJ, Gorvin CM, Cleland SJ, et al. (2013) Mutant prolactin receptor and familial hyperprolactinemia. *N Engl J Med*, 369, 2012-20.
778. Klibanski, A. (2010). Clinical practice. Prolactinomas. *N Engl J Med*, *362*, 1219–1226.
779. Mendelson, J., Mello, N., Teoh, S., et al. (1989). Cocaine effects on pulsatile secretion of anterior pituitary, gonadal, and adrenal hormones. *J Clin Endocrinmol Metab*, *69*, 1256.
780. Tallo, D., & Malarkey, W. (1985). Physiologic concentrations of dopamine fail to suppress prolactin secretion in patients with idiopathic hyperprolactinemia or prolactinomas. *Am J Obstet Gynecol*, *151*, 651–655.
781. Sauder, S., Frager, M., Case, C., et al. (1984). Abnormal patterns of pulsatile luteinizing hormone secretion in women with hyperprolactinemia and amenorrhea: responses to bromocriptine. *J Clin Endocrinol Metab*, *59*, 941.
782. Braverman, P. K., & Breech, L. (2010). American Academy of Pediatrics. Clinical report—gynecologic examination for adolescents in the pediatric office setting. *Pediatrics*, *126*, 583–590.
783. Sane, K., & Pescovitz, O. (1992). The clitoral index: a determination of clitoral size in normal girls and in girls with abnormal sexual development. *J Pediatr*, *120*, 264–266.
784. Rosenfield, R. L. (2003). Menstrual disorders and hyperandrogenism in adolescence. In S. Radovick, & M. H. MacGillivray (Eds.), *Pediatric endocrinology: a practical clinical guide* (pp. 451–478). Totowa, NJ: Humana Press.
785. Rebar, R., & Connolly, H. (1990). Clinical features of young women with hypergonadotropic amenorrhea. *Fertil Steril*, *53*, 804–810.
786. Petakov, M. S., Damjanovic, S. S., Nikolic-Durovic, M. M., et al. (1998). Pituitary adenomas secreting large amounts of prolactin may give false low values in immunoradiometric assays: the hook effect. *J Endocrinol Invest*, *21*, 184–188.
787. Kovacs, K., & Horvath, E. (1983). Tumors of the pituitary gland. In W. Hartmann, & L. Sobin (Eds.), *Atlas of tumor pathology* (pp. 205–209). Washington, DC: Armed Forces Institute of Pathology.
788. Schroeder, I., Johnson, J., & Malarkey, W. (1976). Cerebrospinal fluid prolactin: a reflection of abnormal prolactin secretion in patients with pituitary tumors. *J Clin Endocrinol Metab*, *43*, 1255.
789. Germain, N., Galusca, B., Le Roux, C. W., et al. (2007). Constitutional thinness and lean anorexia nervosa display opposite concentrations of peptide YY, glucagon-like peptide 1, ghrelin, and leptin. *Am J Clin Nutr*, *85*, 967–971.
790. Gardner, D. (1993). Pathogenesis of anorexia nervosa. *Lancet*, *341*, 1631–1634.
791. Molitch, M. E., Thorner, M. O., & Wilson, C. (1997). Management of prolactinomas. *J Clin Endocrinol Metab*, *82*, 996–1000.
792. Colao, A., Di Sarno, A., Landi, M. L., et al. (2000). Macroprolactinoma shrinkage during cabergoline treatment is greater in naive patients than in patients pretreated with other dopamine agonists: a prospective study in 110 patients. *J Clin Endocrinol Metab*, *85*, 2247–2252.
793. Melmed, S., Casanueva, F. F., Hoffman, A. R., et al. (2011). Diagnosis and treatment of hyperprolactinemia: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*, *96*, 273–288.
794. Lock, J., le Grange, D., Agras, W. S., & Dare, C. (2001). *Treatment manual for anorexia nervosa*. New York: Guilford.
795. Misra, M., & Klibanski, A. (2006). Anorexia nervosa and osteoporosis. *Rev Endocr Metab Disord*, 7, 91–99.
796. Katz, R., Mazer, C., & Litt, I. (1985). Anorexia nervosa by proxy. *J Pediatr*, *107*, 247.
797. Rothchild, E., & Owens, R. (1972). Adolescent girls who lack functioning ovaries. *J Am Acad Child Psychiatry*, *11*, 88.
798. Bondy, C. A., for the Turner Syndrome Consensus Study Group (2007). Care of girls and women with Turner syndrome: a guideline of the Turner syndrome study group. *J Clin Endocrinol Metab*, *92*, 10–25.
799. Rosenfeld, R. G., Frane, J., Attie, K. M., et al. (1992). Six-year results of a randomized prospective trial of human growth hormone and oxandrolone in Turner syndrome. *J Pediatr*, *121*, 49–55.
800. Rosenfield, R. L., Devine, N., Hunold, J. J., et al. (2005). Salutary effects of combining early very low-dose systemic estradiol with growth hormone therapy in girls with Turner syndrome. *J Clin Endocrinol Metab*, *90*, 6424–6430.
801. Ross, J. L., Quigley, C. A., Cao, D., et al. (2011). Growth hormone plus childhood low-dose estrogen in Turner's syndrome. *N Engl J Med*, *364*, 1230–1242.
802. Rosenfield, R., Kiess, W., & Keizer-Schrama, S. (2006). Physiologic induction of puberty in Turner syndrome with very low-dose estradiol. In C. Gravholt, & C. Bondy (Eds.), *Wellness for girls and women with Turner syndrome* (pp. 71–79). Amsterdam: Elsevier Science.
803. Taboada, M., Santen, R., Lima, J., et al. (2011). Pharmacokinetics and pharmacodynamics of oral and transdermal 17beta estradiol in girls with Turner syndrome. *J Clin Endocrinol Metab*, *96*, 3502–3510.
804. Gordon, C. M., & Pitts, S. A. (2012). Approach to the adolescent requesting contraception. *J Clin Endocrinol Metab*, *97*, 9–15.
805. Zuckerman-Levin, N., Frolova-Bishara, T., Militianu, D., et al. (2009). Androgen replacement therapy in Turner syndrome: a pilot study. *J Clin Endocrinol Metab*, *94*, 4820–4827.
806. Stanhope, R., Pringle, P., Brook, C., et al. (1987). Induction of puberty by pulsatile gonadotropin releasing hormone. *Lancet*, *2*, 552.
807. Sykiotis, G. P., Hoang, X. H., Avbelj, M., et al. (2010). Congenital idiopathic hypogonadotropic hypogonadism: evidence of defects in the hypothalamus, pituitary, and testes. *J Clin Endocrinol Metab*, *95*, 3019–3027.
808. Devroey, P., Camus, M., Palermo, G., et al. (1990). Placental production of estradiol and progesterone after oocyte donation in patients with primary ovarian failure. *Am J Obstet Gynecol*, *162*, 66–70.

808a. Kawamura K, Cheng Y, Suzuki N, et al. (2013) Hippo signaling disruption and Akt stimulation of ovarian follicles for infertility treatment. *Proc Natl Acad Sci USA*, 110, 17474-9.
809. Jeruss, J. S., & Woodruff, T. K. (2009). Preservation of fertility in patients with cancer. *N Engl J Med*, *360*, 902–911.
810. Krotz, S. P., Robins, J. C., Ferruccio, T. M., et al. (2010). In vitro maturation of oocytes via the pre-fabricated self-assembled artificial human ovary. *J Assist Reprod Genet*, *27*, 743–750.
811. Woodruff, T. K. (2010). The Oncofertility Consortium—addressing fertility in young people with cancer. *Nat Rev Clin Oncol*, *7*, 466–475.
812. Perkins, R. B., Hall, J. E., & Martin, K. A. (1999). Neuroendocrine abnormalities in hypothalamic amenorrhea: spectrum, stability, and response to neurotransmitter modulation. *J Clin Endocrinol Metab*, *84*, 1905–1911.
813. Shaw, R. (1979). Differential response to LHRH following oestrogen therapy in women with amenorrhoea. *Br Obstet Gynaecol*, *86*, 69–75.
814. Weiss, C., Nachtigall, L., & Ganguly, M. (1976). Induction of an LH surge with estradiol benzoate. *Obstet Gynecol*, *47*, 415–418.
815. Misra, M., Miller, K. K., Kuo, K., et al. (2005). Secretory dynamics of ghrelin in adolescent girls with anorexia nervosa and healthy adolescents. *Am J Physiol Endocrinol Metab*, *289*, E347–E356.
816. Lado-Abeal, J., Rodriguez-Arnao, J., Newell-Price, J. D. C., et al. (1998). Menstrual abnormalities in women with Cushing's disease are correlated with hypercortisolemia rather than raised circulating androgen levels. *J Clin Endocrinol Metab*, *83*, 3083–3088.
817. Constantini, N., & Warren, M. (1995). Menstrual dysfunction in swimmers: a distinct entity. *J Clin Endocrinol Metab*, *80*, 2740.
818. Centeno, M. L., Sanchez, R. L., Cameron, J. L., & Bethea, C. L. (2007). Hypothalamic expression of serotonin 1A, 2A and 2C receptor and GAD67 mRNA in female cynomolgus monkeys with different sensitivity to stress. *Brain Res*, *1142*, 1–12.
819. Tofler, I. R., Stryer, B. K., Micheli, L. J., & Herman, L. R. (1996). Physical and emotional problems of elite female gymnasts. *N Engl J Med*, *335*, 281–283.
820. Warren, M. (1992). Amenorrhea in endurance runners. *J Clin Endocrinol Metab*, *75*, 1393–1397.
821. Drinkwater, B., Bruemner, B., & Chestnut, C. I. (1990). Menstrual history as a determinant of current bone density in young athletes. *JAMA*, *263*, 545–548.
822. Frisch, R., Snow, R., Johnson, L., et al. (1993). Magnetic resonance imaging of overall and regional body fat, estrogen metabolism, and ovulation of athletes compared to controls. *J Clin Endocrinol Metab*, *77*, 471–477.
823. Weltman, E., Stern, R., Doershuk, C., et al. (1990). Weight and menstrual function in patients with eating disorders and cystic fibrosis. *Pediatr*, *85*, 282–287.
824. Frisch, R., Wyshak, C., & Vincent, I. (1980). Delayed menarche and amenorrhea in ballet dancers. *N Engl J Med*, *303*, 17.
825. Rakoff, A. (1962). Psychogenic factors in anovulatory women. *Fertil Steril*, *13*, 1.
826. Herzog, A. G. (2006). Menstrual disorders in women with epilepsy. Neurology, 66, S23–S28.
827. Yen, S. S. C. (1978). Chronic anovulation due to CNS-hypothalamic-pituitary dysfunction. In Y. Yen, & R. Jaffe (Eds.), *Reproductive endocrinology*. Philadelphia: WB Saunders Company.
828. Carson, S., & Buster, J. (1993). Ectopic pregnancy. *New Engl J Med*, *16*, 1174.
829. Rochester, D., Jain, A., Polotsky, A. J., et al. (2009). Partial recovery of luteal function after bariatric surgery in obese women. *Fertil Steril*, *92*, 1410–1415.
830. Netzer, N. C., Eliasson, A. H., & Strohl, K. P. (2003). Women with sleep apnea have lower levels of sex hormones. *Sleep Breath*, *7*, 25–29.
831. Maruo, T., Nayashi, M., Matsuo, H., et al. (1987). The role of thyroid hormone as a biological amplifier of the actions of follicle-stimulating hormone in the functional differentiation of cultured porcine granulosa cells. *Endocrinology*, *121*, 1233.
832. Boyar, R. M., Hellman, L. D., Roffwarg, H., et al. (1977). Cortisol secretion and metabolism in anorexia nervosa. *N Engl J Med*, *296*, 190–193.
833. Winters, S. J., & Berga, S. L. (1997). Gonadal dysfunction in patients with thyroid disorders. *Endocrinologist*, *7*, 167–173.
834. Asch, R. H., Smith, C. G., Siler-Khodr, T. M., & Pauerstein, C. J. (1981). Effects of Δ5-tetrahydrocannabinol during the follicular phase of the rhesus monkey (*Mocaca mulatto*). *J Clin Endocrinol Metab*, *52*, 50–55.
835. Dees, W. L., Dissen, G. A., Hiney, J. K., et al. (2000). Alcohol ingestion inhibits the increased secretion of puberty-related hormones in the developing female rhesus monkey. *Endocrinology*, *141*, 1325–1331.
836. Chen, E. C., Samuels, M. H., Luther, M. F., et al. (1998). Cocaine impairs follicular phase pulsatile gonadotropin secretion in rhesus monkeys. *J Soc Gynecol Investig*, *5*, 311–316.
837. Battaglia, D. F., Beaver, A. B., Harris, T. G., et al. (1999). Endotoxin disrupts the estradiol-induced luteinizing hormone surge: interference with estradiol signal reading, not surge release. *Endocrinology*, *140*, 2471–2479.
838. Oerter, K., Kampf, G., Munson, P., et al. (1993). Multiple hormone deficiencies in children with hemochromatosis. *J Clin Endocrinol Metab*, *76*, 357–361.
839. South, S., Asplin, C., Carlsen, E., et al. (1993). Alterations in luteinizing hormone secretory activity in women with insulin-dependent diabetes mellitus and secondary amenorrhea. *J Clin Endocrinol Metab*, *76*, 1048–1053.
840. Lim, V., Kathpalia, S., & Henriquez, C. (1978). Endocrine abnormalities associated with chronic renal failure. *Med Clin N Am*, *62*, 1341.
841. Boyar, R. M., Kapen, S., Weitzman, E. D., & Hellman, L. (1976). Pituitary microadenoma and hyperprolactinemia. *N Engl J Med*, *294*, 263.
842. Shearman, R. (1974). Secondary amenorrhoea after oral contraceptives—treatment and follow-up. *Contraception*, *11*, 123.
843. van der Steeg, N., & Bennink, H. (1977). Bromocriptine for induction of ovulation in normoprolactinaemic post-pill anovulation. *Lancet*, *1*, 502.
844. Ortiz, A., Hirol, M., Stanczyk, F. Z., et al. (1977). Serum medroxyprogesterone acetate (MPA) concentrations and ovarian function following intramuscular injection of depo-MPA. *J Clin Endocrinol Metab*, *44*, 32.
845. Bolognese, R., Piver, S., & Feldman, J. (1967). Galactorrhea and abnormal menses associated with a long-acting progesterone. *JAMA*, *199*, 100.
846. Hirshberg, B., Conn, P. M., Uwaifo, G. I., et al. (2003). Ectopic luteinizing hormone secretion and anovulation. *N Engl J Med*, *348*, 312–317.
847. Valimaki, M. J., Tiitinen, A., Alfthan, H., et al. (1999). Ovarian hyperstimulation caused by gonadotroph adenoma secreting follicle-stimulating hormone in 28-year-old woman. *J Clin Endocrinol Metab*, *84*, 4204–4208.
848. Piaditis, G., Angellou, A., Kontogeorgos, G., et al. (2005). Ectopic bioactive luteinizing hormone secretion by a pancreatic endocrine tumor, manifested as luteinized granulosa-thecal cell tumor of the ovaries. *J Clin Endocrinol Metab*, *90*, 2097–2103.
849. Zimmer, C. A., Ehrmann, D. A., & Rosenfield, R. L. (2010). Potential diagnostic utility of intermittent short-acting GnRH agonist administration in gonadotropin deficiency. *Fertil Steril*, *94*, 2697–2702.
850. Soules, M. R., McLachlan, R. I., Ek, M., et al. (1989). Luteal phase deficiency: characterization of reproductive hormones over the menstrual cycle. *J Clin Endocrinol Metab*, *69*, 804–812.
851. Daya, S. (1989). Optimal time in the menstrual cycle for serum progesterone measurement to diagnose luteal phase defects. *Am J Obstet Gynecol*, *161*, 1009–1011.
852. Hinney, B., Henze, C., Kuhn, W., & Wuttke, W. (1996). The corpus luteum insufficiency: a multifactorial disease. *J Clin Endocrinol Metab*, *81*, 565–570.
853. Seppala, M., Nirvonen, E., & Ranta, T. (1976). Hyperprolactinaemia and luteal insufficiency. *Lancet*, *1*, 229.
854. Wathen, P. I., Henderson, M. C., & Witz, C. A. (1995). Abnormal uterine bleeding. *Med Clin North Am*, *79*, 329–344.
855. Carlson, K. J., & Schiff, I. (1996). Alternatives to hysterectomy for menorrhagia. *New Engl J Med*, *335*, 198–199.
856. Kennedy, S. (1997). Primary dysmenorrhoea. *Lancet*, *349*, 1116.
857. Ammeman, S., Shafer, M.-A., Snyder, D. (1990). Ectopic pregnancy in adolescents: a clinical review for pediatricians. *J Pediatr*, *117*, 677–684.

858. Bachmann, G. A., Rosen, R., Pinn, V. W., et al. (2006). Vulvodynia: a state-of-the-art consensus on definitions, diagnosis and management. *J Reprod Med*, *51*, 447–456.
859. Olive, D. L. (2008). Gonadotropin-releasing hormone agonists for endometriosis. *N Engl J Med*, *359*, 1136–1142.
860. Bulun, S. E. (2009). Endometriosis. *N Engl J Med*, *360*, 268–279.
861. Rosenwaks, Z., Jones, G. S., Henzl, M. R., et al. (1981). Naproxen sodium, aspirin, and placebo in primary dysmenorrhea. *Am J Obstet Gynecol*, *140*, 592.
862. DeVane, G. (1991). Editorial: premenstrual syndrome. *J Clin Endocrinol Metab*, *72*, 250.
863. Grady-Weliky, T. A. (2003). Clinical practice. Premenstrual dysphoric disorder. *N Engl J Med*, *348*, 433–438.
864. Schachter, S. C. (1988). Hormonal considerations in women with seizures. *Arch Neurol*, *45*, 1267–1270.
865. Dalton, K. (1980). Cyclical criminal acts in premenstrual syndrome. *Lancet*, *2*, 1070.
866. Schmidt, P. J., Nieman, L. K., Danaceau, M. A., et al. (1998). Differential behavioral effects of gonadal steroids in women with and in those without premenstrual syndrome [see comments]. *N Engl J Med*, *338*, 209–216.
867. Roca, C. A., Schmidt, P. J., Altemus, M., et al. (2003). Differential menstrual cycle regulation of hypothalamic-pituitary-adrenal axis in women with premenstrual syndrome and controls. *J Clin Endocrinol Metab*, *88*, 3057–3063.
868. Wang, M., Seippel, L., Purdy, R. H., & Backstrom, T. (1996). Relationship between symptom severity and steroid variation in women with premenstrual syndrome: study on serum pregnenolone, pregnenolone sulfate, 5 alpha-pregnane-3,20-dione and 3 alpha-hydroxy-5 alpha-pregnan-20-one. *J Clin Endocrinol Metab*, *81*, 1076–1082.
869. Rutanen, E. M., Teppo, A. M., Stenman, U. H., et al. (1993). Recurrent fever associated with progesterone action and persistently elevated serum levels of immunoreactive tumor necrosis factor-a and interleukin-6. *J Clin Endocrinol Metab*, *76*, 1594–1598.
870. Cannon, J. G., Angel, J. B., Abad, L. W., et al. (1997). Interleukin-1 beta, interleukin-1 receptor antagonist, and soluble interleukin-1 receptor type II secretion in chronic fatigue syndrome. *J Clin Immunol*, *17*, 253–261.
871. Buggs, C., & Rosenfield, R. L. (2005). Polycystic ovary syndrome in adolescence. *Endocrinol Metab Clin North Am*, *34*, 677–705.
872. Rosenfield, R. L. (2012). Polycystic ovary syndrome in adolescents. In B. D. Rose (Ed.), *UpToDate*. Retrieved from www.uptodate.com/index
873. Stein, I. F., & Leventhal, M. L. (1935). Amenorrhea associated with bilateral polycystic ovaries. *Am J Obstet Gynecol*, *29*, 181–191.
874. Goldzieher, M. W., & Green, J. A. (1962). The polycystic ovary. I. Clinical and histologic features. *J Clin Endocrinol Metab*, *22*, 325–338.
875. Hughesdon, P. E. (1982). Morphology and morphogenesis of the Stein-Leventhal ovary and of so-called "hyperthecosis." *Obstet Gynecol Surv*, *37*, 59–77.
876. Zawadzki, J., & Dunaif, A. (1992). Diagnostic criteria for polycystic ovary syndrome: towards a rational approach. In A. Dunaif, J. Givens, F. Haseltine, & G. Merriam (Eds.), *Polycystic ovary syndrome*, (Vol. 4) (pp. 377–384). Cambridge, MA: Blackwell Scientific.
877. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group (2004). Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil Steril*, *81*, 19–25.
878. Azziz, R., Carmina, E., Dewailly, D., et al. (2009). The Androgen Excess and PCOS Society criteria for the polycystic ovary syndrome: the complete task force report. *Fertil Steril*, *91*, 456–488.
879. Johnson, T., Kaplan, L., Ouyang, P., & Rizza, R. (2013). National Institutes of Health evidence-based methodology workshop on polycystic ovary syndrome (PCOS). *NIH EbMW Report* (Vol. 1) (pp. 1–14). Bethesda, MD: National Institutes of Health. Retrieved from http://prevention.nih.gov/workshops/2012/pcos/default.aspx
880. Barber, T. M., Wass, J. A., McCarthy, M. I., & Franks, S. (2007). Metabolic characteristics of women with polycystic ovaries and oligo-amenorrhoea but normal androgen levels: implications for the management of polycystic ovary syndrome. *Clin Endocrinol* (Oxf), *66*, 513–517.
881. Guastella, E., Longo, R. A., & Carmina, E. (2010). Clinical and endocrine characteristics of the main polycystic ovary syndrome phenotypes. *Fertil Steril*, *94*, 2197–2201.
882. Legro, R. S., Arslanian, S. A., Ehrmann, D. A., et al. (2013). Diagnosis and treatment of polycystic ovary syndrome: an Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab*, *98*, 4565–4592.
883. Fauser, B. C., Tarlatzis, B. C., Rebar, R. W., et al. (2012). Consensus on women's health aspects of polycystic ovary syndrome (PCOS): the Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. *Fertil Steril*, *97*, 28–38 e25.
884. Suikkari A-M, MacLachlan, V., Montalto, J., et al. (1995). Ultrasonographic appearance of polycystic ovaries is associated with exaggerated ovarian androgen and oestradiol responses to gonadotropin-releasing hormone agonist in women undergoing assisted reproduction treatment. *Hum Reprod*, *10*, 513–519.
885. Okon, M. A., Laird, S. M., Tuckerman, E. M., & Li, T. C. (1998). Serum androgen levels in women who have recurrent miscarriages and their correlation with markers of endometrial function. *Fertil Steril*, *69*, 682–690.
886. Kirchengast, S., & Huber, J. (2001). Body composition characteristics and body fat distribution in lean women with polycystic ovary syndrome. *Hum Reprod*, *16*, 1255–1260.
887. Manneras-Holm, L., Leonhardt, H., Kullberg, J., et al. (2011). Adipose tissue has aberrant morphology and function in PCOS: enlarged adipocytes and low serum adiponectin, but not circulating sex steroids, are strongly associated with insulin resistance. *J Clin Endocrinol Metab*, *96*, E304–E311.
888. Rosenfield, R. L., Mortensen, M., Wroblewski, K., et al. (2011). Determination of the source of androgen excess in functionally atypical polycystic ovary syndrome by a short dexamethasone androgen-suppression test and a low-dose ACTH test. *Hum Reprod*, *26*, 3138–146.
889. Rosenfield, R. L., Wroblewski, K., Padmanabhan, V., et al. (2012). Antimüllerian hormone levels are independently related to ovarian hyperandrogenism and polycystic ovaries. *Fertil Steril*, *98*, 242–249.
890. Wang, J. G., & Lobo, R. A. (2008). The complex relationship between hypothalamic amenorrhea and polycystic ovary syndrome. *J Clin Endocrinol Metab*, *93*, 1394–1397.
891. Jonard, S., & Dewailly, D. (2004). The follicular excess in polycystic ovaries, due to intra-ovarian hyperandrogenism, may be the main culprit for the follicular arrest. *Hum Reprod Update*, *10*, 107–117.
892. Yen, S. (1980). The polycystic ovary syndrome. *Clin Endocrinol*, *12*, 177–208.
893. DeUgarte, C. M., Bartolucci, A. A., & Azziz, R. (2005). Prevalence of insulin resistance in the polycystic ovary syndrome using the homeostasis model assessment. *Fertil Steril*, *83*, 1454–1460.
894. Lewy, V. D., Danadian, K., Witchel, S. F., & Arslanian, S. (2001). Early metabolic abnormalities in adolescent girls with polycystic ovarian syndrome. *J Pediatr*, *138*, 38–44.
895. Ehrmann, D. A., Barnes, R. B., Rosenfield, R. L., et al. (1999). Prevalence of impaired glucose tolerance and diabetes in women with polycystic ovary syndrome. *Diabetes Care*, *22*, 141–146.
896. Palmert, M. R., Gordon, C. M., Kartashov, A. I., et al. (2002). Screening for abnormal glucose tolerance in adolescents with polycystic ovary syndrome. *J Clin Endocrinol Metab*, *87*, 1017–1023.
897. Leibel, N. I., Baumann, E. E., Kocherginsky, M., & Rosenfield, R. L. (2006). Relationship of adolescent polycystic ovary syndrome to parental metabolic syndrome. *J Clin Endocrinol Metab*, *91*, 1275–1283.
898. Nandalike, K., Strauss, T., Agarwal, C., et al. (2011). Screening for sleep-disordered breathing and excessive daytime sleepiness in adolescent girls with polycystic ovarian syndrome. *J Pediatr*, *159*, 591–596.
899. Hirshfeld-Cytron, J., Barnes, R. B., Ehrmann, D. A., et al. (2009). Characterization of functionally typical and atypical types of polycystic ovary syndrome. *J Clin Endocrinol Metab*, *94*, 1587–1594.
900. Corbould, A., & Dunaif, A. (2007). The adipose cell lineage is not intrinsically insulin resistant in polycystic ovary syndrome. *Metabolism*, *56*, 716–722.

901. Ciaraldi, T. P., Aroda, V., Mudaliar, S., et al. (2009). Polycystic ovary syndrome is associated with tissue-specific differences in insulin resistance. *J Clin Endocrinol Metab*, *94*, 157–163.
902. Du, X., Rosenfield, R. L., & Qin, K. (2009). KLF15 is a transcriptional regulator of the human 17β-hydroxysteroid dehydrogenase type 5 gene: a potential link between regulation of testosterone production and fat stores in women. *J Clin Endocrinol Metab*, *94*, 2594–2601.
903. Franks, S., Webber, L. J., Goh, M., et al. (2008). Ovarian morphology is a marker of heritable biochemical traits in sisters with polycystic ovaries. *J Clin Endocrinol Metab*, *93*, 3396–3402.
904. Rosenfield, R. L. (1997). Current concepts of polycystic ovary syndrome. *Baillieres Clin Obstet Gynaecol*, *11*, 307–333.
905. Barnes, R. B., Rosenfield, R. L., Ehrmann, D. A., et al. (1994). Ovarian hyperandrogenism as a result of congenital adrenal virilizing disorders: evidence for perinatal masculinization of neuroendocrine function in women. *J Clin Endocrinol Metab*, *79*, 1328–1333.
906. Barnes, R. B., Rosenfield, R. L., Burstein, S., & Ehrmann, D. A. (1989). Pituitary-ovarian responses to nafarelin testing in the polycystic ovary syndrome. *N Engl J Med*, *320*, 559–565.
907. Conte, F., Grumbach, M., Ito, Y., et al. (1994). A syndrome of female pseudohermaphroditism, hypergonadotropic hypogonadism, and multicystic ovaries associated with missense mutations in the gene encoding aromatase (P450arom). *J Clin Endocrinol Metab*, *78*, 1287–1292.
908. Toscano, V., Balducc IR, Bianchi, P., et al. (1990). Ovarian 17-ketosteroid reductase deficiency as a possible cause of polycystic ovarian disease. *J Clin Endocrinol Metab*, *71*, 288–292.
909. Kanova, N., & Bicikova, M. (2011). Hyperandrogenic states in pregnancy. *Physiol Res*, *60*, 243–252.
910. Meldrum, D. R., Frumar, A. M., Shamonki, I. M., et al. (1980). Ovarian and adrenal steroidogenesis in a virilized patient with gonadotropin-resistant ovaries and hilus cell hyperplasia. *Obstet Gynecol*, *56*, 216–221.
911. Carbunaru, G., Prasad, P., Scoccia, B., et al. (2004). The hormonal phenotype of Nonclassic 3 beta-hydroxysteroid dehydrogenase (HSD3B) deficiency in hyperandrogenic females is associated with insulin-resistant polycystic ovary syndrome and is not a variant of inherited HSD3B2 deficiency. *J Clin Endocrinol Metab*, *89*, 783–794.
912. Charmandari, E., Kino, T., Ichijo, T., & Chrousos, G. P. (2008). Generalized glucocorticoid resistance: clinical aspects, molecular mechanisms, and implications of a rare genetic disorder. *J Clin Endocrinol Metab*, *93*, 1563–1572.
913. Qin, K., & Rosenfield, R. L. (2011). Mutations of the hexose-6-phosphate dehydrogenase gene rarely cause hyperandrogenemic polycystic ovary syndrome. *Steroids*, *76*, 135–139.
914. Tanaka, Y. O., Tsunoda, H., Kitagawa, Y., et al. (2004). Functioning ovarian tumors: direct and indirect findings at MR imaging. *Radiographics*, *24*(Suppl. 1), S147–S166.
915. Rosenfield, R. L., Cohen, R. M., & Talerman, A. (1987). Lipid cell tumor of the ovary in reference to adult-onset congenital adrenal hyperplasia and polycystic ovary syndrome. *J Reprod Med*, *32*, 363–369.
916. Mandel, F. P., Voet, R. L., Weiland, A. J., & Judd, H. L. (1981). Steroid secretion by masculinizing and "feminizing" hilus cell tumors. *J Clin Endocrinol Metab*, *52*, 779–784.
917. Givens, J. R., Andersen, R. N., Wiser, W. L., et al. (1975). A testosterone-secreting, gonadotropin-responsive pure thecoma and polycystic ovarian disease. *J Clin Endocrinol Metab*, *41*, 845–853.
918. Dunaif, A., Scully, R. E., Andersen, R. N., et al. (1984). The effects of continuous androgen secretion on the hypothalamic-pituitary axis in woman: evidence from a luteinized thecoma of the ovary. *J Clin Endocrinol Metab*, *59*, 389–393.
919. Fragoso, M. C., Latronico, A. C., Carvalho, F. M., et al. (1998). Activating mutation of the stimulatory G protein (gsp) as a putative cause of ovarian and testicular human stromal Leydig cell tumors. *J Clin Endocrinol Metab*, *83*, 2074–2078.
920. Klotz, R. K., Muller-Holzner, E., Fessler, S., et al. (2010). Leydig-cell-tumor of the ovary that responded to GnRH-analogue administration: case report and review of the literature. Exp Clin Endocrinol Diabetes, *118*, 291–297.
921. Basaria, S. (2010). Androgen abuse in athletes: detection and consequences. *J Clin Endocrinol Metab*, *95*, 1533–1543.
922. Escobar-Morreale, H. F., Carmina, E., Dewailly, D., et al. (2012). Epidemiology, diagnosis and management of hirsutism: a consensus statement by the Androgen Excess and Polycystic Ovary Syndrome Society. *Hum Reprod Update*, *18*, 146–170.
923. Rosenfield, R. L. (2005). Clinical practice. Hirsutism. *N Engl J Med*, *353*, 2578–2588. Comment in *N Engl J Med*, *354*, 1533–1535; author reply 1533–1535
924. Dokras, A. (2011). Mood and anxiety disorders in women with PCOS. *Steroids*, *77*, 338–341.
925. Gambineri, A., Patton, L., Vaccina, A., et al. (2006). Treatment with flutamide, metformin, and their combination added to a hypocaloric diet in overweight-obese women with polycystic ovary syndrome: a randomized, 12-month, placebo-controlled study. *J Clin Endocrinol Metab*, *91*, 3970–3980.
926. Geller, D. H., Pacaud, D., Gordon, C. M., & Misra, M. (2011). The use of insulin sensitizers in the treatment of adolescents with polycystic ovary syndrome (PCOS). *Int J Pediatr Endocrinol*. doi:10.1186/1687-9856-2011-9
927. Legro, R. S., Dodson, W. C., Gnatuk, C. L., et al. (2012). Effects of gastric bypass surgery on female reproductive function. *J Clin Endocrinol Metab*, *97*, 4540–4548.
928. Tang, T., Glanville, J., Hayden, C. J., et al. (2006). Combined lifestyle modification and metformin in obese patients with polycystic ovary syndrome: a randomized, placebo-controlled, double-blind multicentre study. *Hum Reprod*, *21*, 80–89.
929. Tang, T., Lord, J. M., Norman, R. J., et al. (2012). Insulin-sensitising drugs (metformin, rosiglitazone, pioglitazone, D-chiro-inositol) for women with polycystic ovary syndrome, oligo amenorrhoea and subfertility. *Cochrane Database Syst Rev*, *5*, CD003053.
930. Hoeger K, Davidson K, Kochman L, et al. (2008) The impact of metformin, oral contraceptives, and lifestyle modification on polycystic ovary syndrome in obese adolescent women in two randomized, placebo-controlled clinical trials. *J Clin Endocrinol Metab*, 93, 4299-306

QUESTIONS

1. Which statement is true about the control of GnRH secretion in the hypothalamus:
   a. Secreted factors from the gonads are necessary for the prepubertal restraint of the GnRH pulse generator.
   b. Prior to the onset of puberty, there is a decrease in the expression of GPR54.
   c. Leptin serves as a signal of sufficient nutritional stores with a threshold level necessary for activation of the GnRH pulse generator at the onset of puberty.
   d. Prepubertal secretion of kisspeptin suppresses GnRH production through activation of its receptor, GPR54.

Answer: c

2. Which statement is false:
   a. Multiple isoforms of LH are produced in the pituitary gland; these isoforms differ in bioactivity, with androgens and estrogens affecting the relative expression of these isoforms.
   b. Prolactin secretion is stimulated by estrogen and TRH, and is inhibited by thyroxine, dopamine and prolactin release-inhibiting factor.
   c. Activins and inhibins are different splice variants of a single gene
   d. Gonadotropin-releasing hormone and prolactin release-inhibiting hormone are unique cleavage products of a common precursor protein.

Answer: c

3. Which statement about sex steroid receptor signaling is false:
   a. There are two estrogen receptors (α and β) that are encoded by separate genes
   b. Estrogen can rapidly stimulate intracellular phosphorylation pathways through binding to membrane-associated estrogen receptors.
   c. The A and B forms of the androgen receptor are produced through differing post-translational modifications
   d. Selective estrogen receptor modulators (SERMs; e.g. tamoxifen and raloxifene) are estradiol agonists in some tissues, but are anti-estrogenic in other tissues, effects predominantly related to their relative affinity for the α versus β isoforms of the estrogen receptor.
   e. The genomic response to estrogen varies depending on the relative expression of co-activators and co-repressors within target cells

Answer: d

4. A 5-year-old girl presents to you after an episode of vaginal bleeding. Her height is at the 75th percentile for age, her weight is at the 90th percentile for age; Her mid-parental height is at the 50th percentile. Her examination was unremarkable except for Tanner II breast development without pubic hair development. Laboratory studies show an LH < 0.2 U/L, FSH < 0.2 U/L, estradiol 115 pg/ml. The best next step in evaluating this child would be:
   a. Brain MRI
   b. Pelvic ultrasound
   c. Exploratory laparotomy
   d. GnRH agonist stimulation test

Answer: b

5. Which statement is true about LH secretion:
   a. In the normal child, there is no LH secretion after infancy until the onset of puberty
   b. In early puberty, LH secretion is pulsatile, which becomes continuous once menstrual cycles are established.
   c. In early puberty, the majority of LH is secreted during the night
   d. An LH level less than 0.5 U/L is sufficient to exclude a diagnosis of central precocious puberty

Answer: c

CHAPTER 16

Turner Syndrome

Paul Saenger, MD • Carolyn A. Bondy, MD

CHAPTER OUTLINE

HISTORICAL BACKGROUND

Turner syndrome is a chromosomal disorder due to complete or partial monosomy for the X chromosome, associated with short stature and primary ovarian failure in phenotypic females. The eponym derives from a study published in 1938 by Henry Turner describing seven women with short stature, sexual immaturity, neck webbing, low posterior hairline, and cubitus valgus[1](Figure 16-1). Several years earlier, Otto Ullrich had described an 8-year-old girl with short stature, lymphedema of the hands and feet, neck webbing, high arched palate, low-set auricles, and several other features now associated with Turner syndrome.[2] Ullrich later recognized that his patient and those of Turner appeared to have the same condition[3] and called attention to the work of Bonnevie, who described cervical distention and malformations of the ears, face, and limb buds in mice secondary to dissection of subcutaneous fetal tissues by fluid. Ullrich suggested that fetal lymphatic obstruction may cause neck webbing and other superficial features of Turner syndrome and proposed the eponym *Bonnevie-Ullrich* to describe this constellation of anomalies. Ullrich's contributions gave rise to the eponym *Ullrich-Turner* syndrome sometimes used in Europe.

Endocrine and pathology studies of the 1940s revealed primary ovarian failure in women with Turner syndrome, associated with elevated gonadotropins, reduced estrogen and "streak" ovaries consisting of connective tissue depleted of germ cells. These early studies also discovered an extraordinary incidence of hypertension and aortic disease in young women with Turner syndrome.[4] The first link between Turner syndrome and sex chromosome anomaly was provided in 1954 by Polani and colleagues, who reported three patients with Turner syndrome and coarctation of the aorta who were sex chromatin negative.[5] Soon thereafter advances in cytogenetic identification of specific chromosomes revealed that Turner syndrome was associated with the presence of a single X chromosome (X monosomy).[6] These observations were paradigm shifting in our understanding of the role of the human sex chromosomes in sex determination, as reviewed by Opitz and Pallister.[7] They also described the significant heterogeneity of patients grouped under the concept of gonadal dysgenesis and pointed out that the terms *dysgenesis* and *agenesis* are inaccurate, as fetal ovarian development seems to be normal in Turner syndrome, with degeneration occurring in most cases around the time of birth. Although eponyms have their disadvantages, the designation *Turner* or *Ullrich-Turner syndrome* is more specific than *gonadal dysgenesis.*

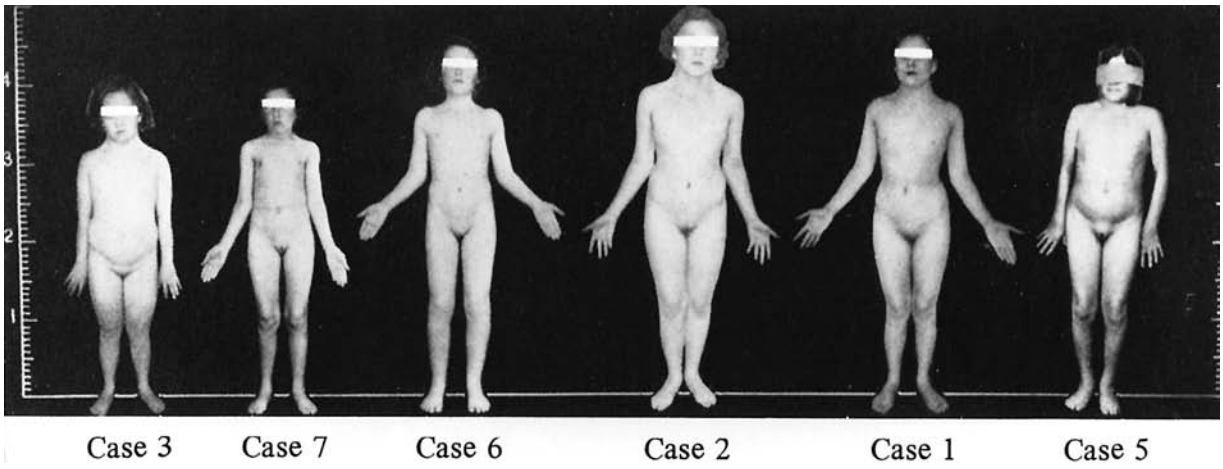


FIGURE 16-1 ■ Patients described by Dr. Henry Turner. Note the height marker at the left indicating the short stature, although with large variation in absolute height among these women. Note also lack of obesity among these women evaluated in the 1930s. (From Turner, H. H. (1938). A syndrome of infantilism, congenital webbed neck and cubitus valgus. *Endocrinology, 23,* 566.)

GENETICS

Chromosomal Origins

Refinements in cytogenetic methodology over the last half of the 20th century promoted the elucidation of the chromosomal basis for Turner syndrome and other aneuploidies. The standard cytogenetic karyotype describes the number and morphologic features of condensed metaphase chromosomes under light microscopy (Figure 16-2A). Karyotype studies of the products of conception and newborns have shown that X monosomy is the only chromosomal monosomy compatible with life, as monosomy for the Y chromosome or autosomes have never been reported.[8] This distinction among chromosomes is explained by the fact that the Y chromosome has relatively few essential genes aside from those involved in male sex determination and spermatogenesis, and the second X chromosome in females is largely inactivated, or silenced early in development. There are, however, a number of genes that escape X inactivation and that have homologs represented on the Y chromosome.[9] Haploinsufficiency for these genes results in short stature and other aspects of the Turner phenotype (discussed later).

The loss or fragmentation of a sex chromosome is most commonly traced to error(s) of recombination and segregation occurring during meiotic divisions.[8] The molecular triggers for such errors are poorly understood but appear to differ for autosomes and sex chromosomes.[10] Thus, in contrast to trisomy 21, Turner syndrome is not preferentially linked to maternal meiotic errors or age and may be more commonly associated with paternal meiotic errors.[11] A less common cause of Turner syndrome is the loss of a sex chromosome due nondisjunction in early embryonic mitotic cell divisions, resulting in a 45,X cell line together with cell lines with normal or supernumerary sex chromosome complement (e.g., 45,X/47,XXX; 45,X/46,XX; 45,X/46,XY).

Epidemiology

Cytogenetic studies found that X chromosome monosomy (45,X) was present in approximately 1/300 spontaneous abortions versus 1/5000 live births,[2-13] indicating that most 45,X gestations do not survive to birth. The ratio of 46,X,i(X)q and 46,X,r(X) to 45,X cases increases from early gestation to birth, leading to the view that X monosomy is incompatible with survival and that surviving 45,X girls started out as 46,X, fragmentary X or Y gestations.[13] It was hypothesized that fragmentary sex chromosomes are lost due to mitotic instability during the course of fetal development, so the girls appear to be 45,X after birth.[13,14] Early in the study of aneuploidy it was thought that monosomy per se would interfere with normal cell proliferation and differentiation.[15] However, it has since been learned that mice with pure X monosomy have normal survival, development, and fertility[16] and that human 45,X cells are able to proliferate and differentiate into various cell types in vitro.[17,18] Thus, it seems that X monosomy need not always be lethal, and the survival and relatively healthy development of some 45,X girls may be related to variations in autosomal genes that compensate for the X chromosome haploinsufficiency. Newborn cytogenetic screening during the 1970s and 1980s found that approximately 1:2500 liveborn females had complete or partial X monosomy consistent with Turner syndrome.[12,13,19] Danish health registry data indicate, however, that only about half the number of cases predicted by the birth incidence receive a clinical diagnosis.[20] This discrepancy may be due to a relatively mild phenotype in some cases. The birth rate for Turner syndrome is going down in some countries,[21] associated with the increasing use of fetal ultrasound screening.[22]

Turner syndrome is reported in all races and countries with similar prevalence. X monosomy does not run in families and is not associated with any known environmental or behavioral factors.[23] There is one known case of an apparently pure 45,X woman with spontaneous puberty and pregnancy giving birth to a 45,X daughter.[24] Epidemiologic observations including more than 1000 girls with Turner and their families suggest that increased parental age modestly increases the risk of having a child with Turner syndrome.[23,25] The single normal X chromosome is identified as maternal in approximately 70%

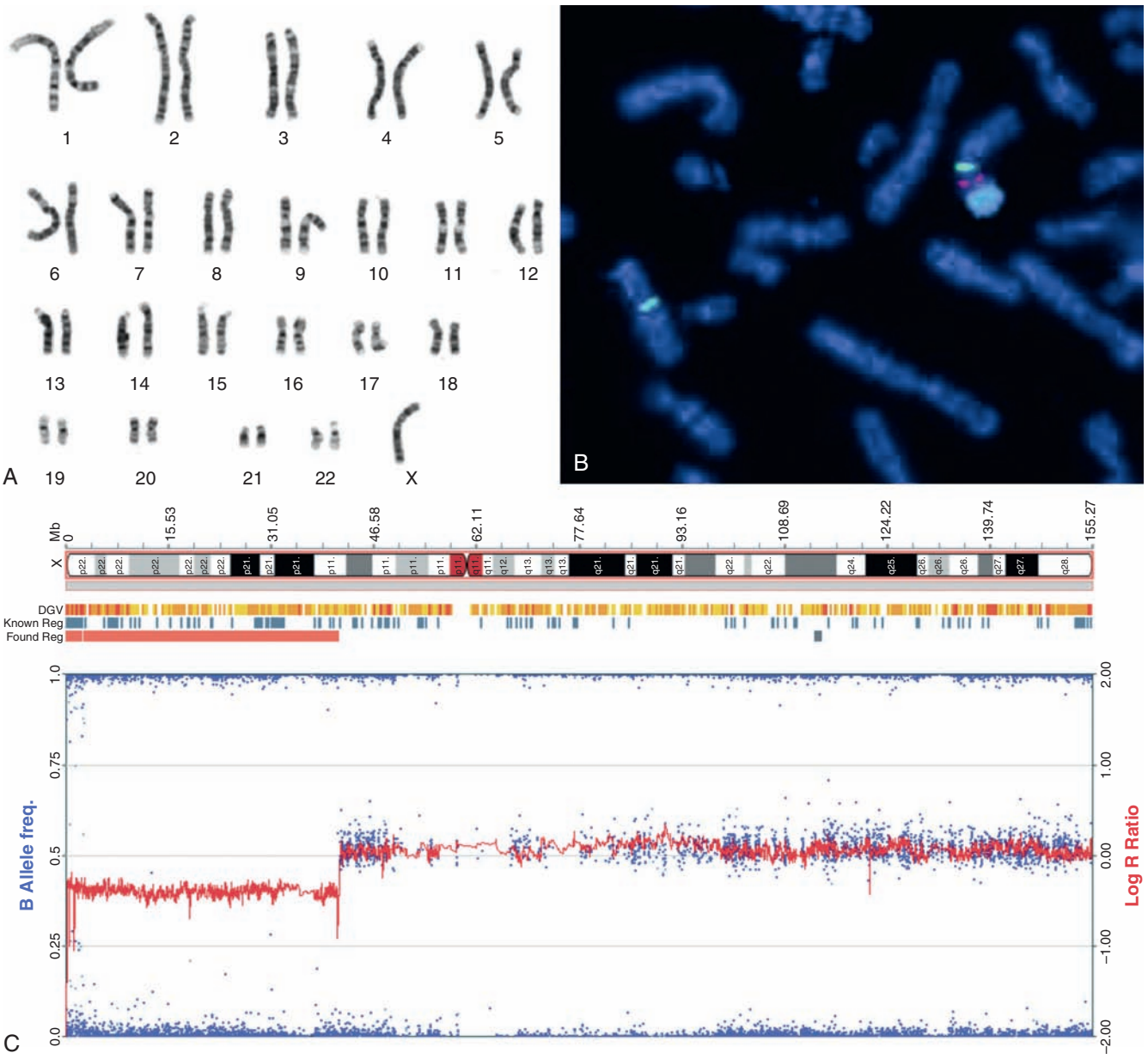


FIGURE 16-2 ■ Sex chromosome analyses. **A,** Standard G-banded metaphase karyotype in which the 22 pairs of autosomes are grouped according to size and the sex chromosomes placed at the end; in this case, there is only one X chromosome. **B,** Fluorescent in situ hybridization (FISH) of metaphase chromosomes showing a normal X chromosome on the left side of the micrograph identified by an X centromere-specific probe (DXZ1; green). The abnormal chromosome seen on the right has an X long arm and centromere joined to a Y chromosome centromere (DYZ3; red bifurcated signal) and long arm (DYZ; large blue signal). This is described in cytogenetic terms as der(X)t(X;Y)(p.11.4;p11.2). **C,** SNP genotyping array of DNA isolated from a Turner patient blood sample. The beta-allele frequency is a measure of the allelic intensity ratio, plotted as (blue) dots with reference to the left y-axis. When there is equal representation of two alternative SNP alleles, the beta allele frequency is 0.5; when only one allele is present, the frequency is 1.0 or 0. The log R ratio is a measure of total signal intensity for the test subject compared to control and is plotted as (red) graph against the y-axis on the right. When test subject signal intensity is equal to that for the control, the ratio is 1:1 and the log is 0. The array results are plotted alongside the X chromosomal ideogram at the top of the panel and show a loss of heterozygosity and reduced signal intensity from chrX: 0-41,500,000, consistent with the deletion of more than half the X chromosome short arm with breakpoint at cytologic band Xp11.4. (**B,** The FISH micrograph was kindly provided by Dr. Marie-France Portnoi of Service de génétique et embryologie médicales, Hôpital Armand-Trousseau, Paris, France. *This image can be viewed in full color online at ExpertConsult*)

and paternal in origin in ~30% of cases with cytogenetically confirmed Turner syndrome.[26-29] The expected ratio, if each parent has an equal probability of contributing a normal X to offspring, is 2:1. The consistent finding of a slightly greater than expected prevalence of X-maternal cases suggests that there may a greater propensity for meiotic errors involving the sex chromosomes during spermatogenesis. Women with Turner syndrome due to partial Xp deletion or translocation who are fertile may pass on the abnormal X chromosome to offspring.[30-32] The possibility of passing on a fragmented sex chromosome underscores the importance of obtaining a full

karyotype in the diagnosis of Turner syndrome, because if such a chromosome is found in the child, the parents need genetic testing and counseling about potential recurrence, or transmission if the daughter is fertile. There does not seem to be an increase in monosomy X or any aneuploidy in assisted pregnancies compared to natural conceptions.[33] Historical case reports have described an association of trisomy 21 and mosaic X monosomy,[34] but this association has not been observed in a large population-based investigation.[23]

Turner Karyotypes

Cytogenetic nomenclature reports the total number of chromosomes followed by the sex chromosomes listed individually, thus the normal female karyotype is "46,XX" and Turner syndrome patients with a single X chromosome are "45,X." The most common karyotype found in Turner syndrome is 45,X.[11,35,36] *Mosaicism* is defined as the presence of two or more cell lines showing different chromosome constitutions. If more than one cell line is detected, the karyotypes are separated by a slash, with the relative cell counts in brackets. For example, 45,X [10]/46,XX[10] describes an individual with 50:50 mosaicism for X monosomic and normal 46,XX cells based on scoring 20 metaphases. This type of cell line mosaicism occurs due to sex chromosome nondisjunction during postzygotic cell divisions. Depending on the developmental timing and cell populations affected, the clinical phenotype may be attenuated by mosaicism for a normal cell line.

The isoXq chromosome is the most common structural abnormality causing Turner syndrome.[11,35,37] It is a mirror image chromosome composed of two copies of the long arm fused head to head with a variable amount of centromeric and proximal Xp sequences in between, most commonly reported as 46,X,i(X)q. Variants that have subtotal Xp deletion are termed *isodicentric* and *pseudo-isodicentric* X chromosomes.[38,39] Individuals with isoXq chromosome are monosomic for the X short arm and trisomic for the X long arm. The presence of an isoXq chromosome is commonly associated with a 45,X cell line, as the abnormal isoXq chromosome is frequently lost during postzygotic cell divisions. This mosaic chromosomal constitution is reported as 45,X/46,X,i(X)q. Because all cells are monosomic for Xp, there is no tendency for moderation of the monosomic phenotype in this type of cell line mosaicism. The isoXq chromosome is not associated with mosaicism for a normal 46,XX cell line.

A ring X chromosome (rX) is fairly common in the etiology of Turner syndrome; the ring results from fusion of broken ends of short and long arms. Most often the ring has lost a major portion of Xp but retains the large part of Xq, and it is functionally equivalent to Xp deletion. The ring X is commonly lost during cell divisions in the course of development, resulting in mosaicism denoted as 45,X/46X,r(X). A ring X missing the X inactivation site (XIST) at Xq13.2 has been associated with severe mental retardation and somatic features atypical of Turner syndrome.[40,41] The XIST locus is required for inactivation of the second X chromosome, and deletion or deficient transcription of XIST may result in failure of X inactivation. The severe phenotype in girls with a small rX or with unbalanced Xp translocation is thought to be due to functional disomy of certain Xp loci that are normally inactivated.[42]

About 5% of clinically diagnosed Turner syndrome patients have a significant Xp deletion, denoted 46,X,del(X)p, with or without mosaicism for a 45,X cell line. Xq deletions are associated with clinical features of Turner syndrome mainly in patients with 45,X/46,X, del(X)q mosaicism; women with terminal Xq deletion without 45,X cells do not usually have short stature or other features aside from premature ovarian failure.[43]

Conventional karyotyping reveals mosaicism for 45,X/46,XY or 46,X,fragmentaryY cell lines in about 10% of clinically diagnosed girls with Turner syndrome.[11] In cases where the Y chromosome is intact, it is likely that the proportion of normal 46,XY cells was too low in the developing gonad to induce normal testis development and male differentiation. Phenotypic males with 45,X/46,XY chromosomal constitutions may have short stature and congenital cardiovascular malformations similar to those with Turner syndrome[44,45] but are considered under the diagnostic category of Disorders of Sexual Differentiation. In girls with Y chromosome structural abnormalities, the sex determining gene SRY may be deleted or inactivated, resulting in absent male differentiation.[46] The presence of Y chromosome material is clinically relevant because of the risk for development of a gonadoblastoma in females with Y chromosome material.[47] The diagnostic issues related to Y chromosome detection and clinical implications with regard to gonadoblastoma are discussed later in sections on "Diagnostic Tests" and "Gonadoblastoma."

X Chromosome Genes and Turner Syndrome

In a classic article published in 1965, Ferguson-Smith analyzed the karyotypes of 307 patients with various forms of gonadal dysgenesis in relation to their clinical findings.[48] He proposed that monosomy for the short arm of the X chromosome was responsible the short stature and congenital malformations found in Turner syndrome, and he noted that Yp deletion was associated with a similar phenotype. Based on this analysis, Ferguson-Smith hypothesized that some Xp genes escape from X-inactivation in 46,XX females and that homologous genes were located on the Y chromosome short arm.[48] In addition, he reported that patients with mosaicism for a normal 46,XX line (i.e., 45,X/46,XX) tended to have fewer phenotypic abnormalities.[48] These observations were confirmed in subsequent chromosomal banding[35] and molecular studies.[49]

As predicted, genes currently implicated in the Turner phenotype are located on the X chromosome short arm, escape the process of X inactivation, and have functional homologs on the Y chromosome.[50] The human sex chromosomes share homologous gene encoding regions located at the termini of the short and long arms. They are termed *pseudoautosomal* because genes in these regions behave like autosomal genes and do not undergo

X inactivation. These regions ensure that X-Y meiotic pairing and recombination takes place, which is essential for male meiosis. The major pseudoautosomal region (PAR1) is located at the Xp and Yp terminal regions and includes at least 25genes.[51] Two groups independently identified a PAR1 gene, which when deleted was associated with skeletal deformities and short stature.[52,53] This gene is termed *SHOX*, for "short stature homeobox" and is located ~500 kb from the telomere of the sex chromosomes at Xp22.3 and at Yp11.3 (Figure 16-3). SHOX encodes a transcription factor that is highly expressed in human bone morphogenetic tissue.[54] SHOX haploinsufficiency due to mutation or microdeletion causes Leri-Weill dyschondrosteosis, characterized by mesomelic short stature and shortening and bowing of the radius with a dorsal subluxation of the distal ulna (Madelung deformity).[55] Homozygous SHOX deficiency causes Langer mesomelic dysplasia, producing extreme short stature, profound mesomelia, and limb deformity. SHOX mutations or deletions are found in 2% to 15% of children with idiopathic short stature, without obvious skeletal dysmorphology.[55,56] These observations have established the view that SHOX haploinsufficiency due to deletion of Xp or Yp terminal regions causes the skeletal anomalies and short stature occurring in Turner syndrome.

Isolated SHOX haploinsufficiency in Leri-Weil or Langer syndromes has not been linked to nonskeletal features of Turner syndrome such as lymphatic obstruction, congenital heart defects, renal anomalies, or hearing loss. It seems likely that haploinsufficiency for other, as yet unknown PAR1 gene(s) contributes to these important defects. This is inferred from the fact that rats and mice that lack PAR1 genes on the sex chromosomes are normal in size, fertile, and without apparent somatic or visceral defects.[16] In contrast, canine, feline, and equine species that share a conserved PAR1 region with humans exhibit dwarfism, infertility, and aortic coarctation in X monosomy.[50] Several Xp genes outside of PAR1 escape X-inactivation and have Y-homologues, and thus may play a role in Turner syndrome, as recently reviewed.[50] Xp deletion mapping data suggested that distinctive neurocognitive characteristics of Turner syndrome are linked to haploinsufficiency for PAR1 or nearby loci.[57] Ogata and colleagues ascertained a "lymphedema critical" region at Xp11.4,[58] although this localization was not confirmed in a more recent study.[59] Bondy and associates have demonstrated that the locus for bicuspid aortic valve and aortic coarctation is telomeric to Xp11.4.[60] Hearing loss also maps to Xp deletion.[61,62] Genes located on the X chromosome long arm (Xq) are not likely candidates given that girls with normal Xq constitution (e.g., 46,X,del(X)p) and girls that actually have an extra copy of Xq (46,X,i(X)q) have the consistent Turner phenotype.[63]

X Chromosome Genomic Imprinting

Although haploinsufficiency for Xp genes is clearly implicated in major features of Turner syndrome,[64] it is also possible that the exclusive expression of maternally or paternally derived X chromosome may differentiate 45,X girls from the 46,XX population that express an equal ratio of X maternal to X paternal genes secondary to random X inactivation. Important male-female differences such as adult height, brain size, risk for autistic spectrum disorders, abdominal adiposity, and atherosclerosis are independent of gonadal effects and could be influenced by X chromosome genomic imprinting.[65] Skuse and colleagues reported impaired social and verbal skills among girls with Turner syndrome who were monosomic for a maternal X chromosome compared to a group monosomic for a paternal X, and they suggested that imprinting of X-linked genes contributes to sex-based differences in risk for autism spectrum disorders.[66] Subsequent studies have not supported this view[67]; however, one MRI study found that brain volumes are greatest in prepubertal girls monosomic for X-maternal, intermediate in 46,XX girls, and smallest in girls monosomic for X-paternal, consistent with a maternal X chromosome dose effect on brain volume.[68]

Adult height is a sexually dimorphic trait; however, there is no apparent height difference between Turner groups monosomic for either parental chromosome.[69-73]

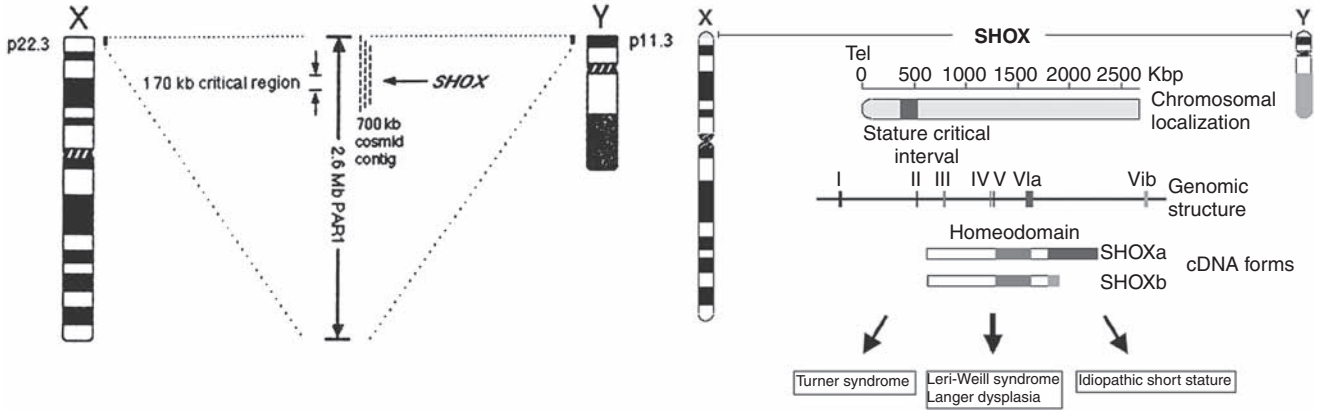


FIGURE 16-3 ■ X and Y chromosome ideograms showing the terminal pseudoautosomal regions (PAR) at Xp22.3 and Yp11.3 where the SHOX gene has been mapped, (A&C) and X chromosome regions historically associated with aspects of phenotype. (From Zinn, A. R. (1997). Growing interest in Turner Syndrome. *Nat Genet, 16,* 3. Reprinted with permission from Macmillan Publisher Ltd.)

Interestingly, adult height in women with Turner syndrome, regardless of X parental origin, tracks maternal but not paternal height.[29,70,72-74] One study suggested that response to growth hormone treatment was influenced by the parental origin of the single X chromosome,[74] but this has not been confirmed in larger, more recent studies.[37,75] Male-pattern abdominal adiposity is observed in females with monosomy for the maternal X chromosome and is associated with an atherogenic lipid profile,[76] which may contribute to greater risk for atherosclerosis among women with Turner syndrome and among the general male population, as males are constitutionally monosomic for a maternally derived X chromosome.

Diagnostic Tests

A chromosomal karyotype has been required for the definitive diagnosis of Turner syndrome.[77,78] This test usually entails obtaining a fresh blood sample from which mononuclear cells are extracted and placed into culture medium. A mitogen such as phytohemagglutinin is used to stimulate lymphocyte proliferation, and after several days, colchicine is used to arrest the cells in metaphase. After fixation, cells are spread on glass slides and treated with Giemsa stain to produce the G-banding pattern, which allows chromosome identification and characterization of major structural anomalies under the light microscope (see Figure 16-2A). Fluorescent-labeled molecular probes corresponding to specific DNA sequences are used to further identify chromosomal deletions or translocations in a process termed fluorescent in situ hybridization or FISH (Figure 16-2B).

For the diagnosis of Turner syndrome, the American College of Medical Genetics recommends scoring a minimum of 20 metaphases from peripheral blood cells.[37] If there is a high degree of clinical suspicion but the initial karyotype is normal, another 10 metaphase spreads should be analyzed. In addition, a standard metaphase karyotype may be performed on cultured fibroblasts or interphase FISH may be used on available cells or tissue, although such tests are not well established. In fact, all the clinical and prognostic information guiding standard practice is based on diagnosis using the standard 20-cell karyotype from peripheral blood mononuclear cells. In some cases the standard karyotype reveals small fragments of chromosomal material known as marker or ring chromosomes that cannot be identified as derived from X or Y chromosomes based on morphology. These fragments require identification using FISH with X and Y chromosome specific probes. Some authors suggest further screening for potential Y chromosome sequences using FISH or polymerase chain reaction (PCR) in girls that have a nonmosaic 45,X karyotype.[37] This approach has not been widely adopted because of uncertainty about the diagnostic reliability and the unclear clinical significance of "cryptic" Y chromosome sequences.

One study showed that measuring the allelic expression of X chromosome single nucleotide polymorphisms (SNPs) by high throughput sequencing of DNA samples from buccal swabs was able to confirm cytogenetic diagnoses of girls with Turner syndrome.[79] The conceptual basis for this approach is that individuals with a single X chromosome will demonstrate lack of heterozygosity for polymorphic X sequences. This method could be used for noninvasive screening of newborns, although the diagnosis would have to be confirmed by standard karyotyping.

A new diagnostic technology relevant to Turner syndrome and other chromosomal disorders uses array-based hybridization to assess both chromosomal sequence copy number and SNP diversity across the entire genome. This new technology provides high resolution virtual, or *in silico* karyotype analysis (Figure 2C) and is performed on stored blood or tissue samples without need for cell culture. These arrays are able to demonstrate X monosomy, X chromosome deletions, and detect Y chromosome presence equivalent to conventional metaphase karyotypes.[80] Their accuracy in detecting low level mosaicism needs further evaluation, however, and additional studies are needed to confirm a correlation between clinical features of Turner syndrome and array-based hybridization diagnoses in a prospective manner.

Indications for Karyotype Testing

There is a distinct biphasic pattern in the diagnosis of Turner syndrome with a substantial proportion being diagnosed around the time of birth and another large group diagnosed around 12 years of age (Figure 16-4). During infancy, the most common indication for karyotype screening is lymphedema. Residual signs of fetal lymphedema include neck webbing, low hairline, malrotated, low-set ears, and drooping eyelids. Swelling or puffiness of the hands and feet accompanies peripheral lymphedema, which is common in infants with Turner syndrome. Skeletal features and growth delay may be present but difficult to appreciate during the first few years. Another important indication for karyotype analysis is the presence of congenital cardiovascular malformation, especially aortic coarctation and aortic valve disease in a female infant or young girl.

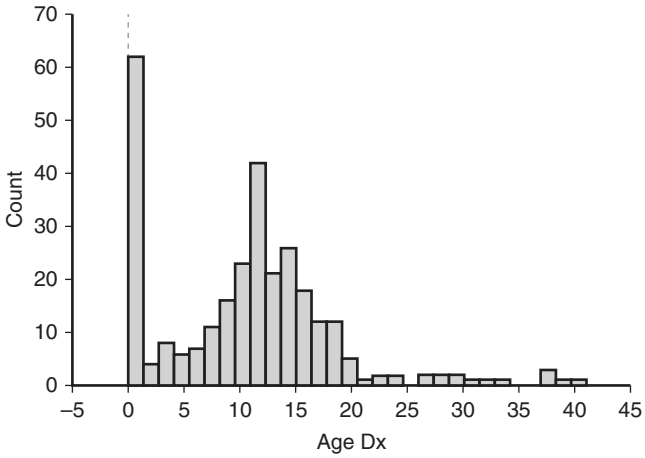


FIGURE 16-4 ■ Age of Turner diagnosis. The histogram shows the age of diagnosis for 292 patients evaluated in the NIH Turner natural history protocol between 2005 and 2010. The median age of diagnosis was 11 years.

A karyotype should be considered in the evaluation of girls with short stature, especially if associated with typical skeletal features such as micrognathia, high-arched palate, short metacarpals, or cubitus valgus as described later (skeletal anomalies and growth failure). Girls and young women presenting with delayed or absent puberty associated with elevation of gonadotropins should have a karyotype analysis. Finally, Turner syndrome is not infrequently discovered by karyotype analysis in women with infertility or premature menopause, as illustrated by the late age of diagnosis for 7% of the National Institutes of Health (NIH) cohort (see Figure 16-4).

Differential Diagnosis

The standard karyotype analysis plays an essential role in differential diagnosis of Turner syndrome. Noonan described male and female children with neck webbing, short stature, and congenital heart disease,[81] and in the past boys with this disorder were classified as "male Turner syndrome." Noonan syndrome is a genetically heterogeneous autosomal dominant disorder with no sex predominance and is associated with a normal karyotype. The Noonan congenital heart defects include pulmonic valve stenosis and hypertrophic cardiomyopathy[81]—in contrast to the Turner cardiovascular phenotype that includes mainly left ventricular outflow tract defects. Puberty may be delayed, but girls are usually fertile whereas boys may have cryptorchidism. The nomenclature, evaluation, genetic counseling, and endocrine management that are appropriate to the girl with Turner syndrome do not apply to patients with Noonan syndrome. Another consideration in the differential diagnosis of a girl undergoing evaluation for short stature with some skeletal features associated with Turner syndrome and similarly affected siblings is SHOX mutation/deletion or Leri-Weill syndrome. Girls with delayed or absent puberty, normal height, and 46,XX or 46,XY karyotypes are thought to have isolated mutations/deletions of genes involved in gonadal differentiation. These patients were historically grouped with girls with Turner syndrome under the category "gonadal dysgenesis," but they clearly have very different genetic, medical, and psychosocial issues.

Prenatal Diagnosis

The prognosis for prenatally detected Turner syndrome depends in large measure on the indications for the testing. When an abnormal fetal ultrasound showing cystic hygroma or fetal hydrops leads to genetic testing and demonstration of nonmosaic 45,X fetal karyotype, there is virtual certainty of a clinically significant diagnosis of Turner syndrome and a high risk of fetal demise.[82] A characteristic ultrasound of a 14-week-old Turner fetus with a cystic hygroma is shown in Figure 16-5. In contrast, an incidental prenatal diagnosis of Turner syndrome with a normal fetal ultrasound is often associated with a mild postnatal phenotype.[83] The postnatal outcomes for fetuses mosaic for 45,X and normal 46,XX or 46,XY cell lines are quite variable and correlate poorly with phenotypes of children ascertained on clinical grounds with Turner syndrome or mixed gonadal dysgenesis.[84] Over 90% of 45,X/46,XY cases ascertained by amniocentesis or villous chorionic sampling appear to be normally developed males at birth.[85] The outcome for fetuses with 45,X/46,XX karyotypes appears to be similarly mild.[83,86] Given these observations, it is important that prenatal counseling inform families that 45,X/46,XY and 45,X/46,XX gestations with normal fetal ultrasound may present a milder phenotype than that of patients diagnosed clinically. Moreover, even the 45,X fetus with cystic hygroma may be viable and enjoy a good quality of life. Importantly, prenatal diagnoses of

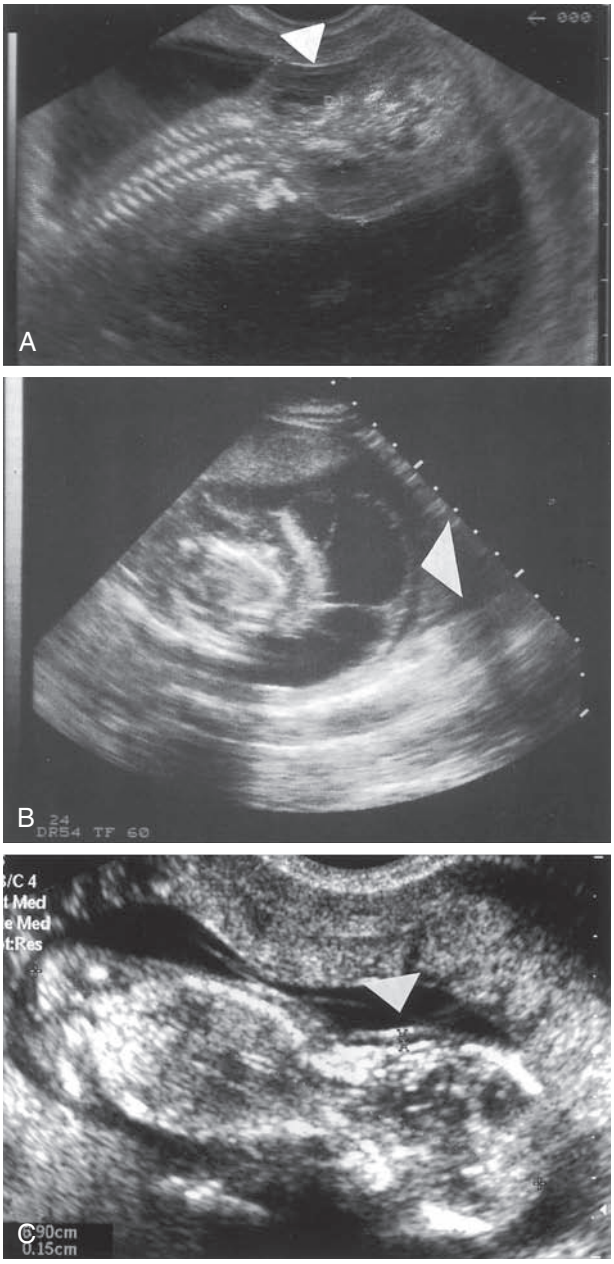


FIGURE 16-5 ■ **A,** A 14-week-old fetus with Turner syndrome and a cystic hygroma (arrow). **B,** A 13-week-old fetus with a normal karyotype and normal nuchal translucency of 1.5 mm (arrow). **C,** Same fetus as in view **A** in transverse plane. Large septated cystic hygroma (arrow) can be seen around the fetal neck. (Courtesy of Pekka Taipale, MD, PhD, Kuppio University Hospital, Finland.)

sex chromosome anomaly should be reevaluated postnatally with a standard peripheral blood karyotype in all cases.

As genomic technology advances, we are seeing new approaches to prenatal screening for genetic abnormalities. Massively parallel genomic sequencing is able to characterize small amounts of cell free fetal DNA in the maternal blood stream by 10 weeks' gestation, and this approach may eventually supplant the use of the maternal "analyte" screen. However, suspicions of aneuploidy based on cell free fetal DNA, maternal analytes or fetal ultrasound still require direct confirmation of the chromosomal complement from in situ fetal tissue obtained from amniocentesis or chorionic sampling. More advances on the genomic technology front may replace the traditional karyotype of fetal tissue. Comparative genomic hybridization (CGH) arrays are equal to traditional karyotype in identification of trisomy 21 and sex chromosome anomalies, including 45X from fetal samples obtained by amniocentesis or chorion samples.[87] CGH is more sensitive to microdeletions or duplications compared to traditional karyotype analysis, but it may not detect balanced translocations or cell line mosaicism associated with Turner syndrome, for example, 45,X/47,XXX. The clinical outcomes of pregnancies identified by the new genomic technologies have not yet been studied.

PHENOTYPIC FEATURES

Since the original description of Turner, it has been recognized that there are a multiplicity of findings in patients with Turner syndrome and that phenotypic features vary remarkably between patients with the same karyotype (Figure 16-6). Table 16-1 summarizes the most common features determined by physical diagnosis and standard medical screening.

FIGURE 16-6 ■ Phenotypic variability in Turner syndrome. Both of these 7-year-old girls with short stature have Turner syndrome with a 45,X karyotype confirmed in analysis of 50 lymphocytes. The girl on the left was diagnosed at birth due to prominent neck webbing and low-set and posteriorly rotated ears. She also has micrognathia and a low posterior hairline. In contrast, the girl on the right was diagnosed at age 7 due to short stature without "classical" stigmata of Turner syndrome, and she is more typical of the clinical presentation of the majority of girls with Turner syndrome diagnosed in the 21st century. *(This image can be viewed in full color online at ExpertConsult)*

TABLE 16-1 **Clinical Features in Girls with Turner Syndrome**

Physical Diagnosis		Percentage Affected
Skeletal	Short stature	100
	Short neck	40
	↑ Upper:lower body ratio	97
	Cubitus valgus	47
	Short metacarpal	37
	Scoliosis	12.5
	Madelung deformity	7.5
	Micrognathia/high palate	60
Lymphatic obstruction	Neck webbing	25
	Low posterior hairline	42
	Edema of hands/feet	22
	Nail dysplasia	13
Other	Strabismus	18
	Ptosis	11
	Multiple nevi	26
Screening Evaluation		
Cardiovascular anomaly	All	44
	Bicuspid aortic valve	30
	Aortic coarctation	12
	Dilated aorta	11
	Other*	12
Renal anomaly	All	18
	Horseshoe kidney	11
	Duplicated collecting ducts	4
	Unilateral agenesis	3
Liver disorder	All	36
	Abnormal liver function tests (LFTs)	27
	Fatty infiltration	19
Hypertension	All	34
	Prehypertension	14
	Overt hypertension	20
Autoimmunity	All	51
	Hashimoto thyroiditis	51
	Graves' disease	1
	Type 1 diabetes	0
	Celiac	5
	Inflammatory bowel	3

"Physical Diagnosis" describes findings from physical examinations of more than 200 girls seen by Drs. Barbara Lippe and Paul Saenger between 1985 and 2000. Information on pubertal development is not included because many girls were not old enough to assess. The "screening evaluation" data are from 100 girls aged 7 to 17 who underwent standardized imaging and lab testing as part of the NIH natural history study performed between 2001 and 2008. The cardiovascular evaluation included MRI and cardiac echo. Under the cardiovascular listing, the "Other" category included partial anomalous pulmonary veins, aberrant right subclavian artery, and atrial septal defects. Blood pressure was measured on 24-hour ambulatory monitors. All patients also had renal and hepatic ultrasound studies. Abnormal liver function was defined as greater than 10% elevation of aminotransferase(s). Hashimoto's was defined by history of clinical hypothyroidism or elevation of circulating thyroid antibodies.

Lymphatic Obstruction

The appearance of the 45,X fetus illustrated in Figure 16-7 dramatically shows the fetal edema that occurs in many conceptuses with Turner syndrome. The edema appears to result from lymphatic malformations and obstruction.[88] The cystic hygromas are due to delayed formation of communications between the jugular and central lymphatics draining into the heart that normally develop between the fifth and sixth weeks of gestation. Peripheral lymphatic hypoplasia or aplasia has also been demonstrated using lymphangiography in adult patients with Turner syndrome.[89]

Webbed neck, or pterygium colli, is the most obvious consequence of nuchal lymphatic obstruction and this is seen in approximately 25% of patients diagnosed currently. The tenting of developing scalp and facial

FIGURE 16-7 ■ A 45,X abortus demonstrating generalized lymphedema. Note the distended cervical region. With resolution of the edema, the redundant skin may cicatrize, resulting in a webbed neck. The edema of the hands and feet may persist and be present at birth. (From Gellis, S. S., & Feingold, M. (1978). Picture of the month. *Am J Dis Child, 132*, 417. Copyright © 1978, American Medical Association.)

structures is believed to cause low-set, rotated auricular structures, down-sloping eyes, drooping eyelids, and low posterior hairline (see Figure 16-6). It has been suggested that this mechanical distention during fetal development may be responsible for lush hair growth, including eyelashes and eyebrows. Edema of the dorsum of the hands and feet at birth signals peripheral lymphedema, which usually resolves, although some patients demonstrate chronic involvement. Others may complain of intermittent or recurrent edema, often after the institution of estrogen replacement therapy.

Skeletal Anomalies and Short Stature

The most common physical abnormality associated with Turner syndrome is short stature. The impairment is most pronounced along the longitudinal body axis. This gives affected individuals the visual appearance of being stocky or squarely shaped and accounts for the predominantly illusory finding of widely spaced nipples.[90] The short neck is secondary to hypoplasia of one or more of the cervical vertebrae.[91] Long bone growth is selectively impaired, resulting in relatively short legs and an increased upper-to-lower segment ratio.[92] Individual bones are affected to varying degrees. For example, cubitus valgus is commonly appreciated and can be measured as the angle of intersection of the long axis of the upper arm with the long axis of the supinated forearm when the elbow is fully extended. Normally, in adult women this angle is approximately 12 degrees—whereas in adult men it is approximately 6 degrees.[93] The major determinant of the angle is the depth of the inner lip of the trochlea of the ulna relative to the outer lip. In many patients with Turner syndrome the angle is between 15 and 30 degrees (Figure 16-8) as a consequence of developmental abnormalities of the trochlear head.

A shortened fourth metacarpal is visible on a bone age radiograph (Figure 16-9A) and is visible on exam by depression of the knuckle when the patient makes a fist. The Madelung deformity is found in 7% to 8% of patients and is caused by bowing of the radius coupled with dorsal subluxation of the distal ulna (Figure 16-10).[94] This anomaly also occurs as part of the Leri-Weill syndrome.

Spinal scoliosis or kyphosis is reported in a significant number of patients[95] and may be secondary to vertebral anomalies or leg-length inequality. Micrognathia or receding chin and high arched palate are common and thought to be due to disturbances of the development of facial bones.

Bones of the hand and wrist on bone age radiographs often demonstrate osteopenia,[96] associated with ballooning of the tips of the terminal phalanges.[97] Both findings are illustrated in Figure 16-9B.This osteoporotic appearance is observed in childhood, suggesting that it may be more related to the developmental role of SHOX than to primary estrogen deficiency. In support of this view,

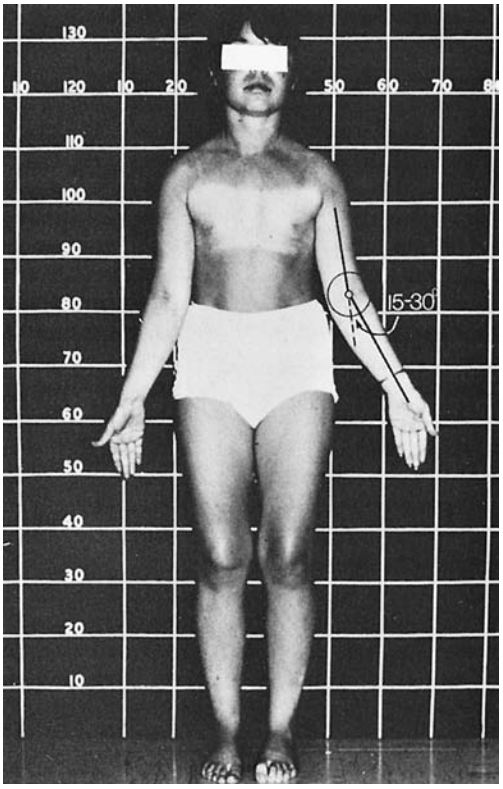


FIGURE 16-8 ■ A 16-year-old girl with Turner syndrome and absence of puberty. Note absence of most characteristic stigmata save short stature and an increased carrying angle (cubitus valgus).

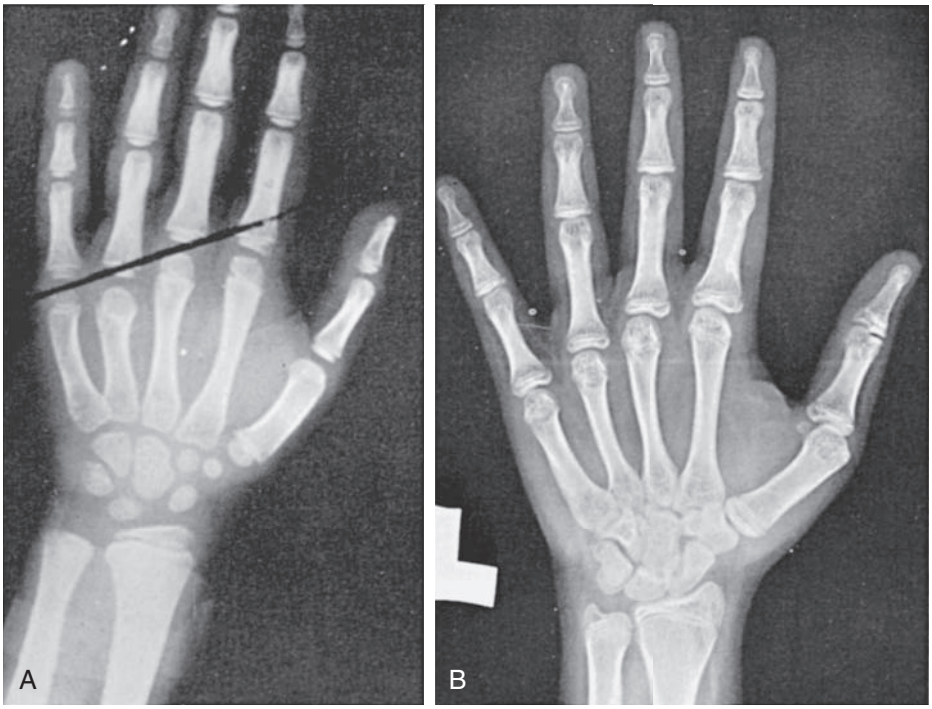


FIGURE 16-9 ■ Two characteristic hand radiographs. **A,** Short fourth metacarpal, the tip falling below a straight line drawn between the third and fifth metacarpals. **B,** Generalized lacy ("fish net") appearance of the carpals and tufting of the distal phalanges, characteristic of the osteoporotic appearance of the bones of patients with Turner syndrome.

FIGURE 16-10 ■ A 19-year-old-patient with Turner syndrome and bilateral bayonet-like Madelung deformities of the wrists.

cortical bone mineralization is selectively reduced in girls and women with Turner syndrome, independent of estrogen exposure.[98] Long bone fractures associated with minimal trauma appear to occur more frequently among girls with Turner syndrome.[99]

Short stature is the most common phenotypic feature of Turner syndrome. The first comprehensive assessment of skeletal growth deficits was reported by Ranke and colleagues in 1983.[100] Patterns of growth are illustrated in Figure 16-11, which shows cross-sectional height and velocity data from a series of 150 Turner children who had not received therapy to promote growth. The observations were supplemented by Davenport and coworkers,[101] who distinguished stages of growth deficit: mild intrauterine growth retardation with average birth length 1 standard deviation (SD) below the mean; a period of mild growth deceleration from birth to age 3.[102] After age 3, there is continued deceleration, so that between ages 3 and 13 years Turner syndrome girls fall farther and farther away from the normal height curves and, if untreated, fail to experience a pubertal growth spurt but continue to grow at a slow rate for several more years.

A positive correlation is found among height at diagnosis,[103] ultimate height achieved, and midparental height.[104] The final height deficit, using comparative data on adult heights in patients with different ethnic backgrounds, is approximately 20 cm.[105] Lyon and associates[106] used the data from Ranke and colleagues and from three other European centers to synthesize a series of growth curves for Turner syndrome. The curves provided mean height and SD values for age and determined a mean adult height 143.1 cm. Lyon and colleagues also noted a strong correlation between the initial height on these Turner curves and the adult height essentially independent of bone age at the time of the first height. Thus, they concluded that one could project the adult height of a girl with Turner syndrome based on her height at an

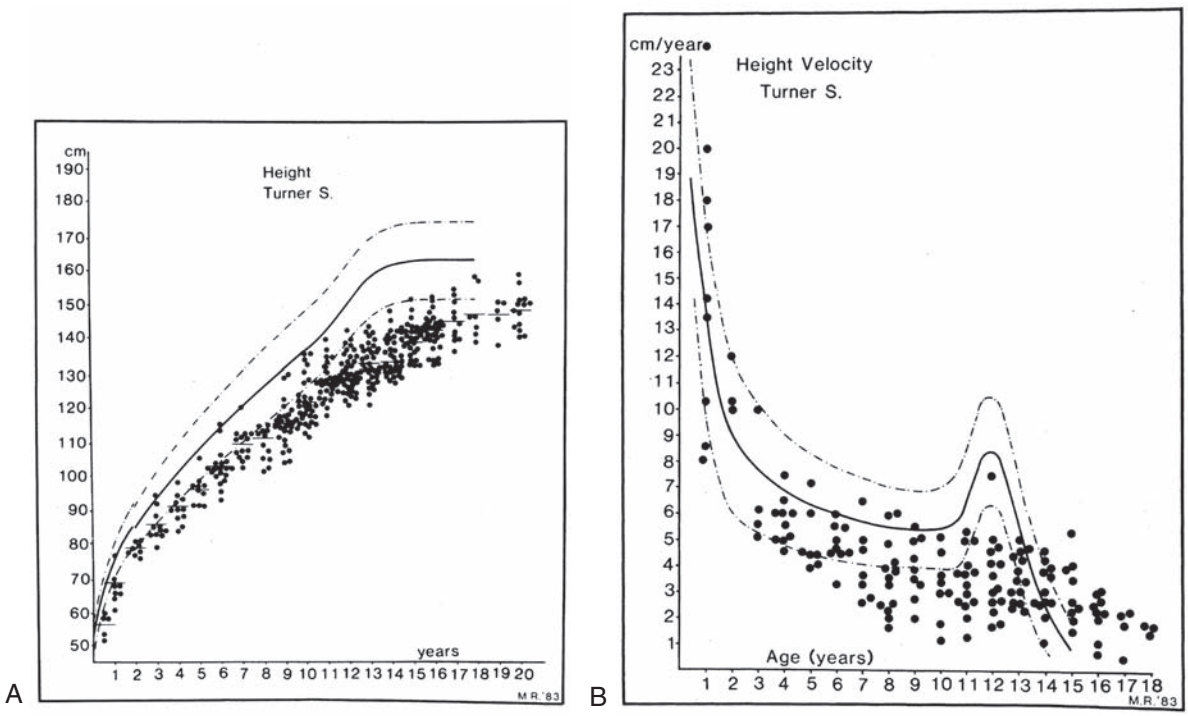


FIGURE 16-11 ■ Height and height velocity in Turner syndrome. **A,** Three hundred eighty-four single measurements of height for 150 children with Turner syndrome. **B,** Height velocity from a total of 159 measurements. The normal ranges are shown by the heavy and dashed lines. (From Tanner, J. M., Whitehouse, R. H., & Takaishi, M. (1965). Standards from birth to maturity for height, weight, height velocity and weight velocity: British children. *Arch Dis Child, 41*, 454, 613; Ranke, M. D., Pfluger, H., Rosendahl, W., et al. [1983]. Turner syndrome: spontaneous growth in 150 cases and review of the literature. *Eur J Paediatr, 141,* 81.)

earlier age. The deficit in longitudinal bone growth in Turner syndrome has been attributed to the deleterious effect of SHOX haploinsufficiency, and a similar deficit is seen in isolated SHOX defects such as Leri-Weill syndrome. Growth hormone deficiency is not implicated in Turner short stature.

Ovarian Insufficiency

The earliest studies of ovarian pathology in women with Turner syndrome described fibrous streaks devoid of oocytes and follicles, and thus initially it seemed that gonads failed to develop or were "dysgenetic." Subsequent studies revealed that early stages of ovarian development appeared normal, with expected numbers of oocytes and primordial follicles at 14 to 16 weeks of gestation in Turner fetuses.[107] However, at later stages of development, Turner ovaries were relatively depleted of oocytes and had few developing follicles, suggesting an accelerated rate of oocyte demise and follicular atresia,[108,109] although follicles in various stages of development are detected in some teenage girls with Turner syndrome.[110] The cause for the high rate of oocyte attrition in most girls with Turner syndrome is unknown. It has been suggested that aneuploidy contributes to oocyte demise due to meiotic mishaps.[111] Another possible explanation for oocyte loss is that diploid expression of unknown X chromosome gene(s) is required for normal oocyte generation or survival. The chromosomal location of putative fertility genes has remained elusive, with gonadal failure frequently observed in Xp deletions with intact Xq complement and in some cases of Xq deletion with normal Xp complement.[112] Terminal Xq deletions have been associated with premature ovarian failure in women who have few if any features of Turner syndrome.[113]

Ovarian function and potential for spontaneous puberty and even pregnancy are variable and sometimes difficult to assess among girls with Turner syndrome. An important Swedish study using ovarian biopsy has shown that a karyotype with mosaicism for 45,X and 46,XX cell lines is the most significant positive predictive factor for the presence of ovarian follicles, whereas karyotypes indicating nonmosaic 45,X or structural defects of one X chromosome were significant negative correlates for the presence of follicles.[114] Clinical factors such as normal follicle stimulating (FSH) and anti-Mullerian hormone (AMH) levels and spontaneous start of puberty were also significant positive predictors of follicle presence, although less robust than the blood karyotype.[114] According to a large, multicenter Italian study that included more than 500 girls with Turner syndrome, spontaneous puberty occurs in about 15% of those with pure 45,X and in 30% of girls with a second cell line with more than one X chromosome (i.e., 45X/46XX; 45X,47XXX).[36] Puberty may fail to progress to menarche in some girls, and menarche may be followed by oligomenorrhea or anovulatory cycles in others, so that the actual percentage of young women that maintain normal menstrual cycles by age 20 is less than 5%.

Spontaneous pregnancy occurs in 2% to 3% of women with Turner syndrome.[36,115,116] This is more common among women with mosaicism for 46,XX or 47,XXX cell lines, but there are several well-documented reports of spontaneous pregnancies in 45,X women who had no evidence of mosaicism despite intensive investigation.[24,116,117] An early case series reported a high frequency of fetal mortality or malformation for spontaneous pregnancies among women with Turner syndrome.[118] However, this has not been observed in more recent, population-based studies[116,119] nor in women with X monosomy.[116,120] If the mother has Turner syndrome due to a sex chromosome structural abnormality, the abnormal chromosome may be passed on to her offspring. The risk of maternal complications with spontaneous or assisted pregnancies is very high for women with Turner syndrome. These concerns are discussed at the end of this chapter in the "Reproductive Options" section.

Gonadoblastoma

The gonadoblastoma is a benign tumor that consists of large germ cells surrounded by small cells with variable granulosa, lutein, or Sertoli-like morphology. This type of tumor strongly resembles the histology of a developing gonad, hence the appellation "gonadoblastoma." This proliferative island of tissue within the dysgenetic gonad has a potential for steroid production and for malignant transformation into dysgerminoma.[47] The gonadoblastoma is found in approximately 40% of females with 46,XY mixed gonadal dysgenesis; the frequency among girls with Turner syndrome and Y chromosomal material is between 10% to 30%.[47] These frequency data are based on a histologic examination of ovaries that were "prophylactically" removed from girls with Y chromosome material in their karyotype. There are no studies showing morbidity or mortality related to gonadoblastoma in Turner syndrome. Analyses of Danish health registry data did not find increased morbidity or mortality related to any type of ovarian tumor among women with Turner syndrome.[20,121] A large British registry study ascertained gonadoblastoma diagnoses in 8% of women with Y chromosome in their peripheral blood karyotype, but it did not report any clinical data associated with the diagnosis.[122]

Prophylactic gonadectomy has been standard practice since the time that an excess of gonadal tumors was first appreciated in females with Y chromosomes and intra-abdominal gonads. Supporting this approach was the view that the gonads in these cases were nonfunctional, essentially contributing nothing to the patient but risk. However, we have learned that spontaneous puberty and even pregnancy may occur in individuals with Turner syndrome and Y chromosome material.[32,123,124] For example, Portnoi and colleagues reported the case of a girl diagnosed with Turner syndrome at age 8 because of short stature who was found to have translocation of Y chromosome material onto the X chromosome (see Figure 16-2B). The patient's family declined the advice for castration and the girl went on to develop spontaneous puberty and eventually had a well-supervised successful pregnancy.[32] All the clinical experience with gonadoblastoma/dysgerminoma in girls with Turner syndrome derives from cases where the karyotype included visible Y chromosome material or the patient had clinical evidence of virilization, hence the recommendation for gonadectomy was applied only to individuals with visible Y chromosome material or virilization.[125,126] With the advent of molecular amplification technology, several studies have reported detection of Y chromosome sequences in 45,X girls without evidence of Y chromosome material on their karyotype, sometimes with a histologic demonstration of gonadoblastoma after gonadectomy. Some authors suggest that all 45,X patients undergo molecular screening for cryptic Y chromosome material. On the other hand, PCR amplification may yield false positive results[127] and immature ovaries may contain nests of germinal cells resembling the benign gonadoblastoma, so the risk of overtreatment is a real concern.

Unfortunately, there is little information on the use of imaging or serum markers for surveillance of potential gonadal tumors in Turner syndrome. In any case where Y chromosomes/sequences are detected and gonadoblastoma is suspected, there needs to be education and counseling of patients or family concerning gender identity, sexual functioning, and reproductive consequences relevant to the decision for gonadectomy. Moreover, preservation of follicles or oocytes may be an option for some patients undergoing gonadectomy. Further discussion of gonadoblastoma issues is found under the "Y Chromosome" section presented later in the chapter.

Cardiovascular System

Spectrum and Etiology of Congenital Cardiovascular Malformations

Congenital cardiovascular malformations (CVM) are the most serious, life-threatening consequences of X chromosome monosomy.[20,122,128] Coarctation of the aorta in Turner syndrome was documented many years ago[5,48,129]; however, early series reporting the spectrum of CVM in Turner syndrome included patients with Noonan syndrome,[130] which has a different cardiovascular phenotype.[81] The frequency and spectrum of Turner-specific CVM was established in more recent studies using chromosomal karyotyping and cardiovascular imaging (Table 16-2). Obvious CVMs occur in approximately 75% of spontaneously aborted Turner fetuses and 30% of living patients. Obstructive lesions of the left ventricular outflow tract predominate, ranging in severity from nonstenotic bicuspid aortic valve to aortic stenosis, coarctation of the aorta, aortic aneurysm, and mitral valve anomalies (see Table 16-2). The most severe form of left-sided hypoplasia (hypoplastic left heart syndrome) also occurs in Turner syndrome and has a very poor prognosis.[131,132] The association of Turner syndrome and left-sided cardiovascular malformation is distinctive among malformation syndromes.

Clark described a significant association between neck webbing and aortic coarctation in girls with Turner syndrome.[133] This association was supported by observations on aborted fetuses[134] and additional clinical studies reporting a significantly higher prevalence of aortic

TABLE 16-2 **Congenital Cardiovascular Malformations in Turner Syndrome**

	Mode	BAV	Coarc	ASD/VSD	PAPVC	ARSC	ETA	Any
Gotzsche[161]	Echo	14%	10%	0	1%	NR	NR	26%
Mazzanti[135]	Echo	12%	7%	1%	5%	NR	NR	23%
Volkl[162]	Echo	18%	18%	8%	5%	NR	NR	30%
NIH[139,163]	MRA/Echo	30%	12%	1%	13%	13%	49%	50%
Kim[164]	MRA	39%	16%	NR	16%	6%	31%	NR

BAV, bicuspid aortic valve; Coarc, aortic coarctation; ASD/VSD, atrial or ventricular septal defects; PAPVC, partial anomalous venous connection; ARSC, aberrant right subclavian artery; ETA, elongated transverse arch of the aorta; NR, not reported

coarctation and bicuspid aortic valve (BAV) in girls with neck webbing.[135,136] Clark proposed that engorged fetal lymphatic channels might compress the ascending aorta and alter intracardiac blood flow, resulting in the spectrum of left ventricular outflow tract (LVOT) defects.[133] However, in a study of 45,X fetuses with severe aortic arch defects and aortic valve abnormality, Miyabara and coworkers described a generalized hypoplasia of fourth branchial arch tissues, which in their view was more consistent with defective migration of neural crest cells, as opposed to mechanical effects due to lymphectasia.[137] Moreover, LVOT defects occur in Turner patients without neck webbing, and neck webbing is found in some girls with normal cardiovascular structure and function demonstrated by advanced imaging,[138] which seems inconsistent with a cause-and-effect relationship. It may be more likely that haploinsufficiency for sex chromosome gene(s) causes central fetal lymphedema *and* aortic heart defects independent of each other, with penetrance conditioned by autosomal genetic variation and possibly environmental effects.

Interestingly, nonsyndromic LVOT defects such as aortic coarctation and BAV in the general population are not associated with lymphedema or neck webbing and are more common in males than in females by a ratio of approximately 3:1. The cardiovascular phenotype is found in patients with Turner syndrome with deletion of just the short arm of the X or Y chromosome,[48,139] suggesting that loci important for LVOT development are present on Xp and Yp. The putative candidate gene would escape X inactivation in females and be expressed from both the X and Y chromosomes in males. The Yp allele may be more prone to disruption in males as a consequence of inherent Y chromosome mutability.[140] PAR1 localization of an LVOT related gene(s) may explain the greater prevalence of these defects among males, as the meiotic recombination rate for this region is sevenfold greater in males than females,[141] increasing risk for Yp gene disruption.

Aortic Complications

Complications of congenital cardiovascular disease are the leading cause of morbidity and premature mortality in Turner syndrome.[128,142,143] The major cardiovascular complications include aortic valve disease and aortic dilation, dissection, or rupture. The aortic valve is congenitally abnormal in approximately 30% of life-born girls with Turner syndrome, but it is not detected in many individuals until the third decade of life or later due to inadequate screening.[139,144] Among 74 patients with bicuspid aortic valve studied at the NIH (median age 30 year, range 7 to 67), 55% had normal aortic valve function, 30% had mild aortic regurgitation, and 15% had moderate to severe aortic regurgitation.[139] Aortic stenosis was present in only 2/74 patients with BAV in this series. BAV prevalence was equal in pediatric and adults groups, but the likelihood of valve dysfunction was higher among adults. Interestingly, the large NIH screening study utilizing advanced imaging confirmed an early small study by Miller and coworkers indicating BAV in 34% of girls with Turner syndrome.[145] It is clearly important to screen for aortic valve pathology in all girls and women with Turner syndrome, because the valve may deteriorate over time, leading to heart failure. Moreover, the presence of aortic valve abnormality is linked to aortic pathology with increased risk for aortic dilation, dissection, and rupture.[146-153]

The presence of an abnormal aortic valve is associated with relative dilation of the ascending aorta, using age and body surface area nomograms, in girls and women with Turner syndrome.[139,151] The degree of dilation is greatest in patients with abnormal aortic valve function[139,151] but may be present in individuals with normal valve function as well. Moreover, mild-moderate ascending aortic dilation is found in approximately 10% of Turner patients who have a normal tricuspid aortic valve and normal blood pressure.[139,151] There is some evidence for a generalized vasculopathy in adults with Turner syndrome with enlarged diameter of carotid and brachial arteries[154] and aneurysms of other arteries.[155] The term *aortopathy* has come into wider use to describe aortic disease of diverse etiologies including Marfan syndrome and related vascular disorders that are not associated with valvular disease, in addition to aortic disease associated with BAV. It is unknown at present whether the risk for aortic complications in Turner syndrome is more similar to the former or the latter. This is a critical issue, because indications for intervention, the most effective type of surgery, and therapeutic responses to pharmacologic treatment are likely to be different in these categories.

The risk for serious aortic complications is increased in girls and women with Turner syndrome by 100-fold or greater compared to the general female population, estimated from an epidemiologic study of Danish registry data.[150] The median age of dissection was 35 years, with

the few pediatric cases clearly associated with severe clinically evident aortic valve disease or coarctation often related to surgical complications. Aortic valve abnormalities and aortic coarctation, with or without surgical repair, are important predisposing factors. Hypertension is a risk factor for aortic disease in the general population as well as in Turner syndrome, and fortunately it is readily diagnosed and treated. The presence of aortic dilation is a clear risk factor for aortic dissection in Turner syndrome, and thus direct measurements of ascending aortic diameters at the sinuses of Valsalva, sinotubular junction, and ascending aorta must be collected and recorded for all patients, together with body surface area and age.[156] Spontaneous and assisted pregnancies are associated with increased risk for catastrophic aortic complications in Turner syndrome, and the French College of Obstetrics and Gynecology[157] and the American Society of Reproductive Medicine[158] have issued stringent guidelines on preconception screening and maternal care aimed at reducing these risks.

Other Cardiovascular Issues

Although aortic valve disease and risk for aortic complications are major concerns in Turner syndrome, other congenital cardiovascular problems are common. Partial anomalous pulmonary venous connection (PAPVC) is increased in Turner syndrome,[159,160] with a prevalence estimated from 1% to 15%,[135,161-164] with the higher prevalence found using contrast cardiovascular magnetic resonance angiography (MRA).[163-164] This diagnosis should be pursued in girls with exercise intolerance, unexplained pulmonary issues, or evidence of pulmonary hypertension or right heart hypertrophy. The significance of incidentally discovered PAPVC in the screening of asymptomatic patients is unclear. Only 1 of the 11 cases reported in the NIH series and 1 of the 7 cases described from Cincinnati Children's Hospital had a clinically significant left-to-right shunt eventuating in surgery.[163,164] Aberrant right subclavian artery is another relatively common anomaly (5% to 10%; Figure 16-12) [163,164] that may compress the esophagus leading to dysphagia and chest pain.[165] Furthermore, the origin of an aberrant right subclavian artery from the descending aorta may mask the presence of aortic coarctation if the right upper extremity alone is chosen to measure upper versus lower extremity blood pressure difference. Atrial or ventricular septal defects and mitral valve disease are more common than in the general female population but less frequent than aortic defects and PAPVC (see Table 16-2).

Minor electrocardiographic findings including right axis deviation, T wave abnormalities, and QTc prolongation are significantly more common among girls and adults with Turner syndrome compared to age-matched female controls.[166-168] These findings are of uncertain significance; however, similar observations are reported in Marfan and other congenital cardiovascular diagnoses. Out of an abundance of caution, we advise screening electrocardiogram (ECG) on all patients with Turner syndrome and recommend those with a prolonged age-specific QTc to avoid medications associated with QTc prolongation.[78] Impaired autonomic responses are suggested by resting tachycardia, reduced heart rate variability, and the absence of the expected nocturnal dip in blood pressure.[169,170] Systemic blood pressure is consistently higher in girls and women with Turner syndrome compared to age-matched female controls,[171,172] and it is in the hypertensive range in approximately 25% of cases.[173] The high prevalence of hypertension appears independent of renovascular or aortic disease[174] and is not adversely impacted by usual estrogen treatment.[173,175] Thus, in the majority of patients the cause of the hypertension is unknown. Further studies are needed to determine ideal systemic blood pressures for girls and women with Turner syndrome given their smaller stature and increased risk for aortic and also atherosclerotic disease. Moreover, further investigation is required to identify beneficial pharmacotherapy for patients with hypertension or BAV and aortic dilation.

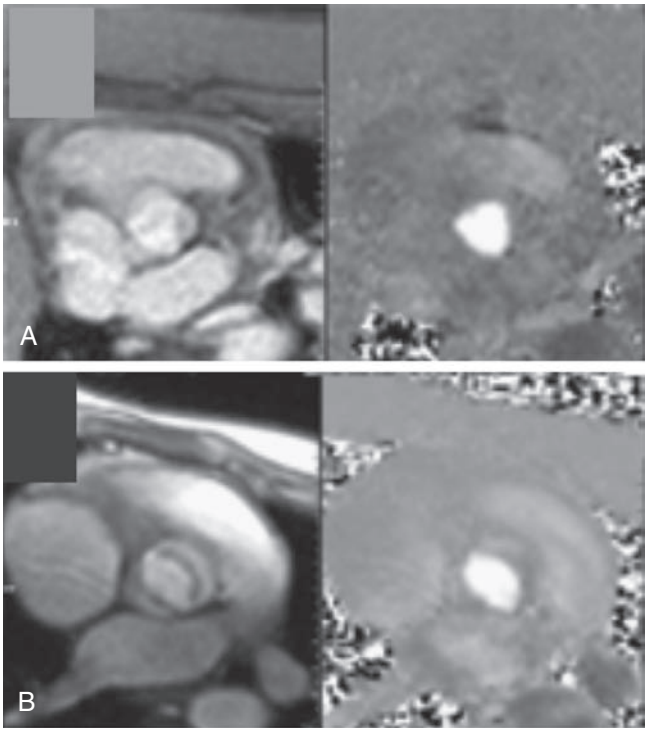


FIGURE 16-12 ■ Aortic valve structure shown by cardiac MRI. Anatomy is shown on the left and blood flow on the right side of each panel. Cardiac MRI is able to visualize the aortic valve more consistently than transthoracic cardiac echo. This figure shows normal tricuspid (A) and bicuspid (B) aortic valves.

Cardiovascular Screening in Turner Syndrome

Girls diagnosed with Turner syndrome associated with CVM in infancy and childhood need ongoing care by the pediatric cardiology team, ideally at a specialized tertiary care center. The mode of imaging and the frequency of follow-up are determined by the clinical situation in each case. Most girls are diagnosed with Turner syndrome in childhood or adolescence due to short stature without

clinically obvious cardiovascular disease. Close cardiovascular examination and consultation with pediatric cardiology are required at the time of diagnosis, with particular attention to blood pressure measurements in all extremities. Transthoracic echocardiography has been the standard approach to screening for congenital cardiovascular disease for decades, but this modality has important limitations related to operator dependency and limited acoustic windows, especially in the context of abnormal chest anatomy and obesity.[176] Cardiovascular magnetic resonance (CMR) imaging provides excellent visualization of cardiovascular anatomy and function, with dimensions of ascending, transverse, and descending aorta, in any desired imaging plane (Figures 16-12 and 16-13). Importantly, CMR consistently detects significant cardiovascular anomalies, including chronic aortic dissection, aortic coarctation and dilation, aortic valve abnormality, and partial anomalous pulmonary venous connection not demonstrated by routine echocardiography in girls and adults with Turner syndrome.[152,163,164,177,178]

In summary, asymptomatic BAV or aortic dilation and some cases of coarctation pose risks for aortic complications but may escape detection with standard evaluation and echocardiography, and hence we recommend more advanced imaging when possible without excessive risks of sedation or radiation exposure, usually by age 12.[78] The detection of asymptomatic BAV and aortic dilation in adolescents is important to assess the individual's risk for aortic complications and the need for continuous cardiac surveillance and care during the adult years. Moreover, knowledge of such underlying defects will enhance education of the young patient and her family about the risks of high-impact or high-resistance exercise and parenting choices.

The most recent consensus meeting agreed that dilation of the ascending aorta in girls and women with Turner syndrome may be defined by BSA-adjusted diameter at the sinus of Valsalva or ascending aorta exceeding two standard deviations (Z-scores) above age- and sex-matched controls.[78] This view was confirmed in large pediatric[151] and adult studies.[148]

Ongoing Cardiac Care

Pediatric patients with known cardiovascular disease require continued care by the pediatric cardiology team and transfer to adult congenital heart clinic when appropriate.[179] Patients who have had aortic coarctation repair in childhood are not "cured" and need continued surveillance for hypertension, aortic valve disease, and imaging of the aorta to monitor for restenosis or aneurysm formation.[180,181] Patients with BAV or aortic dilation need regular and continued monitoring for hypertension, valve function, and potential progression of aortic dilation. The optimal frequency of monitoring is unknown because it is unknown whether there is continuous progressive dilation of the

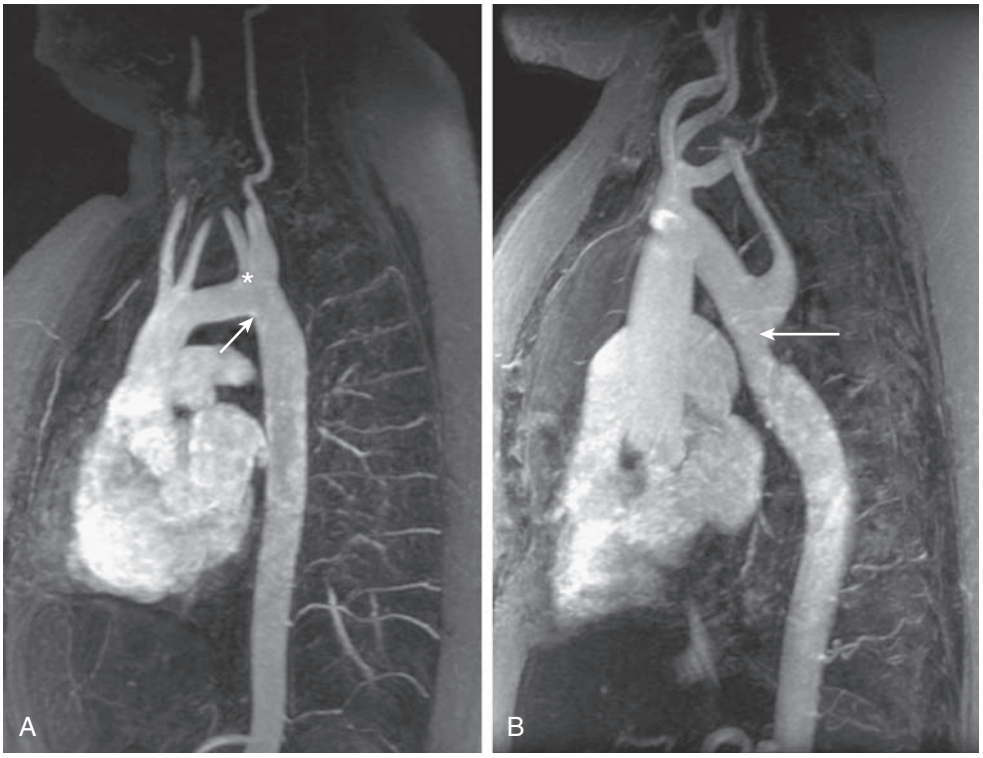


FIGURE 16-13 ■ Cardiac MRI aortograms in Turner syndrome. The image on the left (**A**) shows a squared off, elongated transverse aortic arch (ETA) with a kink in the lesser curvature (arrow). This kink has been called "pseudocoarctation" and is associated with risk for type B dissection. This patient also has a dilated origin of the left subclavian artery (*) and an aberrant origin of the right subclavian artery. The image on the right (**B**) shows a tortuous aortic arch with coarctation just below the take off of the left subclavian. There is moderate dilation of the ascending aorta and poststenotic dilation of the descending aorta as well. This image was obtained from a 41-year-old woman with long-standing hypertension who had been followed with cardiac echo over the years.

aorta as seen in Marfan syndrome, or whether there is a saltatory process,[182] perhaps associated with stressors such as motor vehicle accidents, high-resistance exercise, or pregnancy. Available evidence describes a small annual rate of dilation near the detection limit of current imaging methods.[183,184] Further studies are required to identify parameters that may be more useful in predicting imminent aortic decompensation, such as aortic wall thickness, compliance, or circulating markers such as brain natriuretic peptide (BNP).[185]

Beta-blockers and angiotensin receptor blockade have shown some efficacy in preventing aortic dilation in Marfan syndrome, but neither form of treatment has been investigated in cases of Turner syndrome. Children at increased risk for aortic dissection due to BAV and aortic dilation with or without hypertension and their families should be counseled about possible presenting symptoms (e.g., chest or back pain) and advised to wear a medical identification bracelet indicating the presence of aortic disease. This precaution is advised because delayed diagnosis is the most important cause of fatality in acute aortic dissection. Missed or delayed diagnosis is a major concern for girls and women with Turner syndrome, because the emergency department stereotype for aortic dissection is an adult male in his 60s.

For the patient with no identified cardiovascular defects after adequate cardiovascular evaluation and imaging, routine pediatric care with continued monitoring of blood pressure is advised. It seems also prudent to reevaluate aortic dimension at 5- to 10-year intervals. Heart-healthy moderate aerobic exercise is emphasized and should be encouraged. Eligibility for competitive sports for all those with Turner syndrome should be determined by a cardiologist after a comprehensive evaluation that includes magnetic resonance imaging (MRI) of the aorta.

Risk for Premature Atherosclerotic Disease

Epidemiologic analysis of registry data described a threefold increased risk of coronary and cerebrovascular disease among women with Turner syndrome in Denmark.[186] It was not determined from these data if the coronary disease and stroke were secondary to atherosclerosis or to underlying congenital cardiovascular disease. Adults with nonsyndromic aortic coarctation have increased risk of hypertension, coronary disease, and stroke—despite apparently successful coarctation repair.[140] Total cholesterol levels are elevated in untreated girls with Turner syndrome compared to age-matched females,[187] with improvement in metabolic profile in growth hormone–treated girls.[188] Atherogenic risk factors including abdominal adiposity and lipid profile are higher in women monosomic for the maternal X chromosome versus those with retained paternal X chromosome,[76] but they are not usually elevated into the atherogenic range.[172] Recommendations for preventing atherosclerosis apply equally to girls with Turner syndrome and the general pediatric population and include promoting a healthy lifestyle, monitoring blood pressure and obesity, and initiating metabolic screening by age 9 to 10 years.

Renal Anomalies

Renal abnormalities occur in 20% to 30% of girls with Turner syndrome[63,189] and include most commonly duplication of the pelvocaliceal collecting system, horseshoe kidney, and unilateral renal agenesis. The etiology of the renal anomalies is unknown. There seems to be no correlation between the renal anomalies and other features such as neck webbing or congenital heart defects.[190] The renal anomalies are typically associated with normal renal function during childhood, although pyelonephritis is a frequent complication of collecting system obstruction that requires surgical correction. It is currently recommended that all patients undergo ultrasound imaging of the urogenital system at diagnosis, and as needed in the case of urinary tract infections. Further studies are required to determine if the Turner renal anomalies are associated with deterioration in renal function or the development of proteinuria and hypertension during later life. Certainly renal function should be tested on a regular basis, and renal preservation is an ongoing medical concern for those with a horseshoe or single kidney.

Otologic Disorders

Perhaps the most common medical problem experienced by girls with Turner syndrome is bilateral otitis media. Anderson and coworkers[191] first described medically significant middle ear pathology in girls with Turner syndrome, with many recurrent episodes, spontaneous perforations, frequent need for surgical treatment, and significant hearing loss in about 25%. Additional studies confirmed the problem of recurrent often refractory otitis in Turner syndrome, usually associated with conductive hearing loss and correlated with karyotypes demonstrating loss of the X chromosome short arm, Xp.[61,192-194]

The chronic and severe otitis does not appear to be related to a specific or generalized immunologic dysfunction. Other types of infections and disorders of the mucous membranes do not occur with increased frequency in Turner syndrome. It seems rather that the frequent otitis may be the consequence of abnormalities in growth of the cranial base disturbing the relationship of the middle ear to the eustachian tube, which coupled with abnormalities in the shape of the palate create a predisposition to fluid collection and secondary infection.

Conductive hearing loss is most common and severe in children and is correlated with middle ear pathology,[61] whereas sensorineural hearing loss is more common in adults. This sensory defect is generally bilateral and characterized by symmetrical sensorineural dips in the audiogram in the midfrequency range (Figure 16-14). Thus, the sensorineural hearing deficit appears to progress over time and is not strictly a congenital abnormality. There remains a significant conductive component of hearing loss in adults with Turner syndrome that may signify an ongoing middle ear pathology.[61] The assiduous treatment of ear, nose, and throat (ENT) problems in childhood and avoiding potential injuries to the inner ear may reduce the risk of hearing loss.

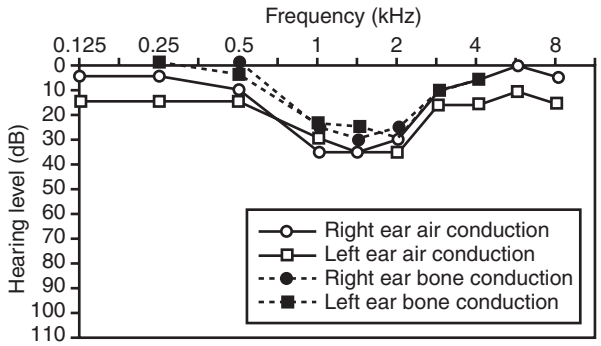


FIGURE 16-14 ■ Audiogram showing the typical dip with the peak of 35 dB in the 1.5-kHz frequency region in a 12-year-old girl with Turner syndrome (karyotype 45,X). This girl has no subjective hearing problems. (From Stenberg, A. E., Nylén, O., Windh, M., & Hultcrantz, M. (1998). Otological problems in children with Turner syndrome. *Hear Res, 124*, 85–90.)

Autoimmunity

Hashimoto lymphocytic thyroiditis is the most prevalent autoimmune disorder in Turner syndrome. Elevated titers of antithyroid antibodies (antithyroid peroxidase, antithyroglobulin) with or without hypothyroidism have been reported in as many as 50% of Turner patients, generally increasing with the patient's age.[120] This condition is most common in patients with the isoX chromosome,[195,196] but it is also prevalent in other karyotype groups.[197,198] The presence of elevated antibody titers may precede the appearance of hypothyroidism and may normalize after the onset of hypothyroidism. The most common finding is a positive antibody screen together with subclinical hypothyroidism with thyroid-stimulating hormone (TSH) mildly elevated and thyroxine levels within normal range.[197,198] The clinical picture of overt hypothyroidism in Turner syndrome may be different from the general population because it has been reported that even severely affected individuals may not show any signs or symptoms of the disease.[197] This, coupled with the high frequency, mandates periodic screening of all Turner syndrome patients.

Graves disease may also be slightly increased among girls with Turner syndrome.[199] In a longitudinal study, 24% of 84 children with Turner syndrome who were followed for about 8 years developed hypothyroidism and 2.5% developed hyperthyroidism.[200] Thyroid disease has been reported as early as 4 years[201]; therefore, all patients with Turner syndrome should be screened annually for autoimmune thyroid disease with a TSH and a free T4 level from 4 years of age onward. In the group of 84 girls followed longitudinally, two had vitiligo and three had alopecia. Other forms of autoimmunity such as Addison disease, hypoparathyroidism, and pernicious anemia are not increased in frequency in Turner syndrome. An association of juvenile rheumatoid arthritis and Turner syndrome was reported by Zulian and colleagues.[202] They surveyed 28 pediatric rheumatology centers and identified 18 girls with Turner syndrome among 15,000 juvenile rheumatoid arthritis patients, which they calculated represents a sixfold increase over what would be expected. A comprehensive clinical and immunologic evaluation of 204 adults with Turner syndrome at the NIH found a significantly higher prevalence of Hashimoto thyroiditis (37%), celiac disease (2.7%), and inflammatory bowel disease (4%) in the Turner group compared to age-matched women with karyotypically normal premature ovarian failure, and to the general female population.[198] There was a trend toward increased Graves disease (2.7%) and psoriasis (3.1%) but no evidence of higher rates of rheumatoid arthritis or any other autoimmune or inflammatory conditions.

Gastrointestinal Disorders

Liver Disease

Minor elevations of hepatic aminotransferases are common in girls and women with Turner syndrome, usually in the absence of signs or symptoms of liver disease. Approximately 20% to 25% of girls[203] and 40% of women[204] demonstrate abnormal liver function tests including aspartate-, alanine-, and gammaglutamyl-transferases and, less commonly, alkaline phosphatase. In some cases, liver enzyme elevations may be associated with pharmacologic estrogen, progestin, or oxandrolone treatments,[203,205] but over the long term estrogen treatment is associated with normalization of hepatic enzymes.[204] Among 100 girls from age 7 to 17 evaluated in the NIH natural history study between 2002-2007, 27% had modest transaminase elevation (see Table 16-1) that was not correlated with growth hormone (GH) or estrogen use, nor with fatty infiltration observed on liver ultrasound.[63] Aside from a rare case report, there does not seem to be any association between autoimmunity and hepatic abnormalities. Liver biopsies in adults with typical enzyme abnormalities have not consistently defined any unique or diagnostic pathology in women with Turner syndrome.[206] Roulot and coworkers have suggested the hepatic disorder may be linked to intrinsic vascular disease. Gravholt and colleagues reported an increased frequency (relative risk 5.7, CI 1.55 to 14.56) of cirrhosis in a Danish National Health registry study, but this has not been observed in clinical series on health in patients with Turner syndrome.

Gastrointestinal Bleeding

A number of reports have called attention to gastrointestinal bleeding occurring in patients with Turner syndrome.[207,208] The bleeding has been ascribed to intestinal telangiectasia, hemangiomatoses, or dilated veins and venules. It is not known which patients may be at risk for developing vascular bleeding, or whether these vascular abnormalities are a consequence of the same obstructive processes that result in the lymphedema. Management of these cases may be extremely difficult; a conservative approach is advised to avoid extensive bowel resections. These patients may require transfusions to maintain a normal hemoglobin concentration. Estrogen treatment may be problematic in such individuals, and treatment has to be individualized.[209,210]

Inflammatory Bowel Disease

As noted previously under "Autoimmunity," girls and women with Turner syndrome have increased risk for Crohn disease and ulcerative colitis.[211-215] Because growth retardation and delayed sexual maturation are characteristic manifestations of inflammatory bowel disease and of Turner syndrome, careful attention must be paid to the review of systems in these patients. Conversely, short girls with inflammatory bowel disease and growth and pubertal delay may need an assessment of gonadal function (measurement of gonadotropins) before their sexual delay is ascribed to their bowel disease alone. The prevalence of celiac disease is also increased in patients with Turner syndrome.[216-218] Considering that growth failure and pubertal delay can be manifestations of celiac disease and Turner syndrome, consideration has to be given to testing short girls with celiac disease with or without pubertal delay (especially if they are on dietary management and have not had catch-up growth) for Turner syndrome. Turner syndrome girls should be screened by measurement of tissue transglutaminase IgA antibodies beginning at age 4 years and repeated every 2 to 5 years.[219,220]

Carbohydrate Intolerance

Carbohydrate intolerance among patients with Turner syndrome was demonstrated in 1963 by Ann Forbes and colleagues at the Massachusetts General Endocrine Clinic.[221] The high rate of glucose intolerance was confirmed repeatedly, but the etiology of the metabolic defect has remained unclear over the years; early studies described insulin deficiency[222] and insulin resistance.[223] A survey of Danish health registry data reported an increased incidence of both type 1 and type 2 diabetes in Turner syndrome,[121] but a higher than usual rate of type 1 has not been observed in other countries or in clinical studies. Clinical studies have consistently shown hyperglycemia in response to glucose challenge with variable insulin responses, and most patients are not insulin dependent or ketosis prone. Elucidation of the metabolic etiology of Turner glucose intolerance has been hampered by the absence of appropriate control groups, as girls and women with Turner syndrome usually have greater adiposity and less exposure to natural ovarian hormones compared to age-matched females, and many use pharmacologic steroids or growth hormone that alter metabolic responses. Another confounding factor is that individuals with a maternally derived X chromosome have excess visceral adiposity whereas those with a paternally derived X appear to have a more beneficial fat distribution,[76] but the two genotypes are routinely lumped together in metabolic studies.

A small study assessed insulin sensitivity via the euglycemic insulin clamp technique in girls with Turner syndrome compared to age but not gender- or body mass index (BMI)–matched controls and found reduced insulin sensitivity in Turner girls.[224] Most recent data generally suggest that glucose intolerance in Turner syndrome is due to insulin deficiency rather than insensitivity,[225-227] although one report noted insulin resistance associated with obesity.[228] The NIH study compared nonobese women with Turner syndrome to age-matched women with 46,XX premature ovarian failure, with both groups off hormone replacement for 2 weeks prior and during the study.[229] Fasting glucose levels were normal in both groups, but glucose levels after oral and IV glucose challenges were significantly higher while fasting and glucose stimulated insulin levels were lower in the Turner group, suggesting that impaired insulin secretion rather than insulin resistance contributes to the risk for diabetes in Turner syndrome.

Of 400 participants evaluated during the NIH study 2001-2010, only one had type 1 diabetes.[230] Type 2 diabetes was not found in any children but was present in 25% of the adults in that study. Interestingly, diabetes was more than twice as frequent among women with an isoXq chromosome compared to those with pure 45,X or with Xp deletion.[230] A cross-sectional cohort study found that girls with Turner syndrome have reduced beta cell function associated with impaired glucose tolerance, and despite greater adiposity and blood pressure, they have normal insulin sensitivity.[231] Thus, it seems that many girls and women with Turner syndrome do not exhibit the typical insulin resistance associated with excess fat in non-Turner individuals.[232]

Neuropsychological Features

The consensus regarding the currently available evidence is that the intelligence of persons with Turner syndrome is normal and girls are similar to their siblings in overall intelligence. Mental retardation has been reported in several cases that were ascertained based on a severe neurodevelopmental phenotype without features typical of Turner syndrome, found to have a ring X chromosome.[40,233-236] It appears that a high risk of mental retardation is caused by the failed inactivation of the ring X from loss of the X inactivation center—thus creating disomy for unknown, normally X-inactivated genes.

Whereas most individuals with Turner syndrome have normal overall intelligence and verbal skills, many have selective impairments in visual-spatial information processing, arithmetic skills, and the coordination of motor and visual-perceptual skills[237-239] coupled in some with a degree of hyperactivity.[240] The discrepancy between verbal and performance IQ is confirmed by many studies and may range between 10 and 15 points—with verbal being higher than performance.[237-239,241-243]

The parental origin of the single normal X chromosome has been implicated in some aspects of the neurocognitive phenotype. Skuse and associates investigated 80 females with Turner syndrome, of whom 25 (the expected proportion) had an X chromosome of paternal origin (Xp). These girls showed satisfactory social adjustment and had higher verbal and executive functional skills than the larger group of girls with the retained X chromosome of maternal origin (Xm).[66]

In an extension of these studies, the Skuse group investigated the relationship of verbal and nonverbal memory with origin of the X chromosome.[244] They observed that 45,Xp Turner females matched controls in verbal memory, whereas this was not the case in the

45,Xm females. In contrast, the results of 45,Xm patients matched those of controls in visual-spatial memory tests, but the 45,Xp group did not. The authors concluded that these data indicate an imprinted locus for social cognition on the X chromosome that is silenced on the maternally derived X. This defect in social cognition may translate into difficulties or lack of understanding of social and nonverbal cues and risk for autism spectrum disorders. The X chromosome parental origin has not been correlated with cognitive or behavioral phenotype in more recent studies,[245-248] although brain size has been correlated with the maternal X chromosome.[68]

The role of estrogen deficiency and estrogen replacement therapy also needs to be considered in the context of organic causes for the cognitive, social, and functional profiles of girls and women with Turner syndrome. Indeed, estrogen replacement appears to enhance motor speed, nonverbal processing, and memory in estrogen-treated Turner syndrome patients compared with placebo-treated patients.[249,250]

The risk for psychiatric illness was systematically investigated in 100 women participating in the NIH natural history study between 2001 and 2003.[251] The incidence of major depression was 5% and that of anxiety disorder was 8%. Women with Turner syndrome reported a higher rate of lifetime depression compared with rates observed in community-based studies but similar to those obtained from gynecologic clinic samples. Affective symptoms among this group of 100 women were prospectively compared to age-matched women with karyotypically normal premature ovarian failure.[247] The two ovarian failure groups had similar levels of shyness and social anxiety that were higher than normal cycling controls, and similar levels of self-esteem that were lower than controls, suggesting that the experience of ovarian failure contributes to the neuropsychological profile of girls and women with Turner syndrome. The first systematic investigation of the personality of girls and women with Turner syndrome was conducted in the 1970s.[252] This and subsequent studies indicate that individuals with Turner syndrome had a high stress tolerance, a tendency toward overcompliance, and a higher degree of dependence and limitations in emotional competence.[240,253,254] Most women with Turner syndrome have a typically female pattern of psychosocial development with unambiguous female gender identification.[255] There may be a delayed sexual debut,[256,257] but sexual fantasies are similar in nature and frequency to average women and sexual activity among Turner women that are married is also similar to that reported by the general female population.[258]

Medical Management

Once the diagnosis of Turner syndrome has been established and a chromosomal confirmation established, additional diagnostic procedures are indicated. These diagnosis and management strategies have been published as recommendations from consensus workshops.[78]

Initial and Follow-up Evaluation

Screening studies at the time of diagnosis and ongoing monitoring for age groups are summarized in Boxes 16-1 and 16-2. All newly diagnosed patients require a thorough cardiovascular evaluation as described previously in this chapter (Box 16-3). Blood pressure should be measured in all four extremities, and 24-hour ambulatory monitoring may be helpful in detecting nocturnal hypertension in girls. Routine renal ultrasound may detect structural abnormalities in renal architecture or collecting system anatomy. If no abnormalities are present, follow-up studies are not routinely indicated. If significant abnormalities are detected, follow-up evaluation and therapy may be indicated and long-term screening for urinary tract infection may be necessary. Scoliosis and kyphosis are evaluated at diagnosis and during growth. If noted, the degree and cause of scoliosis should be determined radiographically.

Otologic and ophthalmologic consultations should begin by age 2 to 3 years and continue as clinically indicated. Otitis media is extremely common and should be treated vigorously. Myringotomy and polyethylene tube placement are considered the primary modes of therapy

BOX 16-1 Screening at Diagnosis of Turner Syndrome in Children and Adults

All Patients
- Cardiovascular evaluation by specialist*
- Renal ultrasound
- Hearing evaluation by an audiologist
- Evaluation for scoliosis/kyphosis
- Evaluation for knowledge of Turner syndrome; referral to support groups
- Evaluation for growth and pubertal development

Ages 0-4 Years
- Evaluation for hip dislocation
- Eye exam by pediatric ophthalmologist (if age ≥ 1)

Ages 4-10 Years
- Thyroid function tests (T_4, TSH) and celiac screen (TTG Ab)
- Educational/psychosocial evaluation
- Orthodontic evaluation (if age ≥ 7)

Ages ≥ 10 Years
- Thyroid function tests (T_4, TSH) and celiac screen (TTG Ab)
- Educational and psychosocial evaluations
- Orthodontic evaluation
- Evaluation of ovarian function/estrogen replacement
- LFTs, FBG, lipids, CBC, Cr, BUN
- BMD (if age 18)

*See Box 16-3.

BMD, bone mineral density; BUN, blood urea nitrogen; CBC, complete blood count; Cr, creatinine; FBG, fasting blood glucose; LFTs, liver function tests.

From Bondy, C. A., for the Turner Syndrome Study Group (2007). Care of girls and women with Turner syndrome. J Clin Endocrinol Metab, 92, *10–25.*

BOX 16-2 Ongoing Monitoring in Turner Syndrome

All Ages
- Cardiology evaluation as indicated*
- Blood pressure annually; consider 24-hour monitor
- ENT and audiology every 1 to 5 years

Girls > 5 Years
- Social skills at age 4 to 5 years

School Age
- Liver and thyroid screening annually
- Celiac screen every 2 to 5 years
- Educational and social progress annually
- Dental and orthodontic as needed

Older Girls and Adults
- Fasting lipids and blood sugar annually
- Liver and thyroid screening annually
- Celiac screen as indicated
- Age-appropriate evaluation of pubertal development and psychosexual adjustment

*See Box 16-3.

From Bondy, C. A., for the Turner Syndrome Study Group (2007). Care of girls and women with Turner syndrome. J Clin Endocrinol Metab, 92, *10–25.*

BOX 16-3 Cardiovascular Screening and Monitoring Algorithm for Girls and Women with Turner Syndrome

Screening (All Patients at Time of Diagnosis)
- Evaluation by cardiologist with expertise in congenital heart disease
- Comprehensive exam, including blood pressure in all extremities
- All require clear imaging of heart, aortic valve, aortic arch, and pulmonary veins
- Echocardiography is usually adequate for infants and young girls
- MRI and echo for older girls and adults
- ECG

Monitoring (Follow-up Depends on Clinical Situation)
- Patients with apparently normal cardiovascular system and blood pressure need reevaluation with imaging at timely occasions (e.g., at transition to adult clinic), before attempting pregnancy, or with appearance of hypertension; girls who have only had echocardiography should undergo MRI when old enough to cooperate with the procedure
- For asymptomatic adults, imaging every 5 to 10 years
- For patients with cardiovascular pathology, treatment and monitoring determined by the cardiologist

From Bondy, C. A., for the Turner Syndrome Study Group (2007). Care of girls and women with Turner syndrome. J Clin Endocrinol Metab, 92, *10–25.*

for serous otitis media in Turner syndrome. The high prevalence of hearing loss, either primary or secondary to residual serous otitis media, mandates that regular ENT evaluation with audiometry is needed for most patients. In infancy, feeding techniques such as those used for cleft palate patients may also be indicated. Because abnormalities in speech may be a consequence of a palatal deformity, speech evaluation may also be indicated. Orthodontic evaluation is needed for most girls by age seven, as the narrow palate and small jaw cause malocclusion and crowded dentition frequently requiring orthodontic treatment.

Ophthalmologic abnormalities including ptosis, strabismus, and amblyopia, are common and require pediatric eye consultation. X-linked red-green color deficiency is present in 8% of girls with Turner syndrome, a percentage similar to that found in males.

Screening for autoimmune thyroid and celiac diseases begins at age 4 and includes thyroxine, thyroid-stimulating hormone, antithyroid antibodies, and tissue transglutaminase antibodies. Subsequently, thyroxine and thyroid-stimulating hormone or thyroid-stimulating hormone alone should be determined at 1- to 2-year intervals. Celiac screen is usually repeated every few years or as indicated by sign or symptoms. Metabolic screening including liver function tests, renal function tests, fasting glucose, or hemoglobin A1c and lipids should commence at about age 10 or earlier in the context of excess adiposity or obesity.

Hypertension is common in Turner syndrome, and blood pressure should be measured at each visit. In addition, 24-hour ambulatory monitoring is ideal for detecting nocturnal or stress-related hypertensive episodes that may support the introduction of antihypertensive treatment.

The decision to seek consultation for plastic surgery to correct the webbed neck deformity or the forwardly displaced ears is individual. It must be pointed out to the patient and family that in addition to the webbing the neck may also be short. Therefore, cosmetic surgery may be somewhat disappointing. In some cases, however, satisfactory results are achieved.

If, or when, psychometric testing should be performed is an individual family decision. Nevertheless, a preschool evaluation to rule out major areas of cognitive dysfunction might be advisable. In light of the previous discussion, school performance should be monitored—and specific problems should be attended to with skilled cognitive specialists. Girls with hearing loss will benefit from preferential seating in the classroom, and those with attention deficit disorder (ADD) may benefit from untimed testing in school when appropriate. The Turner Syndrome Society of the United States (www.turnersyndrome.org) is an excellent resource.

Y Chromosome

As noted earlier in this chapter, the peripheral blood karyotype demonstrating Y chromosome material in a girl with Turner syndrome indicates a risk for development of gonadoblastoma. Pelvic ultrasound may demonstrate an adnexal mass (Figure 16-15), in which case

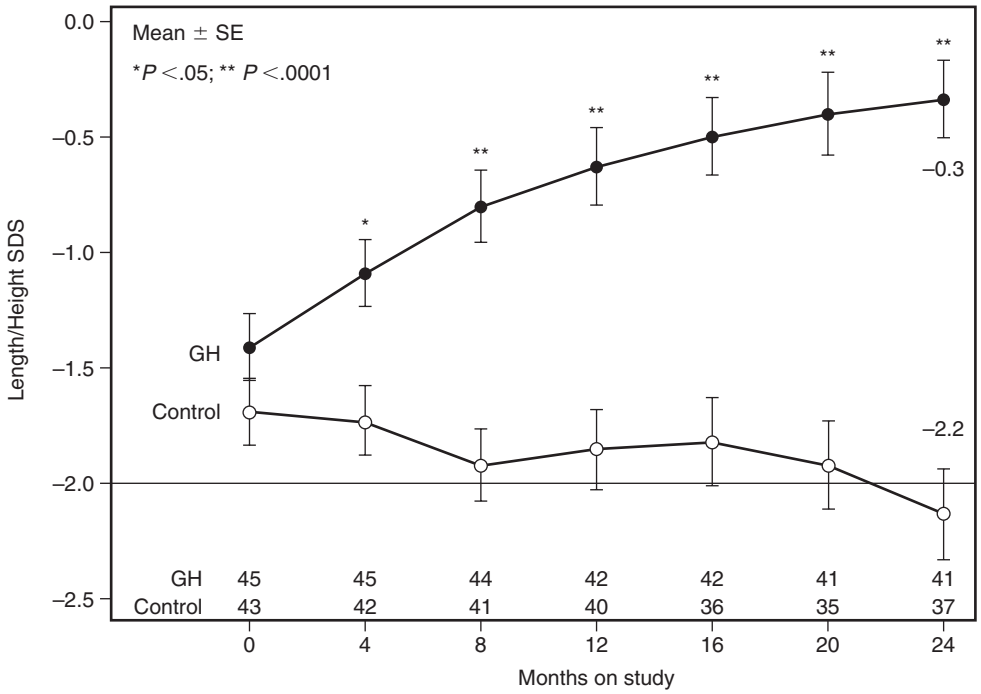


FIGURE 16-15 ■ GH treatment of toddlers with Turner syndrome. Length/height SDS for the untreated control group (open symbols) and the growth hormone treatment group (filled symbols) during the 2-year study. Between-group difference at end point was 1.6 + 0.6 SDS ($p < 0.0001$). Mean age at baseline was 24 + 12.1 months. (From Davenport, M. L., Crowe, B. J., Travers, S. H., et al. [2000]. Growth hormone treatment of early growth failure in toddlers with Turner syndrome: a randomized, controlled, multicenter trial. *JCEM*, *92*, 3406–3416, Figure 1.)

surgical removal of the bilateral adnexal structures is indicated. If pelvic imaging is negative and there is no evidence of virilization, the patient and family must be educated and counseled about the pros and cons of prophylactic gonadectomy versus monitoring by pelvic ultrasound. As discussed in the section on ovarian function, the uncertain risk for a gonadoblastoma that transforms into a malignant tumor must be weighed against possible spontaneous puberty and potential fertility. Cryopreservation of ovarian tissue should be offered if available.

Growth Hormone

In the 1990s, recombinant human growth hormone (GH) was shown to increase growth velocity and final adult height for girls with Turner syndrome. Rosenfeld and coworkers demonstrated a gain in height compared to historical controls,[259] and Sas and colleagues showed a clear dose-response effect with increasing GH doses associated with increasing height.[260] The first randomized study with an untreated contemporaneous control group showed that adult height was increased by about 7.3 cm after an average duration of 5.7 years of treatment, at a dose of 0.3/mg/kg/week,[261] which is lower than the current recommended dose of 0.375 mg/kg/week. Very young girls 9 months to 4 years of age treated with GH maintained normal growth rate compared to untreated Turner controls (Figure 16-16).[262] The results of a randomized, double-blind, placebo-controlled study begun at the NIH in 1987 showed an average gain in adult height of 3 cm with GH alone and 5 cm with GH combined with prepubertal treatment with ultra-low-dose ethinyl estradiol.[263] The ethinyl estradiol dose for girls younger than 12 in the NIH study was less than 1 μg/day, compared with 20 μg in the low-dose birth control pill. These findings are consistent with a growth promoting effect of low, nonfeminizing estradiol levels in the prepubertal years. The growth-enhancing effects of low-dose estradiol are in contrast to the growth-inhibiting effect of feminizing doses of estrogen used to induce pubertal development,[264] which is associated with epiphysial fusion. Ranke and colleagues analyzed height prediction for GH treatment in Turner syndrome.[265] Height at the start of treatment, GH dose, and duration of treatment are identified as important predictive factors.

GH treatment has been evaluated for safety in a group of approximately 5000 girls with Turner syndrome followed for 10 to 20 years in the Genentech National Cooperative Growth Study.[266] An increased frequency of intracranial hypertension (pseudotumor cerebri), scoliosis, and slipped epiphyses was noted, as well as an unexpected higher incidence in the diagnosis of type 1 diabetes. On the beneficial side, GH treatment tends to normalize body proportions, although foot size is disproportionately enlarged.[267] GH treatment has beneficial effects on body composition, as shown in a comparison of age-matched girls who have never received GH (n = 26) to girls treated with GH (N = 76) participating in the NIH natural history study. BMI and visceral adiposity were significantly greater and glucose tolerance significantly worse in the untreated group (off GH treatment for 2 weeks prior to and during the study), with effects apparent years after the discontinuation of therapy.[188]

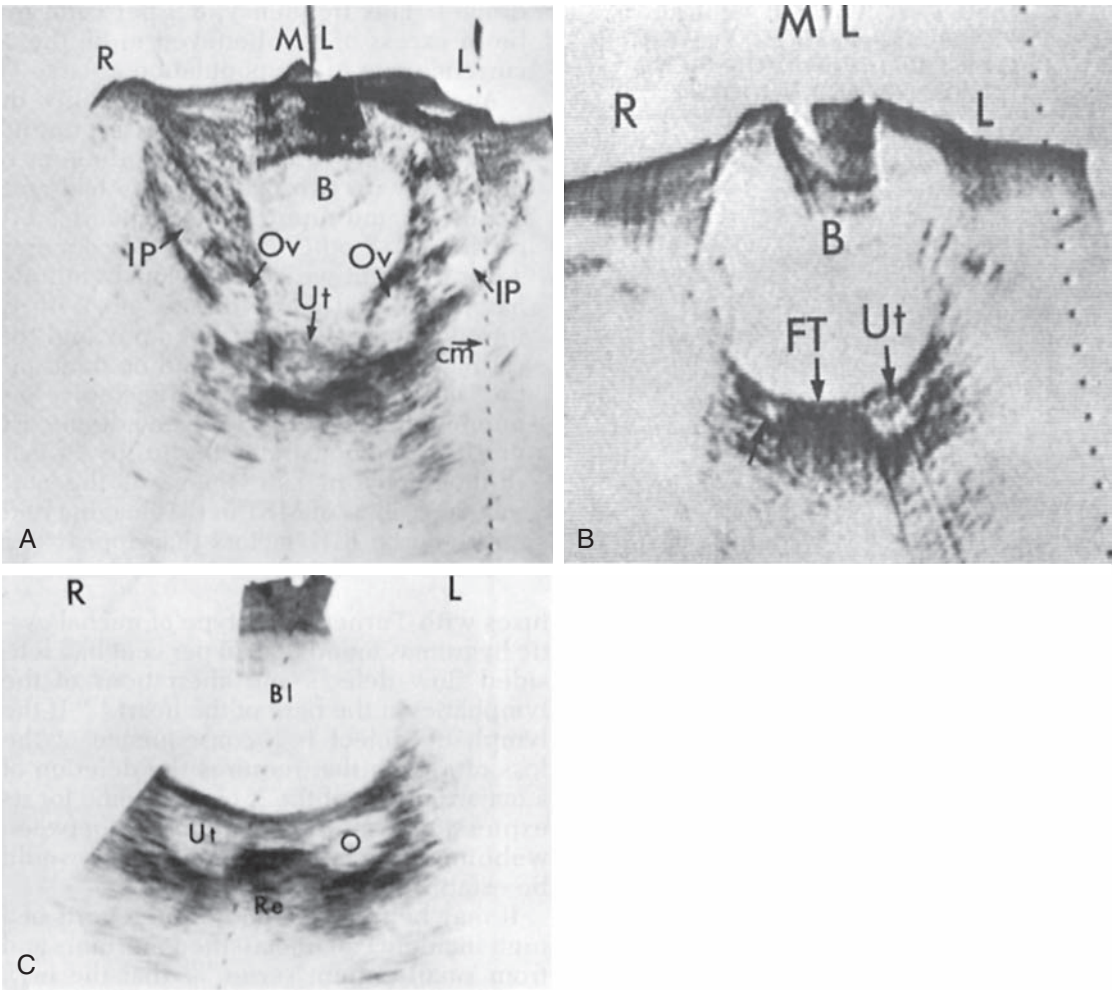


FIGURE 16-16 ■ Examples of pelvic ultrasound studies. **A**, Normal pubertal female demonstrating ovaries of adult size. **B,** Patient with Turner syndrome. The corpus of the uterus is seen slightly to the left of the midline. The fallopian tube can be followed into the right adnexa and observed to terminate in a small structure (arrow) believed to be the fimbriated end of the tube. No ovaries are identified. **C**, Patient with 45,X/46,XY Turner syndrome previously treated with estrogen. The corpus of the uterus is enlarged to adult size. In the left adnexa, a large gonadal mass (O) is seen. Histologically, this was identified as a gonadoblastoma. The images are transverse, oriented right (R) and left (L) of the midline (ML). The dotted scales are in centimeters. **B,** BL, bladder; FT, fallopian tube; IP, iliopsoas; O, gonadoblastoma; OV, ovary; Re, rectum; and Ut, uterus.

GH treatment seems to have no adverse effects on the cardiovascular system over short term follow-up, with body size adjusted ventricular and aortic dimensions similar in treated and untreated patients.[268,269] Moreover, systemic lipids and aortic compliance were relatively better in girls treated with high-dose GH. A comparison of cortical and trabecular bone in GH-treated and age-matched untreated controls with Turner syndrome reveals no apparent effect of GH treatment on bone mineral density.[98]

Late diagnosis of Turner syndrome in girls age 10 and older is a common problem (see Figure 16-4) that compromises attainment of optimal adult stature. In the past, such girls were treated with GH for several years and induction of puberty delayed to age 15 or older. Such a delay of puberty is now viewed as detrimental to social and sexual development and may impair optimal bone mineralization. Statural growth in these girls may be increased by treatment with the nonaromatizable androgen oxandrolone at a dose of 0.05mg/kg/day or less, in addition to GH, which may augment growth by up to 4 cm, usually without virilizing effects.[270,271] Oxandrolone is also used to enhance stature when GH is not available, but patients and families must be warned about potential adverse metabolic effects and diminution of breast development associated with androgen treatment.

GH treatment usually begins at the standard recommended dose of 0.05 mg/kg/day, with close monitoring for height velocity, IGF1 level, and potential adverse effects of intracranial hypertension, scoliosis, and slipped capital epiphyses. Treatment for 3 to 4 years is usually required to experience significant height gain and is discontinued when the target height is attained, when bone age is greater than 14 years, or when growth velocity is less than 1.5 cm per year. It is important to keep in mind that the safety data for GH in Turner syndrome represent intermediate range follow-up of girls in whom treatment

was typically initiated in mid-childhood and continued for several years at the preceding dosage. Although reaching an adult height of 5 feet or greater seems desirable from many points of view, there are as yet no proven medical or psychosocial benefits to this pharmacologic achievement, and hence safety should never be compromised to this end.

Puberty

As noted earlier, spontaneous puberty develops in 10% to 30% of girls with Turner syndrome. The higher rate applies mainly to girls with mosaicism for cells with more than one X chromosome, but a significant number of girls with less favorable chromosomal constitutions will also start puberty on their own. Aside from karyotype, the major indicators of potential natural pubertal onset are normal-range values for FSH and anti-Mullerian hormone (AMH) (Figures 16-17 and 16-18). AMH may be particularly informative, because hormone levels directly reflect the presence of developing ovarian follicles and hence potential fertility.[272] Pelvic ultrasound may be helpful, although failure to visualize ovarian tissue does not preclude the presence of follicles. Patients and families are often very interested in potential fertility, and the clinician needs to have this information to provide counseling about reproductive options (discussed later). In most girls over the age of 10, FSH will have elevated into the menopausal range, and AMH will be undetectable with pelvic ultrasound showing no evidence of ovaries and an immature uterus; these patients will need pubertal induction and maintenance estrogen/progestin treatment.

The aim of estrogen treatment in Turner syndrome is to mimic the beneficial effects of endogenous estrogen in breast and genital development, healthy fat distribution, and bone mineralization, while minimizing the risk for estrogen-associated gynecologic cancers and thrombogenic

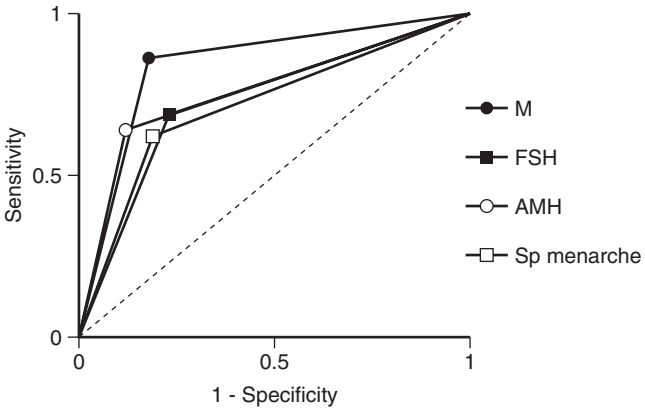


FIGURE 16-17 ■ Receiver operating characteristic curves showing the relationship between sensitivity and specificity regarding the four investigated variables with the highest sensitivity. (From Borgström, B., Hreinsson, J., Rasmussen, C., et al. (2009). Fertility preservation in girls with Turner syndrome: prognostic signs of the presence of ovarian follicles. *J Clin Endocrinol Metab, 94*, 74–80. Erratum in *J Clin Endocrinol Metab, 94*, 1478, Figure 2.)

complications. In the past, the choice for estrogen treatment was limited to metabolized estrogens purified from pregnant mare urine (conjugated equine estrogens [CEE], "Premarin"), and the highly potent, synthetic ethinyl estradiol (EE2). In recent years, transdermal patches containing the natural ovarian product, 17-beta estradiol, have become available and are the preferred delivery method. In addition to replicating the molecular action of the ovary's natural hormone, this formulation is directly absorbed into the venous circulation, bypassing first pass hepatic effects associated with excessive production of prothrombotic proteins. Moreover, the administration of transdermal estradiol allows measurement of estradiol levels in the circulation, which may be helpful for monitoring for adherence. Because estradiol levels vary widely throughout the monthly cycles in eugonadal females, target estradiol levels in pharmacologic treatment are not known. In sexually mature girls, the average daily ovarian estradiol production over the monthly cycle is 100 μg/day, which is approximated by transdermal patches delivering 100 μg/day. Physiologic estradiol repletion is not expected to normalize the FSH level, because FSH secretion is jointly regulated by estrogen and other ovarian products such as the inhibins. Thus, suppression of FSH is not a measure of adequate estrogen therapy.

Current recommendations for estrogen treatment for the induction of puberty suggest beginning the therapy at age 12 for most girls.[126] The suggested doses and rate of escalation are outlined in Table 16-3. The aim of treatment is to initiate development with the lowest possible dose so that sexual maturation will begin on par with peers, while avoiding premature fusion of the epiphyses that would limit growth in stature. Thus, breast development and bone age are evaluated at ~6-month intervals during estrogen treatment for pubertal induction. A sufficient phase of "unopposed" estrogen exposure is required for optimal breast development, and androgen or progesterone treatments may inhibit optimal development. Thus, oxandrolone should be discontinued prior to the initiation of estrogen treatment, and progestins should not be initiated until breast development is satisfactory. Some girls with prominent stigmata of fetal lymphedema may have damaged breast anlage and demonstrate minimal development in response to estrogen. These girls may benefit from breast implantation surgery, if so inclined.

Progestin treatment is necessary to suppress estrogen's proliferative effect on the uterine endometrium and should be introduced after 2 years of unopposed estrogen treatment, or sooner if breakthrough bleeding occurs. This recommendation is given because chronic exposure to estrogen treatment, in the absence of progestin effect, leads to endometrial hyperplasia and risk for hemorrhage and neoplasia. The most physiologic form of progestin is progesterone, which is available in micronized form for oral administration and in cream and gel form. Regrettably, there have been no controlled studies to establish the most effective form of estrogen/progestin treatment for girls with Turner syndrome. The accepted dose of oral micronized progesterone proven to protect the uterus is 200 mg taken at bedtime for the last 10 to 12 days of a monthly cycle, or the last 20 to 30 days of a trimonthly cycle. Progesterone is a soporific and hence

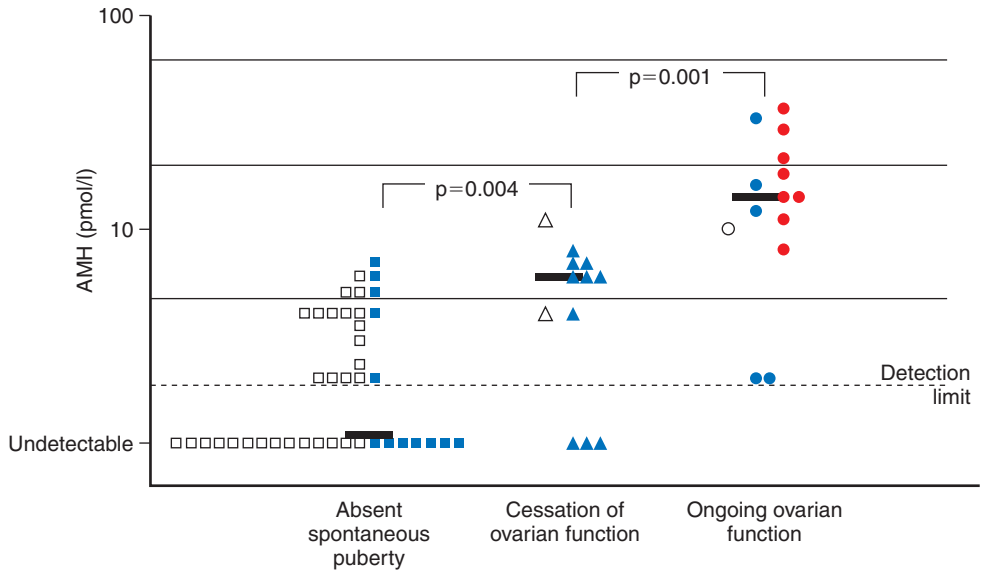


FIGURE 16-18 ■ AMH levels and ovarian function at time of AMH measurement in patients with Turner syndrome, aged 12 to 25 years. Dotted line represents the detection limit of the assay; squares represent patients with absent puberty; triangles represent patients with cessation of ovarian function; circles represent patients with ongoing ovarian function. Thick black bars, median of AMH; black, patients with 45,X; blue, miscellaneous karyotypes; red, 45,X/46,XX. (From Hagen, C. P., Aksglaede, L., Sørensen, K., et al. (2010). Serum levels of anti-Müllerian hormone as a marker of ovarian function in 926 healthy females from birth to adulthood and in 172 Turner syndrome patients. *J Clin Endocrinol Metab, 95*, 5003–5010. Figure 3. *This image can be viewed in full color online at ExpertConsult*)

TABLE 16-3 **Ovarian Hormone Replacement Treatment in Turner Syndrome**

Age (in years)	Age-Specific Suggestions	Comments
10-11	Monitor for spontaneous puberty by Tanner staging, AMH and FSH levels	Low-dose estrogen treatment may not inhibit GH-enhanced growth in stature
12-13	If no spontaneous development and FSH elevated, begin low dose E2	Equivalent initial E2 doses: depot (im) E2, 0.2-0.4 mg/month; transdermal E2, 6.25 μg daily*; micronized E2, 0.25 mg daily by mouth
12.5-15	Gradually increase E2 dose over about 2 years (e.g., 14, 25, 37, 50, 75, 100, 200 μg daily via patch) to adult dose	Usual adult daily dose is 100 μg transdermal E2, 2 mg micronized E2, 20 μg EE2, 1.25 mg CEE
14-16	Begin cyclic progesterone treatment after 2 years of estrogen or when breakthrough bleeding occurs	Oral micronized progesterone best option at present; usual adult dose is 200 mg/day on days 20-30 of monthly cycle or days 100-120 of 3-month cycle
14-30	Continue full doses at least until age 30 because normally estrogen levels are highest between age 15 and 30 years	Some women may prefer using oral or transdermal contraceptive for HRT; monitor endometrial thickness
30-50	The lowest estrogen dose providing full protection versus osteoporosis is 0.625 CEE or equivalent	Monitor osteoporosis risk factors, diet, exercise; obtain BMD and begin regular screening mammography by age 45 years
> 50	Decision on estrogen use based on same considerations as for other postmenopausal women	New HRT options are appearing, and these recommendations may need updating in the near future

*The lowest-dose commercially available, E2 transdermal patches deliver 14 and 25 μg daily; it is not established whether various means of dose fractionation (e.g., administering a quarter patch as directed, or administering whole patches for 7 to 10 days per month) are equivalent.

CEE, conjugated equine estrogens; E2, estradiol; EE2, ethinyl estradiol; HRT, hormone replacement treatment.

the bedtime administration. The efficacy of topical progesterone in preventing uterine hyperplasia is unknown. More androgenic, synthetic progestins such as medroxyprogesterone or norethindrone may inhibit optimal breast and uterine development and have unfavorable metabolic effects in some individuals, although these agents are effective in endometrial protection.

Patients and families must be educated as to the importance of this physiologic hormone treatment regimen for healthy growth and development, especially with regard to building and maintaining strong bones. Discontinuation of hormone therapy during young adulthood is all too common and may result in irreversible loss of bone mineral especially in the spine, leading to

height loss, kyphosis, and chronic pain and disability (Figure 16-19). Families may have legitimate concerns about risks of heart disease and cancer associated with hormone replacement therapy, and it must be explained that these adverse effects were observed in postmenopausal women receiving less physiologic forms of treatment. It is also important to discuss "natural" hormones that are widely touted on women's health sites as alternatives to standard treatments, underlining the fact that these products are not known to be effective or safe. Many older girls and young women may prefer taking oral or transdermal contraceptive formulations for reasons of convenience, tolerability, or financial concerns. Although these are not the most physiologic choices and have greater thrombogenic risk, estrogen/progestin contraceptive formulations are effective in maintaining bone mineralization and protecting the uterus.

It is important to point out that spontaneous puberty, even with menses that appear cyclic, does not always mean that normal ovulatory cycles are occurring or will continue to occur. Some girls have anovulatory cycles that do not achieve normal endometrial maturation and predispose to endometrial hyperplasia, dysfunctional bleeding, or cancer. Irregular cycles may lead to discontinuation of treatment in many cases. Every effort should be made to educate patients and parents as to the importance of continuing hormone replacement, with openness to consultation with experts in adolescent gynecology and adoption of alternative regimens such as oral contraceptive treatment that may ensure continued adherence. Girls with irregular cycles may still have an occasional ovulatory cycle and, if sexually active, are at risk for unplanned pregnancy. For these girls, contraceptive formulations may the best choice for hormone therapy.

Hormone treatment requires regular breast examinations, and patients should be instructed about monthly self-examination. Girls who become sexually active need regular gynecologic follow-up with annual pelvic exams and Pap tests. Regular discussion of issues of concern related to sexual maturation and counseling on the need for ongoing hormone treatment to maintain strong bones will be of paramount importance. There is no need to measure bone mineral density during childhood or adolescence unless there is unusual clinical concern—for example, a low-impact or atraumatic fracture. Short stature is associated with underestimation of bone mineral density obtained by DXA and results need to be normalized for bone size.[273] Size-adjusted vertebral bone mineral density is usually normal in adults with Turner syndrome that have received routine hormone replacement treatment, but it may decline dramatically with discontinuation of treatment (see Figure 16-19).[274] Bone mineral density of the hip and wrist is often lower than normal, reflecting a selective reduction in cortical bone that is not sensitive to estrogen[275] but may reflect the SHOX defect in bone.

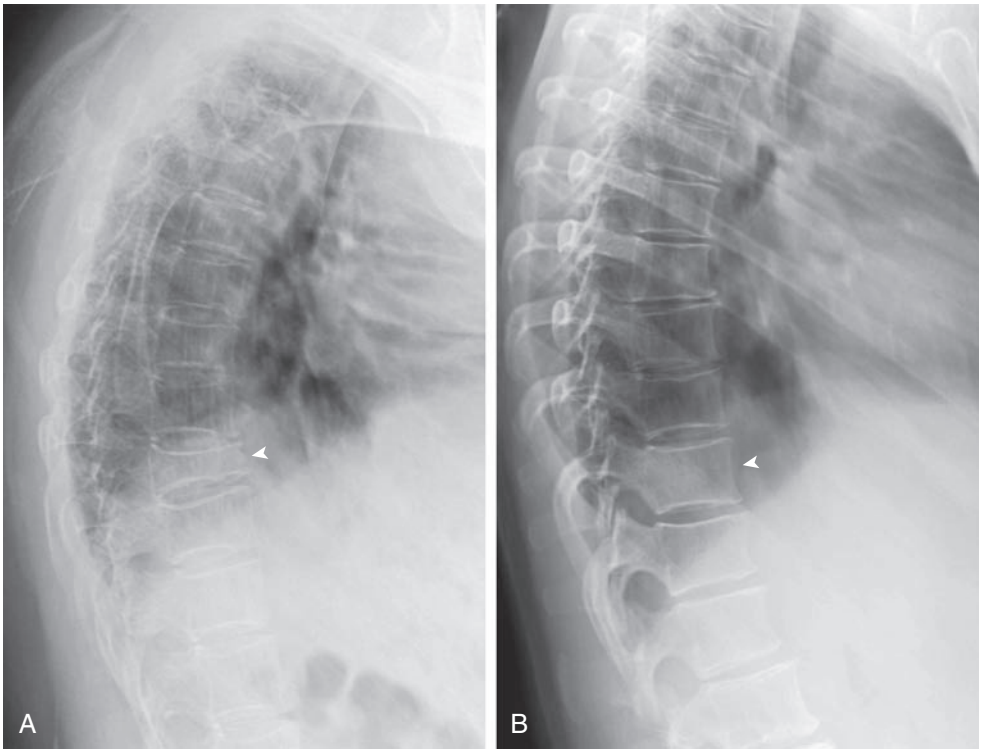


FIGURE 16-19 ■ Estrogen treatment prevents osteoporosis. These lateral chest x-rays are from two 30-year-old women with Turner syndrome. Both had a karyotype of 45,X, both received GH treatment for several years during childhood, and both were started on estrogen treatment at age 12. One patient stopped taking estrogen at age 18 (**A**), and the other adhered to treatment (**B**). The woman who discontinued estrogen treatment experienced height loss, dorsal kyphosis, and chronic pain due to collapse and wedging of vertebral bodies. The arrowhead points to T12 in each woman.

Reproductive Options

As discussed in the "Ovarian Insufficiency" section, about 2% of women with Turner syndrome may have spontaneous pregnancies. Assisted reproduction using donor oocytes with in vitro fertilization has been successful when the uterus has been given adequate hormonal preparation prior to embryo transfer.[276] However, both spontaneous and assisted pregnancies are associated with a high risk for maternal complications, including catastrophic aortic dilation, dissection, and rupture.[277] In most well-documented cases, the women had preexisting risk factors for dissection such as bicuspid aortic valve or aortic coarctation, although these problems were often not detected prior to pregnancy due to inadequate screening.[278] Patients known to have such defects should be counseled about alternatives to pregnancy such as adoption or surrogacy. If not already done, girls and young women with Turner syndrome should have a cardiovascular MRI prior to getting involved in counseling about reproductive options, because significant aortic malformations are often not detected on routine echocardiography and their presence would discourage planning for pregnancy. Girls with ovarian function may be candidates for cryopreservation of recovered oocytes or ovarian tissue obtained by laparoscopy,[276] given the very high probability of premature ovarian failure. This technology has been successful in preserving fertility for girls and women undergoing cancer treatment, although pregnancy resulting from this approach has not yet been reported in Turner syndrome. Girls and families interested in this possibility should be referred to reproductive endocrinology specialists associated with an academic medical center for consultation.

Transition to Adult Care

Pubertal induction treatment should be the occasion for starting the process of involving girls as partners in their care. After years of caring for a young child, a health care provider may overlook the fact that the patient is no longer a child and that new lines of communication need to be established with the patient as an emerging adult. The road to a successful transition involves educating the patient about her health care needs and goals, while developing a schedule for regular thyroid, liver, and renal function testing; annual gynecologic evaluation; and regular cardiology follow-up (see Box 16-2). The patient should be educated about the hormone treatments and other medications she is receiving and the need for regular evaluation of dosage and side effects. Target goals for weight, blood pressure, cholesterol, body fat proportions, and exercise regimen may be collaboratively established, and a portable health record maintained by the patient should be prepared prior to transfer out of pediatric care. Many of the major medical issues for adults with Turner syndrome—such as thyroid function, hormone replacement therapy, hypertension, metabolic status, and bone health—may best managed by endocrinology specialists with a background in internal medicine or gynecology. These providers should also supervise the regular evaluation of otologic/audiologic and cardiovascular systems. Psychosocial aspects of the transition to independence should also be addressed and the patient provided with sources for adult counseling services and support groups.

REFERENCES

1. Turner, H. H. (1938). A syndrome of infantilism, congenital webbed neck, and cubitus valgus. *Endocrinology*, *23*, 566–574.
2. Ullrich, O. (1930). Über typische Kombinationsbilder multipler Abartungen. Z. *Kinderheilk*, *49*, 271–276.
3. Ullrich, O. (1949). Turner's syndrome and status Bonnevie-Ullrich: a synthesis of animal phenogenetics and clinical observations on a typical complex of developmental anomalies. *American J Hum Genet*, *1*, 179–202.
4. Goldman, M. L., Schroeder, H. A., & Futcher, P. H. (1949). Coarctation of the aorta associated with abnormal digits, ovarian insufficiency and shortness of stature. *Clin Endocrinol Metab*, *9*, 622–629.
5. Polani, P. E., Hunter, W. F., & Lennox, B. (1954). Chromosomal sex in Turner's syndrome with coarctation of the aorta. *Lancet*, *267*, 120–121.
6. Ford, C. E., Jones, K. W., Polani, P. E., et al. (1959). A sex-chromosome anomaly in a case of gonadal dysgenesis (Turner's syndrome). *Lancet*, *1*, 711–713.
7. Opitz, J. M., & Pallister, P. D. (1979). Brief historical note: the concept of "gonadal dysgenesis." *Am J Med Genet*, *4*, 333–343.
8. Jacobs, P. A., & Hassold, T. J. (1995). The origin of numerical chromosome abnormalities. In J. C. Hall, & G. Francesco. *Advances in genetics* (Vol. 33, pp. 101–133). Waltham, MA: Academic Press.
9. Carrel, L., Cottle, A. A., Goglin, K. C., & Willard, H. F. (1999). A first-generation X-inactivation profile of the human X chromosome. *Proc Natl Acad Sci USA*, *96*, 14440–14444.
10. Thomas, N. S., Ennis, S., Sharp, A. J., et al. (2001). Maternal sex chromosome non-disjunction: evidence for X chromosome-specific risk factors. *Hum Mol Genet*, *10*, 243–250.
11. Jacobs, P., Dalton, P., James, R., et al. (1997). Turner syndrome: a cytogenetic and molecular study. *Annals Hum Genet*, *61*, 471–483.
12. Cockwell, A., MacKenzie, M., Youings, S., & Jacobs, P. (1991). A cytogenetic and molecular study of a series of 45,X fetuses and their parents. *J Med Genet*, *28*, 151–155.
13. Hook, E. B., & Warburton, D. (1983). The distribution of chromosomal genotypes associated with Turner's syndrome: livebirth prevalence rates and evidence for diminished fetal mortality and severity in genotypes associated with structural X abnormalities or mosaicism. *Hum Genet*, *64*, 24–27.
14. Held, K. R., Kerber, S., Kaminsky, E., et al. (1992). Mosaicism in 45,X Turner syndrome: does survival in early pregnancy depend on the presence of two sex chromosomes? *Human Genet*, *88*, 288–294.
15. Ogata, T., & Matsuo, N. (1995). Turner syndrome and female sex chromosome aberrations: deduction of the principal factors involved in the development of clinical features. *Hum Genet*, *95*, 607–629.
16. Probst, F. J., Cooper, M. L., Cheung, S. W., & Justice, M. J. (2008). Genotype, phenotype, and karyotype correlation in the xo mouse model of Turner syndrome. *J Hered*, *99*, 512–517.
17. Li, W., Wang, X., Fan, W., et al. (2012). Modeling abnormal early development with induced pluripotent stem cells from aneuploid syndromes. *Hum Mol Genet*, *21*, 32–45.
18. Urbach, A., & Benvenisty, N. (2009). Studying early lethality of 45,XO (Turner's syndrome) embryos using human embryonic stem cells. *PLoS ONE*, *4*, e4175–e4175.
19. Nielsen, J., & Wohlert, M. (1991). Chromosome abnormalities found among 34,910 newborn children: results from a 13-year incidence study in Arhus, Denmark. *Hum Genet*, *87*, 81–83.
20. Stochholm, K., Juul, S., Juel, K., et al. (2006). Prevalence, incidence, diagnostic delay, and mortality in Turner syndrome. *J Clin Endocrinol Metab*, *91*, 3897–8902.
21. Iyer, N. P., Tucker, D. F., Roberts, S. H., et al. (2012). Outcome of fetuses with Turner syndrome: a 10-year congenital anomaly register based study. *J Matern Fetal Neonatal Med*, *25*, 68–73.

22. Baena, N., De Vigan, C., Cariati, E., et al. (2004). Turner syndrome: evaluation of prenatal diagnosis in 19 European registries. *Am J Med Genet A*, *129*, 16–20.
23. Hagman, A., Wennerholm, U. B., Källén, K., et al. (2010). Women who gave birth to girls with Turner syndrome: maternal and neonatal characteristics. *Human Reprod*, *25*, 1553–1560.
24. Cools, M., Rooman, R. P., Wauters, J., et al. (2004). A nonmosaic 45,X karyotype in a mother with Turner's syndrome and in her daughter. *Fertil Steril*, *82*, 923–925.
25. Bernasconi, S., Larizza, D., Benso, L., et al. (1994). Turner's syndrome in Italy: familial characteristics, neonatal data, standards for birth weight and for height and weight from infancy to adulthood. *Acta Pædiatr*, *83*, 292–298.
26. Mathur, A., et al. (1991). The parental origin of the single X chromosome in Turner syndrome: lack of correlation with parental age or clinical phenotype. *Am J Hum Genet*, *48*, 682–686.
27. Hassold, T., Benham, F., & Leppert, M. (1998). Cytogenetic and molecular analysis of sex-chromosome monosomy. *Am J Hum Genet*, *42*, 5345–5341.
28. Jacobs, P. A., Betts, P. R., Cockwell, A. E., et al. (1990). A cytogenetic and molecular reappraisal of a series of patients with Turner's syndrome. *Ann Hum Genet*, *54*(Part 3), 209–223.
29. Chu, C. E., Donaldson, M. D., Kelnar, C. J., et al. (1994). Possible role of imprinting in the Turner phenotype. *J Med Genet*, *31*, 840–842.
30. Massa, G., Vanderschueren-Lodeweyckx, M., & Fryns, J. P. (1992). Deletion of the short arm of the X chromosome: a hereditary form of Turner syndrome. *Eur J Pediatr*, *151*, 893–894.
31. Leichtman, D. A., Schmickel, R. D., Gelehrter, T. D., et al. (1978). Familial Turner syndrome. *Ann Intern Med*, *89*, 473–476.
32. Portnoï, M.-F., Chantot-Bastaraud, S., Christin-Maitre, S., et al. (2012). Familial Turner syndrome with an X;Y translocation mosaicism: Implications for genetic counseling. *Eur J Med Genet*, *55*, 635–640.
33. Bettio, D., Venci, A., & Levi Setti, P. E. (2008). Chromosomal abnormalities in miscarriages after different assisted reproduction procedures. *Placenta*, *29*(Suppl. 2), 126–128.
34. Villaverde, M. M., & Da Silva, J. A. (1975). Turner-mongolism poly syndrome: review of the first eight known cases. *JAMA*, *234*, 844–847.
35. Palmer, C. G., & Reichmann, A. (1976). Chromosomal and clinical findings in 110 females with Turner syndrome. *Human Genet*, *35*, 35–49.
36. Pasquino, A. M., Passeri, F., Pucarelli, I., et al. (1997). Spontaneous pubertal development in Turner's syndrome. Italian Study Group for Turner's Syndrome. *J Clin Endocrinol Metab*, *82*, 1810–1813.
37. Wolff, D. J., Van Dyke, D. L., & Powell, C. M. (2010). Laboratory guideline for Turner syndrome. *Genet Med*, *12*, 52–55.
38. Wolff, D. J., Miller, A. P., Van Dyke, D. L., et al. (1996). Molecular definition of breakpoints associated with human Xq isochromosomes: implications for mechanisms of formation. *Am J Hum Genet*, *58*, 154–160.
39. Scott, S. A., Cohen, N., Brandt, T., et al. (2010). Large inverted repeats within Xp11.2 are present at the breakpoints of isodicentric X chromosomes in Turner syndrome. *Hum Mol Genet*, *19*, 3383–3393.
40. Van Dyke, D. L., Wiktor, A., Palmer, C. G., et al. (1992). Ullrich-Turner syndrome with a small ring X chromosome and presence of mental retardation. *Am J Med Genet*, *43*, 996–1005.
41. Migeon, B. R., Luo, S., Jani, M., & Jeppesen, P. (1994). The severe phenotype of females with tiny ring X chromosomes is associated with inability of these chromosomes to undergo X inactivation. *Am J Hum Genet*, *55*, 497–504.
42. Kolomietz, E., Godbole, K., Winsor, E. J., et al. (2005). Functional disomy of Xp: prenatal findings and postnatal outcome. *American J Med Genet A*, *134*, 393–398.
43. Maraschio, P., Tupler, R., Barbierato, L., et al. (1996). An analysis of Xq deletions. *Hum Genet*, *97*, 375–381.
44. De Groote, K., Cools, M., De Schepper, J., et al. (2013). Cardiovascular pathology in males and females with 45,X/46,XY mosaicism. *PLoS ONE*, *8*, e54977.
45. Graham, B. H., & Bacino, C. A. (2003). Male patient with nonmosaic deleted Y-chromosome and clinical features of Turner syndrome. *Am J Med Genet A*, *119A*, 234–237.
46. Lange, J., Skaletsky, H., van Daalen, S. K., et al. (2009). Isodicentric Y chromosomes and sex disorders as byproducts of homologous recombination that maintains palindromes. *Cell*, *138*, 855–869.
47. Cools, M., Drop, S. L., Wolffenbuttel, K. P., et al. (2006). Germ cell tumors in the intersex gonad: old paths, new directions, moving frontiers. *Endocr Rev*, *27*, 468–484.
48. Ferguson-Smith, M. A. (1965). Karyotype-phenotype correlations in gonadal dysgenesis and their bearing on the pathogenesis of malformations. *J Med Genet*, *2*, 142–155.
49. Zinn, A. R., Tonk, V. S., Chen, Z., et al. (1998). Evidence for a Turner syndrome locus or loci at Xp11.2-p22.1. *Am J Hum Genet*, *63*, 1757–1766.
50. Bondy, C., & Cheng, C. (2009). Monosomy for the X chromosome. *Chromosome Res*, *17*, 649–658.
51. Ross, M. T., Grafham, D. V., Coffey, A. J., et al. (2005). The DNA sequence of the human X chromosome. *Nature*, *434*, 325–337.
52. Ellison, J. W., Wardak, Z., Young, M. F., et al. (1997). PHOG, a candidate gene for involvement in the short stature of Turner syndrome. *Hum Mol Genet*, *6*, 1341–1347.
53. Rao, E., Weiss, B., Fukami, M., et al. (1997). Pseudoautosomal deletions encompassing a novel homeobox gene cause growth failure in idiopathic short stature and Turner syndrome. *Nat Genet*, *16*, 54–63.
54. Munns, C. J., Haase, H. R., Crowther, L. M., et al. (2004). Expression of SHOX in human fetal and childhood growth plate. *J Clin Endocrinol Metab*, *89*, 4130–4135.
55. Blaschke, R. J., & Rappold, G. A. (2000). SHOX: growth, Leri-Weill and Turner syndromes. *Trends Endocrinol Metab*, *11*, 227–230.
56. Blum, W. F., Crowe, B. J., Quigley, C. A., et al. (2007). Growth hormone is effective in treatment of short stature associated with short stature homeobox-containing gene deficiency: two-year results of a randomized, controlled, multicenter trial. *J Clin Endocrinol Metab*, *92*, 219–228.
57. Zinn, A. R., Roeltgen, D., Stefanatos, G., et al. (2007). A Turner syndrome neurocognitive phenotype maps to Xp22.3. *Behav Brain Funct*, *3*, 24.
58. Ogata, T., Muroya, K., Matsuo, N., et al. (2001). Turner syndrome and Xp deletions: clinical and molecular studies in 47 patients. *J Clin Endocrinol Metab*, *86*, 5498–5508.
59. Lachlan, K. L., Youings, S., Costa, T., et al. (2006). A clinical and molecular study of 26 females with Xp deletions with special emphasis on inherited deletions. *Hum Genet*, *118*, 640–651.
60. Bondy, C., Bakalov, V. K., Cheng, C., et al. (2013). Bicuspaid aortic valve and aortic coarctation are associated with deletion of the X chromosome short arm. *J Med Genet*, *50*, 662–665.
61. King, K. A., Makishima, T., Zalewski, C. K., et al. (2007). Analysis of auditory phenotype and karyotype in 200 females with Turner syndrome. *Ear Hear*, *28*, 831–841.
62. Verver, E. J., Freriks, K., Thomeer, H. G., et al. (2011). Ear and hearing problems in relation to karyotype in children with Turner syndrome. *Hearing Res*, *275*, 81–88.
63. McCarthy, K., & Bondy, C. (2008). Turner syndrome in childhood and adolescence. *Expert Rev Endocrinol Metab*, *3*, 771–775.
64. Zinn, A. R., & Ross, J. L. (1998). Turner syndrome and haploinsufficiency. *Current Opinion in Genetics & Development*, *8*, 322–327.
65. Bondy, C. A., Hougen, H. Y., Zhou, J., & Cheng, C. M. (2012). Genomic imprinting in Turner syndrome. *Pediatric Endocrinol Rev*, *2*(Suppl.), 728–732.
66. Skuse, D. H., James, R. S., Bishop, D. V., et al. (1997). Evidence from Turner's syndrome of an imprinted X-linked locus affecting cognitive function. *Nature*, *387*, 705–708.
67. Lepage, J. F., Hong, D. S., Hallmayer, J., & Reiss, A. L. (2012). Genomic imprinting effects on cognitive and social abilities in prepubertal girls with Turner syndrome. J Clin Endocrinol Metab, 97, E460–E464.
68. Lepage, J. F., Hong, D. S., Mazaika, P. K., et al. (2013). Genomic imprinting effects of the X chromosome on brain morphology. *J Neuroscience*, *33*, 8567–8574.
69. Mathur, A., et al. (1991). The parental origin of the single X chromosome in Turner syndrome: lack of correlation with parental age or clinical phenotype. *Am J Hum Genet*, *48*, 682–686.

70. Tsezou, A., Hadjiathanasiou, C., Gourgiotis, D., et al. (1999). Molecular genetics of Turner syndrome: correlation with clinical phenotype and response to growth hormone therapy. *Clin Genet*, *56*, 441–446.
71. Sagi, L., Zuckerman-Levin, N., Gawlik, A., et al. (2007). Clinical significance of the parental origin of the X chromosome in Turner syndrome. *J Clin Endocrinol Metab*, *92*, 846–852.
72. Kochi, C., Longui, C. A., Lemos-Marini, S. H., et al. (2007). The influence of parental origin of X chromosome genes on the stature of patients with 45 X Turner syndrome. *Genet Mol Res*, *6*, 1–7.
73. Bondy, C., et al. (2007). The physical phenotype of girls and women with Turner syndrome is not X-imprinted. *Hum Genet*, *121*, 469–474.
74. Hamelin, C. E., Anglin, G., Quigley, C. A., & Deal, C. L. (2006). Genomic imprinting in Turner syndrome: effects on response to growth hormone and on risk of sensorineural hearing loss. *J Clin Endocrinol Metab*, *91*, 3002–3010.
75. Devernay, M., Bolca, D., Kerdjana, L., et al. (2012). Parental origin of the X-chromosome does not influence growth hormone treatment effect in turner syndrome. *J Clin Endocrinol Metab*, *97*, E1241–E1248.
76. Van, P. L., Bakalov, V. K., Zinn, A. R., & Bondy, C. A. (2006). Maternal X chromosome, visceral adiposity, and lipid profile. *JAMA*, *295*, 1373–1374.
77. Saenger, P., Wikland, K. A., Conway, G. S. et al. (2001). Recommendations for the diagnosis and management of Turner syndrome. *J Clin Endocrinol Metab*, *86*, 3061–3069.
78. Bondy, C. A., Turner Syndrome Study Group (2007). Care of girls and women with Turner syndrome: a guideline of the Turner Syndrome Study Group. *J Clin Endocrinol Metab*, *92*, 10–25.
79. Rivkees, S. A., Hager, K., Hosono, S., et al. (2011). A highly sensitive, high-throughput assay for the detection of Turner syndrome. *J Clin Endocrinol Metab*, *96*, 699–705.
80. Prakash, S., Guo, D., Maslen, C. L., et al. (2013). Single-nucleotide polymorphism array genotyping is equivalent to metaphase cytogenetics for diagnosis of Turner syndrome. Genet Med. Advance online publication.
81. Noonan, J. A. (1968). Hypertelorism with Turner phenotype: a new syndrome with associated congenital heart disease. *Am J Dis Child*, *116*, 373–380.
82. Ganapathy, R., Guven, M., Sethna, F., et al. (2004). Natural history and outcome of prenatally diagnosed cystic hygroma. *Prenat Diagn*, *24*, 965–968.
83. Gunther, D. F., Eugster, E., Zagar, A. J., et al. (2004). Ascertainment bias in Turner syndrome: new insights from girls who were diagnosed incidentally in prenatal life. *Pediatrics*, *114*, 640–644.
84. Telvi, L., Lebbar, A., Del Pino, O., et al. (1999). 45,X/46,XY mosaicism: report of 27 cases. *Pediatrics*, *104*(2 Pt. 1), 304–308.
85. Hsu, L. Y. (1994). Phenotype/karyotype correlations of Y chromosome aneuploidy with emphasis on structural aberrations in postnatally diagnosed cases. *American J Med Genet*, *53*, 108–140.
86. Koeberl, D. D., McGillivray, B., & Sybert, V. P. (1995). Prenatal diagnosis of 45,X/46,XX mosaicism and 45,X: implications for postnatal outcome. *Am J Hum Genet*, *57*, 661–666.
87. Wapner, R. J., Martin, C. L., Levy, B., et al. (2012). Chromosomal microarray versus karyotyping for prenatal diagnosis. *N Engl J Med*, *367*, 2175–2184.
88. van der Putte, S. C. (1977). Lymphatic malformation in human fetuses: a study of fetuses with Turner's syndrome or status Bonnevie-Ullrich. *Virchows Arch A Pathol Anat Histol*, *376*, 233–246.
89. Vittay, P., Bösze, P., Gaál, M., & László, J. (1980). Lymph vessel defects in patients with ovarian dysgenesis. *Clin Genet*, *18*, 387–391.
90. Collins, E. (1973). The illusion of widely spaced nipples in the Noonan and the Turner syndromes. *J Pediatr*, *83*, 557–561.
91. Felix, A., Capek, V., & Pashayan, H. M. (1974). The neck in the XO and XX-XO mosaic Turner's syndrome. *Clin Genet*, *5*, 77–80.
92. Neufeld, N. D., Lippe, B. M., & Kaplan, S. A. (1978). Disproportionate growth of the lower extremities: a major determinant of short stature in Turner's syndrome. *Am J Dis Child*, *132*, 296–298.
93. Beals, R. K. (1973). Orthopedic aspects of the XO (Turner's) syndrome. *Clin Orthop Relat Res*, *1973*, 19–30.
94. Kaitila, I., Leisti, J. T., & Rimoin, D. L. (1976). Mesomelic skeletal dysplasias. *Clin Orthop Relat Res*, *1976*, 94–106.
95. Elder, D. A., Roper, M. G., Henderson, R. C., & Davenport, M. L. (2002). Kyphosis in a Turner syndrome population. *Pediatrics*, *109*, e93.
96. Bercu, B. B., Kramer, S. S., & Bode, H. H. (1976). A useful radiologic sign for the diagnosis of Turner's syndrome. *Pediatrics*, *58*, 737–739.
97. Necic, S., & Grant, D. B. (1978). Diagnostic value of hand X-rays in Turner's syndrome. *Acta Paediatr Scand*, *67*, 309–312.
98. Ari, M., Bakalov, V. K., Hill, S., & Bondy, C. A. (2006). The effects of growth hormone treatment on bone mineral density and body composition in girls with Turner syndrome. *J Clin Endocrinol Metab*, *91*, 4302–4305.
99. Ross, J. L., Long, L. M., Feuillan, P., et al. (1991). Normal bone density of the wrist and spine and increased wrist fractures in girls with Turner's syndrome. *J Clin Endocrinol Metab*, *73*, 355–359.
100. Ranke, M. B., Pflüger, H., Rosendahl, W., et al. (1983). Turner syndrome: spontaneous growth in 150 cases and review of the literature. *Eur J Pediatr*, *141*, 81–88.
101. Davenport, M. L., Punyasavatsut, N., Gunther, D., et al. (1999). Turner syndrome: a pattern of early growth failure. *Acta Paediatr Suppl*, *88*, 118–121.
102. Even, L., Cohen, A., Marbach, N., et al. (2000). Longitudinal analysis of growth over the first 3 years of life in Turner's syndrome. *J Pediatr*, *137*, 460–464.
103. Massa, G. G., & Vanderschueren-Lodeweyckx, M. (1991). Age and height at diagnosis in Turner syndrome: influence of parental height. *Pediatrics*, *88*, 1148–1152.
104. Brook, C. G., Mürset, G., Zachmann, M., & Prader, A. (1974). Growth in children with 45,XO Turner's syndrome. *Arch Dis Child*, *49*, 789–795.
105. Ranke, M. B. (1999). Turner syndrome. *Eur J Endocrinol*, *141*, 216–217.
106. Lyon, A. J., Preece, M. A., & Grant, D. B. (1985). Growth curve for girls with Turner syndrome. *Arch Dis Child*, *60*, 932–935.
107. Singh, R. P., & Carr, D. H. (1966). The anatomy and histology of XO human embryos and fetuses. *Anat Rec*, *155*, 369–383.
108. Weiss, L. (1971). Additional evidence of gradual loss of germ cells in the pathogenesis of streak ovaries in Turner's syndrome. *J Med Genet*, *8*, 540–544.
109. Reynaud, K., Cortvrindt, R., Verlinde, F., et al. (2004). Number of ovarian follicles in human fetuses with the 45,X karyotype. *Fertil Steril*, *81*, 1112–1119.
110. Hreinsson, J., Otala, M., Fridstrom, M., et al. (2002). Follicles are found in the ovaries of adolescent girls with Turner's syndrome. *J Clin Endocrinol Metab*, *87*, 3618–3623.
111. Speed, R. M. (1988). The possible role of meiotic pairing anomalies in the atresia of human fetal oocytes. *Hum Genet*, *78*, 260–266.
112. Therman, E., & Susman, B. (1990). The similarity of phenotypic effects caused by Xp and Xq deletions in the human female: a hypothesis. *Hum Genet*, *85*, 175–183.
113. Mercer, C. L., Lachlan, K., Karcanias, A., et al. (2013). Detailed clinical and molecular study of 20 females with Xq deletions with special reference to menstruation and fertility. *Eur J Med Genet*, *56*, 1–6.
114. Borgström, B., Hreinsson, J., Rasmussen, C., et al. (2009). Fertility preservation in girls with turner syndrome: prognostic signs of the presence of ovarian follicles. *J Clin Endocrinol Metab*, *94*, 74–80.
115. Hovatta, O. (1999). Pregnancies in women with Turner's syndrome. *Ann Med*, *31*, 106–110.
116. Hadnott, T. N., Gould, H. N., Gharib, A. M., & Bondy, C. A. (2011). Outcomes of spontaneous and assisted pregnancies in Turner syndrome: the U.S. National Institutes of Health experience. *Fertil Steril*, *95*, 2251–2256.
117. Mortensen, K. H., Rohde, M. D., Uldbjerg, N., & Gravholt, C. H. (2010). Repeated spontaneous pregnancies in 45,X Turner syndrome. *Obstet Gynecol*, *115*, 446–449.
118. Tarani, L., Lampariello, S., Raguso, G., et al. (1998). Pregnancy in patients with Turner's syndrome: six new cases and review of literature. *Gynecol Endocrinol*, *12*, 83–87.
119. Hagman, A., Källén, K., Barrenäs, M. L., et al. (2011). Obstetric outcomes in women with Turner karyotype. *J Clin Endocrinol Metab*, *96*, 3475–3482.

120. Mortensen, K. H., Cleemann, L., Hjerrild, B. E., et al. (2009). Increased prevalence of autoimmunity in Turner syndrome—influence of age. *Clin Exp Immunol*, *156*, 205–210.
121. Gravholt, C. H., Juul, S., Naeraa, R. W., & Hansen, J. (1998). Morbidity in Turner syndrome. *J Clin Epidemiol*, *51*, 147–158.
122. Schoemaker, M. J., Swerdlow, A. J., Higgins, C. D., et al. (2008). Mortality in women with turner syndrome in Great Britain: a national cohort study. *J Clin Endocrinol Metab*, *93*, 4735–4742.
123. Landin-Wilhelmsen, K., Bryman, I., Hanson, C., & Hanson, L. (2004). Spontaneous pregnancies in a Turner syndrome woman with Y-chromosome mosaicism. *J Assist Reprod Genet*, *21*, 229–230.
124. Wei, F., Cheng, S., Badie, N., et al. (2001). A man who inherited his SRY gene and Leri-Weill dyschondrosteosis from his mother and neurofibromatosis type 1 from his father. *Am J Med Genet*, *102*, 353–358.
125. Saenger, P., Wikland, K. A., Conway, G. S., et al. (2001). Recommendations for the diagnosis and management of Turner syndrome. *J Clin Endocrinol Metab*, *86*, 3061–3069.
126. Bondy, C. A., Turner Syndrome Study Group (2007). Care of girls and women with Turner syndrome: a guideline of the Turner Syndrome Study Group. *J Clin Endocrinol Metab*, *92*, 10–25.
127. Nishi, M. Y., Domenice, S., Medeiros, M. A., et al. (2002). Detection of Y-specific sequences in 122 patients with Turner syndrome: nested PCR is not a reliable method. *Am J Med Genet*, *107*, 299–305.
128. Price, W. H., Clayton, J. F., Collyer, S., et al. (1986). Mortality ratios, life expectancy, and causes of death in patients with Turner's syndrome. *J Epidemiol Community Health*, *40*, 97–102.
129. Haddad, H. M., & Wilkins, L. (1959). Congenital anomalies associated with gonadal aplasia; review of 55 cases. *Pediatrics*, *23*, 885–902.
130. Nora, J. J., Torres, F. G., Sinha, A. K., & McNamara, D. G. (1970). Characteristic cardiovascular anomalies of XO Turner syndrome, XX and XY phenotype and XO-XX Turner mosaic. *Am J Cardiol*, *25*, 639–641.
131. Sybert, V. P. (1998). Cardiovascular malformations and complications in Turner syndrome. *Pediatrics*, *101*, e11.
132. Patel, A., Hickey, E., Mavroudis, C., et al. (2010). Impact of noncardiac congenital and genetic abnormalities on outcomes in hypoplastic left heart syndrome. *Ann Thorac Surg*, *89*, 1805–1813.
133. Clark, E. B. (1984). Neck web and congenital heart defects: a pathogenic association in 45 X-O Turner syndrome. *Teratology*, *29*, 355–361.
134. Lacro, R. V., Jones, K. L., & Benirschke, K. (1988). Coarctation of the aorta in Turner syndrome: a pathologic study of fetuses with nuchal cystic hygromas, hydrops fetalis, and female genitalia. *Pediatrics*, *81*, 445–451.
135. Mazzanti, L., & Cacciari, E. (1998). Congenital heart disease in patients with Turner's syndrome. Italian Study Group for Turner Syndrome (ISGTS). *J Pediatr*, *133*, 688–692.
136. Loscalzo, M. L., Van, P. L., Ho, V. B., et al. (2005). Association between fetal lymphedema and congenital cardiovascular defects in Turner syndrome. *Pediatrics*, *115*, 732–735.
137. Miyabara, S., Nakayama, M., Suzumori, K., et al. (1997). Developmental analysis of cardiovascular system of 45,X fetuses with cystic hygroma. *Am J Med Genet*, *68*, 135–141.
138. Bondy, C. A. (2008). Congenital cardiovascular disease in Turner syndrome. *Congenit Heart Dis*, *3*, 2–15.
139. Sachdev, V., Matura, L. A., Sidenko, S., et al. (2008). Aortic valve disease in Turner syndrome. *J Am Coll Cardiol*, *51*, 1904–1909.
140. Bondy, C. A. (2012). Aortic coarctation and coronary artery disease. *Circulation*, *126*, 5–7.
141. Flaquer, A., Fischer, C., & Wienker, T. F. (2009). A new sex-specific genetic map of the human pseudoautosomal regions (PAR1 and PAR2). *Hum Hered*, *68*, 192–200.
142. Gravholt, C. H., Juul, S., Naeraa, R. W., & Hansen, J. (1998). Morbidity in Turner syndrome. *J Clin Epidemiol*, *51*, 147–158.
143. Schoemaker, M. J., Swerdlow, A. J., Higgins, C. D., et al. (2008). Mortality in women with Turner syndrome in Great Britain: a national cohort study. *J Clin Endocrinol Metab*, *93*, 4735–4742.
144. Donadille, B., Rousseau, A., Zenaty, D., et al. (2012). Cardiovascular findings and management in Turner syndrome: insights from a French cohort. *Eur J Endocrinol*, *167*, 517–522.
145. Miller, M. J., Geffner, M. E., Lippe, B. M., et al. (1983). Echocardiography reveals a high incidence of bicuspid aortic valve in Turner syndrome. *J Pediatr*, *102*, 47–50.
146. Lin, A. E., Lippe, B. M., Geffner, M. E., et al. (1986). Aortic dilation, dissection, and rupture in patients with Turner syndrome. *J Pediatr*, *109*, 820–826.
147. Lin, A. E., Lippe, B., & Rosenfeld, R. G. (1998). Further delineation of aortic dilation, dissection, and rupture in patients with Turner syndrome. *Pediatrics*, *102*, e12.
148. Matura, L. A., Ho, V. B., Rosing, D. R., & Bondy, C. A. (2007). Aortic dilatation and dissection in Turner syndrome. *Circulation*, *116*, 1663–1670.
149. Carlson, M., & Silberbach, M. (2007). Dissection of the aorta in Turner syndrome: two new cases and review of 85 cases in the literature. *J Med Genet*, *44*, 745–749.
150. Gravholt, C. H., Landin-Wilhelmsen, K., Stochholm, K., et al. (2006). Clinical and epidemiological description of aortic dissection in Turner's syndrome. *Cardiol Young*, *16*, 430–436.
151. Lopez, L., Arheart, K. L., Colan, S. D., et al. (2008). Turner syndrome is an independent risk factor for aortic dilation in the young. *Pediatrics*, *121*, e1622–e1627.
152. Ostberg, J. E., Brookes, J. A., McCarthy, C., et al. (2004). A comparison of echocardiography and magnetic resonance imaging in cardiovascular screening of adults with Turner syndrome. *J Clin Endocrinol Metab*, *89*, 5966–5971.
153. Hjerrild, B. E., Mortensen, K. H., Sørensen, K. E., et al. (2010). Thoracic aortopathy in Turner syndrome and the influence of bicuspid aortic valves and blood pressure: a CMR study. *J Cardiovasc Magn Reson*, *12*, 12.
154. Ostberg, J. E., Donald, A. E., Halcox, J. P., et al. (2005). Vasculopathy in Turner syndrome: arterial dilatation and intimal thickening without endothelial dysfunction. *J Clin Endocrinol Metab*, *90*, 5161–5166.
155. Polkampally, P. R., Matta, J. R., McAreavey, D., et al. (2011). Aneurysmal dilatation of medium caliber arteries in Turner syndrome. *Congenit Heart Dis*, *6*, 382–383.
156. Bondy, C. A. (2008). Aortic dissection in Turner syndrome. *Curr Opin Cardiol*, *23*, 519–526
157. Cabanes, L., Chalas, C., Christin-Maitre, S., et al. (2010). Turner syndrome and pregnancy: clinical practice. Recommendations for the management of patients with Turner syndrome before and during pregnancy. *Eur J Obstet Gynecol Reprod Biol*, *152*, 18–24.
158. American Society for Reproductive Medicine, Increased maternal cardiovascular mortality associated with pregnancy in women with Turner syndrome. (2012). *Fertil Steril*, *97*, 282–284.
159. van Wassenaer, A. G., Lubbers, L. J., & Losekoot, G. (1988). Partial abnormal pulmonary venous return in Turner syndrome. *Eur J Pediatr*, *148*, 101–103.
160. Bechtold, S. M., Dalla Pozza, R., Becker, A., et al. (2004). Partial anomalous pulmonary vein connection: an underestimated cardiovascular defect in Ullrich-Turner syndrome. *Eur J Pediatr*, *163*, 158–162.
161. Gotzsche, C., & Krag-Olsen, B. (1994). Prevalence of cardiovascular malformations and association with karyotypes in Turner's syndrome. *Arch Dis Child*, *71*, 433–436.
162. Völkl, T. M., Degenhardt, K., Koch, A., et al. (2005). Cardiovascular anomalies in children and young adults with Ullrich-Turner syndrome the Erlangen experience. *Clin Cardiol*, *28*, 88–92.
163. Ho, V. B., Bakalov, V. K., Cooley, M., et al. (2004). Major vascular anomalies in Turner syndrome: prevalence and magnetic resonance angiographic features. *Circulation*, *110*, 1694–1700.
164. Kim, H. K., Gottliebson, W., Hor, K., et al. (2011). Cardiovascular anomalies in Turner syndrome: spectrum, prevalence, and cardiac MRI findings in a pediatric and young adult population. *AJR Am J Roentgenol*, *196*, 454–460.
165. Banerji, D., Martinez, F., Abbara, S., & Truong, Q. A. (2011). Turner syndrome with aberrant right subclavian artery and partial anomalous pulmonary venous return. *J Cardiovasc Comput Tomogr*, *5*, 189–191.
166. Bondy, C. A., Ceniceros, I., Van, P. L., et al. (2006). Prolonged rate-corrected QT interval and other electrocardiogram abnormalities in girls with Turner syndrome. *Pediatrics*, *118*, e1220–e1225.
167. Bondy, C. A., Ceniceros, I., Van, P. L., et al. (2006). Prolonged rate-corrected QT interval and other electrocardiogram abnormalities in girls with Turner syndrome. *Pediatrics*, *118*, e1220–e1225.

168. Dalla Pozza, R., Bechtold, S., Kääb, S., et al. (2006). QTc interval prolongation in children with Ulrich-Turner syndrome. *Eur J Pediatr*, *165*, 831–837.
169. Zuckerman-Levin, N., Zinder, O., Greenberg, A., et al. (2006). Physiological and catecholamine response to sympathetic stimulation in turner syndrome. *Clin Endocrinol*, *64*, 410–415.
170. Gravholt, C. H., Hansen, K. W., Erlandsen, M., et al. (2006). Nocturnal hypertension and impaired sympathovagal tone in Turner syndrome. *J Hypertens*, *24*, 353–360.
171. Nathwani, N. C., Unwin, R., Brook, C. G., & Hindmarsh, P. C. (2000). Blood pressure and Turner syndrome. *Clin Endocrinol* (Oxf), *52*, 363–370.
172. Landin-Wilhelmsen, K., Bryman, I., & Wilhelmsen, L. (2001). Cardiac malformations and hypertension, but not metabolic risk factors, are common in Turner syndrome. *J Clin Endocrinol Metab*, *86*, 4166–4170.
173. Elsheikh, M., Dunger, D. B., Conway, G. S., & Wass, J. A. (2002). Turner's syndrome in adulthood. *Endocr Rev*, *23*, 120–140.
174. Nathwani, N. C., Unwin, R., Brook, C. G., & Hindmarsh, P. C. (2000). The influence of renal and cardiovascular abnormalities on blood pressure in Turner syndrome. *Clin Endocrinol* (Oxf), *52*, 371–377.
175. Gravholt, C. H., Naeraa, R. W., Nyholm, B., et al. (1998). Glucose metabolism, lipid metabolism, and cardiovascular risk factors in adult Turner's syndrome: the impact of sex hormone replacement. *Diabetes Care*, *21*, 1062–1070.
176. Roest, A. A.W, & de Roos, A. (2012). Imaging of patients with congenital heart disease. *Nat Rev Cardiol*, *9*, 101–115.
177. Dawson-Falk, K. L., Wright, A. M., Bakker, B., et al. (1992). Cardiovascular evaluation in Turner syndrome: utility of MR imaging. *Australas Radiol*, *36*, 204–209.
178. Castro, A. V., Okoshi, K., Ribeiro, S. M., et al. (2002). Cardiovascular assessment of patients with Ullrich-Turner's syndrome on Doppler echocardiography and magnetic resonance imaging. *Arq Bras Cardiol*, *78*, 51–58.
179. Bondy, C. A. (2012). Aortic coarctation and coronary artery disease: the XY factor. *Circulation*, *126*, 5–7.
180. Mebus, S., Meierhofer, C., Pringsheim, M., et al. (2011). How late is too late? Giant balloon-like aneurysm of the ascending aorta. *JAMA*, *124*, e3–e4.
181. Oza, N. M., Siegenthaler, M., Horvath, K., et al. (2013). Serious aortic complications in a patient with Turner syndrome. *Eur J Pediatr*, *172*, 703–705.
182. Pleskacova, J., Rucklova, K., Popelova, J., et al. (2010). Aortic dissection and rupture in a 16-year-old girl with Turner syndrome following previous progression of aortic dilation. *Eur J Pediatr*, *169*, 1283–1286.
183. Lanzarini, L., Larizza, D., Prete, G., et al. (2007). Prospective evaluation of aortic dimensions in Turner syndrome: a 2-dimensional echocardiographic study. *J Am Soc Echocardiogr*, *20*, 307–313.
184. Mortensen, K. H., Hjerrild, B. E., Stochholm, K., et al. (2011). Dilation of the ascending aorta in Turner syndrome: a prospective cardiovascular magnetic resonance study. *J Cardiovasc Magn Reson*, *13*, 24.
185. Gutin, L. S., Bakalov, V. K., Rosing, D. R., et al. (2012). N-terminal pro brain natriuretic peptide levels and aortic diameters. *Am Heart J*, *164*, 419–424.
186. Stochholm, K., Juul, S., Juel, K., et al. (2006). Prevalence, incidence, diagnostic delay, and mortality in Turner syndrome. *J Clin Endocrinol Metab*, *91*, 3897–3902.
187. Ross, J. L., Feuillan, P., Long, L. M., et al. (1995). Lipid abnormalities in Turner syndrome. *J Pediatr*, *126*, 242–245.
188. Wooten, N., Bakalov, V. K., Hill, S., & Bondy, C. A. (2008). Reduced abdominal adiposity and improved glucose tolerance in growth hormone-treated girls with Turner syndrome. *J Clin Endocrinol Metab*, *93*, 2109–2114.
189. Lippe, B., Geffner, M. E., Dietrich, R. B., et al. (1988). Renal malformations in patients with Turner syndrome: imaging in 141 patients. *Pediatrics*, *82*, 852–856.
190. Loscalzo, M. L., Van, P. L., Ho, V. B., et al. (2005). Association between fetal lymphedema and congenital cardiovascular defects in Turner syndrome. *Pediatrics*, *115*, 732–735.
191. Anderson, H., Filipsson, R., Fluur, E., et al. (1969). Hearing impairment in Turner's syndrome. *Acta Otolaryngol*, (Suppl.), *247*, 1–26.
192. Barrenas, M. L., Nylen, O., & Hanson, C. (1999). The influence of karyotype on the auricle, otitis media and hearing in Turner syndrome. *Hear Res*, *138*, 163–170.
193. Stenberg, A. E., Nylén, O., Windh, M., & Hultcrantz, M. (1998). Otological problems in children with Turner's syndrome. *Hear Res*, *124*, 85–90.
194. Dhooge, I. J., De Vel, E., Verhoye, C., et al. (2005). Otologic disease in turner syndrome. *Otol Neurotol*, *26*, 145–150.
195. Sparkes, R. S., & Motulsky, A. G. (1963). Hashimoto's disease in Turner's syndrome with isochromosome X. *Lancet*, *1*, 947.
196. Elsheikh, M., Wass, J. A., & Conway, G. S. (2001). Autoimmune thyroid syndrome in women with Turner's syndrome—the association with karyotype. *Clin Endocrinol* (Oxf), *55*, 223–226.
197. Radetti, G., Mazzanti, L., Paganini, C., et al. (1995). Frequency, clinical and laboratory features of thyroiditis in girls with Turner's syndrome. The Italian Study Group for Turner's Syndrome. *Acta Paediatr*, *84*, 909–912.
198. Bakalov, V. K., Gutin, L., Cheng, C. M., et al. (2012). Autoimmune disorders in women with turner syndrome and women with karyotypically normal primary ovarian insufficiency. *J Autoimmun*, *38*, 315–321.
199. Brooks, W. H., Meek, J. C., & Schimke, R. N. (1977). Gonadal dysgenesis with Graves's disease. *J Med Genet*, *14*, 128–129.
200. El-Mansoury, M., Bryman, I., Berntorp, K., et al. (2005). Hypothyroidism is common in turner syndrome: results of a five-year follow-up. *J Clin Endocrinol Metab*, *90*, 2131–2135.
201. Livadas, S., Xekouki, P., Fouka, F., et al. (2005). Prevalence of thyroid dysfunction in Turner's syndrome: a long-term follow-up study and brief literature review. *Thyroid*, *15*, 1061–1066.
202. Zulian, F., Schumacher, H. R., Calore, A., et al. (1998). Juvenile arthritis in Turner's syndrome: a multicenter study. *Clin Exp Rheumatol*, *16*, 489–494.
203. Salerno, M., Di Maio, S., Gasparini, N., Rizzo, M., et al. (1999). Liver abnormalities in Turner syndrome. *Eur J Pediatr*, *158*, 618–623.
204. Koulouri, O., Ostberg, J., & Conway, G. (2008). Liver dysfunction in Turner's syndrome: prevalence, natural history and effect of exogenous oestrogen. *Clin Endocrinol*, *69*, 306–310.
205. Wemme, H., Pohlenz, J., & Schonberger, W. (1995). Effect of oestrogen/gestagen replacement therapy on liver enzymes in patients with Ullrich-Turner syndrome. *Eur J Pediatr*, *154*, 807–810.
206. Roulot, D. (2013). Liver involvement in Turner syndrome. *Liver Int*, *33*, 24–30.
207. Rosen, K. M., Sirota, D. K., & Marinoff, S. C. (1967). Gastrointestinal bleeding in Turner's syndrome. *Ann Intern Med*, *67*, 145–150.
208. Salomonowitz, E., Staffen, A., Pötzi, R., et al. (1983). Angiographic demonstration of phlebectasia in a case of Turner's syndrome. *Gastrointest Radiol*, *8*, 279–281.
209. van Cutsem, E., Rutgeerts, P., & Vantrappen, G. (1990). Treatment of bleeding gastrointestinal vascular malformations with oestrogen-progesterone. *Lancet*, *335*, 953–955.
210. O'Hare, J. P., Hamilton, M., Davies, J. D., et al. (1986). Oestrogen deficiency and bleeding from large bowel telangiectasia in Turner's syndrome. *J R Soc Med*, *79*, 746–747.
211. Arulanantham, K., Kramer, M. S., & Gryboski, J. D. (1980). The association of inflammatory bowel disease and X chromosomal abnormality. *Pediatrics*, *66*, 63–67.
212. Price, W. H. (1979). A high incidence of chronic inflammatory bowel disease in patients with Turner's syndrome. *J Med Genet*, *16*, 263–266.
213. Knudtzon, J., & Svane, S. (1988). Turner's syndrome associated with chronic inflammatory bowel disease: a case report and review of the literature. *Acta Med Scand*, *223*, 375–378.
214. Manzione, N. C., Kram, M., Kram, E., & Das, K. M. (1988). Turner's syndrome and inflammatory bowel disease: a case report with immunologic studies. *Am J Gastroenterol*, *83*, 1294–1297.
215. Hayward, P. A., Satsangi, J., & Jewell, D. P. (1996). Inflammatory bowel disease and the X chromosome. *QJM*, *89*, 713–718.
216. Bonamico, M., Bottaro, G., Pasquino, A. M., et al. (1998). Celiac disease and Turner syndrome. *J Pediatr Gastroenterol Nutr*, *26*, 496–499.
217. Ivarsson, S. A., Carlsson, A., Bredberg, A., et al. (1999). Prevalence of coeliac disease in Turner syndrome. *Acta Paediatr*, *88*, 933–936.

218. Schewior, S., Brand, M., & Santer, R. (1999). Celiac disease and selective IgA deficiency in a girl with atypical Turner syndrome. *J Pediatr Gastroenterol Nutr*, *28*, 353–354.
219. Bonamico, M., Pasquino, A. M., Mariani, P., et al. (2002). Prevalence and clinical picture of celiac disease in Turner syndrome. *J Clin Endocrinol Metab*, *87*, 5495–5498.
220. Hill, I. D., Dirks, M. H., Liptak, G. S., et al. (2005). Guideline for the diagnosis and treatment of celiac disease in children: recommendations of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. *J Pediatr Gastroenterol Nutr*, *40*, 1–19.
221. Forbes, A. P., & Engel, E. (1963). The high incidence of diabetes mellitus in 41 patients with gonadal dysgenesis, and their close relatives. *Metabolism*, *12*, 428–439.
222. AvRuskin, T. W., Crigler, J. F., Jr., & Soeldner, J. S. (1979). Turner's syndrome and carbohydrate metabolism. I. Impaired insulin secretion after tolbutamide and glucagon stimulation tests: evidence of insulin deficiency. *Am J Med Sci*, *277*, 145–152.
223. Neufeld, N. D., Lippe, B., & Sperling, M. A. (1980). Carbohydrate (CHO) intolerance in gonadal dysgenesis: A new model of insulin resistance. *Diabetes*, *25*, 379.
224. Caprio, S., Boulware, S., Diamond, M., et al. (1991). Insulin resistance: an early metabolic defect of Turner's syndrome. *J Clin Endocrinol Metab*, *72*, 832–836.
225. Gravholt, C. H., Naeraa, R. W., Nyholm, B., et al. (1998). Glucose metabolism, lipid metabolism, and cardiovascular risk factors in adult Turner's syndrome. The impact of sex hormone replacement. *Diabetes Care*, *21*, 1062–1070.
226. Bakalov, V. K., Cooley, M. M., Quon, M. J., et al. (2004). Impaired insulin secretion in the Turner metabolic syndrome. *J Clin Endocrinol Metab*, *89*, 3516–3520.
227. Bakalov, V. K., Cheng, C., Zhou, J., & Bondy, C. A. (2009). X-chromosome gene dosage and the risk of diabetes in Turner syndrome. *J Clin Endocrinol Metab*, *94*, 3289–3296.
228. Salgin, B., Amin, R., Yuen, K., et al. (2006). Insulin resistance is an intrinsic defect independent of fat mass in women with Turner's syndrome. *Horm Res*, *65*, 69–75.
229. Bakalov, V. K., Cooley, M. M., Troendle, J., & Bondy, C. A. (2004). The prevalence of diabetes mellitus in the parents of women with Turner's syndrome. *Clin Endocrinol*, *60*, 272–272.
230. Bakalov, V. K., Cheng, C., Zhou, J., & Bondy, C. A. (2009). X-chromosome gene dosage and the risk of diabetes in Turner syndrome. J *Clin Endocrinol Metab*, *94*, 3289–3296.
231. O'Gorman, C. S., Syme, C., Lang, J., et al. (2013). An evaluation of early cardiometabolic risk factors in children and adolescents with Turner syndrome. *Clin Endocrinol*, *78*, 907–913.
232. Ostberg, J. E., Attar, M. J., Mohamed-Ali, V., & Conway, G. S. (2005). Adipokine dysregulation in Turner syndrome: comparison of circulating interleukin-6 and leptin concentrations with measures of adiposity and C-reactive protein. *J Clin Endocrinol Metab*, *90*, 2948–2953.
233. Van Dyke, D. L., Wiktor, A., Roberson, J. R., & Weiss, L. (1991). Mental retardation in Turner syndrome. *J Pediatr*, *118*, 415–417.
234. Grompe, M., Rao, N., Elder, F. F., et al. (1992). 45,X/46,X,+r(X) can have a distinct phenotype different from Ullrich-Turner syndrome. *Am J Med Genet*, *42*, 39–43.
235. Migeon, B. R., Luo, S., Jani, M., & Jeppesen, P. (1994). The severe phenotype of females with tiny ring X chromosomes is associated with inability of these chromosomes to undergo X inactivation. *Am J Hum Genet*, *55*, 497–504.
236. Jani, M. M., Torchia, B. S., Pai, G. S., & Migeon, B. R. (1995). Molecular characterization of tiny ring X chromosomes from females with functional X chromosome disomy and lack of cis X inactivation. *Genomics*, *27*, 182–188.
237. Silbert, A., Wolff, P. H., & Lilienthal, J. (1997). Spatial and temporal processing in patients with Turner's syndrome. *Behav Genet*, *7*, 11–21.
238. Nyborg, H., & Nielsen, J. (1977). Sex chromosome abnormalities and cognitive performance: III. Field dependence, frame dependence, and failing development of perceptual stability in girls with Turner's syndrome. *J Psychol*, *96*(2d Half), 205–211.
239. Waber, D. P. (1979). Neuropsychological aspects of Turner's syndrome. *Dev Med Child Neurol*, *21*, 58–70.
240. McCauley, E., Kay, T., Ito, J., & Treder, R. (1987). The Turner syndrome: cognitive deficits, affective discrimination, and behavior problems. *Child Dev*, *58*, 464–473.
241. Rovet, J., & Netley, C., (1982). Processing deficits in Turner's syndrome. *Dev Psychol*, *18*, 77.
242. Temple, C. M., & Carney, R. A. (1993). Intellectual functioning of children with Turner syndrome: a comparison of behavioural phenotypes. *Dev Med Child Neurol*, *35*, 691–698.
243. Ross, J. L., Stefanatos, G., Roeltgen, D., et al. (1995). Ullrich-Turner syndrome: neurodevelopmental changes from childhood through adolescence. *Am J Med Genet*, *58*, 74–82.
244. Bishop, D. V., Canning, E., Elgar, K., et al. (2000). Distinctive patterns of memory function in subgroups of females with Turner syndrome: evidence for imprinted loci on the X-chromosome affecting neurodevelopment. *Neuropsychologia*, *38*, 712–721.
245. Ross, J. L., Roeltgen, D., Kushner, H., et al. (2000). The Turner syndrome-associated neurocognitive phenotype maps to distal Xp. *Am J Hum Genet*, *67*, 672–681.
246. Russell, H. F., Wallis, D., Mazzocco, M. M., et al. (2006). Increased prevalence of ADHD in Turner syndrome with no evidence of imprinting effects. *J Pediatr Psychol*, *31*, 945–955.
247. Schmidt, P. J., Cardoso, G. M., Ross, J. L., et al. (2006). Shyness, social anxiety, and impaired self-esteem in Turner syndrome and premature ovarian failure. *JAMA*, *295*, 1374–136.
248. Hong, D. S., & Reiss, A. L. (2012). Cognition and behavior in Turner syndrome: a brief review. *Pediatr Endocrinol Rev*, *9*(Suppl. 2), 710–712.
249. Ross, J. L., Roeltgen, D., Feuillan, P., et al. (1998). Effects of estrogen on nonverbal processing speed and motor function in girls with Turner's syndrome. *J Clin Endocrinol Metab*, *83*, 3198–204.
250. Ross, J. L., Roeltgen, D., Feuillan, P., et al. (2000). Use of estrogen in young girls with Turner syndrome: effects on memory. *Neurology*, *54*, 164–170.
251. Cardoso, G., Daly, R., Haq, N. A., et al. (2004). Current and lifetime psychiatric illness in women with Turner syndrome. *Gynecol Endocrinol*, *19*, 313–319.
252. Money, J., & Mittenthal, S. (1970). Lack of personality pathology in Turner's syndrome: relation to cytogenetics, hormones and physique. *Behav Genet*, *1*, 43–56.
253. Downey, J., Ehrhardt, A. A., Gruen, R., et al. (1989). Psychopathology and social functioning in women with Turner syndrome. *J Nerv Ment Dis*, *177*, 191–201.
254. McCauley, E., Sybert, V. P., & Ehrhardt, A. A. (1986). Psychosocial adjustment of adult women with Turner syndrome. *Clin Genet*, *29*, 284–290.
255. Garron, D. C., & Vander Stoep, L. R. (1969). Personality and intelligence in Turner's syndrome: a critical review. *Arch Gen Psychiatry*, *21*, 339–346.
256. Pavlidis, K., McCauley, E., & Sybert, V. P. (1995). Psychosocial and sexual functioning in women with Turner syndrome. *Clin Genet*, *47*, 85–89.
257. Carel, J. C., Elie, C., Ecosse, E., et al. (2006). Self-esteem and social adjustment in young women with Turner syndrome—influence of pubertal management and sexuality: population-based cohort study. *J Clin Endocrinol Metab*, *91*, 2972–2979.
258. Sheaffer, A., Lange, E., & Bondy, C. (2008). Sexual function in women with Turner syndrome. *J Womens Health*, *17*, 27–33.
259. Rosenfeld, R. G., Attie, K. M., Frane, J., et al. (1998). Growth hormone therapy of Turner's syndrome: beneficial effect on adult height. *J Pediatr*, *132*, 319–324.
260. Sas, T. C., de Muinck Keizer-Schrama, S. M., et al. (1999). Normalization of height in girls with Turner syndrome after long-term growth hormone treatment: results of a randomized dose-response trial. *J Clin Endocrinol Metab*, *84*, 4607–4612.
261. Stephure, D. K. (2005). Impact of growth hormone supplementation on adult height in turner syndrome: results of the Canadian randomized controlled trial. *J Clin Endocrinol Metab*, *90*, 3360–3366.
262. Davenport, M. L., Crowe, B. J., Travers, S. H., et al. (2007). Growth hormone treatment of early growth failure in toddlers with Turner syndrome: a randomized, controlled, multicenter trial. *J Clin Endocrinol Metab*, *92*, 3406–3416.
263. Ross, J. L., Quigley, C. A., Cao, D., et al. (2011). Growth hormone plus childhood low-dose estrogen in Turner's syndrome. *New Engl J Med*, *364*, 1230–1242.

264. Chernausek, S. D., Attie, K. M., Cara, J. F., et al. (2000). Growth hormone therapy of Turner syndrome: the impact of age of estrogen replacement on final height. Genentech, Inc., Collaborative Study Group. *J Clin Endocrinol Metab*, *85*, 2439–2445.
265. Ranke, M. B., Lindberg, A., Chatelain, P., et al. (2000). Prediction of long-term response to recombinant human growth hormone in Turner syndrome: development and validation of mathematical models. KIGS International Board. Kabi International Growth Study. *J Clin Endocrinol Metab*, *85*, 4212–4218.
266. Bolar, K., Hoffman, A. R., Maneatis, T., & Lippe, B. (2008). Long-term safety of recombinant human growth hormone in Turner syndrome. *J Clin Endocrinol Metab*, *93*, 344–351.
267. Sas, T. C., Gerver, W. J., de Bruin, R., et al. (1999). Body proportions during long-term growth hormone treatment in girls with Turner syndrome participating in a randomized dose-response trial. *J Clin Endocrinol Metab*, *84*, 4622–4628.
268. Matura, L. A., Sachdev, V., Bakalov, V. K., et al. (2007). Growth hormone treatment and left ventricular dimensions in Turner syndrome. *J Pediatr*, *150*, 587–591.
269. Bondy, C. A., Van, P. L., Bakalov, V. K., & Ho, V. B. (2006). Growth hormone treatment and aortic dimensions in Turner syndrome. *J Clin Endocrinol Metab*, *91*, 1785–1788.
270. Zeger, M. P., Shah, K., Kowal, K., et al. (2011). Prospective study confirms oxandrolone-associated improvement in height in growth hormone-treated adolescent girls with Turner syndrome. *Horm Res Paediatr*, *75*, 38–46.
271. Gault, E. J., Perry, R. J., Cole, T. J., et al. (2011). Effect of oxandrolone and timing of pubertal induction on final height in Turner's syndrome: randomised, double blind, placebo controlled trial. *BMJ*, *342*, d1980.
272. Hagen, C. P., Aksglaede, L., Sørensen, K., et al. (2010). Serum levels of anti-Mullerian hormone as a marker of ovarian function in 926 healthy females from birth to adulthood and in 172 Turner syndrome patients. *J Clin Endocrinol Metab*, *95*, 5003–5010.
273. Bakalov, V. K., Chen, M. L., Baron, J., et al. (2003). Bone mineral density and fractures in Turner syndrome. *Am J Med*, *115*, 257–262.
274. Hanton, L., Axelrod, L., Bakalov, V., & Bondy, C. A. (2003). The importance of estrogen replacement in young women with Turner syndrome. *J Womens Health* (Larchmt), *12*, 971–977.
275. Bakalov, V. K., Axelrod, L., Baron, J., et al. (2003). Selective reduction in cortical bone mineral density in turner syndrome independent of ovarian hormone deficiency. *J Clin Endocrinol Metab*, *88*, 5717–5722.
276. Hovatta, O. (2012). Ovarian function and in vitro fertilization (IVF) in Turner syndrome. *Pediatr Endocrinol Rev*, *9*(Suppl. 2), 713–717.
277. Hadnott, T. N., & Bondy, C. A. (2011). Risks of pregnancy for women with Turner syndrome. *Expert Rev Obstet Gynecol*, *6*, 217–227.
278. Boissonnas, C. C., Davy, C., Bornes, M., et al. (2009). Careful cardiovascular screening and follow-up of women with Turner syndrome before and during pregnancy is necessary to prevent maternal mortality. *Fertil Steril*, *91*, 929 e5–e7.

QUESTIONS

1. Which of the following disorders does not increase in frequency in patients with Turner syndrome?
   a. Autoimmune thyroid disease
   b. Type 1 diabetes mellitus
   c. Chronic otitis media
   d. Celiac disease

Answer: b

2. Which of the following features is not attributed to haploinsufficiency for SHOX?
   a. Cubitus valgus
   b. Short stature
   c. Webbed neck
   d. Madelung deformity

Answer: c

3. A 36-year-old mother underwent amniocentesis to screen for Down syndrome, and the karyotype result showed mosaicism for 46,XY/45,X. The fetal ultrasound at 14 weeks showed an apparently normal male. Which of the following statements is accurate?
   a. The karyotype should be repeated after birth.
   b. The child has "male Turner syndrome."
   c. The likelihood of miscarriage is high.
   d. The mother has a higher than average risk for another gestation with sex chromosome anomaly.

Answer: a

4. A female newborn with neck webbing had a karyotype test showing nonmosaic 45,X. What should further evaluation include?
   a. Renal and cardiac ultrasound
   b. Skeletal x-ray series
   c. Molecular testing for occult Y chromosome sequences
   d. Thyroid autoimmunity screen

Answer: a

5. A karyotype analysis should be carried out for which of the following cases?
   a. A female newborn with neck webbing
   b. A 6-year-old girl with aortic coarctation
   c. An 11-year-old girl with short stature, no sign of puberty, and hypertension
   d. All of the above

Answer: d

6. Spontaneous pubertal development is most likely in Turner girls with which of the following conditions?
   a. Mosaicism for a cell line containing more than one X chromosome (e.g., 46,XX or 47,XXX)
   b. FSH in the age-specific normal range
   c. AMH in the age-specific normal range
   d. All of the above

Answer: d

7. What are the most common cardiovascular anomalies in Turner syndrome?
   a. Bicuspid aortic valve, coarctation, and ventricular septal defect
   b. Bicuspid aortic valve, coarctation, and mitral valve prolapse
   c. Bicuspid aortic valve, coarctation, and partial anomalous pulmonary veins
   d. Bicuspid aortic valve, partial anomalous pulmonary veins, and pulmonic stenosis

Answer: c

8. A girl that is found to have Y chromosome material on her peripheral blood karyotype may have which of the following?
   a. Spontaneous puberty and pregnancy
   b. A high risk for adenocarcinoma of the ovary
   c. A high risk for behavioral disorder
   d. All of the above

Answer: a

9. What is the best treatment for a 12-year-old girl recently diagnosed with Turner syndrome and menopausal FSH?
   a. GH, estrogen, and oxandrolone beginning immediately
   b. GH until age 15 and then pubertal induction
   c. GnRH analog and GH until age 15
   d. GH and low-dose estradiol until height is satisfactory and then full pubertal estrogen

Answer: d

10. What does an annual medical screening for the 10- to 12-year-old with Turner syndrome include?
    a. Blood pressure measurement
    b. Metabolic profile
    c. FSH and AMH levels
    d. Thyroid-stimulating hormone
    e. All of the above

Answer: e

11. Which of the following statements is justified?
    a. Girls need to have a DXA scan for bone mineral density prior to estrogen treatment.
    b. A normal cardiac echo is an adequate cardiac evaluation for the young woman with Turner syndrome who is considering pregnancy.
    c. Girls with surgically repaired coarctation need lifelong cardiovascular follow-up.
    d. Oral contraceptive pills should be used to induce puberty.
    e. All of the above
    f. a, b, and c

Answer: c

CHAPTER 17

Puberty and Its Disorders in the Male

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CHAPTER OUTLINE

Puberty is a period during which children attain adult secondary sexual characteristics and reproductive capability.[1] In humans, two distinct processes of sexual maturation are recognized: gonadarche and adrenarche. Gonadarche is defined as the growth and maturation of the gonads associated with increased sex steroid secretion. Gonadarche requires an intact hypothalamic-pituitary-gonadal (HPG) axis, and any disruption of this axis can result in temporary or permanent disorders of reproductive endocrine function. Adrenarche is defined as maturation of the adrenal cortex associated with increased secretion of dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), and androstenedione. Unlike gonadarche, adrenarche is a phenomenon limited to humans and some

great apes. The physiologic foundations for puberty begin in utero with the development of the neurobiologic structures that govern the hypothalamic-pituitary component of the HPG axis and with the differentiation and the development of the gonads. The entire process, extending from fetal life through achievement of reproductive competence, represents the dynamic and coordinated interactions of an expanding list of genes, proteins, signaling molecules, paracrine factors, and epigenetic events.

PRENATAL NEUROBIOLOGY OF PUBERTY

In postnatal life, the gonadotropin-releasing hormone (GnRH) neurons are located in the hypothalamus and constitute the GnRH pulse generator, which produces an intermittent discharge of GnRH into the hypophysial portal circulation to stimulate gonadotropin synthesis and secretion by the pituitary gonadotropes. The developmental progression of these neurons is governed by multiple regulatory factors; mutations in some of these factors have been associated with disorders of puberty.[2]

However, the 1000 to 2000 GnRH neurons originally differentiate in the olfactory placode and begin their migration along the vomeronasal nerves to the cribriform plate around the sixth week of gestation. This migration can be categorized into four stages. The first stage involves the differentiation of the GnRH neurons from a heterogenous stem cell population in the embryonic olfactory placode. Factors involved in these early stages include the proteins encoded by fibroblast growth factor 8 (*FGF8*), fibroblast growth factor receptor 1 (*FGFR1*), heparin sulfate 6-O-sulfotransferase 1 (*HS6ST1*), chromodomain helicase DNA-binding protein 7 (*CHD7*), and nasal embryonic luteinizing hormone (LH)–releasing hormone (*NELF*). The extracellular domain of FGFR1, a tyrosine kinase receptor, interacts with heparan sulfate proteoaminoglycan, which functions as its coreceptor. Nonrandom modifications of the sugar moieties associated with heparan sulfate proteoaminoglycans such as by HS6ST1 facilitate FGFR1 function. Binding of its cognate ligand, FGF8, to FGFR1, initiates signaling. The FGF8-FGFR1 signal transduction pathway plays an essential role in the neurobiology of GnRH neurons. Several additional genes show spatiotemporal expression patterns similar to *FGF8* and appear to modulate FGF8 signaling through FGFR1. These genes include fibroblast growth factor 17 (*FGF17*), interleukin 17 receptor D (*IL17RD*), dual specificity phosphatase 6 (*DUSP6*), sprout homologue 4 (Drosophila) (*SPRY4*), and fibronectin leucine rich transmembrane protein 3 (*FLRT3*).[3]

The second stage is the onset of GnRH neuron migration, which commences around the sixth week of gestation in humans. This process depends on guidance cues to ensure proper routing of the neurons. Several molecules such as anosmin-1, FGFR1, and prokineticin 2 (PROK2) appear to be involved in this process. Anosmin-1, encoded by the *KAL1* gene, is an extracellular matrix protein; it also binds to heparan-sulfate glycosaminoglycans. Although the precise function of anosmin-1 is unclear, potential functions include providing a guidance platform for migrating neurons, serving as a chemo-attractant for other factors, and interacting with FGFR1. Prokineticin-2 (PROK2), prokineticin receptor-2 (PROKR), NELF, and semaphorin-3A (SEMA3A) appear to influence GnRH neuron migration.

Eventually, these neurons reach the hypothalamus where they extend projections to the median eminence to form a network, which completes the third stage of GnRH neuron migration. The last stage comprises functional activity. By the 15th week of human gestation, the GnRH pulse generator is modulating the function of the fetal gonadotropes. The HPG axis is functionally active for the first time during fetal development, and it continues to function in infancy until it enters a relative quiescent state, often referred to as the juvenile pause or prepuberty. Likely sharing some similarities but also having some differences,[4] the molecular mechanisms responsible for the prepubertal inactivation of the HPG axis and its reactivation at the onset of puberty remain to be characterized.

TESTICULAR DIFFERENTIATION AND DEVELOPMENT

Prenatal Testicular Development

A brief summary of prenatal testicular differentiation and development follows; a more detailed description of testicular differentiation and sex development can be found in Chapter 4. Beginning at approximately 4 to 6 weeks of gestation, the primordial bipotential gonad arises from a condensation of the mesoderm of the urogenital ridge. Genes involved in the development of this bipotential gonad include the Wilms tumor (*WT1*) gene, *GATA4*, chromobox homologue 2 (*CBX2*), and steroidogenic factor-1 (*NR5A1*). During this time, the primordial germ cells proliferate and migrate from the hindgut to colonize the developing gonad. The bipotential gonad is composed of supporting cells, endothelial cells, steroid-secreting cells, and germ cells.

In the usual circumstance, the presence of a Y chromosome bearing the sex-determining region on the Y (*SRY*) gene promotes testicular differentiation. However, novel data emphasize the complexity of gonadal differentiation with involvement of signaling molecules—for example, SRY-box 9 (SOX9) and Forkhead transcription factor 2 (FOXL2)—that activate or repress gonadal determination factors involved in testicular and ovarian development. Sertoli cell differentiation is the earliest manifestation of testicular differentiation. At approximately 7 to 9 weeks of gestation in the human XY gonad, the Sertoli cells envelope the germ cells to form the seminiferous cords. Curiously, germ cells are not required for initial phases of testicular differentiation. Rather, the local environment directs the developmental fate of the primordial germ cells. During the migration though the hindgut, the primordial germ cells proliferate. The stem cell factor (Steel factor) and c-KIT receptor guide the primordial germ cells to the developing genital ridges. This signaling system ensures that viable primordial germ cells travel to their proper environmental niche and that misdirected primordial germ cells undergo apoptosis. Primordial germ cells that escape apoptosis and migrate to other physical locations, such as the mediastinum or central

nervous system, can develop into extragonadal germ cell tumors. Primordial germ cells that occupy the genital ridge become pluripotent gonocytes that express specific stem cell markers including placental/germ cell alkaline phosphatase (PLAP) and octamer binding transcription factor 3/4 (OCT3/4).

Internal genital structures are also bipotential. Sertoli cells secrete anti-Mullerian hormone (AMH), which induces regression of the Mullerian ducts through its actions on the type II AMH receptor. The fetal Leydig cells, initially stimulated by placental hCG, secrete testosterone which stabilizes the Wolffian ducts. In the human male fetus, the testicular compartments, tubular and interstitial components, and specific cell types, Leydig, Sertoli, and germ cells can be visualized by 11 weeks of gestation. The most rapid growth in Sertoli cell number appears to occur during the latter half of the first trimester and the second trimester.[5]

During early gestation, placental hCG governs Leydig cell proliferation and testosterone and insulin-like factor 3 (INSL3) secretion until endogenous LH regulates these activities in midgestation. Due to this role of placental hCG during early gestation, gonadotropin deficiency does not influence male sexual differentiation. LH secretion influences the number of fetal Leydig cells because the number is decreased in anencephalic fetuses and is increased in 46,XY fetuses, with elevated gonadotropin concentrations secondary to complete androgen insensitivity.[6] Follicle-stimulating hormone (FSH) secretion influences Sertoli cell differentiation and AMH and inhibin B secretion.[7]

Androgens induce the genital tubercle, urethral folds, and labioscrotal swellings to develop into the male external genital structures. In target tissues such as genital skin and prostate, testosterone is converted to dihydrotestosterone, which induces the urethral folds to fuse and form the corpus spongiosum and penile urethra. The genital tubercle develops into the corpora cavernosa of the penis and the labioscrotal folds fuse to form the scrotum. By 9 weeks of gestation, a cylindrical 2-mm phallus with genital swellings has developed. By 12 to 14 weeks of gestation, the urethral folds have fused to form the cavernous urethra and corpus spongiosum. By 14 weeks, the external genitalia are clearly masculine apart from testicular location. In the absence of sufficient androgen concentrations, the urethral folds and labioscrotal swellings do not fuse and develop into the labia minora and labia majora, respectively.

Testicular descent occurs in two phases. The transabdominal phase begins at approximately 12 weeks of gestation and is influenced by the Leydig cell product, insulin-like factor 3 (INSL3), and its cognate receptor, leucine-rich repeat-containing G protein–coupled receptor 8 (LGR8). The second androgen-dependent phase, descent of the testes through the inguinal canal, is usually accomplished by the end of week 35.[8]

Postnatal Testicular Development

Following a brief perinatal decline, the hypothalamic-pituitary-testicular axis is active during the first few months of life with testosterone concentrations peaking at 1 to 2 months of age.[9] By approximately 6 months of age, testosterone concentrations decline to prepubertal levels. Despite the increased neonatal HPG activity, sexual hair does not develop and gametogenesis does not occur due to limited androgen receptor (AR) signaling in certain tissues (e.g., Sertoli cells). Throughout infancy, AR is expressed in Leydig cells, but not in Sertoli cells.[10] During infancy and childhood, seminiferous cords are solid and generally filled with immature Sertoli cells. The germ cells are limited to spermatogonia and Leydig cells are rarely visualized.[11] After early infancy, which provides a window of opportunity to assess testicular function, hCG stimulation may be needed to assess Leydig cell function. Inhibin B and AMH continue to be secreted during childhood providing valuable markers of Sertoli cell function.

Reactivation of the GnRH pulse generator at the time of puberty stimulates pituitary LH and FSH secretion (Figure 17-1). The LH stimulation leads to increased testicular testosterone and INSL3 secretion. The increased testosterone acts as a paracrine factor to induce Sertoli cell maturation. Pubertal maturation of the seminiferous tubules is characterized by cytoskeletal rearrangements including development of tight junctions, Sertoli cell polarization, Sertoli cell proliferation, migration of spermatogonia toward the basement membrane, and decreased AMH secretion.[12] FSH stimulates Sertoli cells to secrete inhibin B, which from midpuberty onward serves as the major negative regulator of pituitary FSH secretion. Prior to puberty, inhibin B is germ cell independent. After puberty, inhibin B secretion becomes germ cell dependent and provides a marker of germ cell depletion in adults.[13]

The increase in Sertoli cell number contributes to the increase in testicular volume that marks the onset of gonadarche in boys. As puberty progresses, the seminiferous tubules are larger and develop a lumen; typical Leydig cells become apparent. Although much variation in chronologic age and testicular volume has been described, spermarche precedes peak pubertal linear growth velocity and generally occurs at a median testicular volume of 10 to 12 mL.[14] Beginning in early puberty, testosterone and AMH show an inverse relationship reflecting the paracrine actions of testosterone to repress AMH secretion by Sertoli cells. Intratesticular testosterone and AR expression in Sertoli cells are essential for the decline in AMH concentrations, meiosis, and spermatogenesis.[11] Hence, AMH concentrations provide an indication of Sertoli cell function and androgen action in the testes.

THE ANDROGEN RECEPTOR

The androgen receptor (AR) binds androgens to initiate a signal transduction cascade mediating the effects of testosterone and DHT. The *AR* gene is located at Xq11-q12. Similar to other members of the steroid-thyroid nuclear hormone receptor family, *AR* (also known as NR3C4) has a modular structure with N-terminal regulatory (NTD), DNA binding (DBD), and ligand binding (LBD) domains. The DBD contains the cysteine residues that coordinate zinc atoms to form the zinc finger

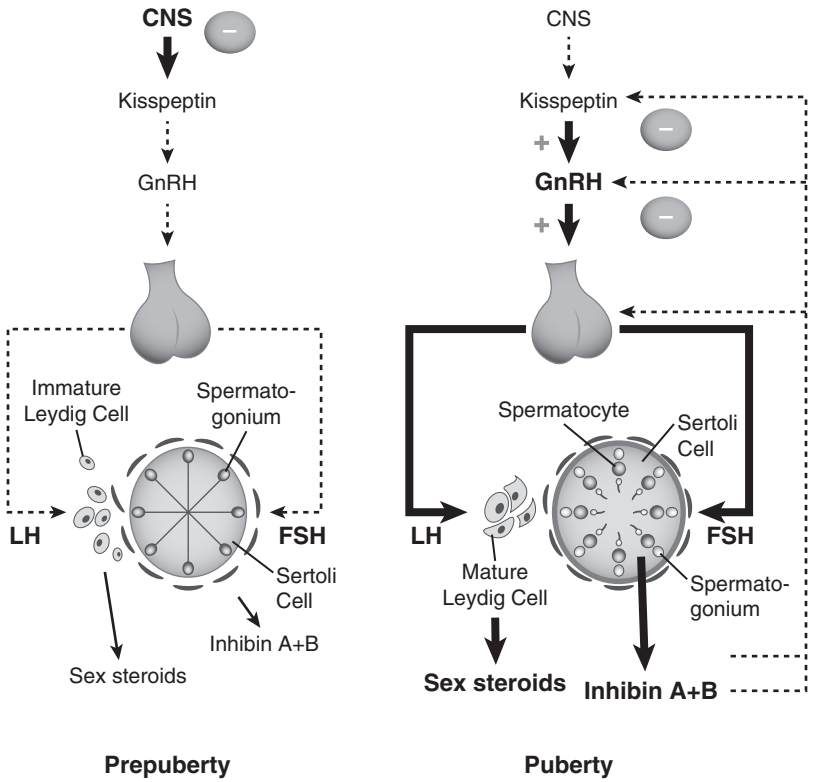

FIGURE 17-1 ■ Testicular products, such as inhibins, play a small role in regulation of the HPG axis prior to puberty, but most of the dampening of the HPG axis after infancy and until the onset of puberty (left panel) derives from central inhibition of the HPG axis. The basis for that inhibition is not fully understood nor is the basis for its diminution, which leads to emergence of central activation (right panel), increased gonadotropin releasing hormone (GnRH) secretion, and the onset of puberty. As pubertal development progresses, lumen develops in Sertoli cells. Inhibin B secreted from the mature testes (right panel) has a more substantial role in regulating/inhibiting hypothalamic-pituitary activity. Weight of lines (from dotted to thin solid to thick solid) indicates increasing strength of the particular signal. LH, luteinizing hormone; FSH, follicle-stimulating hormone.

domains, which bind to DNA. The LBD is composed of 12 α helixes associated with antiparallel β sheets, which form a tripartite sandwich structure.

The three-dimensional structure contains a hydrophobic pocket formed by helixes 4, 5, 7, 11, and 12. Helix 12 can fold like a lid on top of the hydrophobic pocket to capture the ligand and allow for interactions between the LBD and various co-regulator proteins. The AR protein contains several subdomains important for dimerization, protein-protein interactions, and transcriptional regulation. Interactions between the N-terminal domain and the C-terminal LBD stabilize the receptor-ligand complex to slow dissociation of the ligand from the receptor. DHT is a more potent androgen because it dissociates at a slower rate from the receptor than testosterone.

The unliganded receptor is located in the cytoplasm where it is associated with several heat shock proteins (HSPs) such as HSP70 and HSP90. Chaperone proteins such as FKBP52 also associate with this complex. Ligand binding induces dissociation of the cytoplasmic receptor-chaperone protein complex followed by translocation of the receptor-ligand complex into the nucleus where it binds as a homodimer to AR response elements. Additional proteins, coactivators and corepressors, also interact with this complex. Activation of transcription involves two subdomains known as activation function regions. The activation function-1 (AF1) region is located in the NTD. The activation function-2 (AF2) region is located in the LBD and interacts with steroid receptor coactivators through their LXXLL motifs. The LXXLL refers to the amino acids in this motif with L being leucine and X being any amino acid. Steroid receptor coactivators include SRC1, SRC2/TIF2, and SRC3.

PHYSIOLOGY OF PUBERTY

Endocrinology

The onset of puberty is heralded by an increase in the secretion of gonadotropin releasing hormone (GnRH) (see Figure 17-1) from a diffusely distributed network of hypothalamic neurons. Intermittent secretion of GnRH into the hypophysial portal circulation stimulates gonadotropes in the anterior pituitary to synthesize and secrete luteinizing hormone (LH) and follicle stimulating hormone (FSH) that, in turn, bind to ligand specific receptors in the Leydig and Sertoli cells of testes, respectively. Gonadotropin stimulation initiates gonadarche, and the production of sex steroids, most notably testosterone, and at later pubertal stages estradiol as well.[15] Testosterone and estradiol, together with inhibin, activin, and follistatin, provide signals that regulate the subsequent activity of the hypothalamus and pituitary gland. The transition from prepubertal quiescence to the adolescent pattern of

GnRH secretion is a gradual rather than abrupt process. LH and FSH pulsatility has been detected in normal children as young as 4 years of age.[16-19] Throughout childhood, GnRH secretion appears to undergo small but progressive increases until the onset of puberty, when GnRH secretion increases, first at night and eventually throughout the day.[20,21]

Neuroendocrine Regulation of Pubertal Onset

Understanding what factors contribute to the dampening of the HPG axis after infancy and what factors lead to the reemergence of GnRH secretion is critical to understanding the regulation of pubertal timing. Research into kisspeptin (Kiss1) and its receptor (Kiss1R, formerly G protein–coupled receptor 54, GPR54) in both animal and human studies has identified them as critical components of the HPG axis. The first indications of the importance of this signaling complex as a regulator of the HPG axis came in 2003 when two independent groups reported deletions and inactivating mutations of *GPR54* in patients with hypogonadotropic hypogonadism (HH).[22,23] Subsequently, activating mutations in this pathway were associated with central precocious puberty in the case of a female with an autosomal dominant mutation in *GPR54*.[24] Thus, it is clear that activation of Kiss1R by kisspeptins plays a pivotal role in the onset of puberty.

It is not yet known, however, whether this system is the initial trigger of puberty or whether it acts either as a downstream effector or in concert with other regulatory factors.[25,26] For example, the discovery that mutations in *TAC3* (encoding neurokinin B) or its receptor *TACR3* (encoding NK3R)[27] can cause HH has focused much attention on how hypothalamic neurons that coexpress kisspeptin, neurokinin B, and dynorphin (abbreviated as KNDy neurons) regulate the HPG axis in males and females.[28-30] Another excitatory factor in the hypothalamus is glutamate, an important stimulator of GnRH secretion through its actions at n-methyl, D-aspartate (NMDA) and kainate receptors. GnRH secretion is stimulated also by factors such as norepinephrine, dopamine, TGFα, neuregulin signaling via erbB4 receptors, leptin, and galanin-like peptide.[31-35] Many of these factors likely act via a complex, intricate communication network that exists between glial cells and neurons within the hypothalamus.[36] The potential roles that these and other compounds play in regulating the onset of puberty remains an area of active investigation.

Gamma-amino butyric acid (GABA) neurons appear to play a prominent role in inhibiting prepubertal GnRH release.[37] There is evidence that GABA can also stimulate GnRH secretion, but this variable action may be dependent on developmental stage, composition of GABA receptors, and expression of *KCC2* (a protein that can alter the inhibitory and excitatory properties of chloride channels).[38] The finding that neuropeptide Y (*NPY*) mRNA expression in the hypothalamus of juvenile monkeys is higher than neonatal animals suggests that NPY may play a role in the juvenile pause.[4] Other factors that likely inhibit GnRH release include endogenous opioids (i.e., β-endorphin) and melatonin, but neither of these compounds likely plays a major role in regulating the timing of puberty.[35]

Somatic Changes

In boys, the first marker for the onset of puberty is most often the change from Tanner genital stage G1 to stage G2, including enlargement of the testes (i.e., achievement of volume greater than 3 mL or testicular length greater than or equal to 25 mm). Originally Marshall and Tanner reported the mean (SD) onset of puberty in boys to be 11.64 (1.07) years.[39,40] These pubertal stages (Figure 17-2) were based on photographic observation of genital development of a longitudinal but still relatively small sample of 228 boys living in a children's home. Despite the probably poor representative nature of this sample, comparable studies in Switzerland,[41] the United States,[42] and Denmark[43] reported roughly similar mean ages of puberty onset. Although the mean age of onset may be fairly uniform, the onset of puberty occurs across a wide range of ages in normal, healthy adolescents. Several pathologic states influence the timing of puberty either directly or indirectly and contribute to this splay, but the great majority of the variation in pubertal timing cannot be attributed to any clinical disorder. Ninety-five percent of boys experience the onset of genital development between

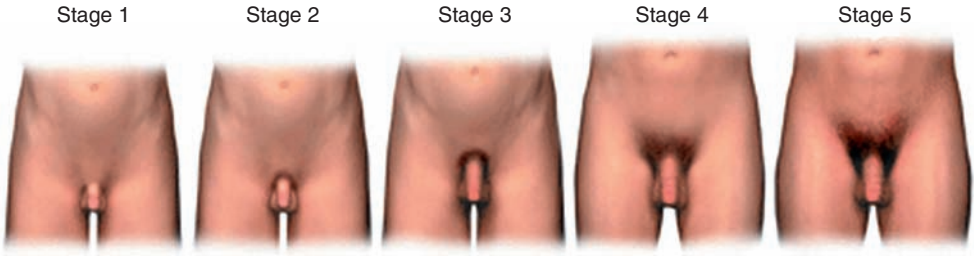

FIGURE 17-2 ■ In boys, genital development is rated from 1 (preadolescent) to 5 (adult); stage 2 marks the onset of pubertal development and is characterized by an enlargement of the scrotum and testis and by a change in the texture and a reddening of the scrotal skin. Pubic hair stages are rated from 1 (preadolescent, no pubic hair) to 5 (adult), and stage 2 marks the onset of pubic hair development. Although pubic hair and genital development are represented as synchronous in the illustration, they do not necessarily track together and should be scored separately. (Reproduced with permission from Carel, J. C., & Leger, J. [2008]. Clinical practice: precocious puberty. *N Engl J Med, 358*, 2366–2377.)

9.5 and 13.5 years,[44,45] data that have led to the traditional definition of sexual precocity in boys as development of secondary sexual characteristics before age 9 years and delayed puberty as lack of testicular enlargement by age 14 years.

Development of secondary sexual characteristics results from both gonadarche and adrenarche. Adrenarche refers to the maturation of the zona reticularis of the adrenal gland, resulting in increased production of adrenal androgens associated with secondary sexual characteristics, such as the development of pubic hair (pubarche), axillary hair, body odor, and acne. Like gonadarche, the onset of adrenarche appears to be a gradual, progressive maturational process that begins in early childhood and is marked by the further increases of production of adrenal androgens (DHEA, DHEA-S, and androstenedione) around the time of puberty.[46] Adrenarche sometimes precedes gonadarche by 1 to 2 years in boys and girls, but the timing of clinical signs can vary. Although adrenarche and gonadarche often overlap, they are separate processes that are independently regulated.[47,48] The triggers for adrenarche remain unknown; however, alterations in body weight and body mass index as well as in utero and neonatal physiology likely modulate this developmental process, perhaps along with intra-adrenal cortisol production.[49-51]

REGULATION OF THE TIMING OF PUBERTY

Genetics

The timing of puberty is influenced by both genetic and environmental factors.[52,53] Evidence for the genetic contribution is provided by correlations in timing within families and between monozygotic twins. These data derive mostly from studies of females and suggest that more than half of the variation in the most commonly used marker for the timing of puberty, age at menarche, is attributable to additive genetic effects and the rest appears to be attributable to nonshared environmental effects.[34,52,54-61] It is important to note that this genetic component does not preclude a significant role for environmental influences that may have changed over time. However, despite changing environmental and secular influences, genetic background still plays a significant role in regulating the variation of pubertal timing within a population at any particular point in time.

Genetic Disorders Causing GnRH Deficiency

The study of genetic disorders that cause GnRH deficiency, such as isolated hypogonadotropic hypogonadism (HH) and Kallmann syndrome (KS), has significantly increased our understanding of development and function of the hypothalamic pituitary gonadal (HPG) axis.[59,62-67] These studies have led to the identification of many genes that play critical roles in the HPG axis. For example, this work has defined roles for the genes that lead to HH (*GNRHR*, *GNRH1*, *GPR54*, *NELF*, *FGFR1*, *FGF8*, *PROK2*, *PROKR2*, *TAC3*, *TACR3*, *CHD7*, and *HS6ST1*), to X-linked (*KAL1*) and autosomal (*NELF*, *FGFR1*, *PROK2*, *PROKR2*, *FGF8*, *WDR11* and *CHD7*) forms of KS, to obesity and HH (*LEP*, *LEPR*, and *PC1*), and to abnormal HPG development (*DAX1*, *SF-1*, *HESX-1*, *LHX3*, and *PROP-1*). Moreover, research in this field permits recognition that, in some cases, HH and KS can be caused by mutations in the same genes and derive from a monogenic or oligogenic basis.[68] In contrast to this great progress, the specific genetic factors that regulate the variation in pubertal timing in the general population are just emerging. In addition to genes identified through genome wide association studies (discussed later), another factor may be the makorin RING-finger protein 3 (MKRN3). Loss-of-function mutations in *MKRN3* were associated with GnRH-dependent precocious puberty.[69]

Normosmic Hypogonadotropic Hypogonadism

Although several HH-related genes have been identified, HH with normal olfaction has been primarily associated with mutations in the genes for the gonadotropin-releasing hormone receptor (GNRHR) and the KISS1 (kisspeptin) receptor (*KISS1R*, formerly *GPR54)*.[22,23,70-74] Estimates regarding the frequency of *GNRHR* mutations in normosmic HH range from 3.5% to 10.4%.[75,76] Mutations in *GNRH1* have also been identified in patients with normosmic HH but only in rare case reports.[77,78] Although these individuals have normal olfaction, they can have other nonreproductive clinical features such as midline facial defects, renal agenesis, and skeletal abnormalities that may provide clues about the underlying pathophysiology.[67] Interestingly, a case of constitutional delay of growth and puberty (CDGP) has also been associated with a homozygous partial loss-of-function mutation in *GNRHR*,[79] and pedigrees of probands with HH can include individuals with delayed but otherwise normal puberty. However, more extensive analyses suggest that genetic variation in neither *GNRH1* nor *GNRHR* nor other HH-related genes is a common cause of late puberty in the general population[80,81]; whether combinations of rare variants in KS or HH-related genes is a cause of some cases of constitutional delay of growth and puberty remains to be determined.

Mutations in genes encoding neurokinin B and its receptor, *TAC3* and *TACR3*, have been identified in HH patients.[27] As noted earlier, these genes are highly expressed in the same neurons that express kisspeptin, further emphasizing the role of kisspeptin in the regulation of pubertal timing.

Kallmann Syndrome

HH associated with abnormal sense of smell (anosmia/hyposmia) is referred to as Kallmann syndrome. As with cases of HH and normal olfaction, other nonreproductive clinical features can be seen in individuals with KS, including ichthyosis, choanal atresia, horseshoe kidneys, and mirror movements of the hands (synkinesia).[67] Several genes critical to HPG axis function and olfactory development have been identified through investigation of Kallmann syndrome. In particular, mutations in Kallmann

syndrome 1 (*KAL-1)*[82,83] and fibroblast growth factor receptor 1 (*FGFR1)*[84] have been implicated in the X-linked and autosomal dominant forms of the disease, respectively, and appear to account for approximately 20% of patients with KS.[85] Mutations in the prokineticin receptor-2 gene (*PROKR2)*, a G protein–coupled receptor, and in its ligand prokineticin-2 *(PROK2)* have also been identified in patients with KS,[85] demonstrating that prokineticin signaling is important for olfactory and HPG axis development. One of the patients in this initial series was heterozygous for both a *PROKR2* mutation and a *KAL1* mutation, suggesting a possible digenic mode of inheritance.[85] Finally, mutations in the nasal embryonic luteinizing hormone releasing hormone factor (*NELF*), which plays a role in migration of GnRH neurons and olfactory axon outgrowth,[86] have also been implicated in the pathogenesis of KS.[87] A heterozygous deletion in *NELF* was initially reported as a component, along with *FGFR1*, of digenic KS,[88] but it has recently been reported that NELF can lead to HH or KS via monogenic as well as digenic inheritance.[89]

The distinction among the different abnormalities of pubertal development is not always clear cut. For example, a comprehensive study of *PROK2* and *PROKR2* in HH and KS patients found mutations in both genes distributed in both groups of patients.[90] Mutations in the *FGF8* gene, which encodes a ligand for FGFR1, have been observed in HH patients accompanied by variable olfactory phenotypes.[91] Mutations in *CHD7*, a gene responsible for CHARGE syndrome, which shares some developmental features with KS, have been identified in patients with both normosmic HH and KS.[92,93] More recently, mutations in *FGFR1*, *FGF8*, and *PROKR2* have been identified among subjects with combined pituitary hormone deficiency (CPHD) and septo-optic dysplasia (SOD), indicating that in some cases HH and CPHD/SOD can share genetic etiologies.[94,95] The distinction between HH and constitutional delay of growth and puberty (CDGP) is also not always clear. Variation in *FGFR1* does not appear to be a major cause of CDGP,[81] although loss-of-function mutations in *FGFR1* can cause delayed puberty in members of HH pedigrees.[96,97] Instances of reversible HH have also been reported,[98] raising the possibility that some cases of HH may represent severe versions of CDGP, further blurring the distinction between HH and CDGP.

Other Genes Associated with HH

Leptin appears to act as a permissive factor in pubertal maturation.[34] HH (accompanied by obesity) can result from defects in the leptin (*LEP*) or the leptin receptor (*LEPR*) genes, highlighting the importance of nutrition in modulating the HPG axis. The neuronal targets for leptin's action are incompletely explained because leptin receptors are not expressed by GnRH neurons, suggesting that the site of leptin action may be upstream of the GnRH neurons. However, association studies have found no substantial association between common polymorphisms in *LEP* and *LEPR* and constitutional delay of growth and puberty (CDGP) or age at menarche in the general population.[81,99]

Mutations in several pituitary transcription factors, including HESX-1, *LHX3*, and *PROP1*, can lead to combinedpituitary hormone deficiencies that include HH as a phenotype. The gene for prohormone convertase-1 (*PC-1*) has been associated with obesity and HH, possibly as a result of defective processing of neuropeptides or prohormones that are components of GnRH secretion.[66,100] Other causes of HH include mutations in genes that are critical to HPG development. This category includes the genes for the orphan nuclear receptors *DAX1* (dosage-sensitive sex reversal adrenal hypoplasia critical [DSS-AHC] region on the X chromosome, gene 1) and steroidogenic factor-1 (*NR5A1*).

Genetic Variation in Normal Puberty

Observations regarding the greater concordance in age at pubertal development in monozygotic twins and correlation at age of menarche in mothers and daughters emphasized the role of genetic influences in the timing of normal pubertal development.[101] Approaches utilized to identify specific genetic factors include candidate genes studies and genome-wide association (GWA) studies.

Candidate Gene-Based Studies

A commonly used approach for identifying the variants that affect complex traits, such as the timing of puberty, in the general population has been candidate gene-based association and sequencing studies.

In an association study that tested for associations between common variants in 10 HH-related genes (*GNRH1*, *GNRHR*, *KISS1R/GPR54*, *KISS1*, *LEP*, *LEPR*, *FGFR1*, *KAL1*, *PROK2*, and *PROKR2*) and age at menarche, only nominally significant associations between SNPs in several of the genes and age at menarche were identified, indicating that genetic variation in these 10 genes does not appear to be a substantial modulator of pubertal timing in the general population.[81]

Other work has also shown no evidence for substantial association between SNPs in *GNRH1* and *GNRHR*[80] or *LEP* and *LEPR*[99] and alterations in pubertal timing. In a direct sequencing study (which assessed variation in *FGFR1*, *GNRHR*, *TAC3*, and *TACR3* in 146 Finnish subjects), variants in the coding regions of these genes were not identified as likely causes of constitutional delay of growth and puberty in the general population.[102]

Genome-Wide Association (GWA) Studies

In large-scale GWA studies, the age of menarche has been the most commonly used marker of the timing of puberty. These studies provide important information regarding genes and pathways that regulate the timing of puberty, but it is unlikely that there is complete overlap among factors that regulate the timing of puberty in males and females. For the most part, further study is still needed in boys and men.

Common variants in *LIN28B* were associated with age at menarche in four independent GWA

studies and one meta-analysis.[103-107] *LIN28B* is a human homologue of *lin-28*, which in *C. elegans* controls the rate of progression from larval stages to adult cuticle formation, indicating conservation of specific microRNA regulatory mechanisms involved in developmental timing.[104] These GWA studies involved between 17,000 and 25,000 individuals, all of European descent. In each case, age at menarche (AAM) was analyzed, but in one study[107] additional phenotypes (breast development in girls, voice breaking and pubic hair development in boys, and tempo of height growth in both boys and girls) were found to associate with variants in *LIN28B*, suggesting that control of pubertal timing in boys and girls shares some common elements. One study found that the signal at *LIN28B* could be split into two haplotypes, suggesting that either multiple variants may associate with AAM at this locus or that a SNP that has not yet been tested for association may represent the true association signal.[106] Effect sizes were estimated at approximately 1.2 months earlier per effect allele for *LIN28B*.[105,107] A second menarche locus was identified in two of the four studies at 9q31.2.[103,105] The biology behind the locus at 9q31.2 remains unknown, but its effect size is similar to the locus in/near *LIN28B*.[105] The associated SNPs lie in an intergenic region with no obvious candidate genes nearby. The closest gene is *TMEM38B*, a transmembrane protein gene, which lies approximately 400 kb away from the signal at 9q31.2.[105] Although these studies were ground breaking, the *LIN28B* and 9q31.2 loci together explain only 0.6% of the variance in age at menarche.[103]

The genetic contribution to the variation in age at menarche was further investigated by a meta-analysis of 32 genome-wide association studies including 87,000 women.[104] Thirty new AAM-associated loci were identified, but pubertal timing among males was not assessed. Despite the large size of the meta-analysis, these loci explained only 3.6% to 6.1% of the variance in the age at menarche.[104] It is important to note that the small effect size does not negate the importance of the discovery. Findings from GWA studies have highlighted biologic pathways involved in a variety of phenotypes, both through "rediscovery" of genes known to be important and through identification of previously unsuspected pathways.[108] This principle also holds true for pubertal timing, with much new biology to explore as the mechanisms through which these newly identified genes modulate pubertal timing are investigated and as future studies determine whether the same genes regulate pubertal timing in boys and in girls.

GWA studies are designed to assess the contribution of common genetic variants to a particular phenotype. However, it is likely that other forms of inheritance also underlie CDGP, including rarer variants (frequency $< 5\%$ in the general population) with large or even small phenotypic effects; combinations of variants within a single gene or multiple genes (oligogenicity); structural variation, such as copy number variants; and epigenetics. Indeed, some of these mechanisms have been identified as causes of hypothalamic amenorrhea.[109]

External Factors and Secular Trends in the Timing of Puberty

Although these genetic advances are exciting, genetic factors cannot explain the reported secular changes in pubertal timing (discussed later) that have occurred since the late 20th century. Clearly, changes in lifestyle or environmental factors must be involved, likely as independent regulators but also as factors that mediate effects through gene by environment (G X E) interactions. Variables such as increased adiposity, insulin resistance, physical inactivity, psychological factors, and changed dietary habits have all been implicated as possible mediators of the observed change in pubertal timing.[52,110,111]

The mean age of menarche in mid-19th century Europe was likely between 17 and 18 years.[52] Starting from the late 19th century to the mid-20th century, a gradual decline in age at puberty has been reported, more convincingly in girls than in boys,[52,112] after which this trend may have slowed. The change in the timing in puberty has likely been the result of better hygiene and nutrition as well as increased stability in socioeconomic conditions.

The extent to which age at puberty has declined in males is controversial. In the mid-1990s, data from the Third National Health and Nutrition Examination Survey (NHANES III), where genital ratings were performed by visual inspection, reported earlier age at puberty in both boys and girls[113-116] than what previously had been reported from the United States.[44,115,117] Using the traditional cutoff of 9 years, these data suggest that an increased number of boys would be classified as having precocious puberty. However, due to lack of data on pubertal onset in the previous population-based study (Third National Health Examination Survey [NHES III]),[44] some controversy remained as how to interpret the NHANES III findings.[113,114,116] Furthermore, questions have been raised regarding the criteria used for genital staging in NHANES III.[118] A subsequent secular trend analysis between NHES III (which lacked data from the early pubertal stages) and NHANES III did not find clear evidence supporting earlier age at puberty, although some indications were present in non-Hispanic white boys.[115] These data were also reviewed by an expert panel, which concluded that the available data are insufficient in quality and quantity to confirm a change in pubertal timing in U.S. boys.[112] Conversely, at the same time in Europe, in comparison with NHANES III studies, some data were reported suggesting older ages at pubertal onset in boys.[41,119-121] Only a few European studies contained data to assess secular trend in the timing of puberty in Europe, and those do not support a substantial enough change in the age at pubertal onset in boys from the mid-1960s to the late 1990s to warrant a change in the age definitions for precocious and delayed puberty.[43,120]

Effect of BMI on Pubertal Timing

Among white American children aged 6 to 11 years, rates of obesity have increased from approximately 5% between 1963 and 1965 to 12% in 1999–2000.[122] The possibility that the increasing rates of obesity contributed to

the secular trend toward early puberty onset was originally highlighted in 1997.[123] However, despite strong evidence in support of a link between increased adiposity and early onset of pubertal markers in girls,[124,124] data in boys have remained somewhat ambiguous, with increased body mass index (BMI) being associated in general with earlier puberty but with obesity being associated with delayed puberty in some cases.[125,126]

Several population studies regarding pubertal timing and BMI have been conducted in boys. A retrospective study of 1520 men with serial height and weight measurements between the ages of 9 and 18 years reported that boys with high childhood BMI tended to have an earlier puberty, whereas boys with a later puberty tended to be taller and were less obese as adults than those who had experienced an earlier puberty.[127] Similarly, high BMI gain during childhood has been related to an earlier onset of puberty and reduced height gain during adolescence.[128]

In a study of 463 Danish choir boys, a significant downward trend in age at voice break was found over a 10-year period (from 14 years to 13.7 years).[129] Age at voice break was significantly different between boys in the different prepubertal BMI quartiles, and a trend toward earlier voice break was associated with increasing BMI SDS. Boys in the heaviest quartile at 8 years of age had an increased likelihood of earlier voice break compared with those in the thinnest quartile, suggesting a relationship between prepubertal BMI and the timing of puberty in boys.[129]

Another Danish study included an evaluation of secular trends in pubertal onset over a 15-year period and their relationship to BMI in boys.[121] In a total of 1528 boys, onset of puberty, defined as age at attainment of a testicular volume $>$ 3 mL, occurred 3 months earlier in 2006–2008 than in 1991–1993. Significantly higher levels of luteinizing hormone but not testosterone were found in boys between 11 and 16 years of age in 2006–2008 compared with boys of the same age in 1991–1993. BMI SD scores increased significantly from 1991–1993 to 2006–2008 as well. Interestingly, pubertal onset and levels of luteinizing hormone were no longer significantly different between study periods after adjustment for BMI. In this study, the estimated mean age at onset of puberty had, therefore, declined during the 15-year interval, and this decline was associated at least partly with an increase in BMI.[121]

In a study from Jamaica, the effects of birth size, growth rates throughout childhood, and body composition on the timing of onset of puberty were assessed in both boys and girls.[130] Fast weight gain from age 0 to 6 months and during childhood, but not large birth size, was associated with advanced puberty in both sexes. In addition, elevated fat mass at 8 years of age was associated with advanced puberty in both sexes. These data support the hypothesis that fast growth throughout childhood, especially with fat-mass accretion, is associated with advanced pubertal development.[130,131]

In a study from Germany, body weight, height, peak height velocity, and pubertal stages were evaluated in 1421 peripubertal children.[132] In contrast to the findings from other studies, the researchers found no significant differences in mean pubic hair stage in girls and boys with obesity when compared with either lean or normal-weight children and, when analysis was restricted to children at pubic hair stage 2, age at this stage was found not to differ significantly between normal-weight and obese individuals. In boys, testicular volume at a given age was also similar across all weight groups.[133]

In summary, research to date highlights inconsistencies in how obesity has been found to affect pubertal timing in boys and emphasizes the need for future research in this area.

Effect of Endocrine Disrupting Chemicals

Some evidence supports associations between human pubertal timing and exposure to environmental modifiers, although much of the data relate to girls. Earlier menarche and pubarche have been associated with exposure to polybrominated biphenyls (PBB) and dichlorodiphenyl dichloroethene (DDT), whereas delayed breast and pubic hair development as well as delayed menarche have been associated with lead exposure.[134,135] In addition, elevated serum levels of a mycotoxin (zearalenone) have been reported in girls with precocious puberty,[136] and phthalate exposure has been associated with changes in pubertal timing. A study in Denmark demonstrated that delayed pubarche, but not thelarche, was associated with high phthalate excretion in urine samples from healthy school girls, which may suggest antiandrogenic actions of phthalates.[137] Greater exposure to pesticides has been reported in boys with cryptorchidism or hypospadias.[138]

The extent to which these effects are associations versus cause-and-effect relationships is unknown as is full understanding of underlying mechanisms. To what extent any effects are shared versus different in girls compared to boys is also not known. Finally, what effects these exposures have in the general population as opposed to isolated examples of associated abnormalities is not clear. Certainly more research is this area is needed.[139,140]

PRECOCIOUS PUBERTY

Traditionally, the onset of secondary sexual characteristics in a boy prior to age 9 years has been defined as precocious, whereas lack of testicular enlargement by age 14 years has been classified as delayed puberty. In clinical settings boys present less often with precocious puberty than with delayed puberty, which is discussed more extensively later in the chapter.

GnRH-Dependent Forms of Precocious Puberty

The most common form of precocious puberty is the activation of pulsatile GnRH secretion 2 to 2.5 SDs earlier than average, recognizing that the normal limits for the onset of puberty can vary by geographic area and by ethnicity. This form of precocious puberty is called

central or gonadotropin-dependent precocious puberty, and it represents true pubertal development. Central precocious puberty (CPP) may result from hypothalamic tumors or central nervous system (CNS) lesions (neurogenic CPP) but in most cases remains unexplained (idiopathic CPP) (Table 17-1).[141,142]

Hypothalamic hamartomas are an example of a cause of neurogenic CPP. The hamartomas are congenital malformations characterized by heterotropic gray matter, neurons, and glial cells, and they are generally located on the floor of the third ventricle or attached to the tuber cinereum. On MR imaging, hamartomas exhibit an isodense fullness. Histologic examination has shown immunoreactivity for GnRH and for astroglial factors such as TGFα. Postulated potential mechanisms include increased GnRH secretion from neurons emancipated from suppression or that TGFα stimulates GnRH secretion from GnRH neurons. Most hypothalamic hamartomas are sporadic, but they may occur in association with Pallister-Hall syndrome, which is due to mutations in the *GLI3* gene. Optic gliomas, which can be associated with NF1, can also cause GnRH-dependent precocious puberty.[143] Other etiologies of neurogenic CPP include pineal tumors, suprasellar cysts, previous head trauma, CNS radiation, and static encephalopathy.[144]

Loss-of-function mutations of the makorin RING-finger protein 3 (MKRN3) located at chromosome 15q11-q13 have been associated with GnRH-dependent precocious puberty. All clinically affected individuals inherited the mutated allele from their fathers, which can be explained because this gene is imprinted and expressed only by the paternal allele. Curiously, almost half of these patients were boys; this differs from the typical female predominance of GnRH-dependent precocious puberty. The finding of MKRN3 as a cause of CPP is also interesting because it calls attention to the importance of inhibitory factors in the regulation of pubertal timing (see Figure 17-1) because loss of function of this gene (and loss of a presumed inhibitory role) leads to CPP.[69]

GnRH-Independent Forms of Precocious Puberty

Precocious development of secondary sexual characteristics may also be caused by mechanisms that do not involve activation of pulsatile GnRH secretion. These forms of precocity are called gonadotropin-independent or peripheral precocity and include gonadal and adrenal tumors, tumors producing human chorionic gonadotropin, mutations activating the gonadotropic pathway, and exposure to exogenous sex steroids (see Table 17-1). Familial male-limited precocious puberty (FMPP, OMIM ID: 176410), also known as testotoxicosis, is a rare dominant form of gonadotropin independent precocity caused by constitutively activating mutations of the human LH choriogonadotropin receptor (*LHCGR*).[145] This disorder usually presents by age 1 to 4 years with physical signs of puberty, rapid virilization, growth acceleration, skeletal (bone age) advancement, and elevated testosterone levels despite prepubertal levels of LH.[146,147] McCune-Albright syndrome (MAS, OMIM ID: 174800) is another rare cause of male sexual precocity of genetic origin. It is caused by a postzygotic somatic activating mutation of the *GNAS1* gene. The mosaic constitutive activation of the GSα protein signaling leads to autonomous cell proliferation and scattered hyperfunction in endocrine organs with a wide phenotypic spectrum.[148,149] The classical features include the clinical triad of bone fibrous dysplasia (BFD), café-au-lait skin spots, and precocious development of secondary sexual characteristics. In addition, excessive pituitary function (such as hyperthyroidism due to activation of thyroid-stimulating

TABLE 17-1 **Common Etiologies of Sexual Precocity in Boys**

Central (GnRH Dependent)	Peripheral (GnRH Independent)
Idiopathic	Congenital adrenal hyperplasia
Central nervous system tumors	McCune-Albright syndrome
—Hamartomas	Testosterone producing tumors
—Astrocytomas	—Adrenal carcinoma or adenoma
—Adenomas	—Leydig cell tumor
—Gliomas	Gonadotropin/hCG producing tumors
—Germinomas	—Choriocarcinoma
Central nervous system infection	—Dysgerminoma
Head trauma	—Hepatoblastoma
Iatrogenic	—Chorioepithelioma
—Low-dose CNS radiation	—Teratoma
—Chemotherapy	—Gonadoblastoma
—Surgical	Exogenous exposure to androgen
Malformations of central nervous system	Familial male limited precocious puberty
—Arachnoid or suprasellar cysts	Hypothyroidism (Van Wyk–Grumbach syndrome)
—Hydrocephalus	

Modified from Nathan BM, Palmert MR (2005). Regulation and disorders of pubertal timing. Endocrinol Metab Clin North Am 34:617-641, ix.

hormone [TSH] secretion and hypercortisolism due to constitutive activation of ACTH secretion), kidney phosphate wasting, cholestasis, and hypertrophic heart disease can be present.[148,150] For reasons that are not clear, MAS leads to sexual precocity more often in girls than in boys.

In 1960, Van Wyk and Grumbach first described a syndrome characterized by breast development, uterine bleeding, and multicystic ovaries in the presence of long-standing primary hypothyroidism.[151] A unique diagnostic feature of the Van Wyk–Grumbach syndrome is the combination of delayed bone age with apparent sexual precocity. Boys with this syndrome have macroorchidism without significant virilization. Testicular histology shows enlargement of the seminiferous tubules without an increase in Leydig cell number.[152,153] Typically, GnRH stimulation shows a prepubertal response with suppressed LH confirming gonadotropin-releasing hormone (GnRH)-independent precocious puberty. Most cases appear to originate from autoimmune thyroid disease, but there are some case reports where the syndrome is secondary to unrecognized congenital hypothyroidism.[154] The pathophysiology of Van Wyk–Grumbach syndrome involves a complex mechanism, which is, at least in part, mediated by the direct action of TSH on FSH receptors. Recombinant human TSH (Rec-hTSH) elicits a dose-dependent cAMP response in cells expressing the human FSH-receptor in vitro; however, the concentration of rec-hTSH required for half-maximal stimulation was several logs greater than that of hFSH.[152] Early recognition and initiation of thyroid hormone replacement can lead not only to resolution of symptoms and improvement in adult height but also to avoidance of further diagnostic procedures, fear of malignancy, and unnecessary surgery.

Another cause of GnRH-independent precocious puberty includes the virilizing forms of congenital adrenal hyperplasia (CAH). These are autosomal recessive disorders that present with premature pubic hair development, axillary hair development, accelerated linear growth, and phallic enlargement in the absence of testicular enlargement. The most common form is 21-hydroxylase deficiency due to loss-of-function mutations in *CYP21A2*. Mutations in 11β-hydroxylase (*CYP11B1*) and 3β-hydroxysteroid dehydrogenase type 2 (*HSD3B2*) account for 5% to 10% of cases. Long-term exposure to androgens in this condition can activate central precocious puberty even when the excessive androgen secretion from the adrenals is suppressed by appropriate cortisol and mineralocorticoid replacement.

Androgen secreting tumors are rare causes of GnRH-independent precocious puberty in boys. Leydig cell tumors secrete testosterone. Because these tumors are usually unilateral, testicular volume may be asymmetric. The majority of Leydig cell tumors are benign. Ultrasound may be useful because the tumor may be too small to palpate. Mutations in the *LHCGR* gene have been identified in some adenomas.[155] In boys, tumors secreting hCG stimulate testicular testosterone secretion. These tumors are generally hepatic in origin. Virilization is a common presentation for adrenal tumors in children.

The classification of precocious puberty is not always clear. As noted earlier, treatment of peripheral forms of sexual precocity, such as CAH or testicular or adrenal tumors, can lead to true central precocious puberty through subsequent activation of pulsatile GnRH secretion. In addition, in some cases of precocious pubertal development among girls, pubertal manifestations will regress or stop progressing, making treatment unnecessary; such cases are not as commonly seen in boys.[156,157] The mechanisms responsible for these nonprogressive forms of precocious puberty are not known. There is evidence that in some cases the HPG axis is intermittently but not fully activated.[157,158] Another form of early development of secondary sexual characteristics occurs when the hypothalamic-pituitary-adrenal (HPA) axis matures 1 to 2 years before the HPG axis, causing premature adrenarche. Premature adrenarche, more common among girls, is not associated with progressive pubertal development and is manifested by pubic and axillary hair with modest elevation of DHEA-S, but without substantial advancement in bone age and hence does not require treatment.

In cases of progressive central or peripheral precocity, the concerns include short adult stature due to early epiphyseal fusion and adverse psychosocial outcomes.[141,159] Several studies have assessed adult height in subjects with a history of precocious puberty. Mean heights range from 151 to 156 cm in boys and from 150 to 154 cm in girls in older patient series, which corresponds to a loss of more than 20 cm in boys and 12 cm in girls from predicted adult height.[160] Height loss due to precocious puberty is inversely correlated with the age at the onset of puberty, and treated patients today tend to have a later onset of puberty than did patients in historical series.[160] It is important to assess carefully the growth rate and skeletal maturation of individuals with central precocious puberty due to CNS lesions (e.g., tumors, cerebral malformations or injury from trauma or radiation) because these lesions may be associated with concomitant growth hormone (GH) deficiency, which can be masked by sex steroid–driven adequate growth rates. In such cases, undiagnosed and untreated GH deficiency may result in severely compromised adult height.

Although available data derive mostly from study of girls with early puberty, it has been suggested that a higher proportion of early-maturing adolescents engage in exploratory risky behaviors (sexual intercourse and legal and illegal substance use) at an earlier age than adolescents maturing within the normal age range or later.[161,162] However, available data regarding potential adverse psychosocial outcomes specific to patients with precocious puberty are limited, as it is not clear that data obtained from individuals with maturation at the early end of the normal spectrum are fully applicable to precocious puberty.

Pathologic entities leading to sexual precocity warrant treatment, but whether and at what ages one should initiate treatment for idiopathic central precocious puberty is less certain. Currently, robust data regarding the short-term and long-term psychosocial sequelae of CPP and data regarding whether treating CPP with gonadotropin releasing hormone analogues (GnRHa) alter these outcomes are lacking.[163] Thus, one needs to be cautious when using psychosocial outcomes as a rationale for

pharmacologic intervention to halt pubertal progression, especially among boys with the onset of puberty close to the normal range.

Evaluation of the Boy with Precocious Development of Secondary Sexual Characteristics

Boys with sexual precocity require careful evaluation because many have underlying disorders.[141] Those with premature pubarche (early development of pubic hair or body odor) need to be assessed for peripheral causes of precocity before determining that the premature pubarche stems simply from premature adrenarche. Premature adrenarche may be distinguished from true precocity over time by lack of progression. Conversely, progressive precocity is marked by significant bone age advancement (> 2 SD for age), a history of growth acceleration, and a progression of secondary sexual characteristics on physical examination.

The evaluation of a boy with sexual precocity is outlined in Box 17-1. Considerations include first verifying that the pubertal development is occurring outside the range of normal development prior to initiating an evaluation. It is also important to note that all tests are not appropriate in each case and that the diagnostic yield of each test is not known. Hence, it is important to allow history and physical examination to guide the evaluation. The child with bilateral enlarged testicles, for example, is most likely to have central precocious puberty, with FSH having led to the expansion of seminiferous tubule volume (although cases of hypothyroidism can also present with bilateral testicular enlargement). Conversely, the child with bilateral prepubertal sized testicles is more likely to have peripheral precocity, whereas the child with unilateral testicular enlargement may well have a testicular tumor. Testing should be directed accordingly.

BOX 17-1 Outline of the Evaluation of Sexual Precocity in Boys

Common Initial Screening Tests

- Careful history, physical exam, and assessment of growth velocity
- Bone age
- LH, FSH
- Testosterone (and in some cases estradiol)
- DHEA-S
- 17-hydroxyprogesterone
- TSH, T4

Secondary Tests to Consider

- Testicular or abdominal ultrasound
- MRI of pituitary and brain
- GnRH agonist stimulation test
- ACTH stimulation test

Modified from Nathan, B. M., & Palmert, M. R. (2005). Regulation and disorders of pubertal timing. Endocrinol Metab Clin North Am, 34, *617–641, ix.*

Peripheral causes of sexual precocity are characterized by suppressed LH and FSH values in the setting of elevated sex steroid levels. Testosterone levels will be elevated in instances of isosexual precocity (secondary sexual characteristics consistent with male gender), whereas estradiol levels may be elevated in the rare instances of contrasexual precocity (secondary sexual characteristics inconsistent with gender, such as marked breast development as the presenting sign in a boy). Determining the underlying mechanism for progressive peripheral precocity is important because all instances will result from pathologic conditions or exogenous exposures.[164] Levels of androgens that are elevated beyond the range expected for the pubertal stage suggest an adrenal or testicular cause of the precocity. DHEA-S is often used as a screen for adrenal tumors or adrenal pathology. Determination of the 17-hydroxyprogesterone concentration is used to screen for congenital adrenal hyperplasia due to 21-OH deficiency. Congenital adrenal hyperplasia and hormone secreting tumors of the adrenal are discussed in detail in Chapter 13.

Historically, the gold standard for the diagnosis of central precocious puberty was GnRH stimulation testing and the demonstration of pubertal gonadotropin responses. However, with the advent of ultrasensitive gonadotropin assays,[165] the recognition that these assays can identify individuals with CPP using unstimulated random samples,[166] and the unavailability of GnRH for testing has led some to forego stimulation testing. GnRH agonists can be used as an alternative to GnRH for stimulation testing, but again the diagnosis can often be made through the combination of clinical features and a baseline LH value in the pubertal range. Although determining the diagnostic cutoff for the basal LH level is difficult because of a lack of normative data and variability among assays, a value of ≥ 0.3 IU/L using an ultrasensitive assay with a detection limit near 0.1 IU/L is commonly cited.[163] If stimulation testing is done, a diagnostic cutoff of 5 IU/L for peak value has been suggested. LH values are more useful than FSH values in the diagnostic evaluation of precocious puberty, but stimulated LH/FSH values can help to identify patients with slowly progressive precocious puberty because these children tend to have FSH predominant responses.[157,167] Whether due to referral bias or to differences in underlying physiology, idiopathic central precocious puberty is a more common cause of precocious puberty among girls than among boys in endocrinology clinics.[168,169] Hence, all boys with CPP should have brain magnetic resonance imaging (MRI) to exclude underlying pathology.[163]

In summary, it is important for physicians evaluating patients with suspected precocious puberty to address these questions: Is pubertal development occurring outside the normal range of development? What is the underlying mechanism, and is that mechanism associated with a risk of a serious condition, such as an intracranial lesion? Is the pubertal development likely to progress, and may this impair the child's normal physical and psychosocial development?

Treatment of the Child with Precocious Puberty

Unless underlying pathology that requires intervention is present, whether the precocious pubertal development is likely to impair the boy's normal physical or psychosocial development is often the feature that determines if treatment is needed.

Central Precocious Puberty

A prerequisite for the consideration of therapy for CPP is the presence of documented, progressive pubertal development over a 3- to 6-month period, although this period of observation may not be necessary if the child is at or beyond Tanner stage 3 genital development. Documentation of progressive development is important because nonprogressive forms of precocious puberty do not require intervention.[163] Because epiphyseal fusion is an estrogen-dependent process, early and progressive production of sex steroids can cause rapid advancement of skeletal maturation and result in compromised adult height. Thus, preservation of adult stature is one of the main reasons to consider treatment of CPP with GnRH agonists, which down-regulate the pituitary-gonadal axis and limit pubertal progression. Although the data derive primarily from studies among girls, it appears that the risk of short stature is most pronounced in those children with earlier onset of symptoms and more shortened prepubertal growth periods.[170] Other factors, such as advanced bone age, may also contribute to poorer height outcomes.[170] In girls, available data suggest that the greatest height gain (preservation) occurs with onset of puberty prior to 6 years of age, with more moderate benefit being seen with onset between 6 and 8 years.[163] However, insufficient data exist to draw similar conclusions regarding age of onset and height outcomes among boys[171,172]; consequently, a consensus conference recommended considering initiation of GnRHa therapy for all boys with onset of CPP before age 9 years who have compromised height potential.[163]

The second main reason to consider therapy in CPP is to mitigate potential psychosocial effects of precocious puberty. However, as noted earlier, additional studies are needed to determine the effects of precocious puberty on quality of life and psychosocial functioning and to evaluate whether treatment with GnRHa therapies affects these outcomes. Thus, the consensus conference concluded that the use of GnRHa therapies solely to influence psychosocial consequences of precocious puberty needs to be considered carefully given the absence of convincing data.[163] These cautions are even more pertinent for boys because data regarding males are even more scarce.

Various formulations (intramuscular, subcutaneous, and intranasal) of short (daily) and long-acting GnRH agonists are available. The depot formulations are preferred, as they are easier for patients and families to administer regularly over a long treatment period. The initial depot medications were 1-month formulations, which have been shown to be well tolerated and effective.[173,174] Subsequently, 3-month depot formulations were developed,[163,175,176] and a 1-year depot formulation (histrelin-acetate) has also become available.[177-179] Which GnRHa to use depends on patient and provider preference, local regulatory approvals, and reimbursement systems.[163]

GnRH agonists are generally well tolerated. Occasionally, an initial, temporary flare of GnRH activity can occur and result in transient advancement of secondary sexual characteristics. Local reactions to the injection can occur in 10% to 15% of patients. If these reactions are persistent, a change in agent should be considered to try to prevent the development of sterile abscesses. Other considerations include long-term effects of GnRH agonists on bone mineral density and reproductive function. These outcomes have largely been studied among girls, but the available data are reassuring.[163] Whether GnRHa use is associated with increased obesity is less clear, and data among boys with CPP are sparse. However, in girls most studies are reassuring and indicate that this concern is not great enough to argue against use of GnRHa in cases of true central precocious puberty, especially because risk factors for obesity may be associated with CPP itself and not necessarily the use of GnRHa.[163]

Monitoring of GnRH agonist therapy should consist of assessment of Tanner stage and growth velocity every 3 to 6 months, with periodic assessment of skeletal maturation. Progression of pubertal development or of rapid skeletal maturation indicates a lack of efficacy, poor adherence, or a misdiagnosis. Such patients warrant reevaluation, including measurement of baseline or stimulated LH levels; conversely, the routine measurement of gonadotropins to monitor suppression during GnRHa treatment is controversial.[142,163] The optimal time to discontinue GnRH agonist therapy is unclear and difficult to determine because of variability in chronologic age, bone age, and degree and duration of secondary sexual characteristics prior to initiation of therapy. Data among boys are limited, but examination of clinical characteristics at the discontinuation of therapy—such as treatment duration, height, growth velocity, bone age, and chronologic age—has failed to identify clear predictors of adult height among girls. Thus, it seems reasonable to time discontinuation based on patient and family preference, often with the intent of having pubertal development resume in parallel with the patient's peers.[163]

Peripheral Precocity

In the past, treatment of GnRH independent causes of sexual precocity such as McCune Albright syndrome or familial male limited precocious puberty included using inhibitors of steroidogenesis (ketoconazole), weak antiandrogen agents (spironolactone), and, subsequently, first-generation aromatase inhibitors (AIs) (testolactone).[180-184] Although these therapies are to some extent effective in slowing growth velocity and reducing virilization,[185-188] the risk of hepatotoxicity and adrenal insufficiency with ketoconazole as well as the requirement of multiple daily dosing are obstacles to achieving a favorable therapeutic outcome.[189] Short-term combination therapy with a potent antiandrogen agent, bicalutamide, and third-generation AIs, anastrozole and letrozole, has also

suggested efficacy in reducing growth rate and virilization and improving predicted adult height.[190-193] This combination therapy provides a more convenient once-daily dosing regimen, although the therapy is more expensive than previous treatments. However, further controlled clinical trials using modern antiandrogens in combination with aromatase inhibitors are needed, including the evaluation of the long-term effects of such therapy on adult height, fertility, metabolic parameters, cognitive functions,[194] and bone health.[195] Until long-term data are available from a larger sample of patients, this combination therapy should be used judiciously and cautiously.

DELAYED PUBERTY

The etiologies, evaluation, and treatment of delayed puberty have been reviewed by the authors.[196] Delayed puberty is defined as the absence of testicular enlargement 2 to 2.5 standard deviations later than the population mean (traditionally age 14 years in boys). As noted previously, because of the downward trend in pubertal timing in some but not all reports from the United States[114,197,198] and other countries,[121,199] some advocate for younger age cutoffs for the general population or perhaps for particular countries or ethnic groups. However, the secular change in the onset of puberty has not been seen in the late developing boys,[121] and hence the need to readjust age definitions for delayed puberty in males may not be necessary. Unlike for sexual precocity, development of pubic hair is usually not considered in the definition of delayed puberty because pubarche may result from maturation of the adrenal glands (adrenarche) and the onset of pubic hair can be independent of HPG axis activation.

Delayed puberty is often quite concerning to patients and families. It can affect psychosocial well-being and peer relationships, and these issues are common reasons for initiating therapy. However, as with precocious puberty, further studies are needed to assess fully the psychosocial distress experienced by individuals with delayed puberty, whether this distress has long-term sequelae, and what impact sex steroid supplementation has on these outcomes.[196] Patients, families, and practitioners are also often worried that delayed puberty may affect adult stature, and many patients present with relative familial short stature along with delayed puberty, which accentuates concerns about adult stature. Adult height can indeed be affected by delayed puberty, but on average it is only slightly below the genetic target.[200] It remains unclear whether adult bone mass is adversely affected by pubertal delay[201] and whether concerns related to bone health represent a medical reason to initiate therapy.

Etiologies of Delayed Puberty

The most common cause of delayed puberty in boys is constitutional delay of growth and puberty (CDGP), which refers to an extreme of the normal spectrum of pubertal timing. In one large series, approximately 65% of boys and 30% of girls with delayed puberty had CDGP[202] (Figure 17-3). However, because the data derive from a tertiary referral center, these percentages likely underestimate of the frequency of CDGP encountered by primary care providers.

Although CDGP represents the single most common cause of delayed puberty in boys, it is a diagnosis of exclusion, and potential pathologic causes of delay must be excluded. Other causes of delayed puberty can be divided into three main categories[196,202] (Figure 17-3 and Table 17-2): permanent hypogonadotropic hypogonadism, which represents approximately 10% of cases among boys and is characterized by low LH and FSH levels due to hypothalamic or pituitary disorders; transient hypogonadotropic hypogonadism or functional hypogonadotropic hypogonadism, which represents approximately 20% of cases and where pubertal delay is due to delayed maturation of the HPG axis secondary to underlying conditions; and hypergonadotropic hypogonadism, which represents approximately 5% to 10% of cases and is characterized by elevated LH and FSH levels due to lack of negative feedback from the gonads.

CDGP

The etiology of CDGP is unknown. Suggested causes include increased total energy expenditure[203] and increased insulin sensitivity,[204] but no definitive etiology has been identified. CDGP does have a strong genetic basis. Fifty percent to 80% of variation of the timing of puberty in humans is due to genetic factors,[101] and 50% to 75% of individuals with CDGP have a family history of delayed puberty.[205,206] The inheritance patterns observed among pedigrees with CDGP are variable but most often consistent with an autosomal dominant pattern, with or without complete penetrance. Inheritance

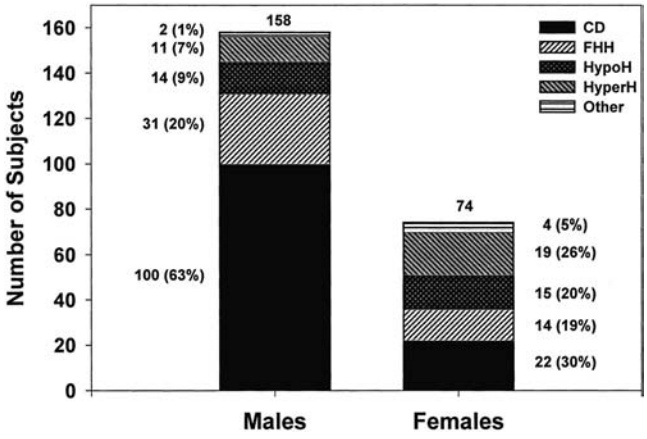

FIGURE 17-3 ■ Distribution of diagnostic categories among males and females with delayed puberty. CD, Constitutional delay of growth and puberty; FHH, functional hypogonadotropic hypogonadism; Hypo H, permanent hypogonadotropic hypogonadism; Hyper H, permanent hypergonadotropic hypogonadism. (Reprinted with permission Sedlmeyer, I. L., & Palmert, M. R. [2002]. Delayed puberty: analysis of a large case series from an academic center. *J Clin Endocrinol Metab, 87,* 1613–1620.)

TABLE 17-2 **Causes of Delayed Puberty Other Than Constitutional Delay of Growth and Puberty**

Hypergonadotropic Hypogonadism	Permanent Hypogonadotropic Hypogonadism	Functional Hypogonadotropic Hypogonadism
Genetic syndromes	CNS tumors/infiltrative diseases	Systemic illness/conditions
—Noonan syndrome and related disorders	—Astrocytoma	—Cystic fibrosis
Cryptorchidism	—Germinoma	—Asthma
Gonadal dysgenesis	—Glioma	—Inflammatory bowel disease
Vanishing testes syndrome	—Craniopharyngioma	—Celiac disease
Trauma/testicular torsion	—Prolactinoma	—Juvenile rheumatoid arthritis
Chemotherapy/radiation therapy	—Langerhans cell histiocytosis	—Anorexia nervosa/bulimia
Gonadal infection	Rathke's pouch cyst	—Sickle cell disease
—Mumps, Coxsackie	Genetic defects*	—Hemosiderosis
Autoimmune orchitis	—Kallmann syndrome *(KAL1, FGFR1, PROK2, PROKR2, FGF8, HS6ST1,* and *CHD7)*	—Thalassemia
Defects in steroidogenesis	—Isolated hypogonadotropic hypogonadism *(KAL1, GNRHR, GNRH1, GPR54, FGFR1, FGF8, PROK2, PROKR2, TAC3, TACR3. HS6ST1, NELF,* and *CHD7)*	—Chronic renal disease
—5-alpha reductase deficiency (SR5A2)	—HPG axis development *(DAX1, SF-1, HESX-1, LHX3,* and *PROP-1)*	—AIDS
—17, 20 lyase deficiency (CYP17A1)	—Obesity and hypogonadotropic hypogonadism (*LEP, LEPR,* and *PC1*) syndromes	Endocrinopathies
—Congenital lipoid adrenal hyperplasia (StAR)	—Prader-Willi	—Diabetes mellitus
—17-hydroxysteroid dehydrogenase deficiency (HSD17B3)	—Bardet-Biedl	—Hypothyroidism
Androgen insensitivity	—CHARGE	—Hyperprolactinemia
Sertoli cell only syndrome (Del Castillo syndrome)	Gaucher disease	—Growth hormone deficiency
	Postcentral nervous system infection	—Cushing syndrome
	Midline defects	Excessive exercise
	—Septo-optic dysplasia	Malnutrition
	—Congenital hypopituitarism	
	Chemotherapy/radiation therapy	
	Trauma	

*Some genes have been identified as causes of both Kallmann syndrome and isolated hypogonadotropic hypogonadism (IHH).
Table reproduced from Palmert, M. R., & Dunkel, L. (2012). Clinical practice. Delayed puberty. N Engl J Med, 366, *443–453 with permission.*

of CDGP is not sex-specific and is characterized by family members having either relatively delayed development (e.g., the average age at menarche among mothers of individuals with CDGP is 14.3 years compared to a mean of 12.7 among controls[19]) or evidence of pubertal timing that would have met clinical criteria for delayed puberty. Review of growth records often shows that linear growth during the first few years of life decelerates followed by growth velocity tracking along the lower percentiles during childhood. Skeletal maturation is usually delayed.

As previously discussed, the investigation of Kallmann syndrome (KS) and isolated hypogonadotropic hypogonadism (IHH) has led to the identification of genes that play critical roles in development and regulation of the HPG axis, but mutations in the genes identified to date do not cause CDGP, except in rare instances[81,102]; however, the genes causing 60% to 70% of cases of KS and IHH remain unknown.[62] Loci have also been identified that are associated with the age of menarche in the general population,[103-107] but these particular loci have likewise not yet been associated with CDGP.[207] Further studies are needed to identify the genes that cause CDGP, and it is likely that identification of these genes will also elucidate factors that regulate the timing of puberty in the general population.

Hypogonadotropic Hypogonadism

GnRH deficiency can be caused by mutations in many genes and is more common in boys than girls, affecting one in 7500 males and only one in 70,000 females.[208] Inheritance patterns include X-linked, autosomal dominant, and autosomal recessive. However, sporadic cases are more common than familial forms. Hypogonadotropic hypogonadism (HH) has been subclassified into three major categories: (1) Kallmann syndrome with anosmia, (2) HH without anosmia, and (3) acquired HH. However, with recognition of phenotypic heterogeneity within families, these classifications may need to be modified.

The classic form of Kallmann syndrome is characterized by isolated gonadotropin deficiency, anosmia, and X-linked inheritance. This disorder is due to mutations in anosmin 1 encoded by the *KAL1* gene resulting in failure of GnRH neurons to migrate to the hypothalamus. MR imaging can confirm the diminished presence or absence of the olfactory bulbs. Nonreproductive features include unilateral renal agenesis, sensorineural hearing loss, synkinesia, cerebellar ataxia, and cleft palate. *KAL1* mutations are also associated with a relatively high incidence of cryptorchidism, microphallus, and decreased inhibin B concentrations.

As noted earlier, the list of genes associated with HH is expanding. Nonreproductive clinical features of patients with *PROK2* and *PROK2R* mutations include fibrous dysplasia, obesity, synkinesia, and epilepsy.[90] To date, numerous mutations have been identified in the *FGFR1* gene; associated features can include skeletal anomalies and synkinesis. Mutations in *FGF8*, the ligand for FGFR1, have also been associated with holoprosencephaly, septo-optic dysplasia, and moebius syndrome.[209] Both normosmia and anosmia have been reported for these four genes.

Loss-of-function mutations in *HS6ST1*, *SEMA3A*, *WDR11*, and *NELF* have been identified in patients with HH.[89,210-212] In families carrying mutations in these genes, phenotypic heterogeneity for anosmia occurs and HH segregates as a complex trait found in association with mutations in other genes. Thus, it remains to be determined whether mutations in these genes are sufficient to lead to GnRH deficiency. Mutations in genes encoding proteins such as *FGF17* and *IL17RD* that modulate the signaling efficiency of FGF8 through FGFR1 have been identified, suggesting the existence of a FGF8 synexpression group that may form an oligogenic basis for HH.[3]

Mutations in genes encoding neurokinin B and its receptors, *TAC3* and *TACR3*, have been identified in HH patients.[27] As noted previously, these genes are highly expressed in the same neurons that express kisspeptin, emphasizing the role of kisspeptin in the regulation of pubertal timing. Mutations in *GNRH1*, *KISS1*, *KISS1R*, *TAC*, and *TACR3* affect GnRH secretion. Curiously, patients have been reported to have reversible HH despite carrying loss-of-function mutations in *FGFR1*, *CHD7*, *TAC3*, or *TACR3* genes. The biologic mechanism for this reversible gonadotropin deficiency remains unclear.[213,214]

Pituitary-Dependent Hypogonadism

Mutations in the GnRH receptor (*GNRHR*) gene have been reported. The phenotype ranges from neonatal presentation with microphallus and cryptorchidism to adolescent presentation for evaluation of delayed puberty.[215] Mutations in the FSH-beta and LH-beta have also been described. Developmental anomalies of pituitary development such as midline defects or septo-optic dysplasia can be associated with gonadotropin and other pituitary hormone deficiencies. Genes associated with developmental anomalies of the pituitary include *HESX1*, *SOX2*, *PITX2*, *LHX3*, and *PROP1* as well as some of the genes known to cause HH such as *FGFR1*, *FGF8*, and *PROKR2*. Nongenetic factors such as maternal cocaine abuse, valproate toxicity, and intrauterine vascular disruptive events may also cause CNS developmental anomalies.

Hypothalamic and Pituitary-Dependent Hypogonadism

Some disorders affect both the hypothalamus and pituitary. Congenital adrenal hypoplasia due to mutations in the DAX1/NROB1 gene is an X-linked disorder characterized by primary adrenal insufficiency and hypogonadotropic hypogonadism. In this disorder, the fetal adrenal cortex develops normally, but the adult zone of the adrenal cortex fails to develop. Affected males may present with adrenal insufficiency at 6 to 8 weeks of age. However, some may present in adolescence with delayed puberty. Decreased hypothalamic GnRH secretion or decreased pituitary responsiveness can occur leading to decreased LH, FSH, and testosterone concentrations. Mutations in this gene may be part of a contiguous gene deletion syndrome with Duchenne muscular dystrophy and glycerol kinase deficiency.[216]

Intracranial tumors can lead to delayed puberty. The most common neoplasm is a craniopharyngioma, which is thought to arise from remnants of Rathke's pouch. Other symptoms can include decreased growth velocity, headache, polyuria, and visual disturbances. Suprasellar or intracellular calcifications may be detected on CT scans. Other tumors include germ cell tumors, epidermoid and dermoid cysts, prolactinomas, and optic gliomas. Histiocytosis X is characterized by infiltration of lipid-laden histiocytes into skin, bone, or visceral organs. Although diabetes insipidus is the most common endocrine manifestation of histiocytosis X, other anterior pituitary hormone deficiencies may occur.

Hyperprolactinemia can lead to hypogonadism. Prolactin can directly suppress pulsatile GnRH secretion. Hyperprolactinemia can be due to microadenomas (adenomas < 10 mm) or macroadenomas (> 10 mm). MRI provides greater anatomic detail that CT scans. Patients with primary hypothyroidism or taking psychotropic medications may have hyperprolactinemia.

Several syndromes are associated with delayed puberty. These syndromes include Prader-Willi, Bardet-Biedl, Alström, and Bloom syndromes. Hereditary hematochromatosis leads to iron overload and iron deposition in endocrine organs leading to HH, impaired glucose tolerance, and diabetes mellitus.

Functional Hypogonadotropic Hypogonadism

Transient causes of hypogonadotropic hypogonadism or functional hypogonadotropic hypogonadism where pubertal delay is due to delayed maturation of the HPG axis secondary to underlying conditions may represent 20% of the etiology of delayed puberty (see Table 17-2). Examples of these conditions include anorexia nervosa, which is a chronic disorder characterized by decreased caloric intake, weight loss, and excessive physical activity. Although considered to be a disorder affecting females, approximately 10% of patients are male. Osteopenia and osteoporosis are potential consequences. In addition to low gonadotropin concentrations, cortisol, GH, and reverse T3 concentrations may be elevated. Chronic disorders such as sickle cell anemia, thalassemia, cystic fibrosis, and chronic renal disease may also be associated with delayed puberty. Intensive physical activity and the need to "make weight" in wrestlers may disrupt pubertal development. For wrestlers, these changes generally reverse within months of the end of the wrestling season.

Hypergonadotropic Hypogonadism

Primary gonadal failure leads to loss of negative feedback inhibition and hypergonadotropic hypogonadism. Although they represent only 5% to 10% of the causes of delayed puberty, etiologies within this category are broad and include sex chromosome anomalies, aberrant gonadal differentiation, gonadal injury, disorders of steroidogenesis, and disorders affecting steroid hormone action. Some disorders such as gonadal dysgenesis, Klinefelter syndrome, and Noonan syndrome affect both Leydig and Sertoli cell function (Figure 17-4). Disorders affecting steroidogenesis predominantly affect Leydig cell function.[11]

Klinefelter Syndrome

Klinefelter syndrome, which is associated with 47,XXY karyotype, is not a cause of delayed puberty per se but can be a cause of a prolonged or stalled pubertal course. The incidence is reported to be 1 in 667 when ascertained by prenatal cytogenetic analysis, but it is much lower when identified solely by clinical features. Thus, incomplete ascertainment of affected individuals likely occurs.[217] Parental origin of the extra X chromosome is approximately equal. In addition to the relative testosterone deficiency, it has been suggested that the extra copy of the short stature homeobox-containing (*SHOX*) gene in the pseudoautosomal region of the X chromosome contributes to the tall stature.[218]

The typical clinical features are small firm testes, tall stature, small penis, and gynecomastia. Both androgen secretion and spermatogenesis are impaired. Inhibin B concentrations are relatively normal during childhood but decline during puberty consistent with the progressive testicular failure. AMH levels follow the normal male pattern but progressively decline from mid-adolescence due to Sertoli cell dysfunction.

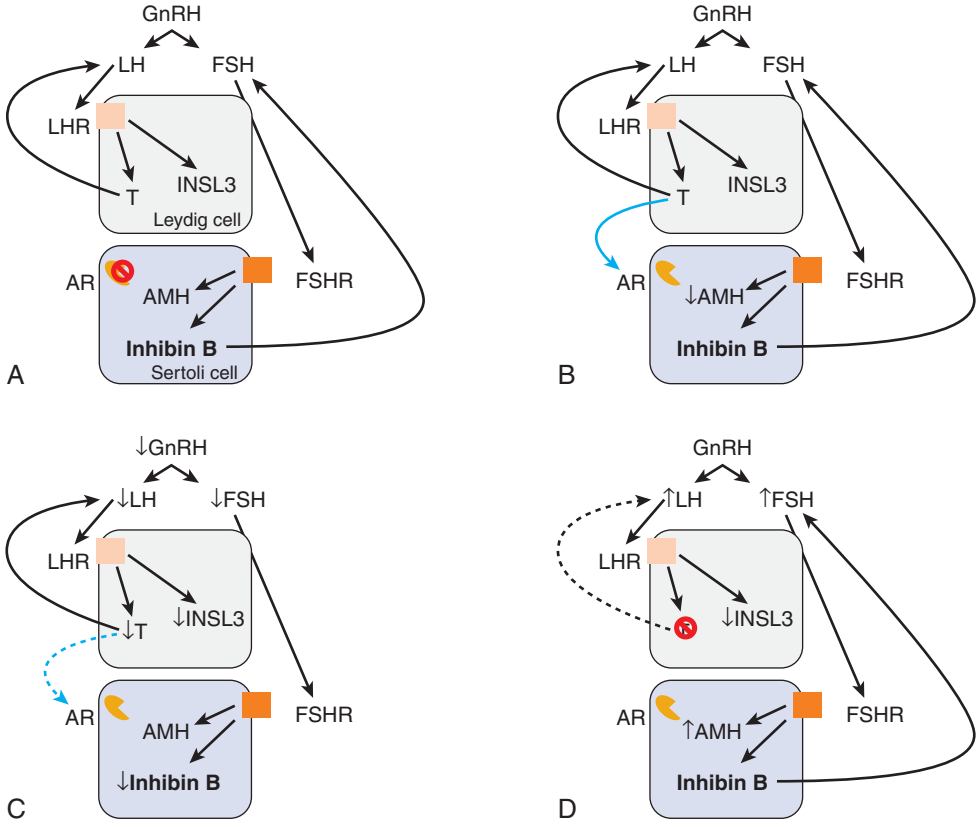

FIGURE 17-4 ■ Testicular compartments and hormone production. In the usual situation, hypothalamic GnRH secretion regulates pituitary LH and FSH secretion. LH acts on the LH receptor expressed on Leydig cells. The Leydig cell secretion of testosterone and INSL3 is influenced by LH secretion. FSH acts on the FSH receptor expressed on Sertoli cells and influences AMH and inhibin B secretion. **A**, Dynamics of HPG axis during gestation, infancy, and pubertal patients with CAIS. LH stimulates testosterone synthesis and FSH promotes AMH secretion. However, Sertoli cells do not express AR during fetal life and infancy. In patients with CAIS, AR is dysfunctional. The lack of Sertoli cell AR expression leads to high AMH production. Inhibin B levels reflect Sertoli cell function even when germ cells are absent. **B,** Dynamics of HPG axis at puberty. Upon reactivation of the GnRH pulse generator, increased LH secretion leads to increased testosterone secretion generating increased intra-testicular testosterone concentrations. INSL3 secretion also increases. Through its paracrine activity, testosterone promotes increased AR expression by Sertoli cells, which leads to decreased AMH secretion. FSH stimulates inhibin B secretion; inhibin B regulates FSH secretion. **C,** Dynamics of HPG axis in patients with hypogonadotropic hypogonadism. The lack of GnRH, LH, and FSH secretion results in testosterone deficiency. INSL3 and inhibin B secretion do not increase. During childhood AMH concentrations may have been lower than normal due to chronic lack of FSH stimulation, Due to the lack of testosterone's inhibitory effect on AMH production, AMH concentrations may be higher than normal during the adolescent years. **D,** Dynamics of HPG axis in patients with LHR mutations and defects in steroidogenesis. At puberty, in the absence of adequate testosterone production, AMH concentrations remain high.

The decreased testicular volume is secondary to degeneration of the seminiferous tubules. Boys with mosaic karyotypes with one normal 46,XY cell line tend to have a milder phenotype. The magnitude of testosterone deficiency and testicular failure varies among affected men. Treatment goals include development of secondary sexual characteristics, increased muscle mass, optimal bone density, and sexual function.

Academic and emotional difficulties are common. Some boys are detected through their learning disabilities. The cognitive phenotype is characterized by deficits in language and executive function. The executive dysfunction is characterized by poor decision making, problem solving, reasoning, and planning issues. Difficulties with self-control, increased distractibility, and reduced fine and gross motor skills are common.[219] Nonreproductive features can include an increased incidence of type 2 diabetes, breast cancer, mediastinal tumors, and vascular disease.[220]

Apart from decreased number of germ cells, testicular architecture is relatively normal in fetal and prepubertal testes. Loss of germ cells beginning in utero culminating in fibrosis and hyalinization of the seminiferous tubules is the proximate cause of the infertility associated with Klinefelter syndrome.[221] Fertility preservation is a relevant topic for the older adolescent boy. Some men with Klinefelter's have oligospermia and are able to father children without medical intervention. For others, paternity has been achieved with testicular sperm extraction combined with intracytoplasmic sperm injection (TESE-ICSI).[222]

XX Males

The frequency of XX males is approximately 1 in 25,000 males. These individuals may come to medical attention for evaluation of delayed puberty, infertility, gynecomastia, or a disorder of sexual development. XX males can be classified as *SRY*-positive or *SRY*-negative. In approximately 80% of XX males, the *SRY* gene can be detected by genetic analysis. In most instances, the *SRY* gene has been translocated to an X chromosome, but translocation to an autosome can occur. The typical phenotype for SRY-positive 46,XX males includes normal male external genital development, small testes, azoospermia, hypergonadotropic hypogonadism, and infertility.

Molecular etiologies are more variable for the SRY-negative 46,XX male. Duplication of *SOX9* was reported in one family.[223] Partial duplication of chromosome 22q13 leading to overexpression of *SOX10* was found in one 46,XX sex reversed patient in association with multiple congenital anomalies.[224] Another genetic etiology of 46,XX sex reversal are mutations in the R-spondin 1 (*RSPO1*) gene. This gene codes for a secreted furin-like domain protein that stabilizes β-catenin in the wingless-type mouse mammary tumor virus integration site-4 (WNT-4) signaling pathway. Patients with *RSPO1* mutations may also have palmoplantar hyperkeratosis with squamous cell carcinoma of skin.[225]

In rare instances, ovotesticular disorder with predominantly male phenotype can occur. This disorder can be diagnosed only with the presence of ovarian tissue containing follicles and testicular tissue containing seminiferous tubules present in the same individual, and often in the same gonad (ovotestis). Most patients with ovotesticular disorder have a 46,XX karyotype.

Gonadal Dysgenesis

This classification encompasses disorders characterized by atypical testicular determination and differentiation, and individuals can present with a disorder of sexual development, sex reversal, or delayed puberty. Approximately 15% of 46,XY patients with gonadal dysgenesis carry mutations in the *SRY* gene. The histologic appearance of the testes can include immature tubules with poorly differentiated Sertoli cells, poorly differentiated Leydig cells, and Sertoli cell only pattern. In some instances, gonadal differentiation appears to be a continuum with more or less differentiation along male or female direction. Carcinoma in situ may be identified. The clinical features are variable and related to the specific disorder and hormone status.

Pure gonadal dysgenesis, also known as Swyer syndrome, is characterized by complete male-to-female sex reversal. At birth, the external genitalia are normal female. Typically, a uterus and fallopian tubes are present, but Wolffian elements are usually absent. Affected individuals present with delayed puberty characterized by absent breast development and hypergonadotropic hypogonadism. Gonadal histology shows "streak" gonads and may show some evidence of testicular development. However, the testicular elements are aberrant. Due to the high risk for gonadal tumors, the gonads are best surgically removed.

Sex chromosome mosaicism is associated with gonadal dysgenesis. The clinical spectrum of internal and external genital development is highly variable. The correlation between the phenotypic appearance, the results of routine peripheral blood karyotyping, and gonadal histology is poor.[226]

The phenotypic spectrum for 45,X/46,XY mosaicism is broad, ranging from female with features of Turner syndrome to normal male appearance, and many live undiagnosed as normal males. However, ambiguous genitalia in a newborn but also mild undervirilization (e.g., hypospadias) in boys or even typical Turner syndrome in girls may be associated with 45,X/46,XY mosaicism.

Syndromes Associated with Delayed Puberty

Noonan syndrome is an autosomal dominant disorder characterized by short stature, ptosis, hypertelorism, mild intellectual disability, and congenital heart disease. Cryptorchidism is common in males. Approximately 50% of cases are due to mutations in the protein-tyrosine phosphatase, nonreceptor type 11 protein (*PTPN11*) gene (also known as *SHP2*). Other genes associated with Noonan syndrome include *KRAS*, *SOS1*, *NRAS*, and *RAF1*. The proteins encoded by these genes are members of the RAS-MAPK signaling pathway.

In one longitudinal study, LH, FSH, testosterone, AMH, and inhibin B concentrations were comparable to healthy boys prior to the onset of puberty. However,

some individuals with Noonan syndrome have delayed puberty, and the adult men with Noonan syndrome had higher LH, FSH, and testosterone concentrations and lower AMH and inhibin B concentrations compared to healthy controls.[227] Because these differences were observed in the total group of men with Noonan syndrome as well as the group with history of cryptorchidism, both Sertoli cells and Leydig cells show impaired function (see Figure 17-4).

Prader-Willi syndrome can also be associated with delayed puberty. Cryptorchidism is common in boys with Prader-Willi syndrome. Inhibin B concentrations are generally normal in infants and prepubertal boys. As puberty progresses, inhibin B concentrations decline and FSH concentrations increase. Testicular histology is variable and is reported to range from normal to complete absence of spermatogonia (Sertoli cell only syndrome).[228] Longitudinal data indicate that the hypogonadism among boys with Prader-Willi syndrome is likely due to primary testicular dysfunction involving both Sertoli and Leydig cell compartments. However, inadequate HPG axis function may also contribute to the hypogonadism.[229]

Defects in Steroidogenesis or Steroid Hormone Action

Although often not among the cardinal manifestations of defects in steroid hormone synthesis or steroid hormone action, delayed puberty can be associated with a broad range of these disorders. Complete loss-of-function mutations may present in the newborn period as a disorder of sexual development or apparent normal female external genital development with labial masses (testes). Importantly, milder variants can be associated with male sex development and delayed or incomplete puberty development.

Smith-Lemli-Opitz syndrome (SLOS) is an autosomal disorder due to mutations in the 7-dehydrocholesterol (*DHCR7*) gene. Clinical features include undervirilization, congenital heart disease, renal anomalies, failure to thrive, and developmental delay. Syndactyly of the second and third toes and proximally placed thumbs are characteristic features of this defect in cholesterol biosynthesis. The diagnosis can be confirmed by finding elevated 7-dehydrocholesterol concentrations.

Steroidogenic factor-1 (*NR5A1)* plays a major role in steroidogenesis, male sexual differentiation, and the development of the hypothalamus, pituitary, adrenal glands, and testes. Two patients were reported to have 46,XY karyotype, female external genitalia, presence of Mullerian structures, and adrenal insufficiency.[230] Haploinsufficiency of *NR5A1* can also manifest as a predominantly gonadal phenotype characterized by hypergonadotropic hypogonadism with normal adrenal function. Penoscrotal hypospadias, bilateral anorchia, and testicular regression syndrome have been described. The phenotypic spectrum extends to 46,XY girls who present with delayed puberty, primary amenorrhea, and low testosterone concentrations in the absence of adrenal insufficiency. Haploinsufficiency of *NR5A1* can also present with delayed puberty or premature ovarian failure in 46,XX individuals. The inheritance pattern is variable, including both autosomal recessive and sex-linked dominant as well as de novo cases. The SF-1 protein contains two zinc finger structures and appears to bind to DNA as a monomer.[230]

Inactivating LH receptor (*LHCGR*) mutations are associated with Leydig cell hypoplasia, testosterone deficiency, undervirilization, and delayed puberty. The phenotype for this autosomal recessive disorder ranges from female external genitalia presenting with absence of puberty to normal male development with hypospadias and microphallus. Mullerian structures are absent because Sertoli cell function and AMH secretion are normal. In the severe forms, microscopic and ultrastructural findings show seminiferous tubules, normal Sertoli cells, and decreased germ cells. Poorly differentiated cells appear in the interstitial spaces; Leydig cells are rare. Mullerian duct derivatives are absent. As would be anticipated, LH concentrations are elevated and testosterone concentrations are low.[231]

Mutations in the steroidogenic acute regulatory protein (*StAR*) gene are associated with congenital lipoid adrenal hyperplasia. In this disorder, loss-of-function *StAR* mutations lead to failed steroidogenesis and accumulation of cholesterol esters in the cytoplasm of steroidogenically active cells. The accumulated lipid droplets cause physical engorgement and chemical damage from the accumulated cholesterol auto-oxidation products. This lipid accumulation damages the fetal Leydig cells but does not affect AMH secretion by the fetal Sertoli cells such that Mullerian duct derivatives are absent. This autosomal recessive disorder is often identified in the neonatal period due to the associated undervirilization and adrenal insufficiency. Milder phenotypes with normal male sex development and non-autoimmune adrenal insufficiency have been described; the magnitude of overt mineralocorticoid deficiency varies.[232] Ethnic groups in which higher incidence of *StAR* mutations occur include Japanese, Korean, and Palestinian Arabs.

Patients with loss-of-function cholesterol side chain cleavage (*CYP11A1*) mutations have been recognized. The phenotype ranges from prematurity associated with undervirilization and early-onset adrenal failure to presentation with adrenal insufficiency in children with normal sexual development.[233] Typically, the normal sized adrenal glands differentiate this genetic disorder from congenital lipoid adrenal hyperplasia. However, *StAR* mutations were detected in sibs with neonatal adrenal insufficiency and normal sized adrenal glands.[234]

Loss-of-function mutations in the 17α-hydroxylase/17, 20-lyase (*CYP17A1*) gene are associated with glucocorticoid and sex steroid deficiencies. Patients may be hypertensive and have hypokalemia because mineralocorticoid biosynthesis is unaffected. Affected males manifest complete or partial male-to-female sex reversal and may seek medical attention for delayed puberty.[235] Characteristic laboratory findings include low or absent 17-hydroxylated C_{21} and C_{19} steroids. There is a subnormal response to cosyntropin (ACTH) stimulation. Because mineralocorticoid secretion is unaffected, serum levels of DOC, corticosterone, 18-hydroxycorticosterone, and

18-hydroxy DOC are elevated and suppress with glucocorticoid treatment.

P450 oxidoreductase (POR) is a protein that functions as an electron donor to microsomal P450 cytochrome (CYP) enzymes. Thus, several enzymes involved in steroidogenesis such as 17α-hydroxylase (P450c17), 21-hydroxylase (P450c21), and P450 aromatase depend on this protein. Mutations that act as loss of function in *POR* are associated with congenital adrenal hyperplasia with combined glucocorticoid and sex steroid deficiency. Although this disorder typically presents in the newborn period with undervirilization, skeletal anomalies (Antley-Bixler syndrome), and glucocorticoid deficiency, males may present with a milder phenotype such as delayed puberty. The skeletal anomalies found in both P450 oxidoreductase deficiency and Antley-Bixler syndrome include brachycephaly and joint contractures.[236]

In 1972, Zachmann and colleagues reported Swiss families with undervirilized males and hormone concentrations suggestive of isolated 17,20-lyase deficiency.[237] No *CYP17A1* mutations were identified when members of the original families were evaluated using the tools of molecular genetics. Rather, deleterious mutations were identified in the aldo-keto reductase family 1, member C2 (*AKR1C2*), and the aldo-keto reductase family 1 member C4 (*AKR1C4*) genes. Further investigations revealed an alternative pathway in which DHT can be synthesized without androstenedione or testosterone as intermediary hormones. This alternative or "backdoor" pathway had been previously described in the tammar wallaby.[238] Thus, loss-of-function mutations in the dihydrodiol dehydrogenase type 2 (*AKR1C2*) and dihydrodiol dehydrogenase type 4 (*AKR1C4*) genes can be associated with undervirilization of males.[239] Typically, adrenal insufficiency does not occur.

In the autosomal recessive 17β-Hydroxysteroid dehydrogenase deficiency, conversion of androstenedione to testosterone in the Leydig cell is impaired. This disorder is due to mutations in the 17β-hydroxysteroid dehydrogenase type 3 (*HSD17B3*) gene. The extent of external genital virilization is variable, ranging from genital ambiguity to microphallus. Wolffian duct development is impaired. At puberty, the increased peripheral conversion of sex steroids (androstenedione) to testosterone induces virilization of affected individuals. Affected "girls" may convert to male gender during puberty. When the diagnosis is made in the newborn period, male gender of rearing is appropriate. Although increased basal androstenedione to testosterone ratios would be expected, hCG stimulation tests and molecular genetic analysis may be necessary to confirm this diagnosis.

Testosterone is converted to dihydrotestosterone (DHT) in specific target cells including the prostate and developing external genitalia. Loss-of-function mutations in the 5α-reductase gene (*SRD5A2*) lead to impaired peripheral conversion of testosterone to DHT. The phenotype of affected 46,XY individuals ranges from varying degrees of male-to-female sex reversal at birth. The classic description of the genitalia is perineoscrotal hypospadias, bilateral labial masses (testes), and absence of Mullerian structures. At puberty, affected individuals typically virilize with phallic enlargement and, if raised as females, may change to a male gender. Thus, patients may present with primary amenorrhea, mild virilization, no breast development, absent uterus, and 46,XY karyotype. Spermatogenesis is often impaired. This autosomal recessive disorder is prevalent in the Dominican Republic, southern Lebanon, southern Turkey, and the eastern highland region of Papua, New Guinea. Curiously, this disorder has been detected in several elite female athletes identified by having primary amenorrhea and elevated testosterone concentrations.[240] Females homozygous for *SRD5A2* mutations generally have no reproductive phenotype. Random testosterone to DHT ratios may be elevated. However, hCG stimulation testing is often necessary to confirm the diagnosis. Urinary steroid metabolite ratios (androsterone/etiocholanolone, 5α-tetrahydrocortisol/tetrahydrocortisol, and 5β-tetrahydrocorticosterone/tetrahydrocorticosterone) may be helpful.[241]

Chemotherapy, Radiation Therapy, and Cancer Survival

Treatment of childhood cancers can lead to gonadal failure, delayed puberty, and infertility. Quality-of-life concerns have become increasingly relevant because many young cancer patients are long-term cancer survivors. Multiple components of the hypothalamic-pituitary-gonadal axis may be affected by chemotherapy and radiation therapy with specific consequences depending on the drugs and fields of irradiation. The germ cells of the gonads are particularly vulnerable to radiation. Discussions regarding fertility preservation are crucial for pediatric patients with cancer and their parents; however, detailed discussion of this relevant subject is beyond the scope of this chapter.

OTHER DISORDERS OF THE MALE REPRODUCTIVE ENDOCRINE AXIS

Several other disorders deserve discussion, albeit not necessarily associated with alterations in pubertal timing.

Androgen Receptor (*AR*) Mutations

The classic presentation of 46 XY individuals with complete androgen insensitivity (CAIS) is primary amenorrhea, breast development, and lack of sexual hair. Labial masses (testes) may be palpated on physical exam. Imaging shows an absence of Mullerian structures because in utero Sertoli cell secretion of AMH is normal. Inheritance is X-linked recessive. Some individuals with CAIS are detected when a gonad is found during repair of an inguinal hernia in a phenotypic female child. As bilateral inguinal hernias are unusual in girls, the diagnosis of androgen insensitivity should be assessed by obtaining a karyotype and checking for the presence of a Y chromosome by fluorescent in situ hybridization (FISH).

The phenotype of 46,XY individuals carrying mutations in *AR* ranges from the classic CAIS to partial forms presenting with undervirilization and male infertility. Individuals with partial androgen insensitivity (PAIS)

may present with gynecomastia, hypospadias, cryptorchidism, micropenis, decreased sexual hair, and infertility. Mild isolated hypospadias has rarely been associated with *AR* mutations; missense mutations were detected in exon 1 that encodes an important regulatory region for the receptor.[242] Phenotypic heterogeneity occurs even within a family. Despite classic features of CAIS, *AR* mutations may not be detected in some individuals. It is speculated that these patients may carry mutations in receptor cofactors or distant regulatory regions of the *AR*.

As noted earlier, the *AR*, located at Xq11-q12, has a modular structure with N-terminal regulatory (NTD), DNA binding (DBD), and ligand binding (LBD) domains. Ligand binding is impaired for mutations located in the ligand binding domain. Mutations located in the DNA binding domain do not affect ligand binding; rather the ligand-receptor complex fails to bind to the promoter region of the gene and shows impaired transcriptional activity. To date, more than 800 *AR* mutations have been described (http://androgendb.mcgill.ca).[243]

Exon 1 of *AR* contains two trinucleotide repeat regions. The CAG repeat encodes for a homopolymeric stretch of glutamines. In vitro studies have demonstrated that the number of CAG repeats is inversely proportional to the transcription activity of the protein. Expansion of the polyglutamine repeat (> 40 repeats) is associated with Kennedy disease, a form of spinal and bulbar muscular atrophy (SBMA). Kennedy disease is an adult-onset lower motor neuron disease characterized by slowly progressive muscle weakness and atrophy of the bulbar, facial, and limb muscles. Men with Kennedy disease may have signs of mild androgen insensitivity. The other trinucleotide repeat encodes a homopolymeric stretch of glycines.

The typical hormone findings during adolescence include elevated LH and testosterone concentrations. Inhibin B and FSH concentrations are usually normal. Estradiol concentrations are elevated due aromatization of testosterone. Sex hormone binding globulin (SHBG) concentrations are generally in normal female range. Surprisingly, testosterone concentrations are not elevated during early infancy in children with CAIS. No explanation for this apparent lack of negative feedback is available.

Once the diagnosis of CAIS is confirmed, full disclosure to the patient and her family is essential. Mothers may feel guilty for transmitting this X-linked disorder. One essential discussion relates to gonadectomy. Some individuals and families prefer to delay gonadectomy until late adolescence to allow for spontaneous breast development. Additional topics for discussion include implications of the 46,XY karyotype, infertility, sexual function, and what information to share with nonfamily members. Individuals with CAIS usually have a blind vaginal pouch, and vaginal dilatation may be the only therapy necessary to create a functional vagina.

Persistent Mullerian Duct Syndrome

Persistent Mullerian duct syndrome (PMDS) is characterized by persistence of Mullerian duct derivatives in the presence of otherwise normal male sex development. PMDS is an autosomal recessive disorder due to loss-of-function mutations in the *AMH* gene or the *AMHRII* gene. Measurement of AMH can be helpful. Low AMH levels are consistent with AMH deficiency and high levels suggest a mutation in the *AMHRII* gene. Common presentations include discovery of a uterus during inguinal hernia repair or orchidopexy. Transverse testicular ectopia with both testes on the same side is virtually pathognomonic for this disorder. Testicular torsion is not uncommon because the testes may not be anchored properly to the bottom of the processus vaginalis.

Testicular differentiation is usually normal, but the male excretory ducts may be embedded in the Mullerian duct remnants or incompletely developed. Infertility may ensue secondary to cryptorchidism, intertwining of vas deferens and uterine wall, or lack of proper communication between the testes and excretory ducts. The close physical relationship of the vas and the uterine wall complicates the surgical excision of the Mullerian remnants. However, because malignancy (adenocarcinoma) has been reported to arise in the Mullerian remnants, surgical excision by experienced surgeons should be considered.[244]

Testicular Regression Syndrome (Anorchia), Cryptorchidism, and Hypospadias

Testicular regression syndrome affects approximately 5% of boys with cryptorchidism.[245] In this instance, one or both testes are nonpalpable despite normal-appearing male external genitalia. Bilateral nonpalpable testes with a normal appearing but small phallus may be considered to be a variant of this syndrome. During early infancy and adolescence, elevated gonadotropin and unmeasurable inhibin B and AMH concentrations indicate anorchia. The lack of a testosterone response to hCG stimulation in the older infant and prepubertal boy is consistent with anorchia. Random inhibin B and AMH concentrations may more helpful than hCG stimulated testosterone concentrations.[246] Imaging studies are generally not informative. Hence, the combination of undetectable plasma AMH and inhibin B, an elevated plasma FSH, and 46,XY karyotype may be sufficient to diagnose anorchia and obviate the need for an hCG stimulation test.[247] Nevertheless, thorough clinical reevaluations are indicated and when warranted hCG stimulation may still be helpful to confirm diagnosis of anorchia.

At operation, nubbins of discrete vascularized fibrosis are found in close proximity to a blind-ending spermatic cord structure. Although the precise etiology is unclear, the testicular regression syndrome has been attributed to in utero testicular torsion. The finding of hemosiderin-laden macrophages and dystrophic calcifications supports a vascular accident etiology.

Cryptorchidism (undescended testes) is the most common disorder of sex development affecting 3% of males. The prevalence decreases to 1% by 6 months of age due to spontaneous descent during infancy. Many conditions are associated with cryptorchidism including hypogonadotropic hypogonadism, aberrant testicular differentiation,

impaired testosterone biosynthesis, androgen insensitivity, holoprosencephaly, abnormal AMH production or action, abnormalities affecting INSL3/LGR8 function, and possibly environmental factors. Other associations include prune belly syndrome, bladder extrophy, and renal anomalies. Cryptorchidism is also a feature of many syndromes (see Chapter 5). Maternal diabetes mellitus, including gestational diabetes, and environmental exposures may be risk factors as well.

In male fetuses, urethral closure occurs during 8 to 14 weeks' gestation. This process reflects continuous ventral fusion in the proximal to distal direction. Hypospadias represents an aberration of this process resulting in abnormal penile and urethral development. Physical examination reveals an abnormal position of the urethral meatus along the ventral portion of the penis, ventral curvature of the penis (chordee), and an abnormal foreskin. Hypospadias can be associated with disorders of sex development and chromosomal anomalies. Hypospadias can be classified into hormone-independent and hormone-dependent etiologies. Genes associated with hypospadias include *FGF8*, *FGFR2*, *BMP4*, and *BMP7*. Insufficient androgen exposure can lead to hypospadias.[248]

Epidemiologic data suggest a possible role for environmental factors in the etiology of both cryptorchidism and hypospadias. Potential mechanisms include binding to hormone receptors, interference with postreceptor signaling pathways, and effects on hormone synthesis, activity, and degradation. Suspected environmental factors include phthalates, bisphenol A, pesticides containing *DDE* (1,1-dichloro-2, 2-bis(p-chlorophenyl)ethylene) and DDT (dichlorodiphenyltrichloroethane), maternal smoking, phytoestrogen intake, low birth weight, and maternal progestin exposure during gestation. Maternal DES exposure is of historical interest. However, studies confirming a causal relationship for environmental disruptors report inconsistent findings.[249]

Testis Tumors

Testis tumors are rare among the pediatric population. Tumors can arise from one of three principal cell types: germ cells, Sertoli cells, and Leydig cells. Rarely, tumors can develop from other elements such as endothelium (hemangioma), muscle (rhabdomyosarcoma), or fibrous tissue (fibroma). Germ cells tumors in children include yolk sac tumor, embryonal carcinoma, seminoma, nonseminoma, dysgerminoma, and gonadoblastoma. For patients with DSDs or undervirilization, the type II germ cell tumors are the most relevant and will be the only type of germ cell tumor discussed in this section.

Germ Cell Tumors

Type II germ cells tumors include seminoma, nonseminoma, and dysgerminoma; these invasive tumors arise from primordial germ cells and occur with increased frequency in patients with DSDs who carry Y chromosomal material. The noninvasive premalignant precursors for type II germ cells tumors are the intratubular germ cell neoplasia unclassified (ITGCNU), previously known as carcinoma in situ (CIS), and the gonadoblastoma. Both reflect delayed germ cell maturation and express the germ cell transcriptional factor, OCT 3/4. A locus mapped to Yp, the testis specific protein Y-encoded (*TSPY*) gene, plays a major role in the pathogenesis of germ cell tumors. Hence, germ cells tumors residing in unfavorable environments may have prolonged expression of OCT 3/4, *TSPY*, placental/germ cell alkaline phosphatase (PLAP), and fail to undergo apoptosis.

ITGCNU is characterized by the presence of germ cells in the seminiferous tubules. Gonadoblastomas are composed of immature germ cells and sex cord–stromal cells. One major difference between these lesions is the local microenvironment. Some degree of Sertoli cell/testicular differentiation favors ITGCNU development, whereas gonadoblastomas develop in streak gonads or poorly differentiated gonadal tissue. Thus, these lesions may represent a continuum with the phenotype reflecting the degree of Sertoli cell differentiation.[250] On histologic examination of the tissue, the origin of the cell, granulosa versus Sertoli, may be indeterminate. Staining of gonadoblastoma for FOXL2 and SOX9 can differentiate granulosa from Sertoli cell, respectively, regarding cell of origin.[251] Dysgerminoma (ovary) and seminoma (testis) are the end-stage invasive tumors.

The risk for the development of germ cell tumors varies among patients with DSD. The risk is negligible for the virilized XX patient with CAH. For patients with complete androgen insensitivity, the risk for germ cell tumors is low in childhood but increases during adulthood. Due to the low risk for neoplastic changes, individuals with CAIS may choose to postpone gonadectomy.[252] Germ cell number generally decreases with advancing age. A retrospective review of MRI findings in 25 patients with CAIS identified benign paratesticular simple cysts in 80%. Histologic evaluation of the testes from these same individuals showed premalignant lesions in 12%; these lesions were too small to be detected on MRI. Until a sensitive and specific screening tool is available, gonadectomy after puberty likely remains the safest option for individuals with CAIS.[253]

In contrast, the risk for a type II germ cell tumor is high in dysgenetic gonads such as in patients with 46,XY gonadal dysgenesis, Frasier syndrome, and partial androgen insensitivity. Curiously, boys with Klinefelter syndrome have an increased risk for mediastinal germ cell tumors, but not testicular germ cell tumors. Intra-abdominal location of the dysgenetic gonad increases the risk for germ cell tumors. Thorough discussion with the patient, if older, and the parents is essential to choose the optimal management ranging from observation to prophylactic gonadectomy.

Nongerm Cell Tumors

As noted previously, Leydig cell tumors may present with peripheral precocious puberty and asymmetry of testicular volume. Reported median age at presentation was 6.5 years. Gonadotropin concentrations are typically low. An activating mutation in the *LHCGR* has been found in some boys.[254]

Tumors can also develop in Sertoli cells. Large cell calcifying Sertoli cell tumors can be found in prepubertal

boys, adolescents, and young adult males. Aromatase expression may be increased such that growth acceleration, advanced skeletal maturation, and gynecomastia may occur. Large cell calcifying Sertoli cell tumors may be bilateral with palpable calcifications due to macrocalcifications within the tumor. The calcifications can be detected on testicular ultrasound. Malignancy occurs in approximately 17% and usually in older men. Peutz-Jeghers syndrome and Carney complex are associated with large cell calcifying Sertoli cell tumors.[255]

Testicular adrenal rest tumors (TARTs) are hypoechoic, well-circumscribed lesions believed to arise from adrenal stem cells that were misdirected and descended into the scrotum with the testes during gestation. These cells express adrenal steroidogenic enzymes. Typically, suppressive glucocorticoid doses decrease the size of the tumor, but TARTs can occur despite ACTH-suppressive glucocorticoid doses. Although more common in men with classical forms of CAH, TARTs have been reported in men with nonclassic CAH. In one pediatric series, the youngest patient was 7 years old.[256] The typical location is in the rete testis. TARTs can obstruct the seminal ducts, with subsequent permanent testicular damage, infertility, decreased sperm count, and decreased testicular testosterone production. Testicular ultrasound screening is helpful to detect TARTs. Importantly, TARTs must be differentiated from other testicular mass lesions to avoid unnecessary surgery.

Gynecomastia

Physical examination is extremely important in the evaluation of gynecomastia to distinguish among true gynecomastia, pseudogynecomastia, and pathologic gynecomastia. With the patient lying supine with his hands clasped beneath his head, the examiner can slowly compress the breast area between forefinger and thumb beginning from the sides of the breast. In true gynecomastia, the breast tissue is located concentrically under the nipple-areolar complex, feels rubbery or firm, and is often bilateral. During the early phase, ductal and epithelial proliferation occurs. This early proliferation is associated with periductal inflammation and edema, which correspond to the tenderness noted by the patient.[257]

The differential diagnosis includes lipomastia or pseudogynecomastia that is characterized by breast fullness and the absence of a nipple-areolar complex mound. Lipomas and neurofibromas can occur in the breast. Although extremely rare in adolescents, the tissue mound in the breast cancer patient feels firm or hard and is located outside the nipple-areolar complex. Nipple discharge, skin dimpling, and nipple retraction do not occur in physiologic gynecomastia.

Pubertal or physiologic gynecomastia is a common midpubertal finding noted at Tanner stage 3 to stage 4 for pubic hair.[258] Pubertal gynecomastia has been attributed to a relative and transient imbalance in testosterone to estradiol ratio. With the progression of puberty and rising testosterone concentrations, the gynecomastia typically resolves.

Medications, exposures, and rare hormone disorders are associated with pathologic gynecomastia. Medications associated with gynecomastia include spironolactone, cimetidine, ketoconazole, estrogens, anti-androgens, growth hormone, GnRH analogs, and 5α-reductase inhibitors.[259] Exposures to estrogen-containing substances, lavender and tea tree oils, and phytoestrogens have been considered to be etiologies. Other drugs associated with gynecomastia include tricyclic antidepressants, chemotherapeutic agents, and cardiovascular medications (e.g., digitalis). Drugs of abuse associated with gynecomastia include marijuana, ethanol, heroin, and amphetamines.

Estrogen excess can cause pathologic gynecomastia. Autosomal dominant mutations in the aromatase (*CYP19A1*) gene result in constitutively increased gene transcription and overexpression of the aromatase enzyme.[260] Feminizing tumors can directly secrete estrogen. Peutz-Jeghers syndrome (PJS) is characterized by mucocutaneous pigmentation, multiple gastrointestinal polyps, and a variety of neoplasms. Gynecomastia was described in prepubertal boys secondary to large cell calcifying Sertoli cell tumors associated with PJS or Carney complex syndrome.[255] Gynecomastia can occur in men with hyperthyroidism and with hypogonadism including Klinefelter syndrome, androgen insensitivity, ovotesticular disorder.[261] Additional associations include obesity, liver disease, radiation exposure, and other causes of hypogonadism.

Gynecomastia in a prepubertal boy is pathologic and warrants investigation. Gynecomastia occurring in a midpubertal boy who is otherwise healthy most likely represents physiologic gynecomastia for which an extensive laboratory evaluation is unwarranted. Physiologic gynecomastia is typically self-limited. If present, treatment of the underlying disorder or removal of environmental causes is appropriate and may lead to regression of the breast tissue. Aromatase inhibitors and estrogen receptor blockers have been used. An open label study concluded that anastrozole reduced breast size.[262] However, the single randomized double placebo controlled pediatric trial noted that anastrozole was no more effective than placebo to reduce gynecomastia.[263]

EVALUATION OF THE CHILD WITH DELAYED PUBERTY

Whether due to a referral bias or to differences in underlying biology that result in a slightly skewed distribution of ages of pubertal timing in boys compared to girls,[52] delayed puberty in general and CDGP in particular are also seen more commonly in boys in endocrinology clinics.[202]

Initial Evaluation

The aim of the initial evaluation is to rule out causes of delayed puberty other than CDGP (Table 17-3 and Figure 17-5). Pubertal development is assessed clinically and biochemically, providing information important for counseling and predicting further pubertal development. Eventual normal progression of puberty verifies a diagnosis of CDGP, whereas absent or slow

TABLE 17-3 **Investigations for Delayed Puberty**

First Line Investigations		
VARIABLE	REQUIREMENTS AND LIMITATIONS	INTERPRETATION
Growth rate	Two or more height measurements, preferably 6-12 months apart.	In early adolescence in both sexes a growth rate less than 3 cm/year is suggestive of a disease specifically inhibiting growth (e.g., GH deficiency, hypercortisolism, hypothyroidism), but such rates can also be seen in CDGP. Boys with delayed puberty who are overweight tend to have height and predicted adult consistent with the genetic height potential.[125,265]
Tanner stages	Pubic hair stages and genital stages should be scored separately because they do not necessarily track together.	Testicular volume of $>$ 3 mL is a more reliable indicator of the onset of puberty than Tanner stage 2 genital development.
Testis volume	Prader's orchidometer or a ruler.	Testicular volume of $>$ 3 mL ($\geq$ 2.5 cm in length) indicates central puberty. Most healthy boys with a testicular volume 3 mL or greater will have a further increase in testicular volume, pubic hair stage, or both, at repeated examination 6 months later.[117]
Bone age	X-ray of left hand and wrist. Greulich and Pyle Atlas. Bone age is also used to predict adult height.	A bone age delay of more than 2 years has arbitrarily been used as a criterion for CDGP but is not specific to that condition. A bone age delay of 4 years has been associated with an average of 8 cm over prediction of adult height, whereas in short stature with no bone age delay, adult height is usually underestimated by the Bayley-Pinneau tables.[293]
Biochemistry	Evaluation varies but common tests include complete blood count, erythrocyte sedimentation rate, creatinine, electrolytes, bicarbonate, alkaline phosphatase, albumin, thyrotropin, and free thyroxine.	To rule out chronic disorders. Should be directed based on history and physical examination. Additional investigations may be necessary based on family history, symptoms, and signs, including screening for celiac disease and inflammatory bowel disease.
Serum luteinizing hormone (LH)	Morning sample. Use immunochemiluminometric (ICMA) or immunofluorometric (IFMA) assays with a lower limit of detection at or below 0.1 IU/L	At low levels, values obtained on ICMA are at least 50% lower than those by IFMA.[270] Values $<$ 0.1 IU/liter are not specific for hypogonadotropic hypogonadism. Value $>$0.6 (IFMA) or 0.2 (ICMA) IU/L are specific but not sensitive markers for the initiation of central puberty, because in early puberty some adolescents have lower values.[270] In delayed puberty, elevated values suggest primary hypogonadism.
Serum follicle-stimulating hormone (FSH)	Morning sample. Use ICMA or IFMA assays with a lower limit of detection at or below 0.1 IU/L if possible.	At low levels, values obtained on ICMA are $\sim$50% lower than those by IFMA. Values $<$0.2 (ICMA) or $<$ 1 (IFMA) IU/L suggest hypogonadotropic hypogonadism but are not diagnostic.[269,270] In delayed puberty, a value above the upper limit of normal for the assay is a marker of inhibin B deficiency and of primary gonadal failure with high sensitivity and specificity.
Serum insulin-like growth factor 1 (IGF-1)	Blood samples should be processed within 2 h to avoid an artifactual increase in results. Only assays that recognize IGF-1 without any interference from IGF binding proteins provide reliable results.	IGF-1 level is used to screen for GH deficiency. An increase in the levels during follow-up or during or after treatment with sex steroids makes the diagnosis of GH deficiency less likely. The IGF-1 concentration shows the greatest change during childhood and puberty and then changes more slowly with advancing age. Therefore, normal values for children and adolescents should include narrow age ranges and Tanner stages. GH provocation tests are needed to diagnose GH deficiency.
Serum testosterone	Morning sample is ideal. Use an assay with a lower limit of detection at or below 10 ng/dL (0.35 nmol/L) if possible. Concentrations show diurnal variation.	An 8 am serum testosterone value of $\geq$ 20 ng/dL (0.7 nmol/L) often predicts the appearance of pubertal signs within 12 to 15 months.[294]

Second-Line Investigations

VARIABLE	REQUIREMENTS AND LIMITATIONS	INTERPRETATION
Gonadotropin-releasing hormone (GnRH) test*	Assay requirements are the same as for basal LH and FSH. Test can be performed any time of day. LH and FSH values vary according to assay used and with the stimulating agent (GnRH or GnRH agonist).	A predominant LH over FSH response after GnRH stimulation or peak LH levels of 5 to 8 IU/L (depending on assay) suggests onset of central puberty. There is an overlap between prepubertal and early pubertal post-GnRH values.[270] A prepubertal response is seen in some patients with CDGP as well as in hypogonadotropic hypogonadism, but a post-GnRH LH value $<$ 0.8 IU (IFMA) and FSH value $<$ 1.1 IU/L (IFMA) may be more consistent with hypogonadotropic hypogonadism in boys.[270]
Human chorionic gonadotropin (hCG) test*	IM or SC injections on several days. There are number of different protocols available. Results vary by the protocol.	Peak testosterone concentrations to both 3-d and 19-d hCG tests have been reported to be significantly lower in patients with hypogonadotropic hypogonadism compared with CDGP. A combination of the GnRH test and hCG test [peak LH cutoff, 2.8 U/L; peak 19-d testosterone cutoff, 275 ng/dL (9.5 nmol/liter)] gave a sensitivity and a specificity of 100% in a small study of CDGP and hypogonadotropic hypogonadism.[295]
Serum inhibin B*	Can be measured any time of day. Has diagnostic value only in boys.	Measurement is used in differentiation of hypogonadotropic hypogonadism from CDGP. Boys with higher baseline inhibin B levels had a higher likelihood of CDGP in one study.[271] In prepubertal boys, sensitivity and specificity of 100% was obtained at concentration of $>$35 pg/mL. With Tanner stage 2 genitalia, sensitivities were 86% and 80%, and specificities 92% and 88%, respectively, for CDGP with an inhibin B value of $>$ 65 pg/mL. In boys, unmeasurable inhibin B indicates primary germinal failure.
Serum prolactin	Measurement is indicated only in some cases. Physiologic states including stress, exercise, and sleep can increase prolactin levels, as can hypothyroidism and some medications.	Elevated levels may indicate hypothalamic-pituitary tumors causing hypogonadotropic hypogonadism. In such cases, additional pituitary-hormone deficiencies may be present. Macroprolactin (physiologically inactive form of prolactin) measurement is recommended in patients with asymptomatic hyperprolactinemia.
Brain magnetic resonance imaging (MRI)	Indicated in any suspicion of CNS pathology (e.g., headache, changes in vision, changes in behavior).	Imaging is performed to rule out underlying disorders of the central nervous system. Imaging in patients with the Kallmann syndrome commonly shows olfactory bulb and sulcus aplasia or hypoplasia and thus may help differentiate the Kallmann syndrome from isolated hypogonadotropic hypogonadism in patients with an apparently normal or difficult-to-evaluate sense of smell. In hypogonadotropic hypogonadism there is a moderate agreement between the MRI of the olfactory bulbs and the UPSIT (overall Kappa 0.5), but in presence of aplastic bulbs and anosmia, there is good agreement (Kappa 0.9).
Olfactory function test	One test, UPSIT,[296] uses microencapsulated odorants, which are released by scratching standardized odor-impregnated test booklets.	Used to assess for hyposmia and anosmia as part of evaluation for Kallmann syndrome.
Genetic testing	In more than 60% of patients with Kallmann syndrome or isolated hypogonadotropic hypogonadism no specific gene defect is found.	Genotyping for known monogenic causes is currently a research procedure and not warranted in routine clinical practice. It may be warranted when there is a positive family history or the patient has phenotypic signs suggestive of a specific mutation. If performed, genetic testing should be accompanied by genetic counseling.
GH testing	Various protocols both for testing and priming are available. The choice of GH stimuli to be used is variable.	Response in GH provocation test is greater after the administration of exogenous (aromatizable) androgens or estrogens (priming). Reliable assay performance and appropriate normative data are critical for appropriate use of GH and IGF-1 measurements.
Karyotype		Diagnostic of Klinefelter syndrome.

*These tests are used to try to differentiate CDGP and IHH. However, validation in larger, independent studies is needed before they can be endorsed for routine clinical use.[267,268] Clinical follow-up is often needed to confirm the diagnosis; no endogenous puberty by age 18 years is diagnostic of IHH.

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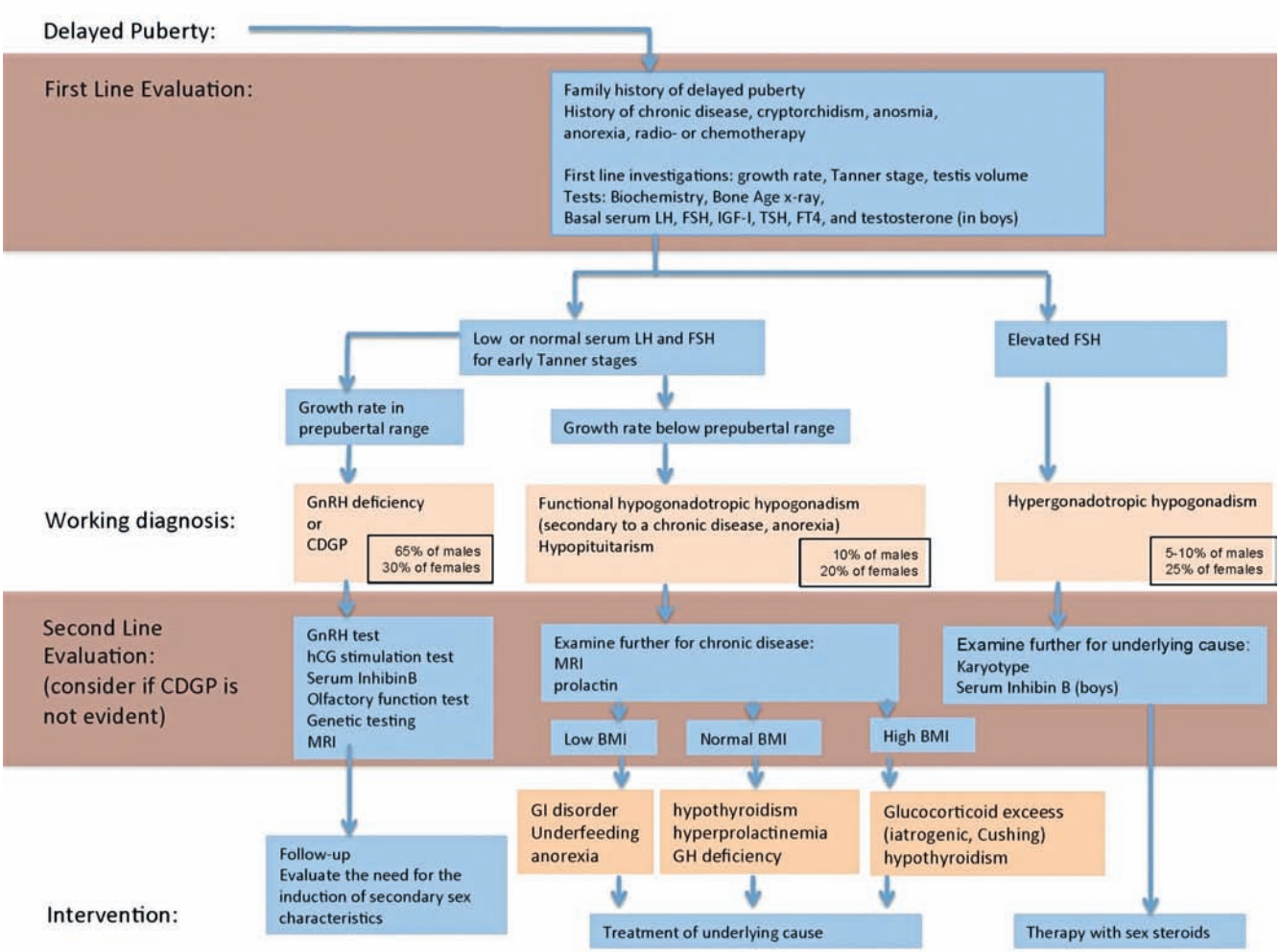

FIGURE 17-5 ■ Flowchart for the evaluation of a patient with delayed puberty. CDGP, constitutional delay of growth and puberty; FSH, follicle stimulating hormone; GH, growth hormone; GnRH, gonadotropin-releasing hormone; hCG, human chorionic gonadotropin; LH, luteinizing hormone. Percentages are from reference 202; they do not add up to 100% because of rounding and because a small percentage of patients have disorders that cannot be classified using this schema. (Reprinted with permission from Palmert, M. R., & Dunkel, L. [2012]. Clinical practice: delayed puberty. *N Engl J Med, 366,* 443–453.)

development, or cessation of development after onset, is consistent with permanent hypogonadism.

History

A family history—including childhood growth patterns, age at pubertal onset of the parents, and infertility—should be obtained. Delayed puberty in a parent or sibling, regardless of gender, followed by spontaneous onset of puberty suggests CDGP. However, if pubertal development was induced by sex steroids in family members, IHH is also possible, as reversal of hypogonadism is noted after discontinuation of sex steroids in about 10% of IHH cases.[98,213]

Chronic diseases, such as inflammatory bowel disease, sickle cell disease, celiac disease, asthma, chronic renal failure, cardiac disease, endocrine disorders such as hypothyroidism and poorly controlled diabetes mellitus, and chronic therapy with glucocorticoids,[264] may delay both linear growth and puberty. Inadequate nutritional intake, when associated with conditions such as cystic fibrosis, anorexia nervosa or excessive energy utilization[203] due to vigorous exercise in athletes or ballet dancers, may result in pubertal delay. Successful intervention in such situations can be followed by catch-up growth and pubertal progression.

Delayed cognitive development associated with obesity or dysmorphic features may suggest an underlying genetic syndrome. Cryptorchidism or small penis at birth, hyposmia, or anosmia may suggest hypogonadotropic hypogonadism. A history of chemo- or radiotherapy may indicate primary gonadal failure. However, some patients receiving treatment for malignancy may have elevated gonadotropin levels during and initially after therapy, but subsequently the gonadotropin levels decrease as these patients experience varying degrees of gonadal recovery. Of special note, although Klinefelter syndrome is a type of hypergonadotropic hypogonadism, males with this condition commonly present with unusually small testes, lack of pubertal progression, or infertility rather than pubertal delay.

Physical Examination

Previous height and weight measurements should be obtained and plotted so that longitudinal growth can be carefully assessed. Height, weight, and body proportions should be determined. Compared with previous data, height and weight can be used to calculate annual growth rates preferably based on a 12-month interval, as intervals less than 4 to 6 months can be inadequate. Delayed puberty is often associated with short stature and slow growth for age, although height and growth rate may be within the prepubertal normal range. Individuals who are underweight for height have a higher likelihood of an underlying condition delaying HPG axis activation. Conversely, in boys, unlike girls, being obese can be associated with later pubertal development.[125,265] Upper-to-lower segment ratio can be determined by measuring sitting height or lower segment (from top of pubic symphysis to floor) and compared with normal for age: ratio decreases with age to 1 or lower depending on racial group. Arm span (finger tip to finger tip) should be within 5 cm of the standing height. Longer limbs or eunuchoid habitus suggest hypogonadism.

The physical examination should also concentrate on identifying markers of chronic disease, malnutrition, neurologic abnormalities, thyroid and other endocrinopathies, and syndromic features. In boys, in addition to determining height, weight, and skeletal proportions, documentation should include stretched penile length, position of urethral opening, testicular size, and whether both testes are present in a normally developed scrotum. Cryptorchidism, bifid scrotum, micropenis, or perineoscrotal hypospadias may indicate defects in gonadal steroidogenesis and disorders of sex development. Small testes, eunuchoid habitus, and gynecomastia suggest Klinefelter syndrome. Obesity and delayed puberty may suggest defects in the prohormone convertase 1 (*PC1*), leptin, or leptin receptor genes. When obesity is associated with delayed puberty and dysmorphic features, it suggests Prader-Willi, Bardet-Biedl, or other genetic syndromes.

In boys, Tanner stage 2 genitalia mark the onset of pubertal development and are characterized by enlargement of scrotum and testes and by a change in the texture and color of the scrotal skin (see Figure 17-2). Testicular volume should also be measured; a volume $>$ 3 cc indicates the initiation of central puberty. In patients with CDGP, both adrenarche and hormonal activation of the gonads often occur later than average, but in isolated hypogonadotropic hypogonadism, adrenarche usually occurs at a normal age.[202,266]

Testing

The history and physical examination should direct the laboratory studies so that a parsimonious, cost-effective evaluation is performed. Certainly, not all tests need to be performed in all individuals.

An individual who is experienced in interpreting these x-rays should review the bone age. A delay in bone age is characteristic but not diagnostic of CDGP and also may occur in individuals with chronic illness, hypogonadotropic hypogonadism, or gonadal failure. Adult height prediction is an important part of counseling if short stature is a component of the presentation, and practitioners need to be aware that the Bayley Pinneau tables overestimate adult height in patients with CDGP if bone age is delayed by more than 2 years (see Table 17-3).

Pubertal onset is characterized by accentuation of diurnal gonadotropin and testosterone secretion before apparent phenotypic changes. Basal LH and FSH levels are low in CDGP and in hypogonadotropic hypogonadism, whereas they are usually elevated in gonadal failure. In general, LH is a better marker of pubertal initiation than FSH, and FSH is a better marker of gonadal failure than LH. Serum IGF-1 concentrations can help in the evaluation of growth hormone deficiency but must be interpreted carefully because levels are often low for chronologic age but within normal range for bone age in patients with delayed puberty. Thyroid function tests are routinely obtained.

Brain and pituitary MRI may be indicated when there are signs or symptoms to suggest a CNS lesion; otherwise, although some clinicians obtain brain imaging routinely, a reasonable strategy is to defer this test until age 15, at which point many patients with CDGP will have spontaneously begun puberty and will require no further evaluation. Full neuroendocrine testing is warranted in patients with hypothalamic-pituitary tumors causing hypogonadotropic hypogonadism, as they may have additional pituitary hormone deficiencies. Assessment of olfactory bulbs on MRI may be helpful in patients with hypogonadotropic hypogonadism and anosmia.

Additional Evaluation

Most boys will not have an apparent alternative cause for delayed puberty on initial evaluation, suggesting CDGP as the likely diagnosis. However, no test can reliably distinguish CDGP from IHH, so the diagnosis of CDGP cannot be made with certitude.[267,268] Observation usually resolves this conundrum; IHH is diagnosed if endogenous puberty has not begun by age 18 years. Several tests have been proposed to distinguish CDGP from IHH (see Table 17-3). If basal gonadotropin levels are inconclusive, some have suggested that stimulation by GnRH or a GnRH agonist may be helpful.[269,270] Stimulated LH levels in the pubertal range indicate reactivation of the HPG axis and suggest that secondary sexual development is likely to occur within 1 year. However, the GnRH test alone often cannot differentiate CDGP from HH because prepubertal values may be observed in HH or in individuals with CDGP who have not yet activated the HPG axis. Data suggest that baseline inhibin B levels may facilitate discrimination between these conditions.[271,272] Low inhibin B levels may predict IHH, but replication is needed before this or other tests can be adopted routinely.[273]

Growth hormone secretion in the basal state, as well as after provocative testing, may be decreased in CDGP. If concerns about growth are sufficient to warrant growth hormone stimulation testing, sex steroid priming with estrogen or testosterone is necessary for

reliable results in patients with delayed puberty; estrogen stimulates endogenous growth hormone secretion, and sex steroid priming facilitates separation of true growth hormone deficiency from physiologic low GH secretion that stems from low estrogen levels. Another strategy of assessing for true growth hormone deficiency is to remeasure IGF-1 levels after the initiation of testosterone therapy. If IGF-1 increases appropriately with administration of testosterone, then true growth hormone deficiency is less likely. If a patient has normal growth rate, GH provocation tests are not necessary, whereas low IGF-1 levels together with reduced growth velocity warrant testing.

TREATMENT OF DELAYED PUBERTY

The options for management of CDGP include expectant observation or therapy with low-dose testosterone (Table 7-4). If puberty has started, clinically or biochemically,

TABLE 17-4 **Medications Used for the Treatment of CDGP and Permanent Hypogonadism**

Drug and Formulation	Treatment of Boys Recommended Dose		Side Effects and Cautions
	CDGP	HYPOGONADISM	
Testosterone*			Erythrocytosis, weight gain, prostate hyperplasia. High doses can cause premature epiphyseal closure. Not for use in boys with bone age < 10 years.
Testosterone enanthate, cypionate, and propionate. Testosterone enanthate has longer duration of effect than testosterone propionate. Intramuscular injection.	Not recommended before 14 years of age. Initial dose 50-100 mg every 4 weeks for 3 to 6 months. Repeated treatment with 25-50 mg increment in dose (not exceeding 100 mg)	Can initiate after age 12 years of age at 50 mg every 4 weeks. Increase with 50 mg increments every 6 to 12 months. After reaching 100-150 mg monthly, decrease interval to every 2 weeks. Adult dose ~200 mg every 2 weeks.	All intramuscular preparations: local side effects (pain, erythema, inflammatory reaction and sterile abscess). Priapism can occur in patients with sickle cell disease.
Testosterone undecanoate. Intramuscular injection	No data available.	Adult dose is 1000 mg every 10-14 weeks.	
Testosterone gel. Transdermal preparations, applied topically at bedtime.	No data available.	Can be started when ~ 50% adult dose with intramuscular testosterone has been achieved. Adult dose 50-80 mg daily.	Local irritation. After applying, avoid close skin contact with others
Aromatase Inhibitors			Not yet approved for this indication. After onset of puberty, may increase gonadotropin secretion and circulating T levels.[297]
Letrozole PO	2.5 mg daily	Not recommended	Decreased level of high-density lipoprotein cholesterol, erythrocytosis, vertebral deformities have been reported.[195]
Anastrozole PO	1 mg daily	Not recommended	Less potent than letrozole.
Pulsatile GnRH Subcutaneous pump†	Not recommended routinely	Initial: 5-25 ng/kg/pulse every 90-120 min; increase to 25-600 ng/kg/pulse	Requires extensive experience. Most physiologic form of replacement.
hCG plus recombinant FSH. Subcutaneous or intramuscular hCG injections Subcutaneous rhFSH injections†	Not recommended routinely	hCG: Dose 500 to 3000 IU twice weekly, increased to every 2 days. Dose adjusted based on serum T levels. rhFSH: Dose 75 to 225 IU 2-3 times weekly.	hCG: Inflammation locally in the testis, may induce apoptosis of germ cells. In hypogonadotropic hypogonadism with prepubertal onset it is necessary to add FSH to induce testicular growth and spermatogenesis. No data on effects on future fertility.

*Testosterone undecanoate tablets or anabolic steroids are not recommended for the induction of secondary sexual characteristics.
†Induction of fertility may be less successful in men who have lower baseline testicular volumes, have received previously testosterone treatment,[288] and have not previously received treatment with GnRH[285,286] or gonadotropins.[285]
CDGP, Constitutional delay of growth and puberty.
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and stature is not a major concern, reassurance with realistic adult height prediction is frequently all that is needed. If therapy is initiated, it is usually to assuage psychosocial difficulties that may derive from negative interactions with peers, decreased self-esteem, and anxiety about growth rate or body habitus.[196] Therapy is usually not initiated solely for medical reasons, such as accrual of bone mass. However, data regarding the effect of pubertal timing on bone mass suggest that additional data are needed to determine definitively whether medical reasons to initiate therapy should be given more consideration.[201]

Since the 1980s, there have been numerous studies of treatment of CDGP in boys, mostly involving treatment with short courses of low-dose androgens. There are limited randomized controlled trials, most with small numbers of subjects,[274-276] but data from these and other studies strongly suggest that treatment leads to increased growth velocity and sexual maturation and positively affects psychosocial well-being without significant side effects, rapid advancement of bone age, or reducing adult height. For boys who elect to be treated, we initiate supplementation with 50 mg testosterone ester intramuscular each month for 3 to 6 months, which can be repeated for another 3 to 6 months with dose escalation (see Table 17-4). Testosterone patches and gels are alternative treatment options, but their use during the initiation of secondary sexual characteristics has been limited by the requirement for low doses that can be delivered reproducibly. If spontaneous puberty has not occurred after 1 year, other diagnoses, such as permanent hypogonadotropic hypogonadism, should be reconsidered, and an MRI of the brain may be indicated.

For a subset of patients with CDGP, short stature can be more concerning than delayed puberty, and indeed in idiopathic short stature (ISS) there is a component of delay in many of the subjects.[277] Although the U.S. Food and Drug Administration (FDA) has approved the use of GH for the treatment of ISS and height SDS ≤ 2.25 for age, this therapy has at best a modest effect on adult height in adolescents with CDGP, and its routine use in CDGP is not recommended.[278] Nor do we use anabolic steroids, such as oxandrolone, for treatment of delayed puberty. Its use for treatment of ISS was not endorsed by the Consensus Statement,[277] and it is rare that boys after age 14 years are not concerned about the lack of pubertal changes, which cannot be induced by oxandrolone because of its weak androgenic effects.

In boys with CDGP and short stature, another potential therapeutic approach is aromatase inhibition, but this treatment requires further study before it should be incorporated into routine practice.[279,280] Aromatase inhibitors (AIs) inhibit conversion of androgens to estrogens. Because estrogen is the predominant hormone needed for epiphyseal closure, AIs could prolong linear growth and potentially increase adult height.[281] In controlled trials in boys with short stature or delayed puberty[279,280] or GH deficiency,[282] AIs have been shown to delay bone maturation and increase predicted adult height. However, adult heights have not yet been reported from all studies, and characteristics of patients who respond and those who do not as well as the optimal timing, dose, and duration of AI treatment remain unresolved.[283]

A complete profile of the potential side effects associated with the use aromatase inhibitors has not been established. Testosterone levels are elevated during AI therapy, and erythrocytosis has been observed in pubertal boys treated with AIs and in men with aromatase deficiency. Theoretical risks include a decline in adiponectin and subsequent development of nonalcoholic hepatic steatosis. Letrozole has no apparent adverse effects on bone mineral density in adolescents. However, estrogen deficiency and letrozole treatment have been associated with impaired trabecular bone development in boys with ISS, and letrozole treatment during prepuberty or early puberty has also been associated with increased risk of vertebral body deformities.[195] Thus, the use of aromatase inhibitors, even in adolescents with compromised predicted adult height, requires further careful study.[284]

In boys with permanent hypogonadism, the initial sex steroid therapy is the same as for CDGP. The difference is that testosterone doses are gradually increased to full adult replacement levels over approximately 3 years (see Table 17-4). During the last year of dose escalation, the interval is decreased from once each month to once every 2 weeks with a typical regimen for adult replacement being 200 mg every 2 weeks. Transdermal preparations can be initiated during the final stages of dose escalation, if preferred. In hypogonadotropic hypogonadism, exogenous testosterone does not induce testicular growth or spermatogenesis. Thus, if male patients with permanent hypogonadotropic hypogonadism wish to father a child or achieve increased testicular volume, gonadotropin therapy can be utilized; induction of testicular growth and fertility can be accomplished through treatment with exogenous gonadotropins[285] or, in hypothalamic disorders, pulsatile GnRH, if available, can be also be used[285-288] (see Table 17-4).

In some cases of hypergonadotropic hypogonadism, individuals may still be able to father children using assisted reproductive technologies, such as intracytoplasmic sperm injection (ICSI), although this technology may be associated with a small increased risk of birth defects.[289] In cases where ICSI is not desired or not feasible, in vitro fertilization using donor sperm and adoption provide additional options for those wanting to have children. As noted earlier, fertility preservation is an important topic for boys with Klinefelter syndrome and malignancies.

TESTOSTERONE: THE HYPOGONADAL MALE ATHLETE AND THE INDIVIDUAL WITH A DSD

Treating the competitive athlete with hypogonadism or a DSD is a potentially problematic issue. Studies by Bhasin and colleagues provided experimental evidence confirming that exogenous testosterone improved athletic performance.[290] These studies ultimately led to a ban on the use of exogenous testosterone in Olympic competition. The World Anti-Doping Agency (WADA) currently maintains a list of substances banned for use by competitive athletes.

Yet it is essential to replace testosterone in the hypogonadal male. The complete ban on testosterone

does make an exception for men with definitive diagnoses of primary gonadal failure and hypogonadotropic hypogonadism. Potential concerns include wrongly accusing an athlete of doping and abuse of testosterone treatment by athletes. Hence, maintaining normal testosterone concentrations is essential for the hypogonadal male athlete.[291]

To prevent men from competing as women, obligatory gender verification testing was conducted from the 1960s until 1999. Such testing confounded the situation, had a negative impact on participation in competitive sports, and exposed some individuals to public humiliation.[292]

CONCLUSION

The pubertal process begins during gestation with the differentiation and development of the components of the hypothalamic-pituitary-gonadal axis. The tools of molecular biology and molecular genetics have repositioned a relatively stagnant conundrum regarding the factors governing the timing and tempo of puberty to the forefront of reproductive endocrinology. The identification of novel genes associated with pubertal disorders has generated more questions regarding the elusive critical components governing GnRH and gonadotropin secretion. Additional new discoveries about HPG axis regulation will further enhance our understanding of normal variation in pubertal timing and provide further insight into the etiology and treatment of pubertal disorders.

REFERENCES

1. Patton, G. C., & Viner, R. (2007). Pubertal transitions in health. *Lancet*, *369*, 1130–1139.
2. Wierman, M. E., Kiseljak-Vassiliades, K., & Tobet, S. (2011). Gonadotropin-releasing hormone (GnRH) neuron migration: initiation, maintenance and cessation as critical steps to ensure normal reproductive function. *Front Neuroendocrinol*, *32*, 43–52.
3. Miraoui, H., Dwyer, A. A., Sykiotis, G. P., et al. (2013). Mutations in FGF17, IL17RD, DUSP6, SPRY4, and FLRT3 are identified in individuals with congenital hypogonadotropic hypogonadism. *Am J Hum Genet*, *92*(2), 725–743.
4. El Majdoubi, M., Sahu, A., Ramaswamy, S., & Plant, T. M. (2000). Neuropeptide Y: a hypothalamic brake restraining the onset of puberty in primates. *Proc Natl Acad Sci USA*, *97*, 6179–6184.
5. O'Shaughnessy, P. J., Baker, P. J., Monteiro, A., et al. (2007). Developmental changes in human fetal testicular cell numbers and messenger ribonucleic acid levels during the second trimester. *J Clin Endocrinol Metab*, *92*, 4792–4801.
6. Wilhelm, D., Palmer, S., & Koopman, P. (2007). Sex determination and gonadal development in mammals. *Physiol Rev*, *87*, 81.
7. Valeri, C., Schteingart, H. F., & Rey, R. A. (2013). The prepubertal testis: biomarkers and functions. *Curr Opin Endocrinol Diabetes Obes*, *20*, 224–233.
8. Bay, K., Main, K. M., Toppari, J., & Skakkebæk, N. E. (2011). Testicular descent: INSL3, testosterone, genes and the intrauterine milieu. *Nat Rev Urol*, *8*, 187–196.
9. Kuiri-Hanninen, T., Seuri, R., Tyrvainen, E., et al. (2011). Increased activity of the hypothalamic-pituitary-testicular axis in infancy results in increased androgen action in premature boys. *J Clin Endocrinol Metab*, *96*, 98–105.
10. Boukari, K., Meduri, G., Brailly-Tabard, S., et al. (2009). Lack of androgen receptor expression in Sertoli cells accounts for the absence of anti-Mullerian hormone repression during early human testis development. *J Clin Endocrinol Metab*, *94*, 1818–1825.
11. Rey, R. A., Grinspon, R. P., Gottlieb, S., et al. (2013). Male hypogonadism: an extended classification based on a developmental, endocrine physiology-based approach. *Andrology*, *1*, 3–16.
12. Josso, N., Picard, J. Y., Rey, R., & di Clemente, N. (2006). Testicular anti-Müllerian hormone: history, genetics, regulation and clinical applications. *Pediatr Endocrinol Rev*, *3*, 347–358.
13. Jensen, T. K., Andersson, A. M., Hjollund, N. H., et al. (1997). Inhibin B as a serum marker of spermatogenesis: correlation to differences in sperm concentration and follicle-stimulating hormone levels: a study of 349 Danish men. *J Clin Endocrinol Metab*, *82*, 4059–4063.
14. Nielsen, C. T., Skakkebaek, N. E., Richardson, D. W., et al. (1986). Onset of the release of spermatozoa (spermarche) in boys in relation to age, testicular growth, pubic hair, and height. *J Clin Endocrinol Metab*, *62*, 532–535.
15. Dunkel, L., Perheentupa, J., & Apter, D. (1985). Kinetics of the steroidogenic response to single versus repeated doses of human chorionic gonadotropin in boys in prepuberty and early puberty. *Pediatr Res*, *19*, 1–4.
16. Dunkel, L., Alfthan, H., Stenman, U. H., & Perheentupa, J. (1990). Gonadal control of pulsatile secretion of luteinizing hormone and follicle-stimulating hormone in prepubertal boys evaluated by ultrasensitive time-resolved immunofluorometric assays. *J Clin Endocrinol Metab*, *70*, 107–114.
17. Dunkel, L., Alfthan, H., Stenman, U. H., et al. (1990). Pulsatile secretion of LH and FSH in prepubertal and early pubertal boys revealed by ultrasensitive time-resolved immunofluorometric assays. *Pediatr Res*, *27*, 215–219.
18. Apter, D., Butzow, T. L., Laughlin, G. A., & Yen, S. S. (1993). Gonadotropin-releasing hormone pulse generator activity during pubertal transition in girls: pulsatile and diurnal patterns of circulating gonadotropins. *J Clin Endocrinol Metab*, *76*, 940–949.
19. Wu, F. C., Butler, G. E., Kelnar, C. J., et al. (1996). Ontogeny of pulsatile gonadotropin releasing hormone secretion from midchildhood, through puberty, to adulthood in the human male: a study using deconvolution analysis and an ultrasensitive immunofluorometric assay. *J Clin Endocrinol Metab*, *81*, 1798–1805.
20. Dunkel, L., Alfthan, H., Stenman, U. H., et al. (1992). Developmental changes in 24-hour profiles of luteinizing hormone and follicle-stimulating hormone from prepuberty to midstages of puberty in boys. *J Clin Endocrinol Metab*, *74*, 890–897.
21. Albertsson-Wikland, K., Rosberg, S., Lannering, B., et al. (1997). Twenty-four-hour profiles of luteinizing hormone, follicle-stimulating hormone, testosterone, and estradiol levels: a semilongitudinal study throughout puberty in healthy boys. *J Clin Endocrinol Metab*, *82*, 541–549.
22. de Roux, N., Genin, E., Carel, J. C., et al. (2003). Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54. *Proc Natl Acad Sci USA*, *100*, 10972–10976.
23. Seminara, S. B., Messager, S., Chatzidaki, E. E., et al. (2003). The GPR54 gene as a regulator of puberty. *N Engl J Med*, *349*, 1614–1627.
24. Teles, M. G., Bianco, S. D., Brito, V. N., et al. (2008). A GPR54-activating mutation in a patient with central precocious puberty. *N Engl J Med*, *358*, 709–715.
25. Seminara, S. B. (2007). Kisspeptin in reproduction. *Semin Reprod Med*, *25*, 337–343.
26. Roth, C. L., Mastronardi, C., Lomniczi, A., et al. (2007). Expression of a tumor-related gene network increases in the mammalian hypothalamus at the time of female puberty. *Endocrinology*, *148*, 5147–5161.
27. Topaloglu, A. K., Reimann, F., Guclu, M., et al. (2009). TAC3 and TACR3 mutations in familial hypogonadotropic hypogonadism reveal a key role for Neurokinin B in the central control of reproduction. *Nat Genet*, *41*, 354–358.
28. Eghlidi, D. H., Haley, G. E., Noriega, N. C., et al. (2010). Influence of age and 17beta-estradiol on kisspeptin, neurokinin B, and prodynorphin gene expression in the arcuate-median eminence of female rhesus macaques. *Endocrinology*, *151*, 3783–3794.
29. Topaloglu, A. K., & Semple, R. K. (2011). Neurokinin B signalling in the human reproductive axis. *Mol Cell Endocrinol*, *346*, 57–64.
30. Navarro, V. M., Gottsch, M. L., Wu, M., et al. (2011). Regulation of NKB pathways and their roles in the control of Kiss1 neurons in

the arcuate nucleus of the male mouse. *Endocrinology*, *152*, 4265–4275.
31. Prevot, V., Rio, C., Cho, G. J., et al. (2003). Normal female sexual development requires neuregulin-erbB receptor signaling in hypothalamic astrocytes. *J Neurosci*, *23*, 230–239.
32. Gottsch, M. L., Clifton, D. K., & Steiner, R. A. (2004). Galanin-like peptide as a link in the integration of metabolism and reproduction. *Trends Endocrinol Metab*, *15*, 215–221.
33. Gottsch, M. L., Cunningham, M. J., Smith, J. T., et al. (2004). A role for kisspeptins in the regulation of gonadotropin secretion in the mouse. *Endocrinology*, *145*, 4073–4077.
34. Plant, T. M., & Barker-Gibb, M. L. (2004). Neurobiological mechanisms of puberty in higher primates. *Hum Reprod Update*, *10*, 67–77.
35. Terasawa, E., & Fernandez, D. L. (2001). Neurobiological mechanisms of the onset of puberty in primates. *Endocr Rev*, *22*, 111–151.
36. Ojeda, S. R., Prevot, V., Heger, S., et al. (2003). Glia-to-neuron signaling and the neuroendocrine control of female puberty. *Ann Med*, *35*, 244–255.
37. Mitsushima, D., Hei, D. L., & Terasawa, E. (1994). gamma-Aminobutyric acid is an inhibitory neurotransmitter restricting the release of luteinizing hormone-releasing hormone before the onset of puberty. *Proc Natl Acad Sci USA*, *91*, 395–399.
38. Rivera, C., Voipio, J., Payne, J. A., et al. (1999). The K+/Cl- co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation. *Nature*, *397*, 251–255.
39. Marshall, W. A., & Tanner, J. M. (1969). Variations in pattern of pubertal changes in girls. *Archives of disease in childhood*, *44*, 291–303.
40. Marshall, W. A., & Tanner, J. M. (1970). Variations in the pattern of pubertal changes in boys. *Archives of disease in childhood*, *45*, 13–23.
41. Largo, R. H., & Prader, A. (1983). Pubertal development in Swiss boys. *Helv Paediatr Acta*, *38*, 211–228.
42. Roche, A. F., Wellens, R., Attie, K. M., & Siervogel, R. M. (1995). The timing of sexual maturation in a group of US white youths. *J Pediatr Endocrinol Metab*, *8*, 11–18.
43. Juul, A., Teilmann, G., Scheike, T., et al. (2006). Pubertal development in Danish children: comparison of recent European and US data. *Int J Androl*, *29*, 247–255; discussion 86–90.
44. Harlan, W. R., Grillo, G. P., Cornoni-Huntley, J., & Leaverton, P. E. (1979). Secondary sex characteristics of boys 12 to 17 years of age: the U.S. Health Examination Survey. *J Pediatr*, *95*, 293–297.
45. Harlan, W. R., Harlan, E. A., & Grillo, G. P. (1980). Secondary sex characteristics of girls 12 to 17 years of age: the U.S. Health Examination Survey. *J Pediatr*, *96*, 1074–1078.
46. Palmert, M. R., Hayden, D. L., Mansfield, M. J., et al. (2001). The longitudinal study of adrenal maturation during gonadal suppression: evidence that adrenarche is a gradual process. *J Clin Endocrinol Metab*, *86*, 4536–4542.
47. Sklar, C. A., Kaplan, S. L., & Grumbach, M. M. (1980). Evidence for dissociation between adrenarche and gonadarche: studies in patients with idiopathic precocious puberty, gonadal dysgenesis, isolated gonadotropin deficiency, and constitutionally delayed growth and adolescence. *J Clin Endocrinol Metab*, *51*, 548–556.
48. Wierman, M. E., Beardsworth, D. E., Crawford, J. D., et al. (1986). Adrenarche and skeletal maturation during luteinizing hormone releasing hormone analogue suppression of gonadarche. *J Clin Invest*, *77*, 121–126.
49. Remer, T., & Manz, F. (1999). Role of nutritional status in the regulation of adrenarche. *J Clin Endocrinol Metab*, *84*, 3936–3944.
50. Ong, K. K., Potau, N., Petry, C. J., et al. (2004). Opposing influences of prenatal and postnatal weight gain on adrenarche in normal boys and girls. *J Clin Endocrinol Metab*, *89*, 2647–2651.
51. Topor, L. S., Asai, M., Dunn, J., & Majzoub, J. A. (2011). Cortisol stimulates secretion of dehydroepiandrosterone in human adrenocortical cells through inhibition of 3betaHSD2. *J Clin Endocrinol Metab*, *96*, E31–E39.
52. Parent, A. S., Teilmann, G., Juul, A., et al. (2003). The timing of normal puberty and the age limits of sexual precocity: variations around the world, secular trends, and changes after migration. *Endocr Rev*, *24*, 668–693.
53. Gajdos, Z. K., Hirschhorn, J. N., & Palmert, M. R. (2009). What controls the timing of puberty? An update on progress from genetic investigation. *Curr Opin Endocrinol Diabetes Obes*, *16*, 16–24.
54. Fischbein, S. (1977). Onset of puberty in MX and DZ twins. *Acta Genet Med Gemellol* (Roma), *26*, 151–158.
55. Sklad, M. (1977). The rate of growth and maturing of twins. *Acta Genet Med Gemellol* (Roma), *26*, 221–237.
56. Kaprio, J., Rimpela, A., Winter, T., et al. (1995). Common genetic influences on BMI and age at menarche. *Hum Biol*, *67*, 739–753.
57. van den Berg, S. M., & Boomsma, D. I. (2007). The familial clustering of age at menarche in extended twin families. *Behav Genet*, *37*, 661–667.
58. Hodges, C. A., & Palmert, M. R. (2007). Genetic regulation of the variation in pubertal timing. In O. H. Pescovitz, & E. C. Walvoord (Eds.), *When puberty is precocious: scientific and clinical aspects* (pp. 83–102). Totowa, NJ: Humana Press.
59. Palmert, M. R., & Hirschhorn, J. N. (2003). Genetic approaches to stature, pubertal timing, and other complex traits. *Mol Genet Metab*, *80*, 1–10.
60. Towne, B., Czerwinski, S. A., Demerath, E. W., et al. (2005). Heritability of age at menarche in girls from the Fels Longitudinal Study. *Am J Phys Anthropol*, *128*, 210–219.
61. Morris, D. H., Jones, M. E., Schoemaker, M. J., et al. (2011). Familial concordance for age at menarche: analyses from the Breakthrough Generations Study. *Paediatr Perinat Epidemiol*, *25*, 306–311.
62. Bianco, S. D., & Kaiser, U. B. (2009). The genetic and molecular basis of idiopathic hypogonadotropic hypogonadism. *Nat Rev Endocrinol*, *5*, 569–576.
63. Bhangoo, A., & Jacobson-Dickman, E. (2009). The genetics of idiopathic hypogonadotropic hypogonadism: unraveling the biology of human sexual development. *Pediatr Endocrinol Rev*, *6*, 395–404.
64. Herbison, A. E. (2007). Genetics of puberty. *Horm Res*, *68*(Suppl. 5), 75–79.
65. Kalantaridou, S. N., & Chrousos, G. P. (2002). Clinical review 148: monogenic disorders of puberty. *J Clin Endocrinol Metab*, *87*, 2481–2494.
66. Silveira, L. F., MacColl, G. S., & Bouloux, P. M. (2002). Hypogonadotropic hypogonadism. *Semin Reprod Med*, *20*, 327–338.
67. Martin, C., Balasubramanian, R., Dwyer, A. A., et al. (2011). The role of the prokineticin 2 pathway in human reproduction: evidence from the study of human and murine gene mutations. *Endocr Rev*, *32*, 225–246.
68. Sykiotis, G. P., Plummer, L., Hughes, V. A., et al. (2010). Oligogenic basis of isolated gonadotropin-releasing hormone deficiency. *Proc Natl Acad Sci USA*, *107*, 15140–15144.
69. Abreu, A. P., Dauber, A., Macedo, D. B., et al. (2013). Central precocious puberty caused by mutations in the imprinted gene MKRN3. *N Engl J Med*, *368*, 2467–2475.
70. Shahab, M., Mastronardi, C., Seminara, S. B., et al. (2005). Increased hypothalamic GPR54 signaling: a potential mechanism for initiation of puberty in primates. *Proc Natl Acad Sci USA*, *102*, 2129–2134.
71. Bedecarrats, G. Y., & Kaiser, U. B. (2003). Differential regulation of gonadotropin subunit gene promoter activity by pulsatile gonadotropin-releasing hormone (GnRH) in perifused L beta T2 cells: role of GnRH receptor concentration. *Endocrinology*, *144*, 1802–1811.
72. Bedecarrats, G. Y., Linher, K. D., Janovick, J. A., et al. (2003). Four naturally occurring mutations in the human GnRH receptor affect ligand binding and receptor function. *Mol Cell Endocrinol*, *205*, 51–64.
73. Bedecarrats, G. Y., Linher, K. D., & Kaiser, U. B. (2003). Two common naturally occurring mutations in the human gonadotropin-releasing hormone (GnRH) receptor have differential effects on gonadotropin gene expression and on GnRH-mediated signal transduction. *J Clin Endocrinol Metab*, *88*, 834–843.
74. Karges, B., & de Roux, N. (2005). Molecular genetics of isolated hypogonadotropic hypogonadism and Kallmann syndrome. *Endocr Dev*, *8*, 67–80.
75. Bhagavath, B., Ozata, M., Ozdemir, I. C., et al. (2005). The prevalence of gonadotropin-releasing hormone receptor mutations in a large cohort of patients with hypogonadotropic hypogonadism. *Fertil Steril*, *84*, 951–957.
76. Beranova, M., Oliveira, L. M., Bedecarrats, G. Y., et al. (2001). Prevalence, phenotypic spectrum, and modes of inheritance of gonadotropin-releasing hormone receptor mutations in idiopathic hypogonadotropic hypogonadism. *J Clin Endocrinol Metab*, *86*, 1580–1588.

77. Chan, Y. M., de Guillebon, A., Lang-Muritano, M., et al. (2009). GNRH1 mutations in patients with idiopathic hypogonadotropic hypogonadism. *Proc Natl Acad Sci USA*, *106*, 11703–11708.
78. Bouligand, J., Ghervan, C., Tello, J. A., et al. (2009). Isolated familial hypogonadotropic hypogonadism and a GNRH1 mutation. *N Engl J Med*, *360*, 2742–2748.
79. Lin, L., Conway, G. S., Hill, N. R., et al. (2006). A homozygous R262Q mutation in the gonadotropin-releasing hormone receptor presenting as constitutional delay of growth and puberty with subsequent borderline oligospermia. *J Clin Endocrinol Metab*, *91*, 5117–5121.
80. Sedlmeyer, I. L., Pearce, C. L., Trueman, J. A., et al. (2005). Determination of sequence variation and haplotype structure for the gonadotropin-releasing hormone (GnRH) and GnRH receptor genes: investigation of role in pubertal timing. *J Clin Endocrinol Metab*, *90*, 1091–1099.
81. Gajdos, Z. K., Butler, J. L., Henderson, K. D., et al. (2008). Association studies of common variants in 10 hypogonadotropic hypogonadism genes with age at menarche. *J Clin Endocrinol Metab*, *93*, 4290–4298.
82. Franco, B., Guioli, S., Pragliola, A., et al. (1991). A gene deleted in Kallmann's syndrome shares homology with neural cell adhesion and axonal path-finding molecules. *Nature*, *353*, 529–536.
83. Legouis, R., Hardelin, J. P., Levilliers, J., et al. (1991). The candidate gene for the X-linked Kallmann syndrome encodes a protein related to adhesion molecules. *Cell*, *67*, 423–435.
84. Dode, C., Levilliers, J., Dupont, J. M., et al. (2003). Loss-of-function mutations in FGFR1 cause autosomal dominant Kallmann syndrome. *Nat Genet*, *33*, 463–465.
85. Dode, C., Teixeira, L., Levilliers, J., et al. (2006). Kallmann syndrome: mutations in the genes encoding prokineticin-2 and prokineticin receptor-2. *PLoS Genet*, *2*, e175.
86. Kramer, P. R., & Wray, S. (2000). Novel gene expressed in nasal region influences outgrowth of olfactory axons and migration of luteinizing hormone-releasing hormone (LHRH) neurons. *Genes Dev*, *14*, 1824–1834.
87. Miura, K., Acierno, J. S., Jr., & Seminara, S. B. (2004). Characterization of the human nasal embryonic LHRH factor gene, NELF, and a mutation screening among 65 patients with idiopathic hypogonadotropic hypogonadism (IHH). *J Hum Genet*, *49*, 265–268.
88. Pitteloud, N., Quinton, R., Pearce, S., et al. (2007). Digenic mutations account for variable phenotypes in idiopathic hypogonadotropic hypogonadism. *J Clin Invest*, *117*, 457–463.
89. Xu, N., Kim, H. G., Bhagavath, B., et al. (2011). Nasal embryonic LHRH factor (NELF) mutations in patients with normosmic hypogonadotropic hypogonadism and Kallmann syndrome. *Fertil Steril*, *95*, 1613–1620, e1–e7.
90. Cole, L. W., Sidis, Y., Zhang, C., et al. (2008). Mutations in prokineticin 2 (PROK2) and PROK2 receptor (PROKR2) in human gonadotrophin-releasing hormone deficiency: molecular genetics and clinical spectrum. *J Clin Endocrinol Metab*, *93*, 3551–3559.
91. Falardeau, J., Chung, W. C., Beenken, A., et al. (2008). Decreased FGF8 signaling causes deficiency of gonadotropin-releasing hormone in humans and mice. *J Clin Invest*, *118*, 2822–2831.
92. Kim, H. G., Kurth, I., Lan, F., et al. (2008). Mutations in CHD7, encoding a chromatin-remodeling protein, cause idiopathic hypogonadotropic hypogonadism and Kallmann syndrome. *Am J Hum Genet*, *83*, 511–519.
93. Jongmans, M. C., van Ravenswaaij-Arts, C. M., Pitteloud, N., et al. (2009). CHD7 mutations in patients initially diagnosed with Kallmann syndrome—the clinical overlap with CHARGE syndrome. *Clin Genet*, *75*, 65–71.
94. Raivio, T., Avbelj, M., McCabe, M. J., et al. (2012). Genetic overlap in Kallmann syndrome, combined pituitary hormone deficiency, and septo-optic dysplasia. *J Clin Endocrinol Metab*, *97*, E694–E699.
95. Reynaud, R., Jayakody, S. A., Monnier, C., et al. (2012). PROKR2 variants in multiple hypopituitarism with pituitary stalk interruption. *J Clin Endocrinol Metab*, *97*, E1068–E1073.
96. Pitteloud, N., Acierno, J. S., Jr., Meysing, A. U., et al. (2005). Reversible Kallmann syndrome, delayed puberty, and isolated anosmia occurring in a single family with a mutation in the fibroblast growth factor receptor 1 gene. *J Clin Endocrinol Metab*, *90*, 1317–1322.
97. Pitteloud, N., Meysing, A., Quinton, R., et al. (2006). Mutations in fibroblast growth factor receptor 1 cause Kallmann syndrome with a wide spectrum of reproductive phenotypes. *Mol Cell Endocrinol*, *254–255*, 60–69.
98. Raivio, T., Falardeau, J., Dwyer, A., et al. (2007). Reversal of idiopathic hypogonadotropic hypogonadism. *N Engl J Med*, *357*, 863–873.
99. Banerjee, I., Trueman, J. A., Hall, C. M., et al. (2006). Phenotypic variation in constitutional delay of growth and puberty: relationship to specific leptin and leptin receptor gene polymorphisms. *Eur J Endocrinol*, *155*, 121–126.
100. Jackson, R. S., Creemers, J. W., Ohagi, S., et al. (1997). Obesity and impaired prohormone processing associated with mutations in the human prohormone convertase 1 gene. *Nat Genet*, *16*, 303–306.
101. Gajdos, Z. K., Henderson, K. D., Hirschhorn, J. N., & Palmert, M. R. (2010). Genetic determinants of pubertal timing in the general population. *Mol Cell Endocrinol*, *324*, 21–29.
102. Vaaralahti, K., Wehkalampi, K., Tommiska, J., et al. (2011). The role of gene defects underlying isolated hypogonadotropic hypogonadism in patients with constitutional delay of growth and puberty. *Fertil Steril*, *95*, 2756–2758.
103. He, C., Kraft, P., Chen, C., et al. (2009). Genome-wide association studies identify loci associated with age at menarche and age at natural menopause. *Nat Genet*, *41*, 724–728.
104. Elks, C. E., Perry, J. R., Sulem, P., et al. (2010). Thirty new loci for age at menarche identified by a meta-analysis of genome-wide association studies. *Nat Genet*, *42*, 1077–1085.
105. Perry, J. R., Stolk, L., Franceschini, N., et al. (2009). Meta-analysis of genome-wide association data identifies two loci influencing age at menarche. *Nat Genet*, *41*, 648–650.
106. Sulem, P., Gudbjartsson, D. F., Rafnar, T., et al. (2009). Genome-wide association study identifies sequence variants on 6q21 associated with age at menarche. *Nat Genet*, *41*, 734–738.
107. Ong, K. K., Elks, C. E., Li, S., et al. (2009). Genetic variation in LIN28B is associated with the timing of puberty. *Nat Genet*, *41*, 729–733.
108. Hirschhorn, J. N. (2009). Genomewide association studies—illuminating biologic pathways. *N Engl J Med*, *360*, 1699–701.
109. Caronia, L. M., Martin, C., Welt, C. K., et al. (2011). A genetic basis for functional hypothalamic amenorrhea. *N Engl J Med*, *364*, 215–225.
110. Biro, F. M., Khoury, P., & Morrison, J. A. (2006). Influence of obesity on timing of puberty. *Int J Androl*, *29*, 272–277, discussion 86-90.
111. Ellis, B. J., & Essex, M. J. (2007). Family environments, adrenarche, and sexual maturation: a longitudinal test of a life history model. *Child Dev*, *78*, 1799–1817.
112. Euling, S. Y., Herman-Giddens, M. E., Lee, P. A., et al. (2008). Examination of US puberty-timing data from 1940 to 1994 for secular trends: panel findings. *Pediatrics*, *121* (Suppl. 3), S172–S191.
113. Karpati, A. M., Rubin, C. H., Kieszak, S. M., et al. (2002). Stature and pubertal stage assessment in American boys: the 1988-1994 Third National Health and Nutrition Examination. *J Adolesc Health*, *30*, 205–212.
114. Sun, S. S., Schubert, C. M., Chumlea, W. C., et al. (2002). National estimates of the timing of sexual maturation and racial differences among US children. *Pediatrics*, *110*, 911–919.
115. Sun, S. S., Schubert, C. M., Liang, R., et al. (2005). Is sexual maturity occurring earlier among U.S. children? *J Adolesc Health*, *37*, 345–355.
116. Herman-Giddens, M. E., Wang, L., & Koch, G. (2001). Secondary sexual characteristics in boys: estimates from the national health and nutrition examination survey III, 1988-1994. *Arch Pediatr Adolesc Med*, *155*, 1022–1028.
117. Biro, F. M., Lucky, A. W., Huster, G. A., & Morrison, J. A. (1995). Pubertal staging in boys. *J Pediatr*, *127*, 100–102.
118. Lee, P. A., Guo, S. S., & Kulin, H. E. (2001). Age of puberty: data from the United States of America. *Apmis*, *109*, 81–88.
119. Sorensen, K., Nielsen, J., Busch, P., et al. (1979). [Measurement of testicular volume with Prader's orchidometer in 1,389 boys 5—15½ years old]. *Ugeskrift for laeger*, *141*, 915–918.
120. Mul, D., Fredriks, A. M., van Buuren, S., et al. (2001). Pubertal development in The Netherlands 1965-1997. *Pediatric Res*, *50*, 479–486.
121. Sorensen, K., Aksglaede, L., Petersen, J. H., & Juul, A. (2010). Recent changes in pubertal timing in healthy Danish boys:

associations with body mass index. *J Clin Endocrinol Metab*, *95*, 263–270.
122. Kaplowitz, P. B. (2008). Link between body fat and the timing of puberty. *Pediatrics*, *121*(Suppl. 3), S208–S217.
123. Herman-Giddens, M. E., Slora, E. J., Wasserman, R. C., et al. (1997). Secondary sexual characteristics and menses in young girls seen in office practice: a study from the Pediatric Research in Office Settings network. *Pediatrics*, *99*, 505–512.
124. Foster, T. A., Voors, A. W., Webber, L. S., et al. (1977). Anthropometric and maturation measurements of children, ages 5 to 14 years, in a biracial community—the Bogalusa Heart Study. *Am J Clin Nutr*, *30*, 582–591.
125. Nathan, B. M., Sedlmeyer, I. L., & Palmert, M. R. (2006). Impact of body mass index on growth in boys with delayed puberty. *J Pediatr Endocrinol Metab*, *19*, 971–977.
126. Kaplowitz, P. (1998). Delayed puberty in obese boys: comparison with constitutional delayed puberty and response to testosterone therapy. *J Pediatr*, *133*, 745–749.
127. Sandhu, J., Ben-Shlomo, Y., Cole, T. J., et al. (2006). The impact of childhood body mass index on timing of puberty, adult stature and obesity: a follow-up study based on adolescent anthropometry recorded at Christ's Hospital (1936-1964). *Int J Obes* (Lond), *30*, 14–22.
128. He, Q., & Karlberg, J. (2001). BMI in childhood and its association with height gain, timing of puberty, and final height. *Pediatr Res*, *49*, 244–251.
129. Juul, A., Magnusdottir, S., Scheike, T., et al. (2007). Age at voice break in Danish boys: effects of pre-pubertal body mass index and secular trend. *Int J Androl*, *30*, 537–542.
130. Boyne, M. S., Osmond, C., Fraser, R. A., et al. (2010). Developmental origins of cardiovascular risk in Jamaican children: the Vulnerable Windows Cohort study. *Br J Nutr*, *104*, 1026–1033.
131. Boyne, M. S., Thame, M., Osmond, C., et al. (2010). Growth, body composition, and the onset of puberty: longitudinal observations in Afro-Caribbean children. *J Clin Endocrinol Metab*, *95*, 3194–3200.
132. Heger, S., Korner, A., Meigen, C., et al. (2008). Impact of weight status on the onset and parameters of puberty: analysis of three representative cohorts from central Europe. *J Pediatr Endocrinol Metab*, *21*, 865–877.
133. Meigen, C., Keller, A., Gausche, R., et al. (2008). Secular trends in body mass index in German children and adolescents: a cross-sectional data analysis via CrescNet between 1999 and 2006. *Metabolism*, *57*, 934–939.
134. Mouritsen, A., Aksglaede, L., Sorensen, K., et al. (2010). Hypothesis: exposure to endocrine-disrupting chemicals may interfere with timing of puberty. *Int J Androl*, *33*, 346–359.
135. Toppari, J., & Juul, A. (2010). Trends in puberty timing in humans and environmental modifiers. *Mol Cell Endocrinol*, *324*, 39–44.
136. Massart, F., & Saggese, G. (2010). Oestrogenic mycotoxin exposures and precocious pubertal development. *Int J Androl*, *33*, 369–376.
137. Frederiksen, H., Sorensen, K., Mouritsen, A., et al. (2012). High urinary phthalate concentration associated with delayed pubarche in girls. *Int J Androl*, *35*, 216–226.
138. Toppari, J., Virtanen, H. E., Main, K. M., & Skakkebaek, N. E. (2010). Cryptorchidism and hypospadias as a sign of testicular dysgenesis syndrome (TDS): environmental connection. *Birth Defects Res A Clin Mol Teratol*, *88*, 910–919.
139. Skakkebaek, N. E., Toppari, J., Soder, O., et al. (2011). The exposure of fetuses and children to endocrine disrupting chemicals: a European Society for Paediatric Endocrinology (ESPE) and Pediatric Endocrine Society (PES) call to action statement. *J Clin Endocrinol Metab*, *96*, 3056–3058.
140. Sharpe, R. M. (2011). Pediatrics: endocrine disruption and human health effects-a call to action. *Nat Rev Endocrinol*, *7*, 633–634.
141. Nathan, B. M., & Palmert, M. R. (2005). Regulation and disorders of pubertal timing. *Endocrinol Metab Clin North Am*, *34*, 617–641, ix.
142. Carel, J. C., & Leger, J. (2008). Clinical practice: precocious puberty. *N Engl J Med*, *358*, 2366–2377.
143. Virdis, R., Street, M. E., Bandello, M. A., et al. (2003). Growth and pubertal disorders in neurofibromatosis type 1. *J Pediatr Endocrinol Metab*, *16*(Suppl. 2), 289–292.
144. Kaulfers, A. M., Backeljauw, P. F., Reifschneider, K., et al. (2010). Endocrine dysfunction following traumatic brain injury in children. *J Pediatr*, *157*, 894–899.
145. Shenker, A., Laue, L., Kosugi, S., et al. (1993). A constitutively activating mutation of the luteinizing hormone receptor in familial male precocious puberty. *Nature*, *365*, 652–654.
146. Schedewie, H. K., Reiter, E. O., Beitins, I. Z., et al. (1981). Testicular Leydig cell hyperplasia as a cause of familial sexual precocity. *J Clin Endocrinol Metab*, *52*, 271–278.
147. Latronico, A. C., & Segaloff, D. L. (1999). Naturally occurring mutations of the luteinizing-hormone receptor: lessons learned about reproductive physiology and G protein-coupled receptors. *Am J Hum Genet*, *65*, 949–958.
148. Shenker, A., Weinstein, L. S., Moran, A., et al. (1993). Severe endocrine and nonendocrine manifestations of the McCune-Albright syndrome associated with activating mutations of stimulatory G protein GS. *J Pediatr*, *123*, 509–518.
149. Mamkin, I., Philibert, P., Anhalt, H., et al. (2010). Unusual phenotypical variations in a boy with McCune-Albright syndrome. *Horm Res Paediatr*, *73*, 215–222.
150. Lala, R., Matarazzo, P., Andreo, M., et al. (2002). Impact of endocrine hyperfunction and phosphate wasting on bone in McCune-Albright syndrome. *J Pediatr Endocrinol Metab*, *15*(Suppl. 3), 913–920.
151. Van Wyk, J. J., & Grumbach, M. M. (1960). Syndrome of precocious menstruation and galactorrhoea in juvenile hypothyroidism: an example of hormonal overlap in pituitary feedback. *J Pediatr*, *57*, 416–435.
152. Anasti, J. N., Flack, M. R., Froehlich, J., et al. (1995). A potential novel mechanism for precocious puberty in juvenile hypothyroidism. *J Clin Endocrinol Metab*, *80*, 276–279.
153. Bruder, J. M., Samuels, M. H., Bremner, W. J., et al. (1995). Hypothyroidism-induced macroorchidism: use of a gonadotropin-releasing hormone agonist to understand its mechanism and augment adult stature. *J Clin Endocrinol Metab*, *80*, 11–16.
154. Durbin, K. L., Diaz-Montes, T., & Loveless, M. B. (2011). Van Wyk and Grumbach syndrome: an unusual case and review of the literature. *J Pediatr Adolesc Gynecol*, *24*, e93–e96.
155. Boot, A. M., Lumbroso, S., Verhoef-Post, M., et al. (2011). Mutation analysis of the LH receptor gene in Leydig cell adenoma and hyperplasia and functional and biochemical studies of activating mutations of the LH receptor gene. *J Clin Endocrinol Metab*, *96*, E1197–E1205.
156. Kaplowitz, P. (2004). Clinical characteristics of 104 children referred for evaluation of precocious puberty. *J Clin Endocrinol Metab*, *89*, 3644–3650.
157. Palmert, M. R., Malin, H. V., & Boepple, P. A. (1999). Unsustained or slowly progressive puberty in young girls: initial presentation and long-term follow-up of 20 untreated patients. *J Clin Endocrinol Metab*, *84*, 415–423.
158. Bordini, B., Littlejohn, E., & Rosenfield, R. L. (2010). LH Dynamics in overweight girls with premature adrenarche and slowly progressive sexual precocity. *Int J Pediatr Endocrinol*. doi:10.1155/2010/724696
159. Klein, K. O. (1999). Precocious puberty: who has it? Who should be treated? *J Clin Endocrinol Metab*, *84*, 411–414.
160. Carel, J. C., Lahlou, N., Roger, M., & Chaussain, J. L. (2004). Precocious puberty and statural growth. *Hum Reprod Update*, *10*, 135–147.
161. Tremblay, L., & Frigon, J. Y. (2005). Precocious puberty in adolescent girls: a biomarker of later psychosocial adjustment problems. *Child Psychiatry Hum Dev*, *36*, 73–94.
162. Michaud, P. A., Suris, J. C., & Deppen, A. (2006). Gender-related psychological and behavioural correlates of pubertal timing in a national sample of Swiss adolescents. *Mol Cell Endocrinol*, *254–255*, 172–178.
163. Carel, J. C., Eugster, E. A., Rogol, A., et al. (2009). Consensus statement on the use of gonadotropin-releasing hormone analogs in children. *Pediatrics*, *123*, e752–e762.
164. Henley, D. V., Lipson, N., Korach, K. S., & Bloch, C. A. (2007). Prepubertal gynecomastia linked to lavender and tea tree oils. *N Engl J Med*, *356*, 479–485.
165. Neely, E. K., Hintz, R. L., Wilson, D. M., et al. (1995). Normal ranges for immunochemiluminometric gonadotropin assays. *J Pediatr*, *127*, 40–46.

166. Neely, E. K., Wilson, D. M., Lee, P. A., et al. (1995). Spontaneous serum gonadotropin concentrations in the evaluation of precocious puberty. *J Pediatr*, *127*, 47–52.
167. Pescovitz, O. H., Hench, K. D., Barnes, K. M., et al. (1988). Premature thelarche and central precocious puberty: the relationship between clinical presentation and the gonadotropin response to luteinizing hormone-releasing hormone. *J Clin Endocrinol Metab*, *67*, 474–479.
168. De Sanctis, V., Corrias, A., Rizzo, V., et al. (2000). Etiology of central precocious puberty in males: the results of the Italian Study Group for Physiopathology of Puberty. *J Pediatr Endocrinol Metab*, *13*(Suppl. 1), 687–693.
169. Cisternino, M., Arrigo, T., Pasquino, A. M., et al. (2000). Etiology and age incidence of precocious puberty in girls: a multicentric study. *J Pediatr Endocrinol Metab*, *13*(Suppl. 1), 695–701.
170. Bar, A., Linder, B., Sobel, E. H., et al. (1995). Bayley-Pinneau method of height prediction in girls with central precocious puberty: correlation with adult height. *J Pediatr*, *126*, 955–958.
171. Mul, D., Bertelloni, S., Carel, J. C., et al. (2002). Effect of gonadotropin-releasing hormone agonist treatment in boys with central precocious puberty: final height results. *Horm Res*, *58*, 1–7.
172. Bertelloni, S., & Mul, D. (2008). Treatment of central precocious puberty by GnRH analogs: long-term outcome in men. *Asian J Androl*, *10*, 525–534.
173. Neely, E. K., Lee, P. A., Bloch, C. A., et al. (2010). Leuprolide acetate 1-month depot for central precocious puberty: hormonal suppression and recovery. *Int J Pediatr Endocrinol*, *2010*, 398639.
174. Lee, P. A., Neely, E. K., Fuqua, J., et al. (2011). Efficacy of leuprolide acetate 1-month depot for central precocious puberty (CPP): growth outcomes during a prospective, longitudinal study. *Int J Pediatr Endocrinol*, *2011*, 2017.
175. Trueman, J. A., Tillmann, V., Cusick, C. F., et al. (2002). Suppression of puberty with long-acting goserelin (Zoladex-LA): effect on gonadotrophin response to GnRH in the first treatment cycle. *Clin Endocrinol* (Oxf), *57*, 223–230.
176. Lee, P. A., Klein, K., Mauras, N., et al. (2012). Efficacy and safety of leuprolide acetate 3-month depot 11.25 milligrams or 30 milligrams for the treatment of central precocious puberty. *J Clin Endocrinol Metab*, *97*, 1572–1580.
177. Eugster, E. A., Clarke, W., Kletter, G. B., et al. (2007). Efficacy and safety of histrelin subdermal implant in children with central precocious puberty: a multicenter trial. *J Clin Endocrinol Metab*, *92*, 1697–1704.
178. Hirsch, H. J., Gillis, D., Strich, D., et al. (2005). The histrelin implant: a novel treatment for central precocious puberty. *Pediatrics*, *116*, e798–802.
179. Rahhal, S., Clarke, W. L., Kletter, G. B., et al. (2009). Results of a second year of therapy with the 12-month histrelin implant for the treatment of central precocious puberty. *Int J Pediatr Endocrinol*. doi:10.1155/2009/812517
180. Almeida, M. Q., Brito, V. N., Lins, T. S., et al. (2008). Long-term treatment of familial male-limited precocious puberty (testotoxicosis) with cyproterone acetate or ketoconazole. *Clin Endocrinol* (Oxf), *69*, 93–98.
181. Babovic-Vuksanovic, D., Donaldson, M. D., Gibson, N. A., & Wallace, A. M. (1994). Hazards of ketoconazole therapy in testotoxicosis. *Acta Paediatr*, *83*, 994–997.
182. Holland, F. J., Fishman, L., Bailey, J. D., & Fazekas, A. T. (1985). Ketoconazole in the management of precocious puberty not responsive to LHRH-analogue therapy. *N Engl J Med*, *312*, 1023–1028.
183. Rajfer, J., Sikka, S. C., Rivera, F., & Handelsman, D. J. (1986). Mechanism of inhibition of human testicular steroidogenesis by oral ketoconazole. *J Clin Endocrinol Metab*, *63*, 1193–1198.
184. Soriano-Guillen, L., Lahlou, N., Chauvet, G., et al. (2005). Adult height after ketoconazole treatment in patients with familial male-limited precocious puberty. *J Clin Endocrinol Metab*, *90*, 147–151.
185. Laue, L., Jones, J., Barnes, K. M., & Cutler, G. B., Jr. (1993). Treatment of familial male precocious puberty with spironolactone, testolactone, and deslorelin. *J Clin Endocrinol Metab*, *76*, 151–155.
186. Laue, L., Kenigsberg, D., Pescovitz, O. H., et al. (1989). Treatment of familial male precocious puberty with spironolactone and testolactone. *N Engl J Med*, *320*, 496–502.
187. Leschek, E. W., Jones, J., Barnes, K. M., et al. (1999). Six-year results of spironolactone and testolactone treatment of familial male-limited precocious puberty with addition of deslorelin after central puberty onset. *J Clin Endocrinol Metab*, *84*, 175–178.
188. Merke, D. P., Keil, M. F., Jones, J. V., et al. (2000). Flutamide, testolactone, and reduced hydrocortisone dose maintain normal growth velocity and bone maturation despite elevated androgen levels in children with congenital adrenal hyperplasia. *J Clin Endocrinol Metab*, *85*, 1114–1120.
189. Best, T. R., Jenkins, J. K., Murphy, F. Y., et al. (1987). Persistent adrenal insufficiency secondary to low-dose ketoconazole therapy. *Am J Med*, *82*, 676–680.
190. Eyssette-Guerreau, S., Pinto, G., Sultan, A., et al. (2008). Effectiveness of anastrozole and cyproterone acetate in two brothers with familial male precocious puberty. *J Pediatr Endocrinol Metab*, *21*, 995–1002.
191. Hero, M., Janne, O. A., Nanto-Salonen, K., et al. (2005). Circulating antiandrogenic activity in children with congenital adrenal hyperplasia during peroral flutamide treatment. *J Clin Endocrinol Metab*, *90*, 5141–5145.
192. Kreher, N. C., Pescovitz, O. H., Delameter, P., et al. (2006). Treatment of familial male-limited precocious puberty with bicalutamide and anastrozole. *J Pediatr*, *149*, 416–420.
193. Tessaris, D., Matarazzo, P., Mussa, A., et al. (2012). Combined treatment with bicalutamide and anastrozole in a young boy with peripheral precocious puberty due to McCune-Albright Syndrome. *Endocr J*, *59*, 111–117.
194. Hero, M., Maury, S., Luotoniemi, E., et al. (2010). Cognitive effects of aromatase inhibitor therapy in peripubertal boys. *Eur J Endocrinol*, *163*, 149–155.
195. Hero, M., Toiviainen-Salo, S., Wickman, S., et al. (2010). Vertebral morphology in aromatase inhibitor-treated males with idiopathic short stature or constitutional delay of puberty. *J Bone Miner Res*, *25*, 1536–1543.
196. Palmert, M. R., & Dunkel, L. (2012). Clinical practice: delayed puberty. *N Engl J Med*, *366*, 443–453.
197. Wu, T., Mendola, P., & Buck, G. M. (2002). Ethnic differences in the presence of secondary sex characteristics and menarche among US girls: the Third National Health and Nutrition Examination Survey, 1988-1994. *Pediatrics*, *110*, 752–757.
198. Susman, E. J., Houts, R. M., Steinberg, L., et al. (2010). Longitudinal development of secondary sexual characteristics in girls and boys between ages 9½ and 15½ years. *Arch Pediatr Adolesc Med*, *164*, 166–173.
199. Aksglaede, L., Sorensen, K., Petersen, J. H., et al. (2009). Recent decline in age at breast development: the Copenhagen Puberty Study. *Pediatrics*, *123*, e932–e939.
200. Albanese, A., & Stanhope, R. (1995). Predictive factors in the determination of final height in boys with constitutional delay of growth and puberty. *J Pediatr*, *126*, 545–550.
201. Gilsanz, V., Chalfant, J., Kalkwarf, H., et al. (2011). Age at onset of puberty predicts bone mass in young adulthood. *J Pediatr*, *158*, *100–105*, 5 e1–e2.
202. Sedlmeyer, I. L., & Palmert, M. R. (2002). Delayed puberty: analysis of a large case series from an academic center. *J Clin Endocrinol Metab*, *87*, 1613–1620.
203. Han, J. C., Balagopal, P., Sweeten, S., et al. (2006). Evidence for hypermetabolism in boys with constitutional delay of growth and maturation. *J Clin Endocrinol Metab*, *91*, 2081–2086.
204. Wilson, D. A., Hofman, P. L., Miles, H. L., et al. (2010). Enhanced insulin sensitivity in prepubertal children with constitutional delay of growth and development. *J Pediatr*, *156*, 308–312.
205. Sedlmeyer, I. L., Hirschhorn, J. N., & Palmert, M. R. (2002). Pedigree analysis of constitutional delay of growth and maturation: determination of familial aggregation and inheritance patterns. *J Clin Endocrinol Metab*, *87*, 5581–5586.
206. Wehkalampi, K., Widen, E., Laine, T., et al. (2008). Patterns of inheritance of constitutional delay of growth and puberty in families of adolescent girls and boys referred to specialist pediatric care. *J Clin Endocrinol Metab*, *93*, 723–728.
207. Tommiska, J., Wehkalampi, K., Vaaralahti, K., et al. (2010). LIN28B in constitutional delay of growth and puberty. *J Clin Endocrinol Metab*, *95*, 3063–3066.
208. Hay, C., & Wu, F. (2002). Genetics and hypogonadotrophic hypogonadism.*Curr Opin Obstet Gynecol*, *14*, 303–308.

209. McCabe, M. J., Gaston-Massuet, C., Tziaferi, V., et al. (2011). Novel FGF8 mutations associated with recessive holoprosencephaly, craniofacial defects, and hypothalamo-pituitary dysfunction. *J Clin Endocrinol Metab*, *96*, E1709–E1718.
210. Tornberg, J., Sykiotis, G. P., Keefe, K., et al. (2011). Heparan sulfate 6-O-sulfotransferase 1, a gene involved in extracellular sugar modifications, is mutated in patients with idiopathic hypogonadotrophic hypogonadism. *Proc Natl Acad Sci USA*, *108*, 11524–11529.
211. Hanchate, N. K., Giacobini, P., Lhuillier, P., et al. (2012). SEMA3A, a gene involved in axonal pathfinding, is mutated in patients with Kallmann syndrome. *PLoS Genet*, *8*, e1002896.
212. Kim, H. G., Ahn, J. W., Kurth, I., et al. (2010). WDR11, a WD protein that interacts with transcription factor EMX1, is mutated in idiopathic hypogonadotropic hypogonadism and Kallmann syndrome. *Am J Hum Genet*, *87*, 465–479.
213. Gianetti, E., Tusset, C., Noel, S. D., et al. (2010). TAC3/TACR3 mutations reveal preferential activation of gonadotropin-releasing hormone release by neurokinin B in neonatal life followed by reversal in adulthood. *J Clin Endocrinol Metab*, *95*, 2857–2867.
214. Laitinen, E. M., Tommiska, J., Sane, T., et al. (2012). Reversible congenital hypogonadotropic hypogonadism in patients with CHD7, FGFR1 or GNRHR mutations. *PLoS One*, *7*, e39450.
215. Bédécarrats, G. Y., & Kaiser, U. B. (2007). Mutations in the human gonadotropin-releasing hormone receptor: insights into receptor biology and function. *Semin Reprod Med*, *25*, 368–378.
216. Peter, M., Viemann, M., Partsch, C. J., & Sippell, W. G. (1998). Congenital adrenal hypoplasia: clinical spectrum, experience with hormonal diagnosis, and report on new point mutations of the DAX-1 gene. *J Clin Endocrinol Metab*, *83*, 2666–2674.
217. Bojesen, A., Juul, S., & Gravholt, C. H. (2003). Prenatal and postnatal prevalence of Klinefelter syndrome: a national registry study. *J Clin Endocrinol Metab*, *88*, 622–626.
218. Ottesen, A. M., Aksglaede, L., Garn, I., et al. (2010). Increased number of sex chromosomes affects height in a nonlinear fashion: a study of 305 patients with sex chromosome aneuploidy. *Am J Med Genet A*, *152A*, 1206–1212.
219. Sokol, R. Z. (2012). It's not all about the testes: medical issues in Klinefelter patients. *Fertil Steril*, *98*, 261–265.
220. Groth, K. A., Skakkebæk, A., Høst, C., et al. (2013). Clinical review: Klinefelter syndrome—a clinical update. *J Clin Endocrinol Metab*, *98*, 20–30.
221. Aksglaede, L., & Juul, A. (2013). Testicular function and fertility in men with Klinefelter syndrome: a review. *Eur J Endocrinol*, *168*, R67–R76.
222. Oates, R. D. (2012). The natural history of endocrine function and spermatogenesis in Klinefelter syndrome: what the data show. *Fertil Steril*, *98*, 266–273.
223. Cox, J. J., Willatt, L., Homfray, T., & Woods, C. G. (2011). A SOX9 duplication and familial 46,XX developmental testicular disorder. *N Engl J Med*, *364*, 91–93.
224. Seeherunvong, T., Perera, E. M., Bao, Y., et al. (2004). 46,XX sex reversal with partial duplication of chromosome arm 22q. *Am J Med Genet A*, *127A*, 149–151.
225. Tomaselli, S., Megiorni, F., De Bernardo, C., et al. (2008). Syndromic true hermaphroditism due to an R-spondin1 (RSPO1) homozygous mutation. *Hum Mutat*, *29*, 220–226.
226. Cools, M., Boter, M., van Gurp, R., et al. (2007). Impact of the Y-containing cell line on histological differentiation patterns in dysgenetic gonads. *Clin Endocrinol* (Oxf), *67*, 184–192.
227. Ankarberg-Lindgren, C., Westphal, O., & Dahlgren, J. (2011). Testicular size development and reproductive hormones in boys and adult males with Noonan syndrome: a longitudinal study. *Eur J Endocrinol*, *165*, 137–144.
228. Vogels, A., Moerman, P., Frijns, J. P., & Bogaert, G. A. (2008). Testicular histology in boys with Prader-Willi syndrome: fertile or infertile? *J Urol*, *180*(Suppl. 4), 1800–1804.
229. Siemensma, E. P., de Lind van Wijngaarden, R. F., Otten, B. J., et al. (2012). Testicular failure in boys with Prader-Willi syndrome: longitudinal studies of reproductive hormones. *J Clin Endocrinol Metab*, *97*, E452–E459.
230. El-Khairi, R., & Achermann, J. C. (2012). Steroidogenic factor-1 and human disease. *Semin Reprod Med*, *30*, 374–381.
231. Salameh, W., Choucair, M., Guo, T. B., et al. (2005). Leydig cell hypoplasia due to inactivation of luteinizing hormone receptor by a novel homozygous nonsense truncation mutation in the seventh transmembrane domain. *Mol Cell Endocrinol*, *229*, 57–64.
232. Baker, B. Y., Lin, L., Kim, C. J., et al. (2006). Nonclassic congenital lipoid adrenal hyperplasia: a new disorder of the steroidogenic acute regulatory protein with very late presentation and normal male genitalia. *J Clin Endocrinol Metab*, *91*, 4781–4785.
233. Tee, M. K., Abramsohn, M., Loewenthal, N., et al. (2013). Varied clinical presentations of seven patients with mutations in CYP11A1 encoding the cholesterol side-chain cleavage enzyme, P450scc. *J Clin Endocrinol Metab*, *98*, 713–720.
234. Gucev, Z. S., Tee, M. K., Chitayat, D., et al. (2013). Distinguishing deficiencies in the steroidogenic acute regulatory protein and the cholesterol side chain cleavage enzyme causing neonatal adrenal failure. *J Pediatr*, *162*, 819–822.
235. Belgini, D. R., Mello, M. P., Baptista, M. T., et al. (2010). Six new cases confirm the clinical molecular profile of complete combined 17α-hydroxylase/ 17,20-lyase deficiency in Brazil. *Arq Bras Endocrinol Metabol*, *54*, 711–716.
236. Idkowiak, J., O'Riordan, S., Reisch, N., et al. (2011). Pubertal presentation in seven patients with congenital adrenal hyperplasia due to P450 oxidoreductase deficiency. *J Clin Endocrinol Metab*, *96*, E453–E462.
237. Zachmann, M., Völlmin, J. A., Hamilton, W., & Prader, A. (1972). Steroid 17,20-desmolase deficiency: a new cause of male pseudohermaphroditism. *Clin Endocrinol* (Oxf), *1*, 369–385.
238. Wilson, J. D., Auchus, R. J., Leihy, M. W., et al. (2003). 5alpha-androstane-3alpha,17beta-diol is formed in tammar wallaby pouch young testes by a pathway involving 5alpha-pregnane-3alpha,17alpha-diol-20-one as a key intermediate. *Endocrinology*, *144*, 575–580.
239. Flück, C. E., Meyer-Böni, M., Pandey, A. V., et al. (2011). Why boys will be boys: two pathways of fetal testicular androgen biosynthesis are needed for male sexual differentiation. *Am J Hum Genet*, *89*, 201–218.
240. Fenichel, P., Paris, F., Philibert, P., et al. (2013). Molecular diagnosis of 5α-reductase deficiency in 4 elite young female athletes through hormonal screening for hyperandrogenism. *J Clin Endocrinol Metab*, *98*, E1055–E1059.
241. Costa, E. M., Domenice, S., Sircili, M. H., et al. (2012). DSD due to 5α-reductase 2 deficiency—from diagnosis to long term outcome. *Semin Reprod Med*, *30*, 427–431.
242. Kalfa, N., Philibert, P., Werner, R., et al. (2013). Minor hypospadias: the "tip of the iceberg" of the partial androgen insensitivity syndrome. *PLOS One*, *8*, e61824.
243. Gottlieb, B., Beitel, L. K., Nadarajah, A., et al. (2012). The androgen receptor gene mutations database: 2012 update. *Hum Mutat*, *33*, 887–894.
244. Farikullah, J., Ehtisham, S., Nappo, S., et al. (2012). Persistent Müllerian duct syndrome: lessons learned from managing a series of eight patients over a 10-year period and review of literature regarding malignant risk from the Müllerian remnants. *BJU Int*, *110*(11 Pt. C), E1084–E1089.
245. Spires, S. E., Woolums, C. S., Pulito, A. R., & Spires, S. M. (2000). Testicular regression syndrome: a clinical and pathologic study of 11 cases. *Arch Pathol Lab Med*, *124*, 694–698.
246. Misra, M., MacLaughlin, D. T., Donahoe, P. K., & Lee, M. M. (2002). Measurement of Mullerian inhibiting substance facilitates management of boys with microphallus and cryptorchidism. *J Clin Endocrinol Metab*, *87*, 3598–3602.
247. Brauner, R., Neve, M., Allali, S., et al. (2011). Clinical, biological and genetic analysis of anorchia in 26 boys. *PLoS One*, *6*, e23292.
248. Kojima, Y., Kohri, K., & Hayashi, Y. (2010). Genetic pathway of external genitalia formation and molecular etiology of hypospadias. *J Pediatr Urol*, *6*, 346–354.
249. Carmichael, S. L., Shaw, G. M., & Lammer, E. J. (2012). Environmental and genetic contributors to hypospadias: a review of the epidemiologic evidence. *Birth Defects Res A Clin Mol Teratol*, *94*, 499–510.
250. Hersmus, R., Kalfa, N., de Leeuw, B., et al. (2008). FOXL2 and SOX9 as parameters of female and male gonadal differentiation in patients with various forms of disorders of sex development (DSD). *J Pathol*, *215*, 31–38.

251. Buell-Gutbrod, R., Ivanovic, M., Montag, A., et al. (2011). FOXL2 and SOX9 distinguish the lineage of the sex cord-stromal cells in gonadoblastomas. *Pediatr Dev Pathol*, *14*, 391–395.
252. Deans, R., Creighton, S. M., Liao, L. M., & Conway, G. S. (2012). Timing of gonadectomy in adult women with complete androgen insensitivity syndrome (CAIS): patient preferences and clinical evidence. *Clin Endocrinol* (Oxf), *76*, 894–898.
253. Nakhal, R. S., Hall-Craggs, M., Freeman, A., et al. (2013). Evaluation of retained testes in adolescent girls and women with complete androgen insensitivity syndrome. *Radiology*, *268*, 153–160.
254. Olivier, P., Simoneau-Roy, J., Francoeur, D., et al. (2012). Leydig cell tumors in children: contrasting clinical, hormonal, anatomical, and molecular characteristics in boys and girls. *J Pediatr*, *161*, 1147–1152.
255. Gourgari, E., Saloustros, E., & Stratakis, C. A. (2012). Large-cell calcifying Sertoli cell tumors of the testes in pediatrics. *Curr Opin Pediatr*, *24*, 518–522.
256. Claahsen-van der Grinten, H. L., Sweep, F. C., Blickman, J. G., et al. (2007). Prevalence of testicular adrenal rest tumours in male children with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Eur J Endocrinol*, *157*, 339–344.
257. Ma, N. S., & Geffner, M. E. (2008). Gynecomastia in prepubertal and pubertal men. *Curr Opin Pediatr*, *20*, 465–470.
258. Kumanov, P., Deepinder, F., Robeva, R., et al. (2007). Relationship of adolescent gynecomastia with varicocele and somatometric parameters: a cross-sectional study in 6200 healthy boys. *J Adolesc Health*, *41*, 126–131.
259. Deepinder, F., & Braunstein, G. D. (2012). Drug-induced gynecomastia: an evidence-based review. *Expert Opin Drug Saf*, *11*, 779–795.
260. Demura, M., Martin, R. M., Shozu, M., et al. (2007). Regional rearrangements in chromosome 15q21 cause formation of cryptic promoters for the CYP19 (aromatase) gene. *Hum Mol Genet*, *16*, 2529–2541.
261. Braunstein, G. D. (2007). Clinical practice. Gynecomastia. *N Engl J Med*, *357*, 1229–1237.
262. Mauras, N., Bishop, K., Merinbaum, D., et al. (2009). Pharmacokinetics and pharmacodynamics of anastrozole in pubertal boys with recent-onset gynecomastia. *J Clin Endocrinol Metab*, *94*, 2975–2978.
263. Plourde, P. V., Reiter, E. O., Jou, H. C., et al. (2004). Safety and efficacy of anastrozole for the treatment of pubertal gynecomastia: a randomized, double-blind, placebo-controlled trial. *J Clin Endocrinol Metab*, *89*, 4428–4433.
264. De Luca, F., Argente, J., Cavallo, L., et al. (2001). Management of puberty in constitutional delay of growth and puberty. *J Pediatr Endocrinol Metab*, *14*(Suppl. 2), 953–957.
265. Lee, J. M., Kaciroti, N., Appugliese, D., et al. (2010). Body mass index and timing of pubertal initiation in boys. *Arch Pediatr Adolesc Med*, *164*, 139–144.
266. Counts, D. R., Pescovitz, O. H., Barnes, K. M., et al. (1987). Dissociation of adrenarche and gonadarche in precocious puberty and in isolated hypogonadotropic hypogonadism. *J Clin Endocrinol Metab*, *64*, 1174–1178.
267. Harrington, J., & Palmert, M. R. (2012). Distinguishing constitutional delay of growth and puberty from isolated hypogonadotropic hypogonadism: critical appraisal of available diagnostic tests. *J Clin Endocrinol Metab*, *97*, 3056–3067.
268. Young, J. (2012). Approach to the male patient with congenital hypogonadotropic hypogonadism. *J Clin Endocrinol Metab*, *97*, 707–718.
269. Grinspon, R. P., Ropelato, M. G., Gottlieb, S., et al. (2010). Basal follicle-stimulating hormone and peak gonadotropin levels after gonadotropin-releasing hormone infusion show high diagnostic accuracy in boys with suspicion of hypogonadotropic hypogonadism. *J Clin Endocrinol Metab*, *95*, 2811–2818.
270. Resende, E. A., Lara, B. H., Reis, J. D., et al. (2007). Assessment of basal and gonadotropin-releasing hormone-stimulated gonadotropins by immunochemiluminometric and immunofluorometric assays in normal children. *J Clin Endocrinol Metab*, *92*, 1424–1429.
271. Coutant, R., Biette-Demeneix, E., Bouvattier, C., et al. (2010). Baseline inhibin B and anti-Mullerian hormone measurements for diagnosis of hypogonadotropic hypogonadism (HH) in boys with delayed puberty. *J Clin Endocrinol Metab*, *95*, 5225–5232.
272. Adan, L., Lechevalier, P., Couto-Silva, A. C., et al. (2010). Plasma inhibin B and antimullerian hormone concentrations in boys: discriminating between congenital hypogonadotropic hypogonadism and constitutional pubertal delay. *Med Sci Monit*, *16*, CR511–CR517.
273. Harrington, J., & Palmert, M. R. (2012). Clinical review: distinguishing constitutional delay of growth and puberty from isolated hypogonadotropic hypogonadism: critical appraisal of available diagnostic tests. *J Clin Endocrinol Metab*, *97*, 3056–3067.
274. Richman, R. A., & Kirsch, L. R. (1988). Testosterone treatment in adolescent boys with constitutional delay in growth and development. *N Engl J Med*, *319*, 1563–1567.
275. Rosenfeld, R. G., Northcraft, G. B., & Hintz, R. L. (1982). A prospective, randomized study of testosterone treatment of constitutional delay of growth and development in male adolescents. *Pediatrics*, *69*, 681–687.
276. Soliman, A. T., Khadir, M. M., & Asfour, M. (1995). Testosterone treatment in adolescent boys with constitutional delay of growth and development. *Metabolism*, *44*, 1013–1015.
277. Cohen, P., Rogol, A. D., Deal, C. L., et al. (2008). Consensus statement on the diagnosis and treatment of children with idiopathic short stature: a summary of the Growth Hormone Research Society, the Lawson Wilkins Pediatric Endocrine Society, and the European Society for Paediatric Endocrinology Workshop. *J Clin Endocrinol Metab*, *93*, 4210–4217.
278. Rosenbloom, A. L. (2009). Idiopathic short stature: conundrums of definition and treatment. *Int J Pediatr Endocrinol*. doi:10.1155/2009/470378
279. Hero, M., Norjavaara, E., & Dunkel, L. (2005). Inhibition of estrogen biosynthesis with a potent aromatase inhibitor increases predicted adult height in boys with idiopathic short stature: a randomized controlled trial. *J Clin Endocrinol Metab*, *90*, 6396–6402.
280. Wickman, S., Sipila, I., Ankarberg-Lindgren, C., et al. (2001). A specific aromatase inhibitor and potential increase in adult height in boys with delayed puberty: a randomised controlled trial. *Lancet*, *357*, 1743–1748.
281. Hero, M., Wickman, S., & Dunkel, L. (2006). Treatment with the aromatase inhibitor letrozole during adolescence increases near-final height in boys with constitutional delay of puberty. *Clinical Endocrinol*, *64*, 510–513.
282. Mauras, N., Gonzalez de Pijem, L., Hsiang, H. Y., et al. (2008). Anastrozole increases predicted adult height of short adolescent males treated with growth hormone: a randomized, placebo-controlled, multicenter trial for one to three years. *J Clin Endocrinol Metab*, *93*, 823–831.
283. Shulman, D. I., Francis, G. L., Palmert, M. R., & Eugster, E. A. (2008). Use of aromatase inhibitors in children and adolescents with disorders of growth and adolescent development. *Pediatrics*, *121*, e975–e983.
284. Wit, J. M., Hero, M., & Nunez, S. B. (2012). Aromatase inhibitors in pediatrics. *Nat Rev Endocrinol*, *8*, 135–147.
285. Warne, D. W., Decosterd, G., Okada, H., et al. (2009). A combined analysis of data to identify predictive factors for spermatogenesis in men with hypogonadotropic hypogonadism treated with recombinant human follicle-stimulating hormone and human chorionic gonadotropin. *Fertil Steril*, *92*, 594–604.
286. Pitteloud, N., Hayes, F. J., Boepple, P. A., et al. (2002). The role of prior pubertal development, biochemical markers of testicular maturation, and genetics in elucidating the phenotypic heterogeneity of idiopathic hypogonadotropic hypogonadism. *J Clin Endocrinol Metab*, *87*, 152–160.
287. Pitteloud, N., Hayes, F. J., Dwyer, A., et al. (2002). Predictors of outcome of long-term GnRH therapy in men with idiopathic hypogonadotropic hypogonadism. *J Clin Endocrinol Metab*, *87*, 4128–4136.
288. Liu, P. Y., Baker, H. W., Jayadev, V., et al. (2009). Induction of spermatogenesis and fertility during gonadotropin treatment of gonadotropin-deficient infertile men: predictors of fertility outcome. *J Clin Endocrinol Metab*, *94*, 801–808.
289. Davies, M. J., Moore, V. M., Willson, K. J., et al. (2012). Reproductive technologies and the risk of birth defects. *N Engl J Med*, *366*, 1803–1813.

290. Bhasin, S., Woodhouse, L., Casaburi, R., et al. (2001). Testosterone dose-response relationships in healthy young men. *Am J Physiol Endocrinol Metab*, *281*, E1172–E1181.
291. Di Luigi, L., Sgrò, P., Romanelli, F., et al. (2009). Urinary and serum hormones profiles after testosterone enanthate administration in male hypogonadism: concerns on the detection of doping with testosterone in treated hypogonadal athletes. *J Endocrinol Invest*, *32*, 445–453.
292. Martínez-Patiño, M. J. (2005). Personal account: a woman tried and tested. *Lancet*, *366*(Suppl. 1), S38.
293. Wit, J. M., & Rekers-Mombarg, L. T. (2002). Final height gain by GH therapy in children with idiopathic short stature is dose dependent. *J Clin Endocrinol Metab*, *87*, 604–611.
294. Wu, F. C., Brown, D. C., Butler, G. E., et al. (1993). Early morning plasma testosterone is an accurate predictor of imminent pubertal development in prepubertal boys. *J Clin Endocrinol Metab*, *76*, 26–31.
295. Segal, T. Y., Mehta, A., Anazodo, A., et al. (2009). Role of gonadotropin-releasing hormone and human chorionic gonadotropin stimulation tests in differentiating patients with hypogonadotropic hypogonadism from those with constitutional delay of growth and puberty. *J Clin Endocrinol Metab*, *94*, 780–785.
296. Doty, R. L., Shaman, P., Kimmelman, C. P., & Dann, M. S. (1984). University of Pennsylvania Smell Identification Test: a rapid quantitative olfactory function test for the clinic. *Laryngoscope*, *94*, 176–178.
297. Wickman, S., & Dunkel, L. (2001). Inhibition of P450 aromatase enhances gonadotropin secretion in early and midpubertal boys: evidence for a pituitary site of action of endogenous E. *J Clin Endocrinol Metab*, *86*, 4887–4894.

QUESTIONS

1. Genetic defects have been identified that cause many cases of all *except* which of the following conditions?
   a. Kallmann syndrome
   b. Constitutional delay of growth and maturation
   c. Isolated hypogonadotropic hypogonadism
   d. McCune-Albright syndrome
   e. Familial male limited precocious puberty

Answer: b

2. The traditional definition of delayed puberty in a boy includes which of the following?
   a. Lack of testicular enlargement by age 13 years
   b. Lack of pubic hair by age 14 years
   c. Lack of testicular enlargement by age 14 years
   d. Lack of pubic hair by age 13 years

Answer: c

3. Among patients seen in pediatric endocrinology clinics, which of the following is true?
   a. Idiopathic central precocious puberty is more common among boys and constitutional delay of growth and puberty is more common among girls.
   b. Idiopathic central precocious puberty is more common among girls and constitutional delay of growth and puberty is more common among boys.

Answer: b

4. GnRH dependence underlies which of the following?
   a. Testicular tumor producing large amounts of testosterone
   b. Adrenal tumor producing large amounts of androgens
   c. Idiopathic central precocious puberty
   d. All of the above

Answer: c

5. Obesity has been associated with earlier onset of puberty in girls. In boys,
   a. The same relationship exists.
   b. There is no relationship between overweight/obesity and pubertal timing.
   c. The relationship between overweight/obesity and onset of puberty is not clear.

Answer: c

6. The decision to treat idiopathic central precocious puberty is based on which of the following considerations?
   a. Preserving growth potential and optimizing adult height
   b. Mitigating psychosocial distress associated with early development
   c. Both
   d. Neither

Answer: c

CHAPTER 18

Disorders of Mineral Homeostasis in Children and Adolescents

Allen W. Root, MD • Frank B. Diamond, Jr., MD

CHAPTER OUTLINE

Disorders of calcium, magnesium, and phosphate metabolism and of bone formation, accrual, and maintenance during the first two decades of life result from suboptimal ingestion, absorption, or retention of constituent nutrients, abnormal vitamin D metabolism or bioactivity, disorders of parathyroid hormone (PTH) synthesis, secretion, or action, and intrinsic aberrations in cartilage and bone cells. The origins of these illnesses may be intrinsic due to pathologic variations in the genes controlling these processes or to acquired insults (Table 18-1). For an integrated overview of calcium, mineral, and skeletal homeostasis and changes in this system through the life cycle, see Chapter 8.

HYPOCALCEMIA

Hypocalcemia in the Neonate and Infant

Clinical manifestations of hypocalcemia occurring in the neonate—defined as values of total calcium < 7.5 to 8 mg/dL and ionized calcium (Ca^{2+}) < 4.4 mg/dL (1.1 mmol/L) in newborns with birth weights > 1500 g and < 7 mg/dL and Ca^{2+} < 3.6 mg/dL (0.9 mmol/L) in newborns with birth weights < 1500 g—are principally those of neuromuscular hyperexcitability: irritability, hyperacusis, jitteriness, tremulousness, facial spasms, tetany, laryngospasm, and focal or generalized seizures.[1,2] Nonspecific symptoms such as apnea, tachycardia, cyanosis, emesis, and feeding problems may also occur. Causes of neonatal hypocalcemia may be considered in relation to the age of onset (before or after 72 hours of life) (Table 18-2).

Early Neonatal Hypocalcemia

In the absence of hypoproteinemia, hypocalcemia occurring within the first 72 hours after birth is considered "early neonatal hypocalcemia." It occurs most commonly in prematurely delivered or small-for-gestational-age, low-birth-weight, or asphyxiated neonates, or in those born to women with gestational or permanent forms of diabetes mellitus, and it is the consequence of impaired PTH secretion and delayed renal tubular phosphaturic response to PTH characteristic of the neonate, prolonged secretion of calcitonin, or hypomagnesemia.[2] Total calcium and Ca^{2+} concentrations decline more rapidly from high intrauterine values to lower nadir levels in preterm than in term neonates. In low-birth-weight (LBW) neonates, hypocalcemia may be further attributed to the rapid accretion of skeletal calcium in the presence of relative resistance to the calcium absorptive and reabsorptive effects of calcitriol on the intestinal tract and bone, respectively. Offspring of severely vitamin D–deficient mothers may become hypocalcemic shortly after birth. Hypocalcemia develops in approximately one third of asphyxiated newborns

TABLE 18-1 **Genetic Origins of Disorders of Mineral, Cartilage, and Bone Metabolism**

Gene	Chromosome	MIM	Disease	MIM
ACVR1	2q24.1	102576	Fibrodysplasia ossificans progressiva	135100
AIRE	21q22.3	607358	Autoimmune polyendocrine syndrome, type I	240300
ALPL	1p36.1	171760	Hypophosphatasia, infantile	241500
			Hypophosphatasia, childhood	241510
			Hypophosphatasia, adult	146300
BAZ1B	7q11.23	605681	Williams-Beuren syndrome	194050
BMP1	8p21.3	112264	Osteogenesis imperfecta type XIII	614856
CA2	8q21.1	611492	Osteopetrosis: renal tubular acidosis	259730
CASR	3q13.3-q21	601199	Hereditary hypocalciuric hypercalcemia	145980
			Neonatal severe hyperparathyroidism	239200
			Hypercalcemic hypercalciuria	601199
			Hypoparathyroidism, familial isolated	146200
			Acquired hypocalciuric hypercalcemia	145980
CDC73	1q31.2	607393	Familial primary hypoparathyroidism	145000
			Hyperparathyroidism jaw tumor syndrome	145001
CDKN1B	12p13.1	600778	Multiple endocrine neoplasia type IV	610755
CLCN5	Xp11.2	300008	X-linked recessive hypophosphatemic rickets	300554
			Dent disease	300009
			Nephrolithiasis, X-linked recessive	310468
CLCN7	16p13,3	602727	Osteopetrosis, autosomal recessive type IV	611490
			Osteopetrosis, autosomal dominant type II	166600
CLDN16	3q28	603959	Type 3 hypomagnesemia	248250
CLDN19	1p34.2	610036	Type 5 hypomagnesemia, hypercalciuria, visual impairment	248190
CNNM2	10q24.32	607803	Type 6 hypomagnesemia with normomagnesuria	613882
COL1A1	17q21.33	120150	Osteogenesis imperfecta type I	166200
			Osteogenesis imperfecta type II	166210
			Osteogenesis imperfecta type III	259420
			Osteogenesis imperfecta type IV	166220
COL1A2	7q22.3	120160	Osteogenesis imperfecta type II	166210
			Osteogenesis imperfecta type III	259420
			Osteogenesis imperfecta type IV	166220
CRTAP	3p22.3	605497	Osteogenesis imperfecta type VII	610854
				610682
CTSK	1q21.3	601105	Pycnodysostosis	265800
CYP2R1	11p15.2	608713	25-Hydroxylase deficiency, selective	600081
CYP24A1	20q13.2	126065	Idiopathic infantile hypercalcemia	143880

Continued

TABLE 18-1 **Genetic Origins of Disorders of Mineral, Cartilage, and Bone Metabolism—cont'd**

Gene	Chromosome	MIM	Disease	MIM
CYP27B1	12q14.1	609506	25α-Hydroxyvitamin D-1α-hydroxylase deficiency (vitamin D–dependent rickets, type I)	264700
DMP1	4q22.1	600980	Hypophosphatemic rickets, autosomal recessive type 1	241520
ELN	7q11.23	120160	Williams-Beuren syndrome	194050
ENPP1	6q23.2	173335	Hypophosphatemic rickets, autosomal recessive type 2	613312
FAM111A	11q12.1	615292	Kenny-Caffey syndrome, type 2	127000
FGF23	12p13.32	605380	Hypophosphatemic rickets, autosomal dominant	193100
			Familial tumoral calcinosis	211900
			Hyperostosis hyperphosphatemia syndrome	211900
FKBP10	17q21.2	607063	Osteogenesis imperfecta type XI	610968
FOXP3	Xp11.23	300292	Immunodysregulation, polyendocrinopathy, enteropathy	304790
FXYD2	11q23.3	601814	Type 2 hypomagnesemia with hypocalciuria	154020
GALANT3	2q24.3	601756	Familial tumoral calcinosis	211900
			Hyperostosis hyperphosphatemia syndrome	610233
GATA3	10p14	131320	Hypoparathyroidism, sensorineural deafness, renal disease (HDR/Barakat syndrome)	146255
GCM2	6p24.2	603716	Hypoparathyroidism, familial isolated	146200
GNAS	20q13.32	139320	Pseudohypoparathyroidism, type 1A	103580
			Pseudohypoparathyroidism, type 1B	603233
			Osseous heteroplasia, progressive	166350
			Fibrous dysplasia/McCune-Albright	174800
GNPTAB	12q23.2	607840	Mucolipidosis type II	252500
GTF21	7q11.23	601679	Williams-Beuren syndrome	194050
HNF1B	17q12	189907	Hypomagnesemia with maturity onset diabetes of youth (5) and renal cysts	137920
HNRNPC	14q11.2	164020	Vitamin D–dependent rickets type 2B	600785
HRPT2	1q24-q31	607393	Familial hyperparathyroidism 2—jaw tumor syndrome	145001
IKBKG	Xq28	300248	X-linked osteopetrosis, ectodermal dysplasia	300301
IFITM5	11p15.5	614757	Osteogenesis imperfecta type V	610967
KCNA1	12p13.32	176260	Hypomagnesemia with myokymia	160120
KCNJ1	11q24	600359	Hypomagnesemia/antenatal Bartter syndrome type 2	600839
KCNJ10	1q23.2	602208	Hypomagnesemia/SESAME syndrome	612780
KL	13q13.1	604824	Familial calcinosis	211900
LEPRE1	1p34.2	610339	Osteogenesis imperfecta type VIII	610915
LRP5	11q13.2	603506	Osteoporosis-pseudoglioma syndrome	259770
			Idiopathic juvenile osteoporosis	259750
			High bone mass variation	601884
			Autosomal dominant osteopetrosis type I	607634
			Van Buchem disease, type 2	607636

TABLE 18-1 **Genetic Origins of Disorders of Mineral, Cartilage, and Bone Metabolism—cont'd**

Gene	Chromosome	MIM	Disease	MIM
MEN1	11q13	613733	Multiple endocrine neoplasia type I	131100
NPR2	9p13.3	108961	Acromesomelic dysplasia (Maroteaux)	602875
OSTM1	6q21	607649	Autosomal recessive osteopetrosis type V	259720
PHEX	Xp22.11	300550	Hypophosphatemic rickets, X-linked dominant	307800
PLEKHM1	17q21.31	611466	Autosomal recessive osteopetrosis type VI	611497
PPIB	15q22.31	123841	Osteogenesis imperfecta type IX	259440
PRKAR1A	17q24.2	188830	Acrodysostosis with/without hormone resistance	101800
PTH	11p15.2	168450	Hypoparathyroidism, autosomnal dominant or negative	146200
PTH1R	3p21.31	168468	Blomstrand osteochondrodysplasia	215045
			Murk-Jansen metaphyseal chondrodysplasia	156400
			Enchondromatosis (Ollier disease)	166000
RET	10q11.2	164761	Multiple endocrine neoplasia type IIA	171400
			Multiple endocrine neoplasia type IIB	162300
			Familial medullary carcinoma of thyroid	155240
SAMD9	7q21.2	610456	Tumoral calcinosis, normophosphatemic	610455
SERPINF1	17p13.3	172860	Osteogenesis imperfecta type VI	613982
SERPINH1	11q13.5	600943	Osteogenesis type X	613848
SLC34A1	5q35.3	182309	Autosomal dominant hypophosphatemia with urolithiasis 1	612286
SLC34A3	9q34.3	609826	Hypophosphatemic rickets with hypercalciuria	241530
SLC9A3R1	17q25.1	604990	Autosomal dominant hypophosphatemia with urolithiasis 1	612286
SLC9A3R1	17q25.1	604990	Autosomal dominant hypophosphatemia with urolithiasis 2	612287
SLC12A1	15q21.1	600839	Antenatal Bartter syndrome type 1	601678
SLC12A3	16q13	600968	Hypomagnesemia/Gitelman syndrome	263800
SLC34A3	9q34	609826	Hypophosphatemic rickets with hypercalciuria, hereditary	241530
SLC7A7	14q11.2	603593	Lysinuric protein intolerance	222700
SNX10	7p15.2	614780	Autosomal recessive osteoporosis type VIII	615085
SOST	17q21.31	605740	Sclerosteosis	269500
			Hyperostosis corticalis generalisata (Van Buchem disease type 1)	239100
SOX3	Xq27.1	313430	Hypoparathyroidism, X-linked	307700
SP7	12q13.13	606633	Osteogenesis imperfecta XII	613849
STX16	20q13.32	603666	Pseudohypoparathyroidism, type 1B	603233
TBX1	22q11.21	602054	DiGeorge syndrome	188400

Continued

TABLE 18-1 **Genetic Origins of Disorders of Mineral, Cartilage, and Bone Metabolism—cont'd**

Gene	Chromosome	MIM	Disease	MIM
TBCE	1q42.3	604934	Sanjad-Sakati (HRD) syndrome	241410
			Kenney-Caffey syndrome, type 1	244460
TCIRG1	11q13.2	604592	Autosomal recessive osteopetrosis type I	259700
TGFB1	19q13.1	190180	Progressive diaphyseal dysplasia	131300
TMEM38B	9q31.2	611236	Osteogenesis imperfecta type IV	615066
TNFRSF11A	18q21.33	603499	Autosomal recessive osteopetrosis type VII	612301
			Hereditary (familial) expansile	
			Polyostotic osteolytic dysplasia	174810
TNFRSF11B	8q24	602643	Paget disease, juvenile	239000
TNFSF11	13q14.11	602642	Autosomal recessive osteopetrosis type II	259710
TRPM6	9q21.13	607009	Type 1 hypomagnesemia with hypocalcemia	602014
VDR	12q13.11	601769	Vitamin D–dependent rickets, type 2A	277440

who are products of complicated and compromised deliveries. In these infants, increased phosphate load due to cellular injury, reduced calcium intake, and hypercalcitonemia are important pathogenetic factors in the development of hypocalcemia. Neonates with severe infections (sepsis) or other critical illness are often hypocalcemic.

Fifty percent of infants of mothers with diabetes mellitus develop early neonatal hypocalcemia; the incidence may be reduced by strict maternal glycemic control.[3] Its causes are multifactorial and include reduced placental transfer of calcium due to substantial maternal urinary excretion of calcium and magnesium, decreased neonatal secretion of PTH, hypercalcitonemia, hypomagnesemia (occurring in 40% of offspring of diabetic women), and limited intake and impaired absorption of ingested calcium.[4] Maternal hypercalcemia due to unsuspected hyperparathyroidism leads to increased transfer of calcium to the fetus and still further increase in the in utero serum calcium concentrations that suppress fetal PTH synthesis and release and stimulate calcitonin secretion, aberrations in homeostatic mechanisms that persist postpartum and may result in hypocalcemic tetany/seizures in offspring. Suppression of PTH secretion may persist for several months and be undetected until symptomatic hypocalcemia develops after weaning of the infant from breast milk to higher phosphate containing cow milk formula. Maternal ingestion of large quantities of calcium carbonate in antacids has also led to neonatal hypocalcemia.[5]

Hypocalcemia has occurred in neonates with hyperbilirubinemia undergoing exchange transfusion due to complexing of calcium by citrate used to store blood and avoid clotting, and in those exposed to phototherapy. Neonates with acute rotavirus infection and severe diarrhea may present with hypocalcemic seizures. Aminoglycoside antibiotics (e.g., gentamycin) increase urinary excretion of calcium and magnesium, thereby facilitating the development of neonatal hypocalcemia. Compounds that complex with and sequester calcium such as citrate (present in transfused blood), phosphates (that alter the calcium × phosphate product), and fatty acids (given as caloric supplements) lower Ca^{2+} levels. Bicarbonate administered to correct acidosis increases calcium binding to albumin and thus lowers Ca^{2+} values. Hypocalcemia may also occur in hyperventilated infants with severe respiratory alkalosis as well as in those with other causes of metabolic alkalosis. Phytates in soy milk bind calcium and phosphate and interfere with their absorption. Neonates and infants with malignant osteopetrosis type II and impaired osteoclastogenesis may present with either early or late neonatal hypocalcemia.[6-8]

Late Neonatal Hypocalcemia

Hypocalcemia developing after 72 hours of postnatal age may be due to an increased intake of phosphate, hypomagnesemia, hypoparathyroidism, or vitamin D deficiency (see Tables 18-2A and B). Often neonatal hypocalcemia develops after 3 days of age in offspring born in the late winter-early spring of the year to multiparous women of lower socioeconomic status with an inadequate intake of vitamin D or exposure to sunlight. Ingestion of excessive phosphate in evaporated milk or modified cow milk formulas forms poorly soluble calcium salts,

TABLE 18-2A Causes of Hypocalcemia

I Neonatal

A Maternal Disorders

1 Diabetes mellitus
2 Toxemia of pregnancy
3 Vitamin D deficiency
4 High intake of alkali or magnesium sulfate
5 Use of anticonvulsants
6 Hyperparathyroidism

B Neonatal Disorders

1 Low birth weight: prematurity, intrauterine growth restriction
2 Peripartum asphyxia, sepsis, critical illness
3 Hyperbilirubinemia, phototherapy, exchange transfusion
4 Hypomagnesemia, hypermagnesemia
5 Acute/chronic renal failure
6 Nutrients/medications: high phosphate intake, fatty acids, phytates, bicarbonate infusion, citrated blood, anticonvulsants, aminoglycosides
7 Hypoparathyroidism
8 Vitamin D deficiency or resistance
9 Osteopetrosis type II

II Hypoparathyroidism

A Congenital

1 Transient neonatal
2 Congenital hypoparathyroidism (see also Table 18-2B)
  a Familial isolated hypoparathyroidism
    (1) Autosomal recessive hypoparathyroidism (*GCMB*, *PTH*)
    (2) Autosomal dominant hypoparathyroidism (*CASR*)
    (3) X-linked hypoparathyroidism (*SOX3*)
  b DiGeorge syndrome (*TBX1*)
  c Sanjad-Sakati syndrome (short stature, retardation, dysmorphism; HRD)
    Kenny-Caffey syndrome 1 (short stature, medullary stenosis) (*TBCE*)
  d Barakat syndrome (sensorineural deafness, renal dysplasia; HDR) (*GATA3*)
  e Lymphedema-hypoparathyroidism-nephropathy, nerve deafness
  f Mitochondrial fatty acid disorders (Kearns-Sayre, Pearson, mitochondrial encephalopathy, lactic acidosis, stroke-like [MELAS])
3 Insensitivity to parathyroid hormone
  a Blomstrand chondrodysplasia (*PTHR1*)
  b Pseudohypoparathyroidism type IA (*GNAS*)
    Pseudohypoparathyroidism type IB
    Pseudohypoparathyroidism type IC
    Pseudohypoparathyroidism type II
    Pseudopseudohypoparathyroidism
  c Acrodysostosis with hormone resistance (*PRKAR1A*)
  d Hypomagnesemia (see Tables 18-7A and B)

B Acquired

1 Autoimmune polyglandular syndrome type I (*AIRE*)
2 Activating antibodies to the calcium sensing receptor
3 Post surgical, radiation destruction
4 Infiltrative—excessive iron (hemochromatosis, thalassemia) or copper (Wilson disease) deposition; granulomatous inflammation, neoplastic invasion; amyloidosis, sarcoidosis
5 Maternal hyperparathyroidism
6 Hypomagnesemia/hypermagnesemia

III Vitamin D Deficiency

IV Other Causes of Hypocalcemia

A Calcium Deficiency

1 Nutritional deprivation
2 Hypercalciuria

B Disorders of Magnesium Homeostasis (see Tables 18-7A and B)

1 Congenital hypomagnesemia
2 Acquired
  a Acute renal failure
  b Chronic inflammatory bowel disease, intestinal resection
  c Diuretics

C Hyperphosphatemia

1 Renal failure
2 Phosphate administration (intravenous, oral, rectal)
3 Tumor cell lysis
4 Muscle injuries (crush, rhabdomyolysis)

D Miscellaneous

1 Hypoproteinemia
2 Hyperventilation
3 Drugs: furosemide, bisphosphonates calcitonin, anticonvulsants, ketoconazole, antineoplastic agents (plicamycin, asparaginase, cisplatinum, cytosine arabinoside, doxorubicin), citrated blood products
4 Hungry bone syndrome
5 Acute and critical illness: sepsis, acute pancreatitis, toxic shock
  a Organic acidemia: propionic, methylmalonic, isovaleric

Modified from Carpenter, T. O. (2006). Neonatal hypocalcemia. In M. J. Favus (Ed.), Primer on the metabolic bone diseases and disorders of mineral metabolism*, (6th ed.) (pp. 224-229). Washington, DC: American Society for Bone and Mineral Research; Shaw, N. (2009). A practical approach to hypocalcaemia in children. In J. Allgrove, & N. Shaw (Eds.),* Calcium and bone disorders in children and adolescents. *Endocr Dev. (Vol. 16, pp. 73–92). Basel: Karger; Levine MA (2006). Parathyroid hormone resistance syndromes. In M. J. Favus (Ed.),* Primer on the metabolic bone diseases and disorders of mineral metabolism *(6th ed.) (pp. 220-224). Washington, DC: American Society for Bone and Mineral Research; Thakker, R. V. (2006). Hypocalcemia: pathogenesis, differential diagnosis, and management. In M. J. Favus (Ed.),* Primer on the metabolic bone diseases and disorders of mineral metabolism, *(6th ed.) (pp. 213-215). Washington, DC: American Society for Bone and Mineral Research; Goltzman, D., & Cole, D. E. C. (2006). Hypoparathyroidism. In M. J. Favus (Ed.),* Primer on the metabolic bone diseases and disorders of mineral metabolism *(6th ed.) (pp. 216-219). Washington, DC: American Society for Bone and Mineral Research.*

TABLE 18-2B **Gene Mutations Associated with Hypoparathyroidism**

Disorder	MIM	Gene(s)	Chromosome MIM	Pathophysiology	Inheritance
Familial isolated hypoparathyroidism	146200	*GCM2*: Glial cells missing, Drosophila, homologue of, 2	6p24.2 603716	Transcription factor essential for parathyroid gland development	AR, AD
		PTH: Parathyroid hormone	11p15.2 168450	Peptide that increases serum Ca^{2+} concentration	AR, AD
		CASR: Calcium sensing receptor	3q21.1 601199	GPCR that monitors ambient Ca^{2+} concentration	AD
Familial isolated hypoparathyroioidism	307700	(*SOX3*: SRY box 3)	Xq26-27 (Xq27.1 313430)	Factor(s) expressed in the developing parathyroid gland	X-linked recessive
DiGeorge syndrome 1 (chromosome 22q 11.2 deletion syndrome)	188400	Continuous gene deletion disorder or mutation in *TBX1*: T-box 1	22q11.21 602054	TBX1: Transcription factor that regulates *GCM2* expression	AD, Sporadic
DiGeorge syndrome 2	601362	Deletion of 10p	10p14-p13		
Chromosome 22q11.2 duplication syndrome	608363	Microduplication involving the same interval deleted in DiGeorge syndrome 1	22q11.2		AD
Barakat syndrome (HDR)	146255	*GATA3*: GATA binding protein 3	10p14 131320	Transcription factor that regulates expression of *GCM2* and development of otic vesicles and kidneys	AD, AR
Sanjad-Sakati syndrome (HRD)	241410	*TBCE:* Tubulin-specific chaperone E	1q42.3 604934	Chaperone protein necessary for formation of the cytoskeleton, protein transport and secretion	AR
Kenny-Caffey syndrome 1 (KCS1)	244460	*TBCE*: Tubulin-specific chaperone E	1q42.3 604934	Chaperone protein necessary for formation of the cytoskeleton, protein transport, and secretion	AR
Kenny-Caffey syndrome 2 (KCS2)	127000	*FAM111A*: Family with early seqence similarity 111	615292	Restriction factor	AD
Kearns-Sayre syndrome	530000	*ATP8*: ATP synthase 8	516070	Mitochondrial gene	Transmitted primarily by females
		MTTL1: Transfer RNA, mitochondrial leucine, 1	590050	Mitochondrial gene	
		RRM2B: Ribonucleotide reductase, M2 B	8p22.3 604712	Enzyme essential for mitochondrial DNA synthesis	AR

Mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS)	540000	*MTTKL*: Transfer RNA, mitochondrial, lysine	590060	Mitochondrial genes	Transmitted primarily by females
		MTTQ: Transfer RNA, mitochondrial, glutamine	590030		
Pearson marrow-pancreas syndrome	557000	Continuous gene deletion/duplication syndrome involving several mtDNA genes		Mitochondrial genes	Transmitted primarily by females
Blomstrand chondrodysplasia	215045	*PTH1R*: Parathyroid hormone 1 receptor	3p21.31 168468	GPCR for parathyroid hormone	AR
Pseudohypoparathyroidism type IA	103580	*GNAS*: GNAS complex locus	20q13.32 139320	Gsα transmits ligand-bound GPCR stimulus to adenylyl cyclase	AD
Pseudohypoparathyroidism type IB	603233	*GNAS*: GNAS complex locus	20q13.32 139320	Gsα transmits ligand-bound GPCR stimulus to adenylyl cyclase	AD
		STX16: Syntaxin 16	20q13.32 603666	Regulates epigenetic control of *GNAS* expression	AD
Pseudohypoparathyroidism type IC	612462	*GNAS*: GNAS complex locus	20q13.32 139320	Gsα transmits ligand-bound GPCR stimulus to adenylyl cyclase	AD
Progressive osseous heteroplasia	1166350	*GNAS*: GNAS complex locus	20q13.32 139320	Gsα transmits ligand-bound GPCR stimulus to adenylyl cyclase	AD
Pseudohypoparathyroidism type II	203330			Biochemical lesion distal to generation of cyclic AMP	
Acrodysostosis	101800	*PRKAR1A*: Protein kinase, cAMP-dependent, regulatory, type I, alpha	17q24.2 188830	Regulatory alpha subunit of protein kinase A controls peripheral cyclic AMP response to PTH and TSH	AD
Autoimmune polyglandular syndrome type 1 (APS1)	240300	*AIRE*: Autoimmune regulator	21q22.3 607358	Transcription factor expressed in medulla of thymus that enables differentiation of self- from foreign antigens	AD
Immunodysregulation, polyendocrinopathy and enteropathy	304790	*FOXP3*: Forkhead box P3	Xp11.23 300292	Transcription factor essential for normal development of naturally occurring T regulatory cells	X-linked recessive

GPCR, G protein–coupled receptor; AD, autosomal dominant; AR, autosomal recessive.

limiting the intestinal absorption of calcium while raising serum phosphate values. Premature introduction of fiber-containing cereals into the infant's diet also decreases calcium absorption. Affected infants may have an associated defect in renal phosphate excretion or coexisting vitamin D deficiency. Hyperphosphatemia and hypocalcemia may initially suggest hypoparathyroidism, but serum PTH concentrations are high in infants with excessive phosphate loading in response to a reciprocal reduction in serum calcium values. Newborns and infants with chronic renal insufficiency due to renal hypoplasia or obstructive nephropathies often are hypocalcemic and hyperphosphatemic with elevated serum PTH levels as well, but they are also azotemic. Hypomagnesemia leads to impaired secretion of PTH and decreased peripheral responsiveness to PTH and may be transient or related to congenital abnormalities of intestinal absorption or renal tubular reabsorption of magnesium.[3] Hypermagnesemia may occasionally be associated with neonatal hypocalcemia.

Hypocalcemia due to fetal/neonatal deficiency of vitamin D occurs in offspring of mothers deprived of vitamin D (either for cultural or socioeconomic reasons); impaired renal 25-hydroxyvitamin D-1α hydroxylase activity or loss-of-function mutations of the vitamin D receptor also lead to hypocalcemia (and hypophosphatemia). Hypovitaminosis D may develop in the breastfed infant of a vegetarian mother who shields herself from sunlight and ingests a diet low in vitamin D. Marginal deficiency of vitamin D in neonates and infants is much more common than has been recognized heretofore.[9-12] "Late-late" neonatal hypocalcemia occurs in premature infants with osteopenia at 3 to 4 months of age in whom the intake of calcium, phosphate, and vitamin D has been marginal; it is perhaps due to an avid deposition of available calcium into bone.[3] Hypocalcemia due to vitamin D deficiency may develop rather acutely and in the absence of clinical or radiographic signs of rickets in the older infant or young child who has ingested an elimination diet low in vitamin D because of severe allergies or who has been maintained indoors with limited exposure to sunlight.

Hypoparathyroidism

Hypoparathyroidism presenting in infancy is often transient and related to delayed developmental maturation of parathyroid gland function; it frequently resolves within the first several weeks of life (see Tables 18-2A and B). When prolonged, hypoparathyroidism is often due to an error in the embryogenesis of the parathyroid glands or in the synthesis or secretion of parathyroid hormone (PTH) or to peripheral unresponsiveness to PTH. Familial isolated congenital hypoparathyroidism may be transmitted as an autosomal recessive, autosomal dominant, or X-linked recessive trait that has been associated with loss-of-function mutations of *GCM2* and *PTH* and possibly *SOX3*, respectively, and gain-of-function mutations in *CASR*. *GCM2* is a gene with five exons that encodes a 506-amino-acid DNA-binding transcription factor whose expression is restricted to the parathyroid glands. "Knockout" of *Gcm2* in mice leads to agenesis of the parathyroid glands and hypoparathyroidism. Intragenic deletions or homozygous missense mutations in exons 2, 3, and 5 of *GCM2* result in hypoparathyroidism in humans.[13-17] Mutations in *GCM2* exons 2 and 3 (encoding DNA binding and transactivation domain 1) lead to impaired protein synthesis and stability and autosomal recessive transmission of congenital hypoparathyroidism, whereas those in exon 5 (encoding transactivation domain 2) lead to mutations with a dominant negative effect and autosomal dominant transmission of this disorder.[17] (Expression of *GCM2* is dependent on normal transcriptional function of *GATA3*, the gene mutated in patients with the Barakat syndrome of hypoparathyroidism-deafness-renal dysplasia [HDR].[18]) Inactivating mutations in *PTH* that interfere with the processing of preproPTH to active PTH result in functional hypoparathyroidism that may be transmitted as an autosomal dominant or recessive characteristic.[15] Depending on the specificity of the immunoassay for PTH, serum levels of PTH may be low, normal, or even high in these patients. Hypoparathyroidism due to heterozygous activating mutations in *CASR* have been found in hypocalcemic neonates.[19] X-linked hypoparathyroidism is associated with agenesis of the parathyroid glands; the disorder has been mapped to Xq26-q27 and may involve a deletion-insertion mutation that adversely affects the position of *SOX3*.[20] Because *Sox3* is expressed in embryonic mouse parathyroid glands, it is likely an important transcription factor for normal embryologic development of these structures.

Hypercalciuric hypocalcemia is an autosomal dominantly transmitted form of hypoparathyroidism that is due to gain-of-function mutations in *CASR* that result in enhanced "sensitivity" to Ca^{2+}. A lowered "set point" enables PTH secretion to be suppressed and renal tubular reabsorption of calcium to be depressed by extremely low concentrations of Ca^{2+}; this disorder may present in the newborn period.[19,21] Mutations may be scattered throughout the gene but occur predominantly in the extracellular domain of the CaSR. Activating mutations (Cys141Trp) of *CASR* may also inhibit function of the renal outer medullary potassium channel (*KCNJ1*, MIM 600359), leading to a Bartter-like syndrome with hypokalemic metabolic alkalosis, hyperreninemia, and hyperaldosteronism as well as hypercalciuric hypocalcemia; the paired metabolic defects are partially responsive to treatment with hydrochlorothiazide and low doses of calcitriol.[22] Children with hypercalciuric hypocalcemia due to gain-of-function mutations in *CASR* are very sensitive to even low doses of calcitriol that can lead to even more marked hypercalciuria and to nephrocalcinosis. Thus, management of these patients has been quite difficult. Administration of recombinant human PTH[1-34] to a 14-month-old hypocalcemic male infant with a de novo nonsense mutation in *CASR* (Leu727Gln) for 17 months partially restored calcium homeostasis with increased but still subnormal serum levels of calcium, whereas urinary excretion of calcium decreased into the normal range.[19] During treatment the child was clinically asymptomatic, did not develop nephrocalcinosis, and tolerated the drug well. Long-term treatment of

children with activating mutations of *CASR* may be feasible.[23,24]

The most common form of dysgenesis of the parathyroid glands in neonates and infants is that associated with the DiGeorge syndrome (DGS), a disorder that occurs with a frequency of 1:4000 births and is present in approximately 70% of children with isolated hypoparathyroidism.[25,26] The DGS is a neurocristopathy that results from the disturbed migration of cervical neural crest cells and consequent maldevelopment of tissues of neural crest origin derived from the third and fourth pharyngeal pouches and first to fifth branchial arches. It is usually associated with microdeletions of chromosome region 22qll.2 (del22q11.2: DGCR), a segment on which more than 35 genes are sited and thus considered a contiguous gene syndrome (a disorder caused by deletion of several adjacent genes that when individually mutated may result in a distinctive clinical feature but when collectively lost lead to a group of apparently unrelated clinical findings). The chromosome 22q11.2 microdeletion is contained within regions of low copy number repeats, and it is this characteristic that results in an unequal segmental exchange between the paired 22nd chromosomes during meiosis.[26] DGS may occur sporadically or be transmitted as an autosomal dominant trait. Although the clinical severity and phenotype of patients with this chromosomal anomaly are variable, characteristically subjects with DGS have the triad of hypocalcemia due to hypoplasia of the parathyroid glands often manifest in the neonatal period but which may not be detected until the child reaches an older age, defective T-lymphocyte function and impaired cell-mediated immunity due to partial or complete absence of thymic differentiation leading to increased frequency of viral and fungal infections, and conotruncal defects of the heart or aortic arch (tetralogy of Fallot, ventricular septal defect, interrupted or right aortic arch, truncus arteriosus, vascular ring).[3,26,27] Immune deficiency is common as are velopharyngeal insufficiency, cleft palate, and developmental challenges.[26] Other features of the DGS include gastrointestinal malformations (esophageal atresia, anal atresia), dysplastic kidneys, cervical spine instability, impaired vision, and ocular malformations.

In addition to the DGS, deletion of chromosome 22q11.2 has been associated with the conotruncal anomaly face and velocardiofacial syndromes. Collectively, these syndromes are associated with similar facial features (ocular hypertelorism, lateral displacement of inner canthi, short palpebral fissures, swollen eyelids, dysmorphic "segmented" nose, small mouth, low-set ears with abnormally folded pinnae, short philtrum, micrognathia, malar hypoplasia, velopharyngeal insufficiency with/without cleft palate), olfactory dysfunction, short stature, nonverbal learning disabilities, and various psychological maladies.[25,28] Takao velocardiofacial syndrome (included in MIM 188440) consists primarily of the typical cardiac defects described earlier that may also be associated with hypocalcemia; Shprintzen velocardiofacial syndrome (MIM 192430) is characterized by craniofacial and palatal defects and cardiac anomalies; Cayler cardiofacial syndrome (MIM 125520) is associated with partial unilateral facial paresis due to hypoplasia of the depressor anguli oris muscle and anomalies of the heart and aorta. These syndromes have been grouped as the CATCH-22 syndromes of *C*ardiac defects, *A*bnormal face, *T*hymic hypoplasia, *C*left palate, *H*ypocalcemia. In addition to the anomalies and clinical findings listed, a litany of additional abnormalities may be seen in patients with del22q11.2.[29] Disparate manifestations of these syndromes may be observed in different members of the same family, indicating the variable clinical expressions that accompany deletions of chromosome 22q11.2.[30]

A two-megabase microdeletion at chromosome 22q11.2 leads to loss of several contiguous genes within this region including *HIRA* (histone cell cycle regulation, MIM 600237), a transcription factor that is expressed in developing heart and upper body neural crest elements and is necessary for normal cardiac development. Also within this region is *TBX1* encoding T-box 1, a transcription factor with a highly conserved DNA binding sequence (the T-box) that is essential for organogenesis and pattern formation and is expressed in the pharyngeal arches and pouches. Experimental disruption of *Tbx1* impairs development of the pharyngeal arch arterial vasculature, whereas introduction of null mutations in *Tbx1* results in anomalies of the cardiac outflow track and hypoplasia of the thymus and parathyroid glands. The transcription factor encoded by *TBX1* is part of a network of gene products (including those encoded by *ISL1*, *SHH*, *FOXA2*, *FOXC2*) that controls development of the parathyroid glands and thymus by regulating expression of *GATA3*, *GCM2* and *PAX9*.[26] Evaluation of patients with clinical characteristics of the CATCH-22 syndromes but intact chromosome 22q11.2 has revealed heterozygous loss-of-function mutations (Phe148Tyr, Gly310Ser) in *TBX1* in patients with the DGS and Shprintzen velocardiofacial syndrome.[25,31] Thus, haploinsufficiency of *TBX1* alone can account for the cardiac defects, abnormal face, thymic and parathyroid hypoplasia, and velopharyngeal insufficiency with cleft palate but not for the developmental delay characteristic of CATCH-22. Another candidate gene for the DGS sited at chromosome 22q11.2 is *UFD1L* (ubiquitin fusion degradation 1-like, MIM 601754), whose product is important for the posttranslational processing of proteins or their degradation by interaction with the ubiquitin fusion protein. Experimentally, the DGS and related disorders have also been linked to genes encoding endothelin-1, vascular endothelial growth factor, and fibroblast growth factor-8 (a target gene for *TBX1*), and to genes within the DGCR at chromosome 22q11.2—*CRKL*—(MIM 602007) and *DGCR6* (MIM 601279). In the mouse hypomorphic for *Fgf8*, there are cardiovascular, craniofacial, parathyroid, and thymic defects—an experimental phenocopy of the human del22q11.2 syndrome.[32] Fgf8 functions through stimulation of transcription of *Crkl*—its product is an adaptor protein that transduces intracellular signals from several tyrosine kinase receptors, one of which is the receptor for Fgf8; interestingly, Fgf8 interacts with Tbx1 as well. The DGS has also been associated with microdeletions of chromosomes 10p13 (DGS2), 18q21.33, and 4q21.2-q25, indicative of the cascade of genes likely involved in the generation of this phenotype. The presence of the DGS should be considered when fetal

ultrasonography reveals an interrupted aortic arch or truncus arteriosus and may be confirmed by appropriate studies (microarray, fluorescent in situ hybridization [FISH]) on samples of chorionic villi or amniotic fluid.

Hypocalcemia has been observed in some subjects with microduplication of chromosome 22q11.2, a copy number variant; clinical characteristics of individuals with this genetic anomaly vary from those who are entirely normal to patients with multiple congenital anomalies, severe developmental delay, autism, and schizophrenia.[33,34] The duplication chromosome 22q11.2 syndrome appears to be transmitted as an autosomal-dominant characteristic whose expression is modified by other factors. The pathophysiology of hypocalcemia in affected subjects is uncertain. In one family in which the proband had the DGS associated with del22q11.2, the normal father had the same anomaly on one of his 22nd chromosomes and dup22q11.2 on his other 22nd chromosome; paternal quantitative expression of the genes located on chromosome 22q11.2 was normal, indicating that the adverse effects of the 22q11.2 deletion were compensated by the 22q11.2 duplication.[35]

Several other syndromes exhibit multisystem involvement and hypoparathyroidism. The Barakat or HDR syndrome of *H*ypoparathyroidism, sensorineural *D*eafness, and *R*enal disease (dysplasia, steroid-resistant nephrosis with progressive renal failure) has been attributed to haploinsufficiency of *GATA3*, a zinc-finger transcription factor that regulates expression of *GCM2* and thus is critical for the embryonic development of the parathyroid glands as well as for the kidneys, otic vesicles, and thymus.[15,18,36] The parathyroid glands of these children are dysgenetic—hypoplastic or absent. Hypocalcemia may be present in the newborn period or unrecognized until later childhood. Malformations of the female genital tract (didelphic uterus, septate vagina) may also occur in patients with HDR.[37] Heterozygous deletions, insertions, missense, and nonsense mutations in *GATA3* have been identified in patients and families with HDR.[38,39] Patients with an isolated loss of *GATA3* function do not have other features common to patients with larger terminal deletions of chromosome 10p such as growth and developmental retardation, dysmorphic facial features, or congenital heart disease.

Biallelic mutations in the gene encoding tubulin-specific chaperone E (*TBCE*) have been identified in the Sanjad-Sakati syndrome of *H*ypoparathyroidism-mental *R*etardation-*D*ysmorphism (HRD) and the Kenny-Caffey syndrome type 1 (KCS1) of hypocalcemia, cortical thickening, medullary stenosis, dysmorphic face, and growth retardation. Children with HRD are short, developmentally delayed, and seizure prone; they have medullary stenosis and other skeletal anomalies; they are microcephalic with faces characterized by deeply recessed eyes or microphthalmia, depressed nasal bridge, beaked nose, long philtrum, thin upper vermillion border, micrognathia, and large earlobes. The HRD syndrome often presents in infancy with symptomatic hypocalcemia associated with low serum concentrations of PTH and normal phosphaturic responses to exogenous PTH. The cardiovascular system of these patients is intact, but as infants they are susceptible to life-threatening pneumococcal infections.[40] Neonates with the closely related disorder KCS1 are often severely hypocalcemic early in the neonatal period. As children they are short, with craniofacial anomalies due to the absence of diploic space in the skull, osteosclerosis, and thickening of the cortices of the long bones with narrowing of the medullary compartment, normal or mildly delayed development, and increased susceptible to recurrent bacterial infections. TBCE is essential for formation of microtubules—cytosolic structures composed of heterodimeric α- and β-tubulin subunits that form the cytoskeleton, mitotic apparatus, cilia, and other cellular components; this chaperonin assists in the correct folding of α- and β-tubulin subunits and the formation of α-β-tubulin heterodimers. The α- and β-tubulin subunits and TBCE are necessary for normal embryogenesis of the parathyroid glands. Mutations in *TBCE* result in lowered microtubule formation and consequently in a decrease in subcellular components such as the Golgi apparatus and endosomal compartments required for normal intracellular movement of proteins. Interestingly, the identical mutation in *TBCE*—a homozygous 12 bp deletion in exon 2—may result in either the HRD or KCS1 phenotype in a specific family.[41] A child with autosomal recessive HRD syndrome and intact TBCE has been identified, suggesting that this disorder is likely to be genetically heterogeneous.[42] In KCS type 2, the phenotype is similar to that of KCS1 but transmission is as an autosomal dominant trait attributable to heterozygous loss-of-function mutations in *FAM111A* (MIM 615292).

Neonates with loss-of-function mutations in *PTH1R*, the gene encoding the GPCR for PTH and PTHrP, are functionally hypoparathyroid despite elevated serum concentrations of PTH.[43] Because of subresponsiveness to PTHrP in utero, fetal bone formation is abnormal, resulting in Blomstrand chondrodysplasia—an osteochondrodystrophy characterized by short extremities and advanced skeletal and dental maturation—abnormalities detectable in utero by fetal ultrasonography. Histologically the proliferative zone of the cartilage growth plate is narrowed with relatively few resting and proliferating chondrocytes, whereas the hypertrophic zone is composed of irregular columns of chondrocytes. Transmitted as an autosomal recessive trait, its clinical characteristics include polyhydramnios, hydrops fetalis, short-limbed dwarfism, facial anomalies, aberrant tooth development, aplasia of the nipples and breasts, hypoplastic lungs, and preductal aortic coarctation. Although Blomstrand osteochondrodysplasia is usually lethal, skeletal malformations may be more (type I) or less severe (type II). Mutations in *PTH1R* that result in complete absence of normal protein (e.g., Arg104Ter) are designated type I, whereas mutations that permit some PTH1R synthesis (Pro132Leu) result in type II Blomstrand osteochondrodysplasia.[44] Heterozygous inactivating mutations of *PTH1R* (Glu155Ter) result in autosomal dominant nonsyndromic failure of tooth eruption (MIM 125350).[45] Eiken chondrodysplasia (MIM 60002) is also due to biallelic loss-of-function mutations in *PTH1R* but is clinically and radiographically distinct from Blomstrand osteochondrodysplasia as affected subjects have mild growth retardation, markedly delayed ossification,

multiple epiphyseal dysplasia, and persistent islands of cartilage in the pelvis.[46] The *PTH1R* mutations in patients with Eiken skeletal dysplasia occur in the carboxyl terminal (e.g., Arg485Ter).

GNAS encodes the stimulatory alpha subunit (Gαs) of G-proteins coupled to seven transmembrane receptors. After activation by its GPCR, the guanosine diphosphate (GDP) moiety on Gαs is replaced by guanosine triphosphate (GTP), Gαs dissociates from its βγ companion subunit complex, and stimulates membrane-bound adenylyl cyclase activity generating cyclic adenosine monophosphate (AMP) and activating protein kinase A, which phosphorylates serine and tyrosine residues of further signal transduction proteins. *GNAS* is composed of 13 exons; it has four major transcripts that arise through splicing of unique first exons onto shared exons 2 to 13 (Figure 18-1).[47] The four transcripts of *GNAS* encode (1) the Gαs transcript is a protein that stimulates adenylyl cyclase and generates cyclic AMP; Gαs is expressed by both maternal and paternal alleles in most tissues; in the proximal renal tubule, thyroid, gonads, and adenohypophysis, however, only the maternal allele of *GNAS* is expressed; (2) XLαs yields a Gαs isoform that is specifically expressed in neuroendocrine and nerve tissues and is identical to Gαs except that it has a very long amino terminal sequence of amino acids; it is expressed only by the paternally acquired allele; (3) the NESP55 (neuroendocrine secretory protein-55) transcript is a chromogranin-like protein that is expressed in neuroendocrine tissues but only by the maternal *GNAS* allele; (4) the alternative first exon A/B (exon 1A) transcript is expressed ubiquitously but only by the paternal GNAS allele and is not translated. The promoters of XLαs, NESP55, and exon 1A transcripts lie within the 5′ differentially methylated region (DMR) of *GNAS*. Methylation of the promoter usually silences expression of that GNAS transcript.

Loss-of-function mutations in *GNAS* or epigenetic aberrations that lead to failure of expression of the Gαs transcript of *GNAS* result in pseudohypoparathyroidism (PHP). PHP type IA is manifested by resistance to protein hormones that signal though GPCRs; resistance to PTH results in hypocalcemia, hyperphosphatemia, and elevated serum concentrations of PTH and impaired urinary excretion of cyclic AMP and phosphate after administration of exogenous PTH. (Resistance to thyroid-stimulating hormone, the gonadotropins, and growth hormone releasing hormone also occurs in patients with PHP IA. PHP IA may be suspected in the neonate with hypocalcemia in whom hyperthyrotropinemia has been detected in the neonatal screening study for congenital hypothyroidism.[48]) When maternally transmitted, PHP IA is coupled with the phenotype of Albright's hereditary osteodystrophy (AHO; short stature; brachydactyly of the 3rd, 4th, and 5th metacarpals; round face; and heterotopic intramembranous subcutaneous calcifications).[49] Although both maternal and paternal *GNAS* alleles are expressed in most tissues including bone, in renal tissue only the maternal *GNAS* allele is expressed. Thus, in renal tissue *GNAS* expression is "imprinted"—that is, there is differential gene expression depending on the parent of origin of the allele. Therefore, clinical manifestations of PHP IA in the affected patient depend on which parent donated the inactive *GNAS* allele. Loss-of-function mutations of the maternal *GNAS* allele result in PHP type IA with end-organ resistance to PTH and the skeletal defects of AHO. PHP IA patients have subnormal expression of Gsα in their erythrocytes. Inactivating mutations of paternal *GNAS* result in the abnormal AHO skeletal phenotype due to haploinsufficiency of *GNAS* expression in bone but intact proximal renal tubular responses to PTH where maternal GNAS is expressed; this clinical complex is designated pseudopseudohypoparathyroidism (PPHP). More than 80 heterozygous loss-of-function mutations in *GNAS* have been described. A four base pair deletion in exon 7 (codons 188 to 189) in *GNAS* that leads to a frameshift and premature stop codon has been found in a number of families with PHP IA and appears to be a "mutational hot spot" as it impairs DNA polymerization and replication. Other mutations alter intracellular movement of GNAS protein (Leu99Pro, Ser250Arg), increase the rate of release of GDP (Arg258Trp, Ala366Ser), or impair coupling of G-protein to PTH1R (Arg385His).

PHP type IB occurs only in the offspring of obligate female carriers in whom loss of maternal *GNAS* expression

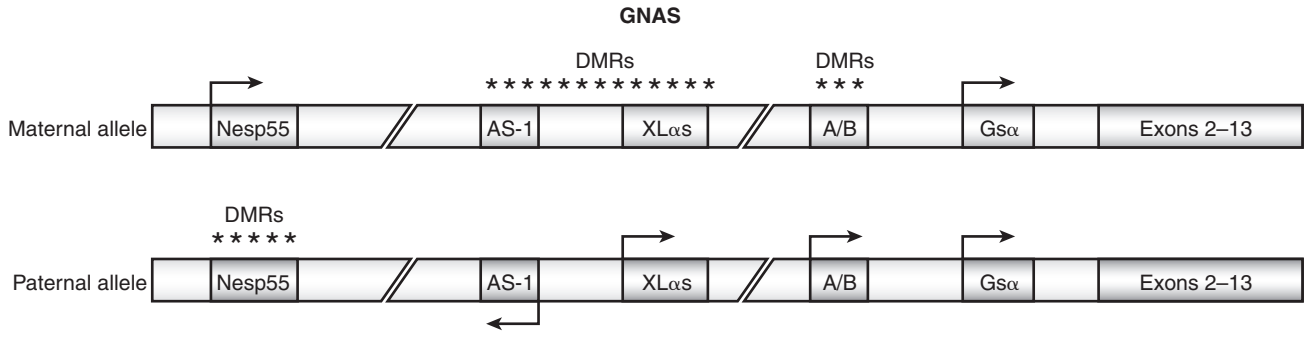


FIGURE 18-1 ■ Schematic view of the *GNAS* gene complex. Three alternate exons 1—NESP55, XLαs, and AS-1—are upstream of the start codon. DMR refers to the differentially methylated region. The pattern of methylation and hence the expression of the alternate exons is dependent on the parent of origin of the allele and the specific tissue in which *GNAS* is expressed. (From Shore, E. M., & Kaplan, F. S. (2010). Inherited human diseases of heterotopic bone formation. *Nat Rev Rheumatol, 6*, 518–527.)

is present in the renal proximal tubule, resulting in selective proximal renal tubular resistance to PTH; skeletal expression of both maternal and paternal *GNAS* is intact and hence bone formation is normal. In some patients, PHP type IB is due to deletions within the DMR (NESP55, XLas, or alternative first exons A/B) of *GNAS* or to loss of a 5′ cis-acting imprinting control center that is essential for methylation of the *GNAS* differentially methylated region that regulates expression of maternal *GNAS* in the proximal renal tubule.[47,49] Within the 5′ upstream region of *GNAS* is *STX16*, a gene that is partially deleted in some patients with PHP IB; mutations of this gene are associated with loss of methylation of *GNAS* exons A/B. Thus, defects in *STX16* and within the DMR of *GNAS* prevent expression of maternal *GNAS* in the proximal renal tubule. Paternal uniparental isodisomy of the long arm of chromosome 20 (site of *GNAS*) may be the cause of PHP IB in some patients. Patients with PHP IB are often resistant to thyroid-stimulating hormone (TSH) as well as to PTH.[49] The phenotype of subjects with PHP type Ic is similar to that of those with PHP IA including AHO, but these patients usually express normal quantities of functional Gsα in their erythrocytes; in some PHP Ic subjects, however, inactivating mutations in *GNAS* have been reported.[49] It must be remembered that there may be overlap in mechanisms that down-regulate expression of *GNAS* in various tissues and that PHP types 1 are heterogeneous in their genetic origins. Indeed, imprinting defects are present in patients with clinical manifestations of either PHP IA or PHP IB, indicating the pathogenetic overlap of these two disorders.[50] Despite elevated levels of PTH and radiographic evidence of bone responsiveness to PTH in patients with PHP IA, bone mineralization is normal in these subjects.[51] In patients with PHP type II, the skeletal phenotype is normal but patients are partially resistant to the renal effects of PTH; after administration of PTH, the urine excretion of cyclic AMP increases but that of phosphate does not; its pathogenesis is unknown as yet. Progressive osseous heteroplasia (MIM 166350) is an autosomal dominant disorder due to loss-of-function mutations in paternal *GNAS*, characterized by dermal ossifications, that begins in infancy and progresses to diffuse bone formation in skeletal muscle and deep fascia.[49,52,53] This disease may lie at one end of the spectrum of PPHP. Although not as yet clinically described, an inactivating mutation in *LRP6* (encoding lipoprotein receptor-related protein 6; MIM 603507) might also be associated with resistance to the biologic effect of PTH. In addition to its primary role in receptor-mediated endocytosis of lipoproteins, LRP6 is essential for the movement of Gαs to the plasma membrane and for its coupling to PTH1R.[54] Experimentally, in vitro "knockdown" or inactivating mutations of *Lrp6* decrease the cellular response to PTH.

Acrodysostosis is a chondrodysplasia with many features of PHP IA (short stature, obesity, brachydactyly, abnormal face with nasal and maxillary hypoplasia, advanced skeletal maturation) that is due to a mutation in *PRKAR1A*. This gene encodes the cyclic AMP–dependent regulatory alpha subunit of protein kinase A (PKA), the protein kinase that is downstream of Gαs and cyclic AMP and whose activation leads to cascades of intracellular signal transduction pathways that regulate cell division, differentiation, metabolism, and apoptosis. Paradoxically, prolonged activation of the regulatory alpha subunit of PKA results in a decline in the functional activity of the catalytic subunit of PKA. In a patient with acrodysostosis, a de novo germline gain-of-function mutation (Arg368Stop) in *PRKAR1A* was identified that led to functional resistance to PTH and TSH.[55] Mutant PRKAR1A was a shortened protein whose binding avidity to the catalytic subunit of PKA was increased because it lacked one of two cyclic AMP binding domains; hence, it could only be slowly released from the catalytic subunit of PKA by cyclic AMP, thereby maintaining the catalytic subunit of PKA in the inactive state.

Deletions and mutations of the (maternal) mitochondrial genome (composed of 16,569 base pairs) have been associated with hypoparathyroidism. Patients with the Kearns-Sayre syndrome manifest ophthalmoplegia, retinal pigmentation, and cardiomyopathy; muscle biopsy may reveal ragged red fibers. Hypoparathyroidism has been observed in patients with the syndromes of *M*itochondrial encephalopathy, *L*actic *A*cidosis, and *S*troke-like episodes (MELAS), *M*itochondrial *T*rifunctional *P*rotein *D*eficiency *S*yndrome (MTPDS, MIM 609015), and Pearson marrow-pancreas syndrome.[15,43,56] Point mutations, duplications, and deletions of various length of the mitochondrial genome have been found in these patients.

Evaluation and Management

Evaluation of the neonate with hypocalcemia begins with review of the maternal, gestational, peripartum, postnatal, and family histories and physical examination (Figure 18-2).[1-3] Historical data include those related to maternal parity and complications of pregnancy such as maternal diabetes mellitus (gestational, types I or II), toxemia of pregnancy or ingestion of agents that may cause maternal hypercalcemia (excessive alkali), intrauterine growth restriction, abnormalities of delivery, neonatal sepsis, or other early postpartum illnesses. The family history is examined for members with abnormalities of mineral metabolism such as renal calculi, rickets, or hypocalcemia (e.g., seizure disorders). The social history provides information about the socioeconomic status of the mother and her cultural milieu that may have impacted maternal diet and exposure to sunlight during gestation. Physical examination of the neonate (abnormal face, cardiac murmur) may suggest a complex form of hypocalcemia. Determination of a complete blood count, serum concentrations of total calcium, Ca^{2+}, magnesium, phosphate, creatinine, intact PTH, calcidiol, and calcitriol, and urinary calcium and creatinine concentrations in a spot urine should precede initial therapy of the hypocalcemic newborn whenever possible. Decreased serum concentrations of PTH are common in neonates with early onset hypocalcemia, but persistently low PTH levels suggest impaired PTH secretion. High PTH concentrations are present in patients with vitamin D deficiency or insensitivity, PTH resistance due to loss-of-function mutations in *PTH1R*, *GNAS*, or *PRKAR1A* or impaired renal function. Low levels of calcidiol signify decreased maternal (and hence fetal) vitamin D stores or

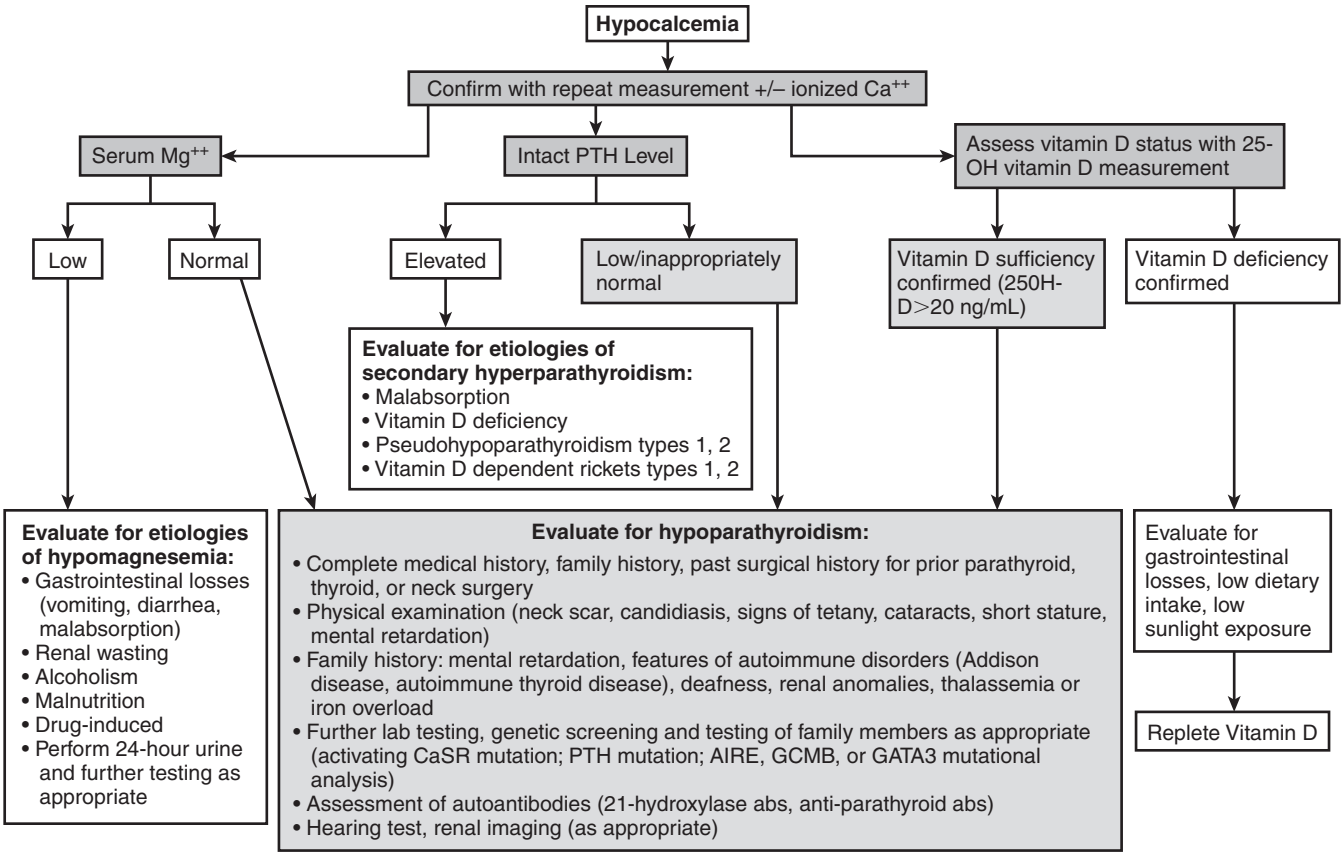


FIGURE 18-2 ■ **Evaluation of hypocalcemia.** (From Bilezikian, J. P., Khan, A., Potts, J. T., Jr, et al. (2011). Hypoparathyroidism in the adult: epidemiology, diagnosis, pathophysiology, target-organ involvement, treatment, and challenges for future research. *J Bone Mineral Res, 26,* 2317–2337.)

rarely a defect in vitamin D-25 hydroxylase, whereas calcitriol concentrations are inappropriately low in subjects with severely compromised renal function, hypoparathyroidism, or those with a deficiency of 25OHD-1α-hydroxylase. Elevated calcitriol values suggest vitamin D resistance due to an abnormality in *VDR*, a disorder that may be associated with alopecia. Skeletal radiographs may disclose osteopenia, whereas chest x-ray may not identify a thymic shadow (although this is an unreliable sign in a severely ill or stressed neonate). Serum levels of calcium, Ca^{2+} phosphate, and intact PTH should be measured in the mothers of neonates with unexplained hypocalcemia.

In neonates with hypocalcemia not otherwise explained, evaluation for possible DGS should be undertaken, particularly when physical examination reveals an abnormal face, and a congenital anomaly of the outflow tract of the heart is present. The white blood and T (CD4) lymphocyte counts are low in DGS and the thymic shadow often absent. The diagnosis of the DGS is confirmed by the presence of a microdeletion of chromosome 22q11.2 as demonstrated by microarray or fluorescent in situ hybridization (FISH). Occasionally, sequence analysis of *TBX1* may be needed to establish this diagnosis, if the other studies are normal. Because the DGS may be heritable, examination of the karyotype of the parents of a DGS infant is indicated as well as those of the siblings if the parent also has deletion of chromosome 22q.11.21. It should be noted that the majority of neonates and infants with DGS are recognized primarily because of cardiac anomalies and that subjects without these lesions may not be identified until mid or late childhood or adolescence.[57] Neonates with PHP IA may present with elevated serum levels of TSH in the neonatal metabolic screening survey but usually do not have the characteristic skeletal phenotype (brachymetacarpals) of AHO; if no cause of congenital hypothyroidism is identified, the diagnosis of PHP IA should be suspected, and measurement of serum calcium levels and, if appropriate, genotyping and methylation studies of *GNAS* are indicated.[49]

Early neonatal hypocalcemia is often asymptomatic, but nevertheless treatment is indicated when the total serum calcium concentration is below 6 mg/dL in the preterm infant and less than 7 mg/dL in the term infant.[3] Asymptomatic neonates are most easily managed by increasing the oral intake of calcium and establishing an overall ratio of calcium:phosphate intake of 4:1 (including that in feedings with a low phosphate formula such as Similac PM 60/40^{R}—calcium:phosphate ratio 1.6:1) with calcium glubionate or calcium carbonate administered in divided doses every 4 to 6 hours (Table 18-3). Eucalcemia is almost always restored in these subjects within 3 weeks after birth and often earlier. In hypocalcemic infants with tetany or frank seizures, 10% calcium gluconate

TABLE 18-3 **Preparations of Vitamin D, Calcium, Magnesium, and Phosphate**

Content	Elemental Mineral	
Vitamin D		
Vitamin D		
Calciferol	8000 IU/mL	
	50,000 IU/capsule	
Calcidiol	20 or 50 μg/tablet	
Calcitriol		
Rocaltrol	1 μg/mL (oral solution)	
	0.25 μg or 0.5 μg/capsule	
Vectical	3 μg/g (ointment)	
Dihydrotachysterol		
Hytacherol	0.2 mg/5 mL 0.125, 0.2, 0.4 mg/tablet	
Calcium		
Calcium acetate		
Phoslyra	667 mg/5 mL	667 mg/capsule
Calcium gluconate (iv)	93 mg/10 mL	93 mg/g
	500, 650 mg/tablet	
Calcium glubionate (solution)	64 mg/g	115 mg/5 mL
Calcium carbonate	400 mg/g	500, 600, 750, 1250 mg/ tablet
Titralac	420 mg tablet	168 mg/tablet
	780 mg tablet	300 mg/tablet
Oscal	650 mg tablet	260 mg/tablet (vitamin D)
	1250 mg tablet	500 mg/tablet (vitamin D)
Tums	500 mg tablet	200 mg/tablet
	750 mg tablet	300 mg/tablet
	1000 mg tablet	400 mg/tablet
	1250 mg tablet	280 mg/tablet
Caltrate	1500 mg tablet	600 mg/tablet (vitamin D)
Calcium carbonate (suspension)	1250 mg/5 mL	500 mg/5 mL
Calcium chloride	100 mg/mL	
Calcium citrate	200 mg/g	210 mg/tablet
Citracal	950 mg tablet	200 mg/tablet
Calcium lactate	84 mg/g	130 mg/tablet
Magnesium		
Magnesium sulfate	49 mg/mL (50% intramuscular solution)	
	0.32, 0.64, 4 mEq/mL (intravenous solution)	
Magnesium oxide	603 mg/g	241 mg/tablet
Magnesium gluconate	54 mg/g	27 mg/tablet
Magnesium chloride	120 mg/g	64 mg/tablet
Phosphorus		
Sodium phosphate (Phospha-Soda)		127 mg/mL
Sodium/potassium phosphate (Phos-NaK)		250 mg/packet (powder)
Potassium phosphate (Neutraphos-K)		250 mg/packet (powder)
Potassium phosphate (K-Phos Original)		114 mg/tablet
Sodium/Potassium phosphate (K-Phos #2)		250 mg/coated tablet
Sodium/Potassium phosphate (K-Phos Neutral)		250 mg/tablet

Compiled from Alon US (2006). Hypophosphatemic vitamin D–resistant rickets. In M. J. Favus (Ed.), Primer on the metabolic bone diseases and disorders of mineral metabolism, *(6th ed.) Washington, DC: American Society for Bone and Mineral Research, 342-345; Shoback, D. (2008). Hypoparathyroidism. N Engl J Med 359:391-403.*

(elemental calcium 9.3 mg/mL) at a dose of 1 to 3 mg/kg and at a rate of less than 1 mL/minute with the total dose not to exceed 20 mg of elemental calcium/kg may be administered by intravenous infusion over 15 minutes; often seizures will cease after 1 to 3 mL of 10% calcium gluconate have been administered.[3,58] Cardiac rate and rhythm must be carefully monitored to prevent bradycardia and asystole. Further intravenous bolus doses of calcium (~10 mg/kg at 6-hour intervals) should be used sparingly, as they result in wide excursions in serum calcium values. After initial treatment of neonatal hypocalcemia, 500 mg of calcium gluconate/kg/24 hours may be administered by continuous intravenous infusion taking care to prevent extravasation or infiltration as extracellular calcium will precipitate in soft tissues.[59] These infants may receive supplemental oral calcium if necessary (as discussed previously). Depending on the cause of the hypocalcemia, supplemental vitamin D or calcitriol may also be needed. In a neonate with hypocalcemia due to hypoparathyroidism, administration of synthetic PTH[1-34] may occasionally be required to restore eucalcemia.[60] Serum and urine calcium and creatinine levels should be determined frequently and treatment modified to maintain eucalcemia and the urine calcium/creatinine ratio < 0.2 - < 0.5 in an effort to avoid iatrogenic hypercalcemia, hypercalciuria, nephrocalcinosis, and renal insufficiency. In neonates who require parenteral alimentation, 50 mg of elemental calcium/kg/24 hours should be incorporated into the infused solution; elemental phosphate must also be administered as permitted and indicated.

After restoration of eucalcemia in the infant with DGS, other components of this disorder must be addressed. Cardiac anomalies often require surgical correction, as do palatal clefts. In DGS infants who are immunocompromised and experience recurrent infections due to thymic aplasia, appropriate anti-infectious therapy is mandatory. Transplantation of fetal or cultured postnatal thymic tissue, bone marrow, or peripheral blood mononuclear cells has restored immune function in infants with the DGS. Supplemental calcitriol (20 to 60 ng/kg/day) and calcium are necessary for restoration and maintenance of eucalcemia in infants with hypoparathyroidism. Poor growth due to feeding difficulties and learning disabilities due to developmental delay must be managed on an individual basis and illustrate the need for a multidisciplinary approach to the care of DGS patients.[57] Hypocalcemia due to hypomagnesemia is managed acutely by the intravenous infusion over 1 to 2 hours or by the intramuscular injection of 50% magnesium sulfate at a dose of 0.1 to 0.2 mL/kg while carefully monitoring cardiac status.

Hypocalcemia in the Child and Adolescent

Etiology

Causes of hypocalcemia in the child and adolescent include many that also result in neonatal hypocalcemia and are listed in Tables 18-2A and B. Hypocalcemia is defined by the norms of the analytic laboratory and its parameters dependent on the age of the subject (total calcium concentrations: 1 to 5 years 9.4 to 10.8; 6 to 12 years 9.4 to 10.2; $>$ 20 years 8.8 to 10.2 mg/dL).[58] Total calcium levels are low in the hypoalbuminemic patient; a correction for hypoalbuminemia may be calculated by adding 0.8 mg/dL to the recorded total calcium concentration for every decrease in albumin concentration of 1 g/dL. Thus, it is appropriate to measure both total and Ca^{2+} values when evaluating the hypocalcemic child, but reliance on Ca^{2+} determinations alone is discouraged given the technical difficulties with this assay. Hypocalcemia develops as a consequence of either too little inflow of calcium from the gastrointestinal tract, bone, or kidney into the extracellular and vascular spaces or an excessive loss of calcium from these spaces into urine, stool, or bone. Thus, hypocalcemia may be due to decreased intake or absorption or excessive loss of calcium, decreased production of bioactive PTH due to congenital abnormalities of parathyroid gland development or PTH synthesis or of the CaSR, destruction of parathyroid glands by autoantibodies, metal overload (copper, iron), surgical or radiation insults, granulomatous infiltration, or impaired cellular responsiveness to PTH. Restricted exposure to sunlight or reduced intake, absorption, metabolism, or activity of vitamin D leads to hypocalcemia. Hypomagnesemia impairs the secretion (but not the synthesis of PTH) and blunts tissue responsiveness to PTH. Hypocalcemia occurs in the very ill child and after exposure to a number of drugs and medications. Hypocalcemic tetany may develop after the administration of phosphate containing enemas by rectum or laxatives by mouth.[61] At times, the hypocalcemic child or adolescent may be asymptomatic and identified by chemical screening for an unrelated problem, or she or he may present with intermittent muscular cramping either at rest or during exercise (when the increase in systemic pH due to hyperventilation further lowers the concentration of Ca^{2+}); paresthesias of fingers, toes, or circumoral regions; tetany (carpopedal spasm, laryngospasm, bronchospasm); or seizures (grand mal, focal, petit mal, adynamic, or syncopal). Prolonged and severe hypocalcemia may lead to congestive heart failure.[62] Physical examination often reveals a positive Chvostek or Trousseau sign (carpopedal spasm) and hyperreflexia. However, a Chvostek sign is commonly present in normal adolescents also.

Hypoparathyroidism may occur as a solitary disorder, as part of a multidimensional autoimmune polyendocrinopathy, or as one manifestation of a group of complex congenital anomalies (DGS, HDR, HRD, KCS1, Blomstrand, and other syndromes).[62] There are sporadic and familial forms of hypoparathyroidism; when familial, hypoparathyroidism may be transmitted as an autosomal dominant, autosomal recessive, or X-linked recessive trait (see Tables 18-2A and B). Abnormalities in the development of the parathyroid glands, in the transcription of *PTH*, and in the processing of the translated product have been associated with inherited forms of hypoparathyroidism. Autosomal dominant dyshormonogenic hypoparathyroidism due to a monoallelic T $\Rightarrow$ C transition in codon 18 (Cys18Arg) of the 25 amino acid signal peptide of prepro-PTH impaired efficient transport of protein from the ribosome and interaction of prepro-PTH with the signal

recognition particle, movement of the precursor peptide into and exit from the rough endoplasmic reticulum, its cleavage by a signal peptidase, and its incorporation into a secretory granule.[63] Autosomal recessive dyshormonogenic hypoparathyroidism has been associated with a homozygous G ⇒ C transversion in nucleotide 1 of intron 2 of *PTH* within the signal sequence that prevented normal cleavage of prepro-PTH and decreased secretion of PTH. However, inactivating mutations in *PTH* are uncommon in patients with sporadic idiopathic hypoparathyroidism.[64] Isolated hypoparathyroidism may be found in patients with deletion of chromosome 22q11.2 without other signs or symptoms of the DGS or the velocardiofacial syndrome.

Autosomal dominant hypoparathyroidism (MIM 146200) due to heterozygous gain-of-function mutations in the extracellular, transmembrane, and intracellular domains of *CASR* that transcribe a CaSR that is not intrinsically constitutively active but is exceptionally sensitive to and easily activated by very low serum Ca^{2+} concentrations may be identified in infancy or in older subjects (Figure 18-3). Even at hypocalcemic levels, Ca^{2+} binds avidly to the CaSR and activates phospholipase C-β1, increasing cytosolic levels of inositol phosphate and Ca^{2+} and stimulating the mitogen-activated protein kinase (MAPK) signal transduction pathway in parathyroid chief cells—suppressing PTH synthesis and secretion—and in the kidney—decreasing renal tubular calcium and magnesium resorption leading to urinary wasting of these cations (hypercalciuric hypocalcemia); urinary concentrating ability is also depressed. In these subjects, serum levels of phosphate are increased and magnesium values decreased; PTH concentrations are low or inappropriately normal. Affected patients frequently have symptomatic hypocalcemia such as tetany and seizures. They are very sensitive to vitamin D; the dose of calcitriol must be limited to that which raises serum calcium to asymptomatic values even if not within the normal range, as larger doses lead to hypercalciuria (sometimes even when serum calcium levels remain subnormal), nephrocalcinosis, and functional renal insufficiency. Administration of recombinant human (rh)PTH[1-34] restores calcium homeostasis in this disorder but does not necessarily prevent nephrocalcinosis.[19,23,24] Although there has been reluctance to administer rhPTH to children because there is an increased incidence of bone tumors in young rats receiving very large amounts of this agent, primates appear to be less susceptible to PTH-induced bone tumor formation than do rodents.[65] Development of stimulatory autoantibodies to the CaSR results in an acquired variant of spontaneous hypoparathyroidism that may be isolated or part of a complex autoimmune endocrinopathy.[66] Indeed, in perhaps as many as one third of patients with acquired isolated idiopathic hypoparathyroidism, antibodies directed against epitopes in the extracellular domain of the CaSR may be present. This form of acquired hypoparathyroidism may be reversible, as these antibodies do not destroy the parathyroid glands. In patients with other forms of autoimmune hypoparathyroidism, the antibodies are cytotoxic and accompanied by lymphocytic infiltration, atrophy, and fatty replacement of parathyroid tissue.

In mid-childhood and adolescence, acquired hypoparathyroidism may be a late manifestation of a congenital abnormality (e.g., DGS), but it is also likely to be the result of destruction of the parathyroid glands by autoimmune disease or surgical removal or operative trauma to the vascular supply of these structures. Unusual causes of acquired hypoparathyroidism in this age group include infiltration by iron (hemochromatosis, thalassemia) or copper (Wilson's hepatolenticular degeneration), granulomatous diseases, or radiation (mantle radiation for Hodgkin/non-Hodgkin lymphoma or radioiodine therapy of hyperthyroidism).[2,67] Autoimmune hypoparathyroidism may occur as an isolated disorder or as part of the complex of autoimmune polyendocrinopathy syndrome type I.[67,68] Development of an autoimmune endocrinopathy is initiated by failure to recognize a peptide specific for a target organ by a subgroup of regulatory T cells that ordinarily recognize that peptide. When immunologic tolerance for that peptide is lost, clones of CD4 regulatory T cells for the peptide expand; type 1 helper T cells secrete inflammatory cytokines such as interferon-γ, whereas type 2 helper T cells stimulate B cell function and lead to autoantibody-mediated inflammation. Loss of immune tolerance may be the consequence of the postinfectious inflammatory state due to activation of the innate immune system or due to a gene mutation that depresses immune tolerance and permits expansion of a CD4+ regulatory T cell clone after exposure to quantitatively small amounts of antigen. Genetic variations within the major histocompatibility complex (HLA-DQ, HLA-DR) that determine peptide (antigen) presentation to CD4+ regulatory T cells join with genetic abnormalities in immune regulation to induce autoimmune disease. In 30% of patients with isolated idiopathic hypoparathyroidism; the disorder is due to antibodies to the extracellular domain of the CaSR and thus is functionally inhibitory but potentially reversible; in approximately 33% of patients, serum antibodies to other components of the parathyroid chief cell may be present.[66] There are several types of autoimmune polyendocrine syndromes (APS), but only in type I is hypoparathyroidism a major component. Autoimmune polyendocrine syndrome type II is a polygenic disorder manifested by hypoadrenalism, type 1 diabetes mellitus, and autoimmune thyroid disease (and gastritis, celiac disease, vitiligo) and is related to the major histocompatibility complexes.[69] The syndrome of *I*mmunodeficiency, *P*olyendocrinopathy, and *E*nteropathy—X-linked (IPEX) is associated with autoimmune enteropathy, insulin dependent neonatal diabetes mellitus, autoimmune thyroid disease, eczema, glomerulonephritis, thrombocytopenia, and hemolytic anemia that occurs only in males as it is due to loss-of-function mutations in *FOXP3* (MIM 300292; chromosome Xp11.23). FOXP3 is a transcription factor that is essential for normal development of naturally occurring T regulatory cells.

Autoimmune polyendocrinopathy syndrome type I is an autosomal recessive disorder with the classic triad of *A*utoimmune *P*olyendocrinopathy, mucocutaneous *C*andidiasis, and *E*ctodermal *D*ystrophy (APECED) that is due to loss-of-function mutations in *AIRE* encoding the autoimmune regulator.[69] In a Finnish cohort of 91 patients with APS1/APECED, the cardinal manifestations were mucocutaneous candidiasis involving the nails

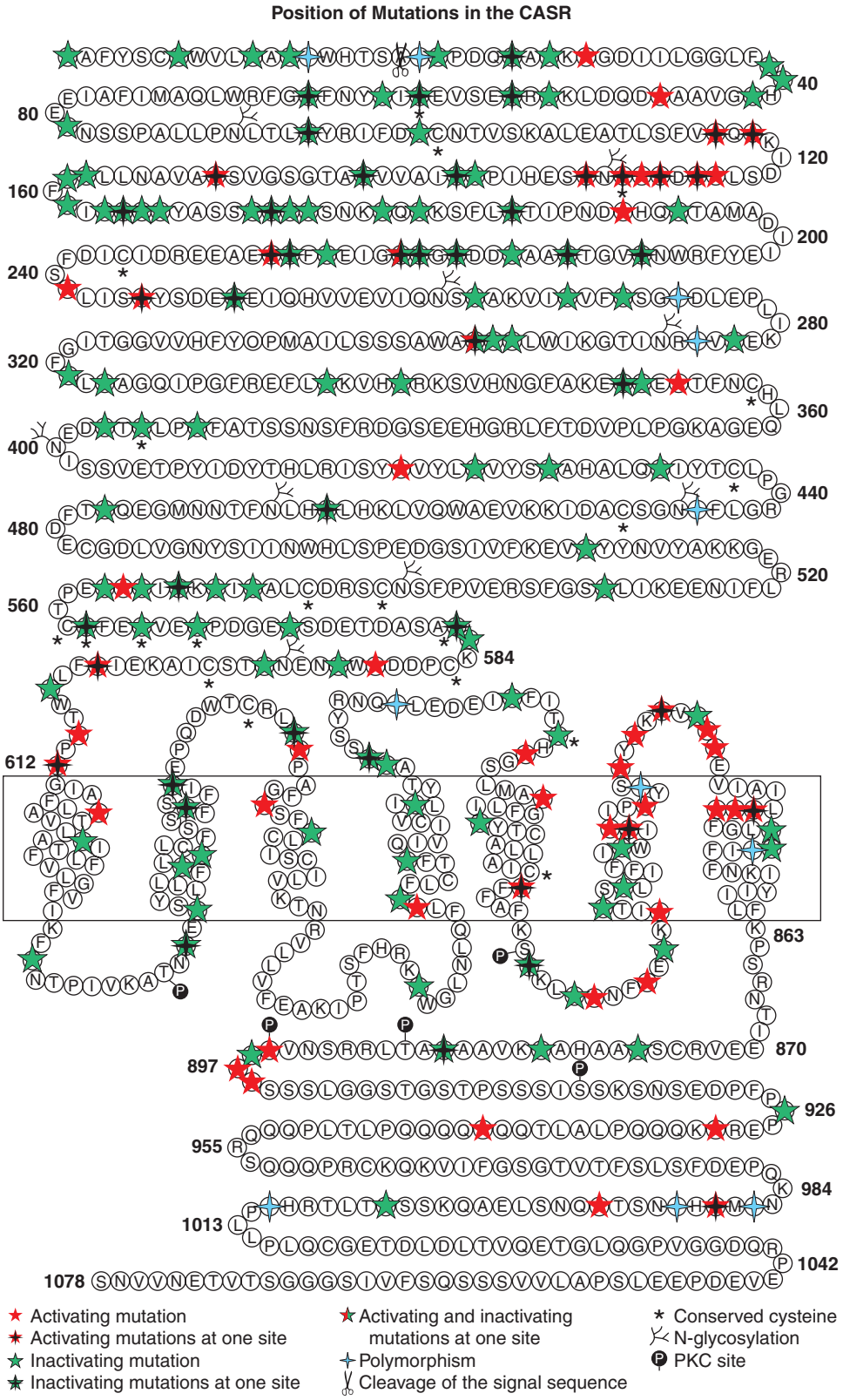


FIGURE 18-3 ■ Schematic view of the calcium sensing receptor (CaSR) depicting inactivating (hereditary/familial hypocalciuric hypercalcemia) and activating (autosomal dominant hypoparathyroidism) mutations. Note that mutations at adjacent codons may result in either a gain or a loss of function of the mutant *CASR* product (e.g., codons 565, 567, 568). (Courtesy of Drs. Lilia D'Souza-Li and Geoffrey N. Hendy, McGill University, Montreal, Quebec, www.casrdb.mcgill.ca/?Topic=CasrGraph2&v=new.) *This image can be viewed in full color online at ExpertConsult.*

and mouth occurring in 100% of patients (often in the first 2 years of life), hypoparathyroidism, and hypoadrenocorticism, both of which illnesses developed in 80% to 90% of affected subjects.[70] Hypoparathyroidism occurred most often in patients who were between 2 and 10 years old, and hypoadrenocorticism developed in patients who were between 5 and 15 years of age. Almost all females with APECED developed hypoparathyroidism, whereas 80% of affected males did so. Hypomagnesemia, often severe and recalcitrant to therapy, was common in patients with hypoparathyroidism due to APECED. The most frequent presenting manifestations of APECED were mucocutaneous candidiasis (60%), hypoparathyroidism (32%), and hypoadrenocorticism (5%); the disease first became apparent when patients were between 2 months and 18 years of age. However, 10% of patients presented with another manifestation of APECED. In addition to hypoparathyroidism and hypoadrenocorticism, other endocrinopathies encountered in APECED included autoimmune oophoritis leading to ovarian failure (70%), orchitis resulting in testicular failure (30%), diabetes mellitus (30%), thyroiditis (30%), and hypophysitis (4%). Besides mucocutaneous candidiasis, dermatologic manifestations and complications of APECED in the Finnish cohort included alopecia (40%), vitiligo (30%), and rashes with fever (15%). Keratoconjunctivitis developed in 20% of affected subjects, pernicious anemia in 30%, hepatitis in 20%, and chronic diarrhea in 20%. In a Norwegian population of 36 patients with APECED, 13 had clinical evidence of disease at or before 5 years of age and an additional 15 subjects presented at or before 15 years of age.[71] In a Russian population of 46 patients, mucocutaneous candidiasis was present in 70%, hypoparathyroidism in 83%, and adrenal insufficiency in 54%.[72] Other common problems were alopecia (27%), thyroid dysfunction (20%), and malabsorption syndromes (18%). Unusual findings in this cohort were retinitis pigmentosa and metaphyseal dysplasia, both occurring in 7% of patients. Clinical manifestations of APS1/APECED and their age of onset vary among subjects and even among siblings. Later manifestations of APECED include esophageal and oral squamous cell carcinoma, asplenia, and interstitial nephritis. The diagnosis of APECED usually requires the presence of two of three of its major diseases (mucocutaneous candidiasis, hypoparathyroidism, hypoadrenocorticism), but occasionally chronic candidiasis, hypoparathyroidism, or primary adrenal insufficiency alone may be the only manifestation of an inactivating mutation in *AIRE*.[72] In patients with autoimmune hypoparathyroidism, antibodies to PTH and to the CaSR have been identified; some CaSR antibodies have been activated, leading to a clinical picture that mimics that of patients with activating mutations in *CASR*.[73] Antibodies to NACHT leucine-rich-repeat proteins (NALP5), another antigen present in the parathyroid gland, have also been found in patients with autoimmune hypoparathyroidism and APS1.[74]

Autoimmune polyendocrinopathy syndrome type I is the result of homozygous or compound heterozygous loss-of-function mutations in *AIRE* (autoimmune regulator), a 14-exon gene encoding a 545-amino-acid protein with two zinc-finger motifs that is expressed in nuclei of thymic medullary epithelial cells and in lymph nodes, spleen, monocytes.[69] AIRE also serves as an E3 ubiquitin ligase, an essential component of the ubiquitin-proteasomal system for protein modification and destruction involved in cellular division and differentiation, protein transport, and intracellular signaling.[75] Structurally, AIRE contains two plant homeodomains: an amino acid sequence composed of an octet of cysteines and histidines that coordinate two zinc ions; the first plant homeodomain is essential for the E3 ubiquitin ligase activity of the protein and the second plant homeodomain is required for its transcription-regulating action. AIRE also contains a SAND domain, a structure that permits it to bind DNA. Functionally, AIRE promotes expression of peripheral antigens in the thymus, thereby increasing immune tolerance.[68]

Development of an immune system that is capable of distinguishing between "self" and "foreign" antigens is essential in order to prevent autodestruction of an organism's cells and tissues. To this end, thymic stromal medullary epithelial cells locally transcribe the majority of protein encoding genes expressed in various peripheral tissues in order to permit their recognition by developing T cells prior to their release from the thymus and thus prevent an autoimmune response when these proteins are encountered by T cells in the periphery. The autoimmune regulatory gene *AIRE* enables the thymic transcription and expression of these genes by interacting with nuclear transporters (e.g., exportin), chromatin binding proteins (e.g., histones), transcription factors, and postinitiation transcription processes (e.g., cyclic AMP response element binding protein; complex of DNA-dependent protein kinase-polymerase- topoisomerase), and pre-mRNA splicing and processing factors.[76] AIRE does not function as a specific transcription factor. Inasmuch as nuclear AIRE binds to the unmethylated tails of histone-3 in chromatin, it has been proposed that AIRE activates quiescent genes by attracting to their site the transcription apparatus that enables initiation of transcription, elongation, and pre-mRNA processing.[76] In the Finnish and Russian populations with APS1, the most common loss-of-function mutation in *AIRE* was a homozygous truncating mutation at codon 257 (Arg257Ter). Pathogenic mutations in *AIRE* detected in patients with APECED include missense, nonsense (Arg139Ter), insertions, and deletions (e.g., 13 base pair deletion; 964del13, NT 1094, exon 8) that alter the subcellular distribution of AIRE or decrease its transcriptional activation capacity or its E3 ubiquitin ligase activity.[72,75,77]

Pseudohypoparathyroidism is a disorder with variable clinical manifestations associated with resistance to the action of PTH, classified as types IA, IB, IC, and II, and pseudopseudohypoparathyroidism (PPHP), whose clinical manifestation and pathophysiology were discussed previously. Deficiency of vitamin D intake, aberrant metabolism of cholecalciferol, or decreased biologic responsiveness of the vitamin D receptor (VDR) may result in hypocalcemia.[56] In the subject with skeletal demineralization due to marked vitamin D deficiency, serum calcium concentrations may fall precipitously after administration of even small amounts of vitamin D as renewed mineralization of bone matrix due to accelerated osteoblastic

activity consumes calcium and phosphate (the "hungry bone" syndrome). After parathyroidectomy for primary hyperparathyroidism, calcium concentrations often decline rapidly by the same mechanism. Drugs that inhibit PTH secretion (excessive magnesium), osteoclast resorption of bone (bisphosphonates), or renal resorption of calcium (furosemide) may lead to hypocalcemia. Intravenous infusion or rectal administration of phosphate (in enemas), acute cellular destruction by tumor cell lysis or rhabdomyolysis, and acute and chronic renal failure increase serum phosphate levels and lead to reciprocal decline in calcium values. Serum calcium concentrations decline in patients receiving multiple transfusions of citrated blood or during plasmapheresis. In subjects with acute pancreatitis, calcium complexed with free fatty acids generated by pancreatic lipase is deposited in necrotic tissue.[78] Acute severe illness of diverse pathogenesis is often associated with hypocalcemia; this has been attributed to hypoalbuminemia, functional hypoparathyroidism, hypercalcitonemia, hypomagnesemia, decreased calcitriol synthesis, alkalosis and increased serum concentrations of free fatty acids (the latter increase binding of Ca^{2+} to albumin), and increased cytokine activity.

Evaluation

Figure 18-2 outlines the evaluation of the child/adolescent with hypocalcemia. Hypocalcemia may be asymptomatic until detected by a multiassay chemical profile obtained for another purpose. It may be first identified during evaluation of a long QT interval noted by electrocardiography obtained for evaluation of a functional heart murmur or arrhythmia.[79] Clinical symptoms that suggest hypocalcemia include paresthesias, circumoral numbness, muscular cramping (particularly during vigorous exercise), tetany (uncontrollable muscular contractions including laryngospasm and bronchospasm), muscle twitching, carpal-pedal spasm (flexion of the elbow and wrist, adduction of the thumb, flexion of the metacarpal/metatarsal-phalangeal joints, and extension of the interphalangeal joints), and seizure.[62] Review of the past medical history may reveal symptoms consistent with or illnesses associated with hypocalcemia (recurrent infections, congenital cardiac anomalies, surgical procedures in the neck, cervical radiation, infiltrative diseases), or the family history may identify members with hypoparathyroidism, dysmorphic physical characteristics, autoimmune endocrinopathies, or hypomagnesemia. Physical examination will disclose characteristic abnormalities in children with PHP type IA in whom the AHO phenotype is present (short stature, round face, subcutaneous hard nodules, brachymetacarpals), the DGS (typical face, recurrent infections, cardiac murmur), and familial autoimmune polyendocrinopathy syndrome type I (chronic mucocutaneous candidiasis and other ectodermal abnormalities such as vitiligo, alopecia, keratoconjunctivitis), whereas rachitic deformities in the hypocalcemic child imply the presence of a form of hypovitaminosis D. Most commonly, however, the physical examination reveals no striking abnormality in the hypocalcemic child other than those of increased neuromuscular irritability such as hyperreflexia, positive Chvostek sign (twitching of the circumoral muscles when tapping lightly over the seventh cranial nerve) or Trousseau sign (carpal pedal spasm when maintaining the blood pressure cuff 20 mm Hg above the systolic blood pressure for three minutes) and occasionally cataracts, papilledema, or abnormal dentition. (Tetany also occurs in subjects with hypo- and hypernatremia, hypo- and hyperkalemia, and hypomagnesemia, and a positive Chvostek sign may be found in many normal adolescents.)

After confirmation of the hypocalcemic state (by measuring serum total calcium and Ca^{2+} levels, the latter to exclude hypoalbuminemia as a cause of a low total calcium value), urine calcium excretion is determined; in most hypocalcemic patients, urine calcium excretion is low (see Figure 18-2). If the urine calcium excretion is inappropriately normal or high, disorders such as autosomal dominant (gain-of-function mutation in *CASR*) hypoparathyroidism may be considered; *CASR* may then be analyzed or antibodies to CaSR determined as clinically indicated. The frequency with which antibodies to the CaSR are detected in patients with autoimmune hypoparathyroidism depends on the method employed for their identification. Thus, antibodies to the CaSR have been found in 86% of subjects with APS1/APECED employing an immunoprecipitation assay with the full-length CaSR expressed in human embryonic kidney cells and in 50% of the same subjects utilizing a flow cytometry assay but in none of these patients applying a radiobinding assay.[73,80] The serum levels of intact PTH, calcidiol, magnesium, phosphate, creatinine, and alkaline phosphatase are also to be measured. Determination of the serum concentration of intact PTH (by ultrasensitive assay) permits differentiation between low (or inappropriately "normal") and high PTH secretory states. The child/adolescent with hypocalcemia, hypocalciuria, hyperphosphatemia, and low or undetectable serum PTH concentration (and normal or only slightly low serum magnesium level) likely has hypoparathyroidism due to a primary defect in PTH synthesis or secretion related to congenital malformation or acquired destruction of the parathyroid glands. Patients with hypoparathyroidism often have low serum concentrations of calcitriol, normal levels of calcidiol, decreased excretion of urinary nephrogenous cyclic AMP, and increased renal tubular reabsorption of phosphate.[67] The DGS may be identified by microarray or FISH analysis of chromosome 22q11.2 or *TBX1* genotyping. Analysis of *GCM2* or *SOX3* and genes associated with syndromic hypoparathyroidism (*TBCE GATA3*) is indicated in the appropriate clinical context (see Table 18-2B). The diagnosis of autoimmune polyendocrinopathy syndrome type I is based on clinical and laboratory findings and genotyping of *AIRE*. The presence of two of three of its major manifestations (candidiasis or ectodermal dystrophy, hypoparathyroidism, hypoadrenocorticism) is the accepted criterion for its clinical diagnosis, but isolated hypoparathyroidism, adrenal insufficiency, or chronic mucocandidiasis may occasionally be its only manifestation.[72] Antibodies to the CaSR or other cellular components of the parathyroid glands, the adrenal glands (side-chain cleavage, 21-hydroxylase, 17α-hydroxylase enzymes), neurotransmitters (aromatic

L-amino acid decarboxylase, tryptophan hydroxylase), and interferons-α and -ω may be determined; antibodies to interferon-ω are commonly present in patients with APS1/APECED.[71,81] Genotyping of *AIRE* and identification of the mutations confirm the diagnosis of APECED. There is wide variability in the clinical expression of APECED both between families and among siblings, as the phenotype is not directly related to the genotype. In patients with isolated, idiopathic hypoparathyroidism, a search for antibodies to the CaSR or to parathyroid tissue is warranted. In hypomagnesemic subjects, magnesium and PTH levels are quite low, and PTH secretion increases rapidly after intravenous administration of magnesium. Primary hypomagnesemia should be considered when hypocalcemia and hypercalciuria coincide and serum PTH and magnesium values are low; urinary magnesium excretion should then be quantitated and *CLDN16* genotyped (see Tables 18-7A and B, presented later in the chapter). Because hypomagnesemia may also be due to a selective small intestinal defect in magnesium absorption, mutations in *TRPM6* should be examined as warranted. Primary hypomagnesemia must be differentiated from that due to the Gittleman and Bartter syndromes.

An elevated serum concentration of PTH in a hypocalcemic subject suggests that the patient is either secreting an abnormal PTH molecule or is resistant to PTH or there is a compensatory (secondary) PTH secretory response to hypocalcemia (see Figure 18-2). Physicochemical characterization of the PTH molecule and analysis of *PTH* enable one to define the abnormality in PTH synthesis, posttranslational processing, secretion, or activity leading to the functionally hypoparathyroid state. Analysis of *GNAS* and its pattern of imprinting permits identification of the specific genetic defect in the majority of patients with clinical PHP type IA and PPHP. Skeletal and renal responsiveness to PTH may be assessed if warranted by measurement of changes in serum calcium, phosphate, cyclic AMP, and calcitriol concentrations and urinary nephrogenous cyclic AMP and phosphate excretion following administration of biosynthetic $PTH^{1\text{-}34}$ (Elsworth-Howard test). In the normal subject and in the patient with primary hypoparathyroidism, urinary cyclic AMP excretion increases 10- to 20-fold and that of phosphate several fold; in the patient with PHP types IA and IB there is less than a 3-fold increase in the excretion of cyclic AMP after administration of $PTH^{1\text{-}34}$. The diagnosis of PHP type IB may be established by examining the imprinting patterns of *GNAS* and analyzing *STX16* and the 5′ sequences of *GNAS* as indicated. In the child with vitamin D deficiency, serum levels of calcidiol are low. In patients with decreased renal $25OHD_3$-1α-hydroxylase activity, serum concentrations of calcidiol are normal, whereas those of calcitriol are inappropriately low. Increased concentrations of calcitriol suggest the presence of a defect in the nuclear vitamin D receptor. The patient with renal failure is recognized by an increased serum creatinine value. Other findings in hypocalcemic subjects include prolongation of the Q-T interval by electrocardiography and calcification of the basal ganglia by cranial computerized tomography.

Management

The primary goal in the care of the hypocalcemic child and adolescent is to increase serum calcium concentrations to levels at which the patient is asymptomatic and as close to the lower range of normal as possible; the secondary goal is to identify the cause of hypocalcemia as quickly as possible in order to provide disease-specific management.[56,62,67] Asymptomatic hypocalcemia (total calcium > 7.5 mg/dL) may not require immediate intervention. With lower serum calcium levels or when hypocalcemia is symptomatic (tetany, seizures, laryngospasm, bronchospasm), acute management may require the intravenous administration of calcium gluconate (93 mg of elemental calcium/10 mL vial) at a slow rate (not greater than 2 mL [1.86 mg of elemental calcium]/kg over 10 minutes) while closely monitoring pulse rate (and the QT interval). Acutely, intravenous administration of calcium is intended to ameliorate the more serious consequences of hypocalcemia such as seizures, bronchospasm, or laryngospasm, not to restore and maintain the eucalcemic state. Intravenously, calcium should not be administered with phosphate or bicarbonate, because these salts may coprecipitate. Extravascular extravasation of calcium is to be avoided, as it may precipitate and cause local tissue injury. After the acute symptoms have resolved, calcium gluconate (10 mL = 93 mg of elemental calcium in 100 mL 5% dextrose/0.25 normal saline) may be infused intravenously at a rate sufficient to maintain calcium levels in the asymptomatic low-normal range while the cause of the hypocalcemia is identified and more specific therapy for persistent hypocalcemia prescribed. The evaluation should proceed as rapidly as possible, and oral therapy should begin reasonably quickly. In the child with marked hyperphosphatemia as a cause of hypocalcemia, in addition to parenteral calcium administration, infusion of normal saline sufficient to maintain urine output at or above 2 mL/kg per hour is necessary.[61] Frequent measurement of serum calcium and phosphate concentrations permits rapid adjustment of fluid and electrolyte therapy.

After stabilization, patients with hypoparathyroidism or PHP may be treated with calcitriol (20 to 60 ng/kg/day) and supplemental calcium (calcium glubionate or calcium citrate 30 to 75 mg elemental calcium/kg/day in divided doses; see Table 18-3) to restore and maintain eucalcemia. The serum calcium concentration should be maintained within the low-normal range. Each patient must be carefully monitored to avoid hypercalcemia, hypercalciuria (calcium excretion greater than 4 mg/kg/24 hours), nephrocalcinosis, and nephrolithiasis. Basal and periodic measurements of serum concentrations of calcium, phosphate, and creatinine and urinary calcium and creatinine excretion and renal sonography are mandatory. Children with autosomal dominant hypoparathyroidism due to gain-of-function mutations in *CASR* are extremely sensitive to vitamin D and its metabolites. Even small doses of calcitriol may lead to hypercalciuria with a minimal increase in serum calcium levels; in that instance, the addition of hydrochlorothiazide (0.5 to 2.0 mg/kg/day) may increase renal tubular reabsorption of calcium and lower the calcitriol requirement. Administration of

recombinant human PTH[1-34] has been beneficial in individual subjects with this disorder.[19,23,24] In adults and children with hypoparathyroidism due to a variety of causes, the use of rhPTH[1-34] (0.4 to 0.5 μg/kg every 12 hours subcutaneously) together with calcium carbonate and vitamin D (1200 mg/day of elemental calcium and 800 IU/day of cholecalciferol in four equally divided doses) has proven effective and safe for as long as 3 years.[24] rhPTH[1-34] led to an acceleration of bone turnover as reflected by increases in serum alkaline phosphatase and osteocalcin and urinary excretion of pyridinoline and deoxypyridinoline, whereas accrual of bone mineral mass, linear growth, and weight gain were not adversely impacted in treated children.[24] Continuous infusion of rhPTH[1-34] has also proven effective in maintaining reasonable serum calcium concentrations in adults with postsurgical hypoparathyroidism with fewer fluctuations in calcium levels and lower urine calcium values when compared to twice-daily subcutaneous injections of rhPTH[1-34].[82]

Because PHP type IA is associated with resistance to a number of peptide hormones that act through GPCRs, periodic assessment of pituitary-thyroid and pituitary-ovarian function and growth hormone secretion is necessary and hormone replacement therapy should begin as indicated.[49] In general the short stature of patients with PHP type IA reflects the AHO phenotype and not GH deficiency. Transient hypoparathyroidism of infancy may be the initial manifestation of later onset hypoparathyroidism; thus, it is important to assess calcium homeostasis in such subjects throughout childhood. Patients with apparently isolated hypoparathyroidism of unknown etiology should be reevaluated periodically to identify the development of autoimmune disorders in the patient or family. Assessment of thymic function is important in subjects with findings suggestive of the DGS. The management of patients with vitamin D deficiency or resistance is discussed in subsequent sections.

When hypomagnesemia is symptomatic, administration of magnesium sulfate parenterally may be necessary (50% solution—0.1 to 0.2 mL/kg intramuscularly—repeated after 12 to 24 hours if needed). The patient with primary hypomagnesemia may require daily parenteral (intramuscular, intravenous) doses of magnesium sulfate in order to prevent tetany, seizures, and other neurologic symptoms (slurred speech, choreoathetoid movements, weakness) and to enable normal growth and development.[83] Calcitriol alone raises serum calcium levels in hypomagnesemic subjects but is ineffective in the prevention of tetany. Continuous overnight nasogastric infusion of magnesium may help alleviate the gastrointestinal side effects of multiple large doses of oral magnesium. More mild and transient forms of hypomagnesemia may be treated with oral magnesium gluconate or tribasic magnesium citrate (see Table 18-3).

HYPERCALCEMIA

Hypercalcemia in the Neonate and Infant

Hypercalcemia in neonates and very young infants is defined as total blood calcium concentration > 10.8 to 11.3 mg/dL (depending on the analytic laboratory). However, substantial symptoms (impaired growth, anorexia, gastroesophageal reflux and emesis, constipation, and lethargy and hypotonia or irritability and seizures) usually do not occur until the total calcium level exceeds 12.5 to 13 mg/dL.[2,58,84,85] These infants frequently have polyuria and may become dehydrated because of renal resistance to antidiuretic hormone if oral fluid intake is restricted. Due to the vasoconstrictive effect of calcium, the hypercalcemic infant may be hypertensive. Hypercalcemia also shortens the S-T segment and can lead to heart block and ultimately asystole. In older infants and young children with chronic hypercalcemia, poor growth and failure to thrive are often presenting manifestations. Hypercalcemia also leads to polyuria due to hypercalciuria, nephrocalcinosis, and nephrolithiasis.

Etiology

Neonatal/infantile hypercalcemia may be iatrogenic in origin, e.g., administration of excessive calcium or vitamin D (at times to the mother who secretes cholecalciferol in her breast milk); use of thiazide diuretics that increase renal tubular absorption of calcium; or purposeful restriction of phosphate (Tables 18-4A and B). Hypercalcemia, hypophosphatemia, hyperphosphatasemia, and radiographic evidence of rickets may develop in very premature infants receiving intravenous alimentation deficient in phosphate or in those fed only human breast milk, the phosphate content of which is low. In this situation, hypophosphatemia leads to increased synthesis of calcitriol by two mechanisms: (1) direct stimulation of renal tubular 25OHD-1α hydroxylase activity and (2) depressing secretion of fibroblast growth factor 23 (FGF23), a factor that inhibits its activity of this enzyme—the two processes additively increase 25OHD-1α hydroxylase activity, calcitriol synthesis, and intestinal calcium absorption.[85] The problem may be circumvented by increasing the amount of parenteral phosphate administered to the extent possible or by the use of breast milk fortified with phosphate. (Adequate extrauterine mineralization of the preterm skeleton requires intakes of both calcium and phosphate of approximately 200 mg/kg/day.) Extracorporeal membrane oxygenation may also be associated with hypercalcemia in neonates.[86,87]

Hypervitaminosis D may be due to prolonged feeding of an improperly prepared formula or commercial dairy milk containing excessive vitamin D, iatrogenic prescription of vitamin D, calcidiol, or calcitriol, or increased endogenous production of calcitriol from inflammatory sites.[84] In infants with severe birth trauma or perinatal asphyxia, subcutaneous fat necrosis may develop in tissues that have sustained direct trauma and be manifested by indurated, extremely firm, violaceous nodules on the cheeks, trunk, buttocks and legs.[58] Hypercalcemia may be present when the lesions first appear or develop as the nodules resolve several weeks later. Histologically, the skin lesions are composed of adipocytes, an inflammatory lymphohistiocytic infiltrate, and multinucleated giant cells in a bed of calcium crystals. The hypercalcemia of subcutaneous fat necrosis is attributable not only to

TABLE 18-4A **Causes of Hypercalcemia**

I Neonate/Infant

A Maternal Disorders
1 Excessive vitamin D ingestion, hypoparathyroidism, pseudohypoparathyroidism

B Neonate/Infant
1 Iatrogenic: excessive intake of calcium, vitamin D, vitamin A
2 Phosphate depletion
3 Subcutaneous fat necrosis
4 Williams-Beuren syndrome (del7q11.23/*BAZ1B*)
5 Neonatal severe hyperparathyroidism (*CASR*)
6 Metaphyseal chondrodysplasia, Murk-Jansen type (*PTH1R*)
7 Idiopathic infantile hypercalcemia (*CYP24A1*)
8 Persistent parathyroid hormone related protein
9 Lactase/disaccharidase deficiency (*LCT*)
10 Infantile hypophosphatasia (*TNSALP*)
11 Mucolipidosis type II (*GNPTAB*)
12 Blue diaper syndrome
13 Antenatal Bartter syndromes types 1 and 2 (*SLC12A1*, *KCNJ1*)
14 Distal renal tubular acidosis
15 IMAGe syndrome (*CDKN1C*)
16 Post bone marrow transplantation for osteopetrosis
17 Endocrinopathies: primary adrenal insufficiency, severe congenital hypothyroidism, hyperthyroidism

II Hyperparathyroidism

A Sporadic
1 Parathyroid hyperplasia, adenoma, carcinoma

B Familial
1 Neonatal severe hyperparathyroidism (*CASR*)
2 Multiple endocrine neoplasia, type I (*MEN1*)
3 Multiple endocrine neoplasia, type IIA (*RET*)
4 Multiple endocrine neoplasia, type IIB (*RET*)
5 Multiple endocrine neoplasia, type IV (*CDKN1B*)
6 McCune-Albright syndrome (*GNAS*)
7 Familial isolated hyperparathyroidism 1(*CDC73*)
8 Familial isolated hyperparathyroidism 2 (jaw tumor syndrome) (*CDC73*)
9 Familial isolated hyperparathyroidism 3
10 Jansen's metaphyseal dysplasia (*PTH1R*)

C Secondary/Tertiary
1 Postrenal transplantation
2 Chronic hyperphosphatemia

D Hypercalcemia of Malignancy
1 Ectopic production of PTHrP
2 Metastatic dissolution of bone

III Familial Hypocalciuric Hypercalcemia

A Familial Hypocalciuric Hypercalcemia I (CASR)
1 Loss-of-function mutations in *CASR*
a Monoallelic: familial benign hypercalcemia
b Biallelic: neonatal severe hyperparathyroidism

B Familial Hypocalciuric Hypercalcemia II (GNA11)
C Familial Hypocalciuric Hypercalcemia III, Oklahoma Variant (AP2S1)
D CASR blocking autoantibodies

IV Excessive Calcium or Vitamin D

A Milk-Alkali Syndrome
B Exogenous Ingestion of Calcium or Vitamin D or Topical Application of Vitamin D (calcitriol or analog)
C Ectopic Production of Calcitriol Associated with Granulomatous Diseases (sarcoidosis, cat scratch fever; tuberculosis, histoplasmosis, coccidioidomycosis, leprosy; human immunodeficiency virus; cytomegalovirus; chronic inflammatory bowel disease)
D Neoplasia
1 Primary bone tumors
2 Metastatic tumors with osteolysis
3 Lymphoma, dysgerminoma
4 Tumors secreting PTHrP, growth factors, cytokines, prostaglandins, osteoclast activating factors

E Williams-Beuren Syndrome (del7q11.23)

V Immobilization
VI Other Causes

A Drugs: Thiazides, Lithium, Vitamin A and Analogs, Calcium, Alkali, Antiestrogens, Aminophylline
B Total Parenteral Nutrition
C Endocrinopathies: Hyperthyroidism, Hypoadrenocorticism, Pheochromocytoma
D Vasoactive Intestinal Polypeptide-Secreting Tumor
E Acute or Chronic Renal Failure/Administration of Aluminum
F Hypophosphatasia
G Juvenile Rheumatoid Arthritis: Cytokine Mediated

Adapted from Lietman, S. A., Germain-Lee, E. L., & Levine, M. A. (2010). Hypercalcemia in children and adolescents. Curr Opin Pediatr, 22, *508–515; Benjamin, R. W., Moats-Staats, B. M., Calikoglu, A., et al. (2008). Hypercalcemia in children.* Pediatr Endocrinol Rev, 5, *778–784; Davies, J. H. (2009). A practical approach to the problems of hypercalcaemia. In J. Allgrove, & N. Shaw (Eds.),* Calcium and bone disorders in children and adolescents. *Endocr Dev. (Vol. 16, pp. 93–114). Basel: Karger.*

reabsorption of precipitated calcium but also to extrarenal synthesis of calcitriol by local macrophages and resultant hyperabsorption of ingested calcium. The 25OHD-1α-hydroxylase activity of these inflammatory macrophages is not under the control of PTH, calcium, or phosphate, but it is suppressible by glucocorticoids.[58] Excessive production of prostaglandin E and interleukins (IL)-1 and -6 further contributes to hypercalcemia in this disorder by increasing the rate of bone turnover. Hypercalcemia attributable to subcutaneous fat necrosis is managed by the ingestion of a low-calcium formula, avoidance of vitamin D, and administration of fluids, furosemide, calcitonin, glucocorticoids, or bisphosphonate (etidronate, pamidronate) as needed.[2] Hypercalcemia due to subcutaneous fat necrosis has also been observed in older children with major trauma or disseminated varicella.[84] Congenital lactase and other disaccharidase deficiencies have been associated with infantile hypercalcemia, likely due to increased intestinal absorption of calcium promoted by disaccharides.

TABLE 18-4B **Gene Mutations Associated with Hypercalcemia**

Disorder	OMIM	Gene(s)	Chromosome OMIM	Pathophysiology	Inheritance
Hyperparathyroidism					
Multiple endocrine neoplasia type 1 (MEN1)	131100	*MEN1*	11q13.1 613733	Menin: inhibitor of transcription; tumor suppressor	Germline and somatic inactivating mutations sum to produce MEN1, AD
Multiple endocrine neoplasia type 2A	171400	*RET*	10q11.21 164761	Tyrosine kinase receptor mediating cell differentiation and growth	AD
Multiple endocrine neoplasia type 2B (3)	162300	*RET*	10q11.21 164761	Tyrosine kinase receptor mediating cell differentiation and growth	AD
Multiple endocrine neoplasia type 2C (isolated: medullary carcinoma of thyroid)	155240	*RET*	10q11.21 164761	Tyrosine kinase receptor mediating cell differentiation and growth	AD
Multiple endocrine neoplasia type 4	610755	*CDKN1B*	12p13.1 600778	Inhibitor of cyclin dependent kinase-mediated cell division, tumor suppressor	AD
Familial isolated primary hyperparathyroidism (FIHP) type 1	145000	*CDC73*	1q31.2 607393	Cell division cycle protein	AD
Hyperparathyroidism jaw tumor syndrome (FIHP type 2)	145001	*CDC73*	1q31.2 607393	Cell division cycle protein	AD
Familial isolated primary hyperparathyroidism (FIHP) type 3	610071		2p14-p13.3		AD, AR
Familial isolated primary hyperparathyroidism (FIHP)		*MEN1*	11q13.1 613733	Menin: inhibitor of transcription; tumor suppressor	AD
Neonatal severe hyperparathyroidism	239200	*CASR*	3q13.3-q21 601199	GPCR for Ca^{2+}	AD, AR
Familial hypocalciuric hypercalcemia, type I	145980	*CASR*	3q21.1 601199	GPCR for Ca^{2+}	AD
Metaphyseal chondrodysplasia, Murk-Jansen type	156400	*PTH1R*	3p21.1 168468	GPCR for PTH and PTHrP	AD
Williams-Beuren syndrome (WBS)	194050		Delq7q11.23	Contiguous gene deletion syndrome	AD with variable manifestations
Williams-Beuren syndrome (WBS)	194050	*BAZ1B*	7q11.23 605681	WBS transcription factor (WSTF) complex regulates transcriptional responses to VDR	
Williams-Beuren syndrome (WBS)	194050	*ELN*	7q11.23 130160	Elastin required for normal formation of supravalvular aorta	
Williams-Beuren syndrome (WBS)	194050	*GTF21*	7q11.23 601679	TF influences neurocognition and behavior	
Williams-Beuren syndrome (WBS)	194050	*GTF21RD1*	7q11.23 604138	TF influences neurocognition and behavior	
Dup7q11.23 syndrome	609757		Duplication of chromosome 7q11.23	Contiguous gene duplication syndrome	AD with variable penetrance
Idiopathic infantile hypercalcemia	143880	*CYP24A1*	20q13.2 126065	Renal enzyme that hydroxylates vitamin D metabolites at C-24	AR
Intrauterine growth retardation, metaphyseal dysplasia, adrenal hypoplasia, congenita, genital anomalies (IMAGE syndrome)	614732	*CDKN1C*	11p15.4 600856	Inhibitor of G1 cyclin/cyclin dependent kinase (Cdk) complexes that interferes with cell cycle	AD through mother; imprinted with expression of only the maternal allele

TF, transcription factor.

Neonatal severe hyperparathyroidism (NSHPT) is a potentially lethal form of familial (hereditary) hypocalciuric hypercalcemia (HHC). It is most commonly due to homozygous or compound heterozygous inactivating mutations of *CASR* that greatly increase the serum concentration of Ca^{2+} needed to suppress PTH synthesis and secretion. However, in several infants with NSHPT, there have been heterozygous inactivating mutations of *CASR* (e.g., Arg185Gln, Arg227Leu), suggesting that the products of these mutations exert a dominant-negative effect on normal CaSR, perhaps by interfering with the migration of wild-type receptor to the cell surface or inactivation of wild-type receptor by linking to the mutated CaSR once embedded in the cell membrane, or by sequestration of G-proteins.[88] In addition, the fetus bearing a heterozygous mutation in *CASR* that has been inherited from an affected father but residing in the womb of a normal mother may be relatively "hypocalcemic" in utero leading to hyperplasia of the fetal parathyroid glands that persists after birth giving rise to NSHPT. Occasionally, NSHPT may be transmitted as an autosomal recessive disorder by clinically and biochemically normal parents.[89,90] Subjects with homozygous mutations near the amino terminal of *CASR* (e.g., Leu13Pro) may not become manifest until mid-childhood or even adulthood.[91] The clinical spectrum of NSHPT ranges from mild (constipation, polyuria) with calcium concentrations ranging from 11 to 13 mg/dL to severe and life-threatening (dysrhythmia, respiratory distress due to hypotonia, demineralization and fractures of the ribs) when calcium levels exceed 15 mg/dL.[85,92] NSHPT may present within the first few days of life to several months of age depending on the degree of hypercalcemia. Search of the family history may identify members with mild hypercalcemia due to autosomal dominant HHC1. Physical examination often reveals hypotonia, difficulties swallowing, and respiratory distress. The serum calcium concentration is frequently markedly elevated (average 14 mg/dL, range 11 to > 20 mg/dL) as is the PTH value (average 540 pg/mL, range 55 to > 1000 pg/mL).[93] There is hypophosphatemia, hypermagnesemia, hyperphosphatasemia, and elevated calcitriol values, low renal tubular reabsorption of phosphate, and relative hypocalciuria. Radiographically, there is evidence of hyperparathyroid bone disease: osteopenia, metaphyseal widening and irregularity, subperiosteal resorption, varus angulation of the hips, and fractures. Lowering of the elevated serum calcium concentration present in NSHPT may be effected by induction of sodium diuresis by infusion of fluids and by administration of furosemide to increase urinary calcium excretion, and calcitonin and a bisphosphonate (e.g., pamidronate) to suppress resorption of bone calcium. The allosteric calcimimetic cinacalcet hydrochloride that acts directly on the CaSR has also been effective for lowering serum calcium concentrations in infants with NSHPT.[94] (However, in 2013 the U.S. Food and Drug Administration suspended clinical trials of cinacalcet in children because of safety concerns.) Parathyroidectomy may be a requisite lifesaving measure at times. Children with NSHPT who remain hypercalcemic are anorectic, fail to thrive, and are at risk for developmental delay.[58]

Secondary hyperparathyroidism in the neonate may be the result of maternal hypocalcemia due to hypoparathyroidism, PHP, renal tubular acidosis, or vitamin D deficiency.[58,85] Maternal hypocalcemia reduces placental transport and net delivery of calcium to the fetus, resulting in relative fetal hypocalcemia and leading to hyperplasia of the fetal parathyroid glands and secondary hyperparathyroidism proportional to the maternal calcium deficit. Although only 25% of infants of hypocalcemic mothers are hypercalcemic, most have skeletal changes that reflect PTH excess that vary from severe demineralization with fractures to osteopenia detectable only by bone mineral densitometry. Secondary hyperparathyroidism usually resolves within a few weeks after birth as the infant ingests adequate calcium and phosphate. Mucolipidosis type II (MIM 252500) is a second cause of neonatal secondary hyperparathyroidism; this Hurler-like disorder is characterized by facial abnormalities (asymmetry, flat nasal bridge), hepatosplenomegaly, skeletal deformities (dysostosis multiplex), and developmental delay and is due to inactivating mutations in a gene (*GNPTAB*) encoding N-Acetyglucosamine-1-phosphotransferase, α/β subunits, a lysosomal phosphotransferase required for synthesis of mannose 6-phosphate.[95,96] In this disease, maternal calcium concentrations are normal, but placental histology is abnormal, suggesting impaired placental transport of calcium and fetal hypocalcemia leading to compensatory increase in the in utero PTH generation. In turn, skeletal evidence of PTH excess (osteopenia, fractures) develops; secondary hyperparathyroidism and its adverse effects remit within the first several weeks to months after birth. Transient neonatal distal renal tubular acidosis has also been associated with hypercalcemia due to secondary hyperparathyroidism.

Murk-Jansen metaphyseal chondrodysplasia is an autosomal dominant chondrodystrophy associated with marked hypercalcemia as a consequence of heterozygous mutations that lead to constitutive, ligand-independent activation of *PTH1R* expressed in the kidney, bone, and growth plate chondrocytes.[84,85] Phenotypically, there is short-limbed dwarfism; deformities of the long bones, digits, spine, and pelvis; choanal atresia; highly arched palate; micrognathia; widely open cranial sutures (in infancy); sclerosis of the basal cranial bones; disorganization of the metaphyses (delayed chondrocyte differentiation, irregularly calcified cartilage protruding into the diaphysis); and excessive loss of cortical bone but normal trabecular bone. Interestingly, birth length and physical appearance are often normal in these neonates, although radiographic evidence of the chondrodysplasia is present. In affected neonates and infants, there are hypercalcemia, hypophosphatemia, increased serum concentrations of calcitriol, and elevated urinary excretion of nephrogenous cyclic adenosine monophosphate but low or undetectable serum levels of PTH and PTHrP. (Adults with this disorder are very short [adult height 100 cm], skeletal maturation is delayed, metaphyses of the long bones are long and histologically disorganized, and cranial bones are sclerotic.) Constitutively activating mutations of *PTH1R* in these subjects include His223Arg at the junction of the first intracellular loop and second transmembrane domain and Thr410Pro in the sixth transmembrane domain—sites that are specifically important

in conferring ligand-independent activity upon PTH1R.[84] Excessive secretion of PTHrP and resultant hypercalcemia have been observed in infants with neonatal iron storage disease and embryonal renal tumors (Wilms, mesoblastic nephroma).

Williams-Beuren syndrome (WBS) is a hemizygous contiguous gene deletion syndrome (involving 26 to 28 genes on chromosome 7q11.23) that is transmitted as an autosomal dominant disorder and whose prevalence is 1:7500 to 1:15,000 live births.[97,98] WBS is characterized by intrauterine and postnatal growth retardation, hypercalcemia in infancy in 15% of patients that usually resolves by 2 years of age but may occasionally persist into adulthood; consistent hypercalciuria; supravalvular aortic stenosis in 30% of subjects; narrowing and stenoses of the pulmonary, renal, mesenteric, and celiac arteries; microcephaly; "elfin" face (epicanthal folds, stellate iris pattern, esotropia, short nose with full nasal tip, arched upper and prominent lower lips, long philtrum, full cheeks with flattened malar eminences, dental malocclusion); hoarse voice; hyperacusis in childhood leading to nerve deafness, radioulnar synostosis, renal hypoplasia or unilateral agenesis; hypertension, and developmental delay (poor visual-motor integration, attention deficit disorder, mean IQ 58, range 20 to 106).[58] Although most patients with WBS are developmentally challenged, they have unique and proficient verbal skills with a large vocabulary and enhanced auditory memory particularly for names, adept social language skills, and exceptional musical aptitude including the ability to memorize and sing many musical compositions and play many instruments.[84] Children with WBS prefer to be in the company of adults rather than with their peers and are not shy or fearful of strangers.[97]

Hypercalcemia in WBS subjects may be pathogenetically related to a loss of function of the Williams syndrome transcription factor (WSTF) encoded by *BAZ1B*. WSTF is a nuclear protein that is part of a multimeric, ATP-dependent, chromatin remodeling complex termed WINAC (WSTF including nucleoside assembly complex).[99] Independently of ligand-binding, the VDR interacts with WINAC. Under usual circumstances, binding of calcitriol to the VDR-WINAC complex (1) represses expression of renal tubular *CYP27B1*, the gene encoding 25-hydroxyvitamin D-1α-hydroxylase, and (2) enhances transcription of *CYP24A1*, the gene encoding 25-hydroxyvitamin D-24-hydroxylase. Hence, haploinsufficiency of WSTF leads to an increased and relatively unregulated synthesis of calcitriol while slowing its rate of degradation; in response to the increase in calcitriol generation, the intestinal absorption of calcium increases and hypercalcemia ensues. Other endocrinopathies associated with WBS include glucose intolerance and hypothyroidism.[100]

WBS is the consequence of the deletion of chromosome 7q11.23 due to unequal meiotic recombination (unequal crossover of genes between chromosome 7 homologues during meiosis); this genetic error occurs with equal frequency in the gametes of both parents and results in the loss of 26 to 28 genes.[97] Hemizygosity of *ELN* (MIM 130160) encoding elastin is the likely cause of the cardiovascular malformations and hypertension observed in WBS, as partial loss of *ELN* leads to a compensatory increase in the number of rings of smooth muscle and elastic lamellae resulting in arterial thickening and increased risk of obstruction as well as softening of the skin and an aged appearance. Functional hemizygosity for *GTF2I* (MIM 601679) and *GTF2IRD1* (MIM 604318) may underlie the neurocognitive deficits and strengths that characterize WBS and the distinctive personalities of these patients.[97] Hemizygous loss of *STX1A* (MIM 186590) may be pathogenetically related to development of glucose intolerance in WBS subjects.[98] The WBS phenotype has also been associated with interstitial deletion of chromosome 6q22.2-q23 as well as defects in chromosomes 4, 11, and 22, implying that the syndrome is genetically heterogeneous involving a number of genetic systems. Diagnosis of the WBS syndrome is suspected on the basis of the characteristic clinical phenotype (with/without hypercalcemia) and confirmed by demonstration of the microdeletion at chromosome 7q11.23 by microarray or FISH, although a normal chromosome analysis does not entirely eliminate this diagnosis. Specific genotyping may also prove diagnostically useful on occasion. Hypercalcemia is managed by ingestion of a low-calcium, vitamin D–free formula; occasionally short-term glucocorticoid therapy may be necessary to restore eucalcemia.

Duplication of chromosome 7q11.23 results in a syndrome characterized by heart defects, diaphragmatic hernia, cryptorchidism, and neurocognitive and behavioral aberrations.[101] Additional findings in patients with dup7q11.23 include short stature and minor dysmorphic facial features (prominent forehead, long nasal tip, short philtrum, thin lips, retrognathia, folded ear helices). Neurocognitively, there is severe speech delay and often autistic behavior but intact visuocognitive skills—characteristics that are opposite to those present in WBS patients. Interestingly, duplication of *GTF2I*, one of the genes lost in del7q11.23 (WBS), reportedly results in separation anxiety in mice and humans.[102]

Idiopathic infantile hypercalcemia is manifested clinically by impaired growth, febrile episodes, polyuria and vomiting leading to dehydration, hypercalciuria, and nephrocalcinosis associated with hypercalcemia in the presence of decreased serum concentrations of PTH.[103] *CYP24A1* encodes 24-hydroxylase, an enzyme to which heme is bound that converts 25-hydroxyvitamin D to 24,25 dihydroxyvitamin D and 1,25-dihydroxyvitamin D to 1,24,25-trihydroxyvitamin D and increases their excretion in bile and urine. In some infants with idiopathic infantile hypercalcemia, biallelic loss-of-function mutations in *CYP24A1* have been demonstrated.[103] Delaying the degradation of 1,25-dihydroxyvitamin D prolongs its effective biologic life. Inactivating mutations in *CYP24A1* have also been found in adults with hypercalcemia, hypercalciuria, and nephrolithiasis.[104,105] *CYP24A1* mutations may either decrease the binding of the enzyme to its substrate or interfere with the interaction of heme and enzyme protein; either type of mutation decreases function of the enzyme protein.[106] In most patients hypercalcemia resolves within the first several years of life, but it may persist to older ages. Avoidance of vitamin D, low dietary calcium intake, and glucocorticoids to reduce

intestinal absorption of calcium are therapeutic modalities for this disorder.

Antenatal Bartter syndromes, type 1 (MIM 601678) and type 2 (MIM 241200) are clinically and biochemically identical and are due to biallelic loss-of-function mutations in genes controlling transepithelial transport of chloride (*SLC12A1*) and potassium (*KCNJ1*), respectively, into the lumen of the renal tubular thick ascending limb of the loop of Henle (TALH). Affected fetuses develop polyhydramnios leading to premature delivery with postnatal salt wasting, hypokalemic metabolic acidosis, hypercalciuria and occasionally hypercalcemia, nephrocalcinosis, osteopenia, failure to thrive, and often death. The type 1 disorder is due to inactivating mutations of the gene encoding sodium/potassium/chloride cotransporter-2 (*SLC12A1*), the mediator of active reabsorption of sodium chloride in the TALH, whereas the type 2 syndrome is due to an inactivating mutation in the gene encoding an inwardly rectifying apical potassium channel (*KCNJ1*).[107] Hypokalemia (occasionally transient hyperkalemia in the type 2 syndrome), reduced intravascular volume, and increased levels of angiotensin sum to increase renal and systemic production of prostaglandin E2 that further inhibits sodium and chloride reabsorption in the TALH and enhances juxtaglomerular renin release. Hypochloremic, hypokalemic alkalosis, hyperprostaglandin E, hypercalciuria leading to nephrocalcinosis and osteopenia, and hypercalcemia suggest neonatal Bartter syndrome. Replacement of fluid and electrolytes and administration of potassium sparing diuretics and the cyclooxygenase inhibitors (indomethacin or a specific inhibitor of cyclooxygenase type 2) may be effective in ameliorating the biochemical and clinical manifestations of the disease.

The "blue diaper" syndrome (MIM 211000) is named for the color noted on the diaper of affected infants; it is due to a defect in intestinal absorption of tryptophan that is then metabolized to indoles by bacteria, absorbed, excreted in urine, and turned blue when oxidized. It is associated with hypercalcemia and nephrocalcinosis, but its pathogenesis has not as yet been elucidated.

Infantile hypophosphatasia (MIM 241500) is an often fatal, autosomal recessive disorder that is recognized clinically by 6 months of age and is characterized by rickets, demineralization of the calvarium and peripheral skeleton, increased intracranial pressure (due to premature cranial synostosis), flail chest, vitamin B_6-dependent seizures, hypercalcemia, and hypercalciuria.[108,109] Radiographically, spurs of cartilage and bone extend from the sides of the knee and elbow joints.[110] The disease is due to defective osteoblast synthesis of tissue nonspecific alkaline phosphatase because of loss-of-function missense, nonsense, and donor splice site mutations of *ALPL*. The lethal homozygous or compound heterozygous mutations of *ALPL* are located within or near the enzyme domain or the homodimer and tetramer interfaces.[109] Decreased alkaline phosphatase activity results in a deficit in phosphate ions and impaired hydroxyapatite formation resulting in rickets, whereas continued intestinal calcium absorption leads to hypercalcemia. Inappropriately low serum bone alkaline phosphatase activity differentiates this condition from other rachitic or osteopenic states (osteogenesis imperfecta congenita or achondrogenesis type 1A) in which alkaline phosphatase activity is usually normal or elevated. Increased urine phosphoethanolamine and serum inorganic pyrophosphate (an inhibitor of hydroxyapatite crystallization that also impairs skeletal mineralization) and pyridoxal-5′-phosphate values are consistent with the diagnosis of hypophosphatasia, whereas analysis of *ALPL* identifies the gene mutation(s) itself. Hypophosphatasia can be diagnosed prenatally by *ALPL* genotyping.[108] The hypercalcemia of infantile hypophosphatasia is managed by hydration, diuretics that act at the TALH (e.g., furosemide), and the administration of bisphosphonates (pamidronate), calcitonin, or glucocorticoids as necessary. Dietary calcium intake should be restricted and vitamin D and its metabolites avoided. Bone marrow transplantation and "stem cell boosts" of transfused donor osteoblasts have also been used to treat affected patients. Administration of recombinant TNSALP has been employed successfully to treat life-threatening hypophosphatasia.[111] A clinical trial (NCT00744042) of the efficacy and safety of biosynthetic human recombinant tissue nonspecific alkaline phosphatase fusion protein in 1-year-old patients with infantile hypophosphatasia has been completed; 9 of 11 subjects demonstrated radiographic response after 24 weeks of treatment, but many adverse effects were recorded in this study.

Evaluation and Management

After historical review (the family history is explored for members with mineral disorders and maternal status considered in depth, and the infant's intake of calcium, phosphate, and vitamin D is estimated) and physical examination (nonspecific manifestations of hypercalcemia include hypertension, subnormal growth, hypotonia, weakness, lethargy, stupor, occasionally seizures; specific findings associated with hypercalcemia include facial and cardiovascular signs of WBS, subcutaneous nodules consistent with fat necrosis, deformities of metaphyseal chondrodysplasia or infantile hypophosphatasia) have been completed, evaluation of the hypercalcemic neonate and infant continues with total serum calcium and Ca^{2+}, phosphate, alkaline phosphatase, PTH, calcidiol, and calcitriol measurements and determination of urine calcium and creatinine levels (see Table 18-6, presented later in the chapter, and Figure 18-4). Hypocalciuria in the presence of hypercalcemia suggests the presence of NSHPT. Occasionally one may encounter a neonate in whom the total calcium concentration is elevated due to hyperproteinemia but the Ca^{2+} value is normal, a state termed "pseudohypercalcemia" by some authors.[85,112] In infants with suspected WBS, microarray or FISH analysis searching for deletion of chromosome 7q11.23 should be undertaken. In those in whom NSHPT is suspected, determination of parental serum and urine calcium and creatinine values is often helpful unless the disorder has been transmitted as an autosomal recessive disease.[89,90] Genotyping of CASR should be undertaken when *NSHPT* is being considered.

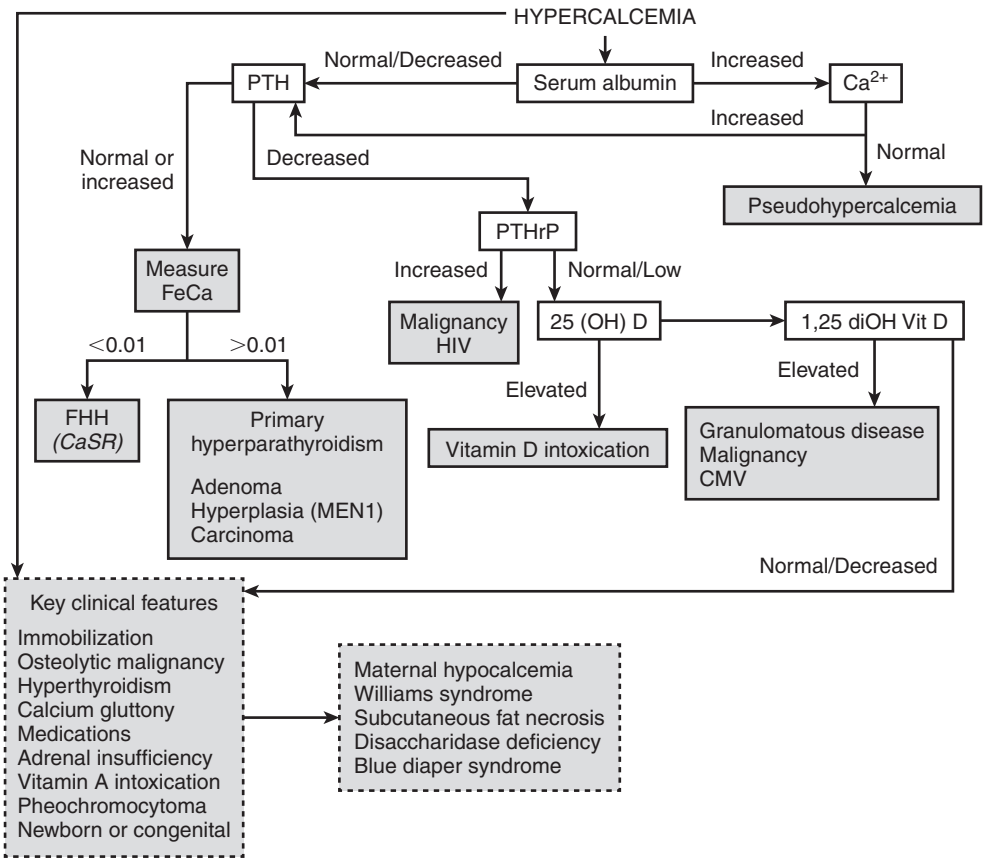


FIGURE 18-4 ■ Evaluation of hypercalcemia. FeCa, fractional excretion of urinary calcium. (From Lietman, S. A., Germain-Lee, E. L., & Levine, M. A. (2010). Hypercalcemia in children and adolescents. *Curr Opin Pediatr*, *22*, 508–515.)

Treatment of hypercalcemia in neonates and infants focuses on preventing its progression, identifying its cause, and assessing its severity. Use of a formula low in calcium and avoidance of vitamin D (excessive intake or sunlight) are helpful in the majority of neonates with modest hypercalcemia.[58] Infants with significantly elevated serum calcium levels, particularly if long standing, are often dehydrated. Immediate treatment consists of infusion of 0.9% sodium chloride with 30 mEq/L of potassium chloride (10 to 20 mL/kg over 1 hour); after dehydration has been corrected and adequate urine flow established, and if substantial hypercalcemia persists, an intravenous bolus injection of furosemide (1 to 2 mg/kg) may be administered. Currently, bisphosphonates, analogs of pyrophosphate that adsorb to the surface of hydroxyapatite crystals and are incorporated into and then disable the osteoclast and thereby inhibit osteoclast dissolution of bone mineral and matrix, are the agents of choice for the treatment of substantial hypercalcemia in infants. Etidronate (5 mg/kg twice daily orally) and pamidronate (0.5 to 2 mg/kg in 30 mL normal saline intravenously over 4 hours) have been successfully employed in infants with hypercalcemia due to vitamin D intoxication, subcutaneous fat necrosis, NSHPT, and other causes.[85,113,114] The effects of bisphosphonate last for several weeks to months and when administered in excess can lead to hypocalcemia, hypophosphatemia, and hypomagnesemia.[85] Other agents that may be considered in the treatment of a hypercalcemic infant include hydrocortisone (l mg/kg intravenously every 6 hours) to reduce intestinal calcium absorption and salmon calcitonin (2 to 4 units/kg subcutaneously every 12 hours) to inhibit calcium mobilization from bone. Inasmuch as the type II calcimimetic—cinacalcet—has been demonstrated experimentally to improve the function of many inactive mutant forms of the CASR and to be of clinical benefit in an older subject with familial hypocalciuric hypocalcemia, a therapeutic trial of this agent may be considered in a patient with NSHPT with appropriate safeguards and permissions.[85,115] In the newborn with life-threatening NSHPT, total parathyroidectomy may be urgently required.[114] Postoperatively, the patients may become hypocalcemic—as calcium is avidly deposited in bone ("hungry bone syndrome")—and require large amounts of supplemental calcium until stabilized.

Infants with hypercalcemia due to WBS (and other forms of hypercalcemia as well) may be managed by ingestion of a low-calcium formula (Calcilo XD, < 10 mg of calcium per 100 calories; no vitamin D) and withholding of supplemental vitamin D. Hypercalcemia in WBS subjects usually remits by 2 years of age. Infants with "idiopathic" hypercalcemia of infancy due to inactivating mutations of *CYP24A1* should receive the low-calcium formula and supplemental vitamin D should be withheld.

Mineral homeostasis must be monitored closely in infants receiving a low-calcium formula and from whom vitamin D is being withheld in order to prevent deficiencies of both nutrients.

Hypercalcemia in the Child and Adolescent

Etiology

Causes of hypercalcemia in infants, children, and adolescents are listed in Tables 18-4A and B. In the presence of a normal serum protein concentration, hypercalcemia occurs when the "set point" for serum Ca^{2+} is increased due to a loss-of-function mutation in *CASR* or the set point is reversibly increased by lithium, or when the rate of entry of calcium into the extracellular and circulatory compartments from bone, the intestinal tract, or the kidney exceeds its rate of loss.[112] The resorption rate of bone mineral may be increased by excessive secretion of PTH or PTHrP, constitutive activation of PTH1R, excessive vitamin D or metabolites, increased production of osteoclast-activating inflammatory cytokines or localized osteolytic processes such as metastatic neoplasms, and dissociation of the rates of bone formation and resorption (e.g., immobilization). The absorption rate of intestinal calcium may be increased by excessive intake of calcium, by hypervitaminosis D of exogenous or endogenous origin, or by increased secretion of PTH or PTHrP. Augmented renal tubular reabsorption of filtered calcium may occur with the administration of calcium-sparing diuretics such as thiazides, an effect that may "unmask" hypercalcemia in a previously eucalcemic patient with hyperparathyroidism.[116] The total serum calcium concentration but not the Ca^{2+} concentration increases in the presence of hyperalbuminemia (pseudohypercalcemia). Venous stasis (e.g., by tourniquet) results in spuriously altered local pH and Ca^{2+} values.

Familial hypocalciuric hypercalcemia type 1 (HHC1) is an autosomal dominant disorder with 100% penetrance at all ages characterized by PTH-dependent usually asymptomatic (total and ionized) hypercalcemia with hypocalciuria (and, therefore, an absence of nephrocalcinosis or calcium-containing renal calculi), hypermagnesemia, hypomagnesuria, and hypophosphatemia that is due to heterozygous loss-of-function mutations in *CASR*.[117-119] Consequently, higher serum concentrations of Ca^{2+} are required to inhibit synthesis and secretion of PTH and to suppress renal tubular reabsorption of calcium. Serum concentrations of PTH may be normal or slightly elevated but inappropriately high for the Ca^{2+} level; calcidiol and calcitriol values are normal. In children, HHC1 is most commonly suspected initially by the presence of unexpected hypercalcemia (11 to 13 mg/dL) in a chemistry profile or through family screening of a parent or other relative with hypercalcemia. Older subjects with HHC1 may complain of fatigue, weakness, or polyuria; there is a slightly increased incidence of relapsing pancreatitis, cholelithiasis, chondrocalcinosis, and premature vascular calcification in subjects with HHC1, but bone mass and fracture rate are normal. Because of decreased parathyroid chief cell membrane CaSR number or function, the set point for Ca^{2+} suppression of PTH secretion is reset upward.[115,117] The parathyroid glands are slightly hyperplastic. In renal tubular cells, decreased CaSR number and activity lead to increased renal tubular reabsorption of filtered calcium and relative hypocalciuria (ratio of calcium clearance/creatinine clearance < 0.01 in 80% of patients with HHC1); renal tubular reabsorption of magnesium is also increased; urinary concentrating ability and other measures of renal function are normal; in hypercalcemia of other pathogenesis, urinary calcium excretion is increased and renal concentrating ability depressed. Usually, HHC1 requires no therapy but must be differentiated from mild primary hyperparathyroidism in which hypomagnesemia and hypercalciuria are present. Subtotal parathyroidectomy in HHC1 does not lower calcium levels as the residual parathyroid glands hypertrophy; total parathyroidectomy is unnecessary except in some infants with NSHPT. More than 100 nonsense, missense, insertion, and deletion mutations in *CASR* associated with HHC1 or NSHPT have been identified, mostly in the receptor's extracellular Ca^{2+} binding domain; these mutations either decrease receptor affinity for Ca^{2+} or alter intracellular processing of the CaSR (glycosylation, dimerization [e.g., Arg66His, Asn583Stop]) in the endoplasmic reticulum, preventing its translocation to the surface of the cell membrane[115,119,120] (Figure 18-5). Many of the mutations are located in the extracellular domain of CaSR between codons 39 to 300, a region rich in aspartate and glutamate residues in which Ca^{2+} may nestle.[43] Glycosylation is necessary for both dimerization and trafficking of the CaSR. Missense mutations involving arginine at codon 66 (Arg66His, Arg66Cys) result in a product that is able to be only partially glycosylated; it can form homodimers in the endoplasmic reticulum but cannot enter the Golgi apparatus and be transported to the cell plasma membrane.[120] Experimentally, approximately 50% of the products of inactivating mutations of *CASR* can be "escorted" to the plasma membrane and functionally stabilized by the allosteric calcimimetic cinacalcet (NPS R-568), proffering a potentially therapeutic agent for the management of patients with symptomatic hypercalcemia due to loss-of-function *CASR* mutations, especially those with NSHPT, if it proves safe to do so (as noted earlier).[115] Mutations in *CASR* tend to be unique to the affected family. In approximately one third of families with HHC1, no mutations in the coding region of *CASR* have been identified; they may have a mutation in a noncoding region of *CASR* or an abnormality in genes associated with the HHC1 phenotype that have been identified on chromosomes 19p13.3 (HHC2, MIM 145981) and 19q13 (HHC3, MIM 600740).[117] Autoantibodies against the amino terminal extracellular domain of the CaSR that reversibly inhibit receptor activity have been identified in patients with acquired HHC1; this disorder may be responsive to glucocorticoid therapy.[73,121] In some patients with primary or uremic secondary hyperparathyroidism, there is reduced expression of *CASR* and an increased set point for suppression of PTH secretion.

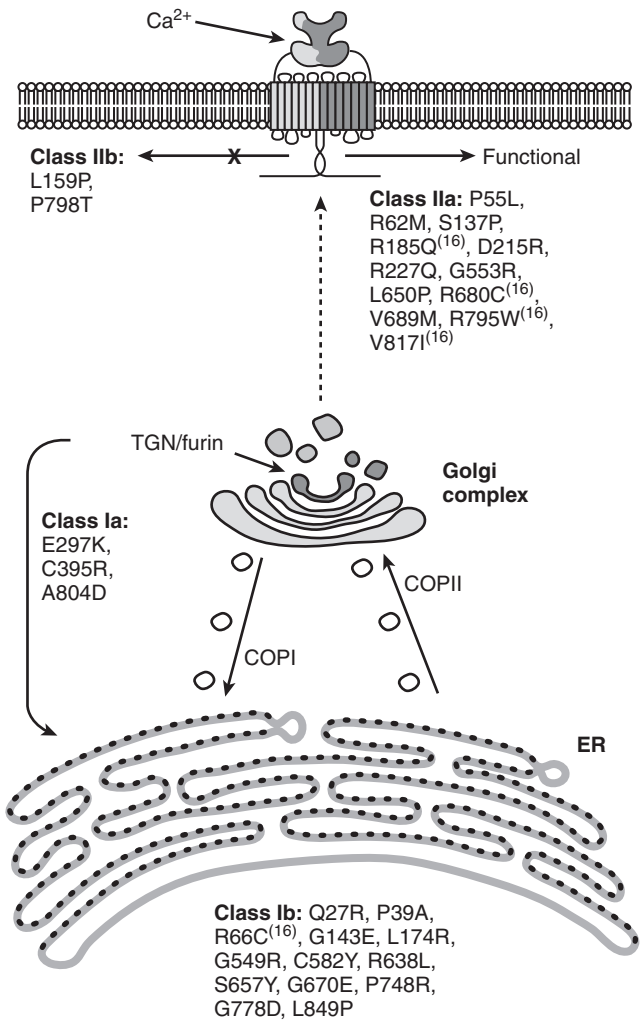


FIGURE 18-5 ■ Movement of the calcium sensing receptor through the endoplasmic reticulum (ER) and Golgi complex to the cell membrane. Inactivating mutations of *CaSR* may arrest movement of CaSR at various sites along this pathway including the cell membrane. Depending on the site of arrested movement, the mutations are designated classes Ia (Golgi), Ib (endoplasmic reticulum), and II (plasma membrane). Functional activity of class IIa CaSR mutants can be "rescued" by a pharmacochaperone inserted into the plasma membrane and attain functionality, whereas class IIb CaSR mutants are resistant to pharmacochaperone administration and remain inert. (From White, E., McKenna, J., Cavanaugh, A., & Breitwieser, G. E. (2009). Pharmacochaperone-mediated rescue of calcium sensing receptor loss-of-function mutants. *Molec Endocrinol, 23,* 1115–1123.)

Primary hyperparathyroidism is an unusual childhood disorder with an incidence of 2 to 5/100,000 as compared with that in adults of approximately 100/100,000.[122,123] In adults with hyperparathyroidism, females outnumber males 3:1; in children the female-to-male ratio is closer to 1. In older children and adolescents, primary hyperparathyroidism is most often a sporadic disease and usually the result of a single parathyroid adenoma. It also occurs as an autosomal dominant disorder in familial isolated primary hyperparathyroidism (MIM 145000) due to germline mutations in the genes responsible for multiple endocrine neoplasia (MEN) type I (*MEN1*) or the hyperparathyroidism-jaw tumor syndrome (*CDC73*).[84,85,114,122,124,125]

The majority of children and adolescents with hypercalcemia due to hyperparathyroidism are symptomatic at the time of diagnosis (personality and behavioral changes, particularly depression, headache, malaise, proximal muscle weakness, anorexia, abdominal cramping, nausea and vomiting, constipation, polydipsia and polyuria) or present with symptoms reflecting the consequences of this disorder such as flank pain and hematuria due to renal calculi (hypercalciuria), abdominal pain (pancreatitis), or pathologic fractures (through areas of osteopenia or lesions of osteitis fibrosa cystica).[93,114,123] Although the physical examination may reveal slight weakness of proximal musculature, it is unusual to palpate a cervical mass in these patients. Hypercalcemia, hypophosphatemia, and elevated serum concentrations of intact PTH are present in the majority of children with hyperparathyroidism. Ultrasonography, magnetic resonance imaging, computed tomography, three-dimensional imaging, and radionuclide scans (^{99m}Technetium-labeled sestamibi-single photon emission CT scan) have been employed to localize the abnormal parathyroid gland(s) prior to surgical excision (as discussed earlier). (Sestamibi is methoxyisobutyl isonitrile, a lipophilic cation.) Occasionally, the parathyroid tumor may be located ectopically in the thymus, thyroid gland, or mediastinum.

In subjects with primary hyperparathyroidism, hypercalcemia is the result of increased secretion of PTH due to loss of the normal relationship between the set point of serum Ca^{2+} and PTH synthesis and release and to Ca^{2+}-independent (constitutive) PTH secretion related to the mass of parathyroid tissue.[122] Pathologically, hyperparathyroidism in children is most often due to a chief cell adenoma involving one parathyroid gland, but adenomas in several parathyroid glands and diffuse hyperplasia (particularly in patients with multiple endocrine neoplasia type 1) and rarely carcinoma of the chief cells may also occur.

The majority of parathyroid adenomas are monoclonal in origin—that is, a single mutant cell develops into a tumor. In adolescents, parathyroid tumors may rarely develop after external radiation of the neck for treatment of lymphoma. In some parathyroid tumors, increased expression of cyclin D1 (encoded by *CCND1*, MIM 168461, chromosome 11q13.3) has been demonstrated; cyclins are intracellular proteins that regulate cyclin-dependent protein kinases that control the rate of cellular transition from G1 to S in the cycle of cell division.[43] Overexpression of *CCND1* in parathyroid chief cells is at times the result of a somatic chromosome mutation—inversion (rotation) of regions 11p15 and 11q13 in which the promoter region of *PTH* is repositioned to serve as a promoter for *CCND1*, thereby increasing the rate of chief cell division whenever the (hypocalcemic) stimulus for PTH generation is received and leading ultimately to (benign) tumor formation. However, in many parathyroid adenomas there is increased activity of cyclin D1 without this chromosomal rearrangement.[122] In patients with parathyroid adenomas and carcinomas, overexpression of the retinoblastoma and p53 tumor-suppressor genes, whose products normally inhibit the cell cycle at

the G1/S step, has also been demonstrated as have somatic mutations in *CDC73*.[126] In approximately 35% of sporadic parathyroid adenomas, a somatic loss-of-function mutation of the tumor suppressor factor menin—the germline mutation in patients with MEN type 1—can be identified together with loss of heterozygosity on chromosome 11.[127] Germline loss-of-function mutations in *MEN1* and *CASR* have also been detected in patients with isolated primary hyperparathyroidism in association with multiglandular involvement; in this instance, patients with *CASR* mutations did not have the typical biochemical findings of HHC1 (Table 18-4B).[124,128] Chronic renal insufficiency leads to secondary hyperparathyroidism due to hyperplasia of the parathyroid glands and also to monoclonal parathyroid tumors (tertiary hyperparathyroidism) associated with somatic chromosomal deletions in some instances.

Isolated autosomal dominant familial primary hyperparathyroidism (HRPT1) or in association with multiple ossifying fibromas of the jaw (HRPT2) is due to loss-of-function heterozygous germline mutations in the tumor suppressor gene encoding parafibromin *(CDC73R)*.[114,129] Hyperparathyroidism occurs in 80% of subjects with a mutation in *CDC73* at a mean age of 32 years, but it may also appear in children younger than 10 years of age.[130] In these patients, the parathyroid lesion may be an atypical, potentially premalignant cystic adenoma (65%), hyperplasia (20%), or even carcinoma (15%); the parathyroid tumor may be an isolated finding or it may be associated with maxillary or mandibular bone tumors composed of ossified fibrous tissue; renal (Wilms tumor, papillary renal cell carcinoma, hamartoma, polycystic kidney), pancreatic (carcinoma), and uterine (tumor) lesions may also develop in these patients.[43,124] Lesions within the parathyroid glands may develop asynchronously. *CDC73* is a 17 exon gene that encodes parafibromin, a 531-amino-acid nuclear protein that is a component of a complex of accessory factors that modulates the activity of RNA polymerase II and of a histone methyltransferase (SUV39H1) and hence regulates gene expression and cell proliferation—functioning as both a transcriptional activator and repressor.[131,132] Parafibromin is also a component of the WNT signaling system; experimentally, overexpression of *CDC73* increases WNT signaling activity through β-catenin.[133] For a parathyroid tumor to develop, a "second hit" must occur that results in a loss of heterozygosity—that is, the germline inactivating mutation of *CDC73* on one allele must be matched by a mutation in or deletion of *CDC73* in the remaining normal allele.[134] When a germline mutation in *CDC73* has been detected, screening of family members for this mutation and longitudinal evaluation of affected subjects is recommended as asymptomatic individuals with atypical adenoma or carcinoma of the parathyroid glands may be so identified.[135] Somatic mutations in *CDC73* have also been identified in atypical parathyroid adenomas and many parathyroid carcinomas (MIM 608266), but they are unusual in the typical sporadic parathyroid adenoma.[136] Familial isolated hyperparathyroidism type 3 (HRPT3, MIM 610071) has been linked to chromosome 2p14-p13.3, but a specific mutated gene in this region has not as yet been identified.

The syndromes of multiple endocrine neoplasia (MEN) are familial autosomal dominant diseases of high penetrance associated with the development of tumors in two or more endocrine glands within a single individual.[124,125] There are four defined MEN syndromes: 1, 2A, 2B, and 4 (Table 18-5). MEN1 is characterized by development of tumors of the parathyroid glands (adenoma), pituitary (prolactinoma, somatotropinoma), and enteropancreatic unit (gastrinoma, insulinoma, pancreatic polypeptide-oma, carcinoid). In addition, neoplasms of the adrenal cortex, neuroendocrine tumors of the bronchopulmonary tree and thymus, lipomas, angiofibromas, collagenomas, and meningiomas develop in affected subjects.[125] The postmortem incidence of MEN1 is 0.25%; in hyperparathyroid subjects it is 1% to 18%; in patients with gastrinoma it is 16% to 38%.[125] Hyperparathyroidism (due to a solitary parathyroid adenoma or tumors within several parathyroid glands or hyperplasia of all four parathyroid glands) is the most common manifestation of MEN1 occurring in more than 90% to 95% of affected patients; it is the most frequent endocrinopathy in children with MEN1, at times developing before the child is 10 years of age. Unlike other forms of hyperparathyroidism, equal numbers of males and females are affected in MEN1. Pituitary tumors secreting prolactin or growth hormone often (30% to 40% of MEN1 subjects) develop as do gastrin- (Zollinger-Ellison syndrome), insulin-, and glucagon-secreting tumors of the pancreatic islets and gastrointestinal system (30% to 70% of patients); these neoplasms also occur in children and adolescents with MEN1.[43,124] Hypercortisolemia in patients with MEN1 may be due to excessive secretion of adrenocorticotropin by a pituitary adenoma or ectopically by a neoplasm or to a primary adrenal tumor. Thyroid neoplasms occur in 25% of patients with MEN1. Nonendocrine tumors such as lipomas (34%), intestinal and bronchial carcinoids, and other intestinal neoplasms are reasonably common in subjects with MEN1. Indeed, the dermatologic manifestations of MEN1 (angiofibromas, 85%; collagenomas, 70%; more than three angiofibromas and any collagenomas) are extremely sensitive and specific indicators of this disease.[137] Patients with MEN1 may also develop a schwannoma or a pheochromocytoma, the latter a tumor most often present in patients with MEN types 2A and 2B. Although most of the tumors that develop in MEN1 are benign but functionally hyperactive, those of pancreatic, intestinal, and foregut origin may be malignant.

Germline mutations in *MEN1*, a 10 exon gene that encodes a 610-amino-acid cytoplasmic and nuclear protein termed menin, have been demonstrated in the majority of patients with familial and sporadic forms of MEN1. Menin regulates cell growth, division, and demise in two sites and through multiple pathways. In the cytoplasm, menin binds AKT1 (protein kinase B—MIM 164730, chromosome 14q32.33), preventing its translocation to the cell membrane where it is ordinarily activated by phosphorylation enabling AKT1 to exert proliferative and antiapoptotic effects; by binding of menin to AKT1, both the cytosolic level of AKT1 and its kinase activity are depressed, thereby suppressing its effects on cell division and survival, a property lost when

TABLE 18-5 **Multiple Endocrine Neoplasia Syndromes**

Subtype	Gene	Chromosome	Site of Frequent Tumors	Mutations
MEN 1	*MEN1*	11q13.1	Parathyroid (90%)	Intron 4 N:5168 G->A (10%)
			Enteropancreatic (30%-70%): gastrinoma,	Codons 83-84 (4%)
			Insulinoma, pancreatic polypeptide,	Codons 119 (3%)
			nonfunctional	Codons 209-211 (8%)
			Anterior pituitary (30%-40%): prolactinoma	Codon 418 (4%)
			GH, ACTH, nonfunctional	Codon 516 (7%)
			Adrenocortical (40%): diffuse and nodular hyperplasia, adenoma, carcinoma	
			Intestinal: gastric neuroendocrine (10%),	
			Thymic neuroendocrine (2%)	
			Other: facial angiofibroma (85%), collagenoma (70%),	
			lipoma (30%), meningioma (8%)	
MEN 2	*RET*	10q11.21		
MEN 2A			MCT (90%)	Codon 634 (Cys->Arg, 85%)
			Pheochromocytoma (50%)	
			Parathyroid hyperplasia (20%-30%)	
			Cutaneous lichen amyloidosis	
MEN 2B (3)			MCT (> 90%)	Codon 918 (Met->Thr, > 95%)
			Pheochromocytoma (40%-50%) Associated:	
			Marfanoid habitus	
			Mucosal neuromas (90%)	
			Medullated corneal nerves	
Megacolon				
MEN 2C			MCT (100%)	Codon 618 (> 50%)
MEN 4	*CDKN1B*	12p13.1	Parathyroid	
			Pituitary	
			Testicular/cervical	
			Renal/adrenal	

MCT, medullary carcinoma of thyroid.

Adapted from Marx, S. J., & Simonds, W. F. (2005). Hereditary hormone excess: genes, molecular pathways, and syndromes. Endocr Rev, 26, *615–661; Thakker, R. V., Newey, P. J., Walls, G. V., et al. (2012). Clinical practice guidelines for multiple endocrine neoplasia type 1 (MEN1).* J Clin Endocrinol Metab, 97, *2990–3011.*

MEN1 sustains an inactivating mutation.[138] Menin enters the nucleus with the guidance of two nuclear localization signals in its carboxyl terminus (amino acids 479 to 498, 589 to 608) where it is involved directly in the regulation of transcription, replication, and survival. By binding directly to JunD, menin blocks JunD-mediated inhibition of transcription of activating protein-1 (and consequently cell division); many of the mutations in *MEN1* in patients with MEN1 cluster in exon 4 and interrupt the binding of these two proteins (between menin amino acids 139 to 142 and 323 to 428). By interacting with SMAD3 (MIM 603109, chromosome 15q22.3), menin inhibits signaling by transforming growth factor (TGF) β and impairs TGFβ-mediated inhibitory control of cell replication.[139] Interaction of menin with the SMAD 1/5 complex inhibits signaling by bone morphogenetic protein (BMP) 2; menin also inhibits the transcription regulating protein—nuclear factor κB (NFκB).[43,140] Further, menin interacts directly with a histone methyltransferase complex and with genes that regulate DNA repair and cell replication and apoptosis such as tumor suppressor *TP53* (p53), *CDKN1A*, *CDKN1B*, *GADD45A*, *POLR2A*, *BBC3*, *TP5313*, and *FAS*.[141]

In subjects with heterozygous germline loss-of-function mutations in *MEN1*, unregulated cell growth and tumor formation occur when a second insult leads to loss of *MEN1* on the normal allele within susceptible tissues. As with *CDC73* (discussed previously), the "two hit"

hypothesis of tumorigenesis in MEN1 denotes that the patient inherits germline susceptibility to neoplasia superimposed on which is a second insult leading to loss of heterozygosity for chromosome segment 11q13 and biallelic loss of *MEN1*; the "second hit" may be deletion of a segment of chromosome 11 that includes 11q13 or a mutation (missense, frameshift) within the wild-type *MEN1* allele itself, an observation that also extends to MEN type 1 tumors with somatic mutations in *MEN1*.[124] Several hundred germline mutations in *MEN1* have been identified in patients with MEN1; 25% have been nonsense mutations, 15% missense mutations, 45% frameshift insertions or deletions, with more than 80% leading to the synthesis of an inactive product due to loss of the nuclear localization signal or the ability to bind to JunD or other downstream factor. Especially susceptible germline mutational "hot spots" in *MEN1* are nucleotide 5168 G ⇒ A transitions, which result in a novel splice site in intron 4, codons 83 to 84, 118 to 119, 209 to 211, 418, and 516 where collectively mutations have been identified in 37% of patients with MEN1.[142] On either side of many of these sites are segments of repeat DNA sequences of single nucleic acids or of dinucleotides to octanucleotides; this configuration may lead to increased susceptibility to "replication-slippage" because of misalignment of the nucleotide repeat segments during DNA replication permitting deletion or insertion of nucleotides at inappropriate sites. Mutations within the exonic/intronic splice site coding region of *MEN1* have been detected in approximately 70% of patients with familial MEN1; likely in the remaining 30% of subjects there is either deletion of one *MEN1* allele or a mutation in one of its noncoding regions.[143] New mutations of *MEN1* occur sporadically in 10% of patients with MEN1. In only 7% of patients with a variant of MEN1 in which only tumors of the parathyroid gland and the adenohypophysis are present has a mutation in *MEN1* been identified.[144] Familial autosomal dominant, isolated primary hyperparathyroidism has been variably associated with germline mutations (e.g., Val184Glu, Glu255Lys, Gln260Pro) in *MEN1* as well. Somatic mutations in *MEN1* have also been identified in patients with sporadic, isolated tumors of the parathyroid glands, pancreatic islet cells, anterior pituitary, and adrenal cortex.[43] Clinically apparent disease due to mutations in *MEN1* increases with advancing age: at 10 years of age, 7% of children with a mutation in *MEN1* have a detectable endocrinopathy (primarily hyperparathyroidism); 52% of affected 20-year-old subjects manifest one or more tumors; penetrance increases to 87% by 30 years, to 98% by 40 years and to 100% by 60 years.[125]

There are three forms of multiple endocrine neoplasia type 2: types 2A, 2B (also termed type 3), and 2C. Medullary carcinoma of the thyroid (MCT) is the most common neoplasm encountered in MEN 2A occurring in 90% of patients (MIM 171400) (see Table 18-5).[125,143] It evolves from antecedent parafollicular C-cell hyperplasia within the thyroid gland. These subjects also develop adrenal medullary pheochromocytomas (50%), parathyroid hyperplasia or adenoma (20% to 30%), localized cutaneous lichen amyloidosis (suprascapular pruritic deposits of subepidermal keratin), and partial or complete megacolon. Calcitonin, the secretory product of thyroid C cells, is also produced in large amounts by MCT; its measurement aids in the diagnosis of MCT and in monitoring the response of this tumor to therapy. In addition to MCT and pheochromocytoma, patients with MEN 2B (MIM 162300) have a marfanoid habitus, mucosal neuromas of the lips and tongue, gastrointestinal ganglioneuromas, medullated corneal nerve fibers, and megacolon, but they do not develop parathyroid disease.[124,125,143] Familial isolated MCT is a variant of these disorders. Germline heterozygous gain-of-function mutations in the *RET* proto-oncogene underlie the pathogenesis of the type 2 hereditary multiple endocrine neoplasias (Figure 18-6). *RET* is a 20-exon gene encoding an 860 amino acid glycosylated cell membrane tyrosine kinase receptor with extracellular, transmembrane, and intracellular domains that is expressed in tissue of neural crest origin (sympathetic ganglia, adrenal medulla, thyroid parafollicular cells). The natural ligand of this receptor is glial cell line–derived neurotrophic factor (GDNF—MIM 600837, chromosome 5p13.2). Constitutively activating mutations of one of six cysteine residues in the extracellular domain of RET—codons 609, 611, 618, 620, 630, and particularly codon 634 (Cys -> Arg)—are present in patients with MEN 2A. Loss of but one cysteine residue facilitates receptor homodimerization without ligand binding, thereby activating the RET intracellular tyrosine kinase domain resulting in autophosphorylation of critical tyrosine residues (particularly at codons 1015 and 1062) and subsequent signal transduction.[124] An activating mutation has been identified at codon 918 (Met -> Thr) in more than

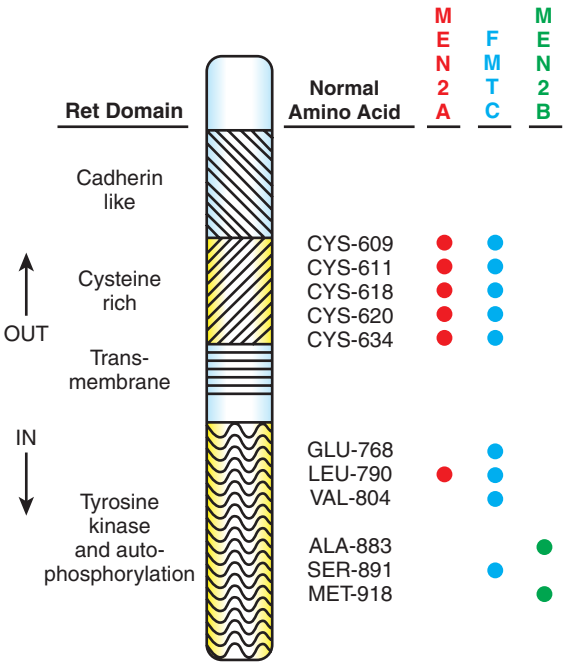


FIGURE 18-6 ■ Schematic depiction of the domains of the *RET* protooncogene with sites of activating mutations in *RET* associated with multiple endocrine neoplasia types 2A and 2B and familial medullary carcinoma of the thyroid. (From Marx, S. J., & Simonds, W. F. (2005). Hereditary hormone excess: genes, molecular pathways, and syndromes. *Endocr Rev, 26*, 615–661.) *This image can be viewed in full color online at ExpertConsult.*

95% of patients with MEN 2B; this site lies within the tyrosine kinase domain and this mutation permits signal transduction and neural cell transformation and differentiation in the absence of both ligand binding *and* receptor homodimerization. The Met918Thr MEN 2B-associated *RET* mutation often arises de novo from sporadic mutations that occur in the germline of an older father, because this mutation confers a "selective advantage" upon the mutated spermatocyte.[145] Other missense mutations of *RET* associated with MEN 2B have been identified at codons Val804, Ala883, and Ser904. Missense mutations at *RET* codons 609, 611, 618, 620, and 634 within the extracellular domain and at codons 768, 790, 804, and 891 within the intracellular tyrosine kinase domain have been found in patients with familial MCT. Two coreceptors, GFRA1 and GFRA2, interact with RET protein, but no role of the coreceptors in the pathogenesis of MEN 2 has yet been identified. In addition to the disorders designated "multiple endocrine neoplasia," other familial syndromes associated with the development of tumors of the endocrine and other systems include Von-Hippel-Lindau (adrenal medulla, pancreas, neuroendocrine), Beckwith Wiedemann (adrenal cortex, rhabdomyosarcoma, Wilms, hepatoblastoma), Carney complex (adrenal cortex, testes, pituitary, thyroid), Cowden (thyroid, endometrial, breast), McCune-Albright (pituitary, adrenal cortex), neurofibromatosis type I (adrenal medulla, thyroid, pancreatic, carcinoid), tuberous sclerosis (pituitary, parathyroid, pancreatic, adrenal medulla, carcinoid), Peutz-Jeghers (thyroid, ovarian, Sertoli cell, pancreas), Li-Fraumeni (adrenal cortex, bone, breast, brain), and familial polyposis coli (thyroid, pancreatic, adrenal cortex, hepatoblastoma, sarcoma, medulloblastoma).[124,146] In children, hyperparathyroidism is an unusual manifestation of the McCune-Albright syndrome due to a postzygotic activating mutation of *GNAS*.[147]

Ingestion of excessive amounts of vitamin D or calcitriol for therapeutic reasons (treatment of rickets, hypoparathyroidism, or other causes of hypocalcemia), megavitamin intake, manufacturing errors in the preparation of vitamin supplements, or inappropriate fortification of milk are major causes of hypervitaminosis D.[148,149] Topical application of creams containing vitamin D or an analog (e.g., 22-oxacalcitriol) for treatment of psoriasis might also lead to hypercalcemia, particularly if the urinary excretion of calcium is compromised.[150] Patients with granulomatous diseases (noninfectious: sarcoidosis, berylliosis, eosinophilic granuloma, subcutaneous fat necrosis, inflammatory bowel disease; infections: tuberculosis, histoplasmosis, coccidioidomycosis, candidiasis, cat-scratch disease) and neoplastic disorders (B-cell lymphoma, Hodgkin disease, dysgerminoma) develop hypercalcemia due to the associated monocytic (macrophage and other cells) expression of $25OHD_3$-1α-hydroxylase activity and the production of calcitriol.[151] Unlike renal tubular cells in which this enzyme is within mitochondria and the transcription of *CYP27B1* is closely regulated by PTH, calcitriol, calcium, and phosphate, in monocytes $25OHD_3$-1α-hydroxylase is microsomal in location and its gene is constitutively expressed and calcitriol synthesis quantitatively determined by the amount of substrate. The monocytic expression of *CYP27B1* is sensitive to stimulation by interferon-γ and its postreceptor signal transducer nitric oxide, as well as by leukotriene C_4; it is easily suppressed by glucocorticoids, ketoconazole, and chloroquine. Subjects with acquired immunodeficiency disease may become hypercalcemic by infection with granuloma-forming organisms or by osteoclast-activating cytokines elaborated during the course of this disorder. Elevated serum calcium concentrations have also been recorded in children with congenital hypothyroidism, primary oxalosis, congenital lactase deficiency, trisomy 21, and juvenile idiopathic (rheumatoid) arthritis.[152] In some hypercalcemic children, excessive prostaglandin production may be of pathogenetic significance. Hypercalcemia develops frequently in small subjects with the infantile form of hypophosphatasia, likely a consequence of the dissociation of the rates of low bone formation and normal bone resorption. Hypercalcemia may follow successful bone marrow transplantation in infants with osteopetrosis as functional osteoclasts rapidly reabsorb excess bone mineral.

Oncogenic or malignancy-associated hypercalcemia may be the consequence of synthesis and secretion of osteoclast activating agents such as PTHrP (rarely PTH), calcitriol, or cytokines (interleukins, TNF, TGFβ), or it may be due to direct invasion and destruction of bone by the neoplasm.[85,114,153] Although hypercalcemia occurs in less than 1% of children with cancer, it may develop even in very young patients with acute lymphatic and monocytic leukemias, Hodgkin and non-Hodgkin lymphoma, rhabdomyosarcoma, hepatoblastoma, neuroblastoma, and Ewing sarcoma.[154] Acute immobilization of the rapidly growing child with a femoral fracture or a spinal cord injury results in decreased bone mineral accretion and uncoupling of the interaction of osteoblasts and osteoclasts with increased rate of bone resorption leading to hypercalciuria and "acute disuse osteoporosis."[155] When the rate of bone resorption exceeds the renal tubular capacity for excretion of calcium, hypercalcemia ensues. Acute disuse osteoporosis and hypercalcemia can even occur in the immobilized hypoparathyroid or vitamin D depleted individual. Increased intake of calcium and absorbable alkali (milk or calcium containing antacids such as calcium carbonate) for peptic ulcer disease or as dietary supplements lead to absorptive hypercalcemia, hypercalciuria, and nephrocalcinosis. Parenteral nutrition with excessive calcium or aluminum or too little phosphate can also result in hypercalcemia. Hypophosphatemia of various etiologies leads to hypercalcemia as the body attempts to maintain the calcium x phosphate product over 30. Drugs causing hypercalcemia include: thiazide diuretics increase renal tubular resorption of calcium and decrease plasma volume; vitamin D and analogs increase intestinal absorption of calcium; vitamin A and its retinoic acid analogues stimulate bone resorption; lithium increases the set point for PTH secretion, thereby increasing serum calcium concentrations while lowering urinary calcium excretion and thus mimicking HHC1. In the thyrotoxic subject, hypercalcemia is the result of thyroid hormone-mediated stimulation of osteoclast function and the subsequent increase in the rate of bone resorption.[114] Pheochromocytomas and some islet cell tumors may be associated with hypercalcemia, in some instances

because of cosecretion of PTHrP. Hypercalcemia in the hypoadrenal patient is of uncertain pathogenesis but is not related to increased serum concentrations of PTH, calcidiol, or calcitriol, or with augmented bone resorption.

During recovery from acute renal failure, serum calcium levels may increase due to mobilization of calcium from ectopic sites such as muscle in which it had been deposited during the hyperphosphatemic phase of the illness from which it is released by rhabdomyolysis.[114] Hypercalcemia can develop in patients with chronic renal failure due to a combination of factors including immobilization, aluminum toxicity, excessive ingestion of calcium antiacids or vitamin D or its analogs, and secondary hyperparathyroidism. After renal transplantation hypercalcemia is often the result of secondary hyperparathyroidism due to hypertrophy and hyperplasia of parathyroid chief cells that occurred in response to the PTH stimulatory effects of hyperphosphatemia, hypocalcemia, and decreased synthesis of and response to calcitriol during the period of chronic renal insufficiency. In patients with compromised renal function, mild hypocalcemia and calcitriol deficiency develop when the glomerular filtration rate falls below 80 to 60 mL/min/1.73 m^2, whereas phosphate retention occurs after the glomerular filtration rate has fallen to 60 to 30 mL/min/1.73 m^2.[156,157] The secretion of PTH rises in these patients in an effort to increase the synthesis of calcitriol, raise calcium levels, and lower phosphate values. Prolonged, uncontrolled secondary hyperparathyroidism can lead to relatively autonomous parathyroid hyperfunction ("tertiary hyperparathyroidism") and hypercalcemia, primarily in patients with chronic renal failure. Hyperplasia of chief cells is followed by defects in function of the CaSR and loss of effective down-regulation of PTH secretion refractory to increased serum concentrations of Ca^{2+}. There is an expanded number of monoclonal chief cells in which the expression of *CASR* and the number of vitamin D nuclear receptors have declined. Secondary and tertiary hyperparathyroidism have occurred in patients with prolonged nutritional vitamin D deficiency rickets and in subjects with X-linked hypophosphatemic rickets receiving large amounts of phosphate.[156] Secondary hyperparathyroidism (in which by definition serum calcium concentrations are normal) also develops in patients with inadequate dietary calcium, impaired intestinal absorption of calcium (lactose intolerance, ingestion of phytates, malabsorption syndromes due to pancreatic insufficiency or celiac disease), or excessive calcium loss in the urine or soft tissues.[156] Enhanced but transient secretion of PTH may accompany the administration of GH to adolescents with chronic renal failure, likely the result of superimposing upon a high basal rate of PTH secretion further increase in the rate of bone remodeling related to GH and sex hormones. In acutely ill adults, GH administration has been associated with hypercalcemia as well.[155]

Isolated hypercalciuria in the eucalcemic child may be idiopathic or due to renal medullary or tubular dysfunction or increased intestinal absorption of calcium including mutations in genes encoding the vitamin D and calcium sensing receptors and soluble adenylyl cyclase (MIM 605205), demineralizing disorders such as juvenile idiopathic (rheumatoid) arthritis, hyperalimentation, metabolic acidosis, excessive protein ingestion, and diabetes mellitus; hypercalciuria with/without hypercalcemia may be observed in patients with familial forms of hypomagnesemia, several types of Bartter syndrome, distal renal tubular acidosis, and other disorders.[158]

Evaluation

When hypercalcemia is mild (total calcium concentration < 12 mg/dL), there may be few, if any, symptoms; affected children and adolescents are often identified by a multichannel screening study obtained for some other purpose. Hypercalcemia may also be detected during studies for renal calculi, abnormal bone mass, pathologic fractures, or during screening of families for associated problems. It should also be recognized that an elevated serum (total or ionized) calcium concentration in a single specimen may reflect assay variability and must be verified by repeated determinations in a reliable laboratory. Pseudohypercalcemia is the presence of persistently elevated total calcium concentrations in the presence of normal Ca^{2+} values and is found in hyperalbuminemia and other dysproteinemic states.[152] Symptoms attributable to hypercalcemia are independent of its cause, are related to the degree of hypercalcemia, and include intestinal symptoms such as anorexia, nausea, vomiting, abdominal pain (peptic ulceration, acute pancreatitis), and constipation; urinary symptoms such as polydipsia, nocturia, and polyuria (calcium acts as an osmotic diuretic while hypercalcemia impairs the concentrating function of the distal renal tubule); skeletal conditions such as bone pain; and neural symptoms such as headache, muscular weakness, impaired ability to concentrate, increased requirement for sleep, and altered consciousness (ranging from lethargy and confusion to irritability, delirium, stupor, and coma); on occasion, depression may be the major presenting concern in an adolescent with hypercalcemia.[112,114,116] In the toddler and young child, hypercalcemia is manifested by anorexia, constipation, poor weight gain, and impaired linear growth ("failure to thrive"). In a series of 52 children and adolescents with hypercalcemia due to primary hyperparathyroidism, 80% were symptomatic; the most common symptoms were fatigue/lethargy (35%), headache (35%), nausea (29%), vomiting (23%), and polydipsia (21%).[123] Bone involvement (low bone mass, fractures) was present in 30%, and 14% of these subjects were depressed. All of the children (N = 17) with nephrolithiasis in this series were symptomatic. In another series of 44 children and adolescents (26 girls) with primary hyperparathyroidism, mean age at diagnosis was 13 years (ranging from 6 to 18 years), overall 37 were symptomatic (anorexia, weight loss, malaise, depression), and there were 18 patients with nephrolithiasis.[93] At surgery, 29 patients had a parathyroid adenoma and 11 had hyperplastic parathyroid glands, two of whom had MEN.

Evaluation of the hypercalcemic child begins with the historical review during which the family/patient is queried not only about symptoms related to hypercalcemia and its consequences (renal calculi) but also about possibly excessive intake of vitamin D, vitamin A and related

compounds (such as Retin A for treatment of acne), calcium (perhaps to "prevent" osteoporosis), and alkali, or drugs that affect calcium metabolism (thiazide diuretics may "unmask" hyperparathyroidism by increasing renal tubular resorption of calcium and thereby raising borderline calcium concentrations into the hypercalcemic range) (see Figure 18-4). The family history is explored for members with known disorders of calcium metabolism (HHC1, hyperparathyroidism, renal calculi) or familial neoplasms (galactorrhea as a sign of a prolactinoma, severe peptic ulcer disease as an indicator of a gastrinoma and the Zollinger-Ellison syndrome). Except in extreme instances when hypertension (if normally hydrated) or bradycardia, dehydration, decreased muscular strength, or altered consciousness may be present or in the "marfanoid" subject with MEN 2B, physical examination of the hypercalcemic child and adolescent is usually normal. Rarely is a paratracheal (parathyroid) mass palpable in the hyperparathyroid patient. (Subjects with hypercalcemia due to subcutaneous fat necrosis have firm to hard, irregular, movable masses scattered about the trunk and extremities. Those with WBS have a typical face, whereas those with Jansen metaphyseal chondrodysplasia have characteristic skeletal deformities.)

After confirming the presence of total and ionized hypercalcemia, the urinary excretion of calcium is measured (Table 18-6). If the PTH concentration is normal or elevated and the calcium excretion is low, it is most probable that the patient has HHC1; this diagnosis can be substantiated by the finding of asymptomatic hypocalciuric hypercalcemia in one of the parents and further defined by identification of the inactivating mutation in *CASR*. If the patient is hypercalciuric, other causes of hypercalcemia should be sought. With highly sensitive and specific immunoradiometric and immunochemiluminometric assays for "intact" PTH[1-84] in comparison to serum calcium values, separation of patients with hyperparathyroidism from those with other causes of hypercalcemia in whom PTH values are low or normal is usually possible. In the absence of secondary hyperparathyroidism (chronic renal insufficiency, malabsorption syndromes, ingestion of thiazide diuretics or lithium), consistently elevated PTH concentrations in the hypercalcemic, hypophosphatemic, hypercalciuric child or adolescent are consistent with primary hyperparathyroidism. Although the diagnosis of primary hyperparathyroidism is usually apparent in children and adolescents, there is an occasional patient in whom serum calcium or PTH values may not be elevated in a single specimen and in whom repeated measurements of serum and urine calcium and PTH values are necessary before this diagnosis can be established. In a series of 52 children/adolescents with hyperparathyroidism, serum calcium values were normal in 10% and PTH levels in 15%; however, in all subjects the PTH concentration was inappropriately increased relative to the calcium level.[123] Hypercalcemia, hypophosphatemia, and elevated PTH concentrations were recorded in all 44 children and adolescents (6 to 18 years of age at diagnosis) with primary hyperparathyroidism in another series.[93] Osteitis fibrosa cystica, brown tumors (localized non-neoplastic areas of bone resorption composed of osteoclast-like multinuclear giant cells, fibroblast-like spindle shaped cells, and hemorrhagic infiltrates), and subperiosteal and endosteal bone resorption can be detected radiographically in most children with hyperparathyroidism, whereas cortical (distal radial) bone mineral density is likely to be decreased in these subjects. Nonspecific findings in the hypercalcemic subject of diverse etiology include shortening of the QT interval by electrocardiography because the rate of cardiac repolarization is accelerated by increased calcium levels, bradycardia, and first-degree atrioventricular block, and nephrocalcinosis and renal calculi detected by abdominal ultrasonography. Serum concentrations of PTHrP should be measured when clinical and laboratory findings are consistent with primary hyperparathyroidism, but PTH values are low and humoral hypercalcemia of malignancy is suspected. When PTH concentrations are low in the hypercalcemic patient, metabolites of vitamin D (calcidiol, calcitriol) should be measured and other causes of hypercalcemia sought (discussed later).

TABLE 18-6 **Laboratory Findings in Hypercalcemic Patients**

Disorder	Calcium	Phosphate	Urine Calcium	PTH	PTHrP	25OHD	$1,25(OH)_2$ D
NSHPT	⇑⇑⇑	⇓	⇓	⇑⇑	⇓	N	⇑
FHH/HHC	⇑, ⇑⇑	⇓	⇓	⇑	⇓	N, ⇓	⇑
Primary hyperparathyroidism	⇑, ⇑⇑	⇓	⇑	⇑,⇑⇑	⇓	N	⇑
Vitamin D intoxication	⇑	⇑	⇑⇑	⇓	⇓	⇑⇑	⇑
WBS	⇑	⇑	⇑	⇓	⇓	N	⇑
Immobilization	⇑, ⇑⇑	⇑	⇑⇑	⇓	⇓	N	⇓
Malignancy	⇑⇑	⇓	⇑	⇓	⇑⇑	N	N, ⇑, ⇓
Granulomatous disease	⇑	⇑	⇑⇑	⇓	⇓	N	⇑⇑
Subcutaneous fat necrosis	⇑	⇑	⇑	⇓	⇓	N	⇑⇑

N, normal; ⇑ increased; ⇑⇑ greatly increased; ⇓ decreased; NSHPT, neonatal severe hyperparathyroidism; FHH, familial hypocalciuric hypercalcemia; PTH, parathyroid hormone; PTHrP, PTH-related peptide.

Adapted from Lietman, S. A., Germain-Lee, E. L., & Levine, M. A. (2010). Hypercalcemia in children and adolescents. Curr Opin Pediatr, 22, *508–515; Benjamin, R. W., Moats-Staats, B. M., Calikoglu, A., et al. (2008). Hypercalcemia in children.* Pediatr Endocrinol Rev, 5, *778–784.*

Preoperatively, a parathyroid adenoma may be localized by high-resolution ultrasonography, computed tomography, magnetic resonance imaging, or radionuclide scanning with ^{99m}Tc-sestamibi. The latter radionuclide is taken up by both the thyroid and parathyroid glands but quickly "washed out" from the thyroid gland; thus, two scans obtained 2 hours apart permit differentiation of parathyroid from thyroid tissue (Figure 18-7).[122] Alternatively, a simultaneously administered second radionuclide selectively accumulated by the thyroid (^{123}iodine) may be employed to visualize parathyroid tissue. Scans may be obtained by conventional two-dimensional or computed tomographic techniques, the latter offering a three-dimensional image. Rarely is it necessary to undertake selective venous catheterization with sampling of local PTH levels or arteriography to identify the site of a parathyroid adenoma. Evaluation for associated endocrine tumors is necessary if the family history suggests the possibility of MEN 1 or MEN 2A or if there are clinical findings (galactorrhea, excessive growth, hypertension) to suggest the presence of a prolactinoma, somatotropinoma, or pheochromocytoma. Patients at risk for MEN may be screened by determining basal and stimulated serum concentrations of prolactin, GH, IGF-I, gastrin, glucagon, pancreatic polypeptide, calcitonin, catecholamines, and other substances as warranted. Indeed, it is reasonable to consider screening children with hyperparathyroidism preoperatively for an associated pheochromocytoma or tumors of the maxilla and mandible and for mutations in *RET*, *MEN1*, and *CDC73*.[124]

In the patient with hypercalcemia due to ingestion of excessive amounts of vitamin D, serum concentrations of calcidiol are markedly elevated; in those receiving exogenous calcitriol or in hypercalcemic patients with granulomatous, chronic inflammatory, and lymphomatous diseases, serum levels of calcitriol are increased. Other disorders associated with hypercalcemia (see Tables 18-4A and B) should be eliminated by appropriate historical findings and laboratory and genetic studies.

Management

Appropriate management of the patient with hypercalcemia depends on the severity and the cause of the high calcium levels. It is important to distinguish the patient with pseudohypercalcemia (due to hyperproteinemia) from one with a pathologic cause of hypercalcemia so that effective medical and surgical therapy may be initiated promptly. It is equally important to recognize the child/adolescent with HHC1 so that aggressive therapy is avoided. When the calcium concentration is < 12 mg/dL in the asymptomatic subject, treatment may be delayed until the cause of the hypercalcemia is understood; in the interim, it is reasonable to recommend that the patient increase fluid intake, avoid calcium and vitamin D supplements, and discontinue drugs associated with hypercalcemia if relevant. The child with HHC1 often has serum total calcium concentrations between 11 to 13 mg/dL but no clinical symptoms and requires no therapy under usual circumstances. In the absence of HHC1, if the total serum calcium concentration exceeds 12 mg/dL or if the child is symptomatic (anorexia, nausea, altered sensorium, polydipsia, polyuria, dehydration, muscular weakness), efforts to lower its level are usually necessary because of the adverse effects of hypercalcemia on cardiac, central nervous, renal, and gastrointestinal function. In these children and adolescents, diagnostic studies as outlined here and treatment to lower the serum calcium concentration should begin simultaneously. The tools of therapy of severe hypercalcemia are usually employed sequentially as follows: (1) hydration—with 0.9% saline (twice maintenance volume over 24 to 48 hours)—restores intravascular volume, dilutes and decreases serum Ca^{2+} levels, increases glomerular filtration of Ca^{2+}, decreases reabsorption of Ca^{2+} in the proximal and distal renal tubules, and promotes calciuresis; hydration alone usually lowers the total serum calcium concentration 1 to 3 mg/dL; (2) calciuresis—intravenous infusion of the loop diuretic furosemide (1 mg/kg slowly) initiated only

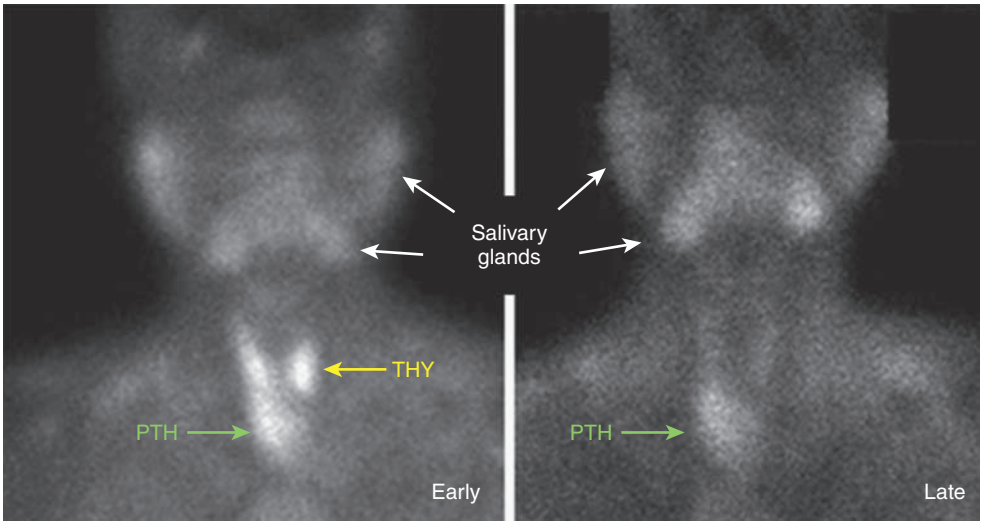


FIGURE 18-7 ■ Visualization of a parathyroid adenoma with technetium-99m sestamibi scanning. (From Steenkamp, D., & Lee, S. L. (2011). Hypercalcemic crisis in a young woman. Endocrine Today, October. Reprinted with permission from SLACK Incorporated.) *This image can be viewed in full color online at ExpertConsult.*

after restoration of extracellular fluid volume with saline—further lowers calcium levels by inhibiting resorption of calcium (and sodium) by the TALH (thiazide diuretics are to be avoided as they increase renal tubular reabsorption of calcium and increase serum calcium concentrations and are clearly contraindicated in the management of hypercalcemia); (3) inhibition of bone resorption—if hypercalcemia does not respond to the preceeding measures, specific inhibitors of osteoclast function may be employed. Bisphosphonates (pamidronate, zoledronic acid) are the agents of choice in the acute management of hypercalcemia in children and adolescents. Bisphosphonates are nonhydrolyzable, phosphatase-resistant analogues of pyrophosphate; in vivo by binding to and coating the hydroxyapatite crystal beneath the osteoclast, they interfere with osteoclast attachment, and by incorporation into the osteoclast, they interfere with its cellular metabolism thereby inhibiting its functional ability to dissolve bone and hastening its death. Pamidronate (0.5 to 1 mg/kg/dose by intravenous infusion over 4 to 6 hours) effectively lowers serum calcium concentrations in hypercalcemic infants and children.[85,113] The hypocalcemic effect of bisphosphonates is variable in duration (days to weeks); transient systemic side effects (fever, myalgia) may accompany the administration of pamidronate and other bisphosphonates. Salmon calcitonin also acts rapidly but transiently to lower serum calcium concentrations by inhibiting osteoclast activity and increasing urinary calcium excretion; it must be administered by multiple daily subcutaneous injections (2 to 4 U/kg/injection every 6 to 12 hours). However, calcitonin tachyphylaxis develops several days after its administration, thus limiting its usefulness.[159] Calcitonin may be paired with a bisphosphonate at the beginning of treatment of hypercalcemia in order to lower serum calcium levels more rapidly.[116] Glucocorticoids do not lower calcium levels in patients with hyperparathyroidism or solid tumor malignancies but are quite effective in the management of hypercalcemia due to excess vitamin D ingestion or calcitriol production by activated monocytes or hematologic malignancies. In adults with hypercalcemia of malignancy, gallium nitrate has also been employed to lower serum calcium values, but it must be administered over 5 days; gallium inhibits osteoclastic bone resorption directly.[159] Rarely, it may be necessary to dialyze (peritoneal or hemodialysis with low- or zero-calcium dialysate) the severely hypercalcemic patient resistant to conventional therapy. During acute treatment of hypercalcemia, Ca^{2+} concentrations may be assessed indirectly by monitoring the (shortened) Q-T interval by electrocardiography, but serial measurements of total and Ca^{2+} are necessary to monitor the efficacy of therapy and to prevent hypocalcemia.

Although in older adults (> 50 years) with asymptomatic primary hyperparathyroidism (without bone disease or renal stones), no immediate intervention may be advised at times, in younger adults, children, and adolescents with hyperparathyroidism, surgical intervention is recommended when the diagnosis is established. Although hyperparathyroidism in youth is most often (> 60%) due to an adenoma, hyperplasia of the parathyroid glands is also common (~30%).[93,123] As discussed, prior to surgical intervention in the pediatric population with hyperparathyroidism it is important to localize a parathyroid adenoma employing one of several imaging techniques such as echography with Doppler analysis, magnetic resonance, computed tomography, or ^{99m}Technetium-sestamibi combined with computed tomography. It is also possible to construct a three-dimensional virtual model of the neck by pairing iodine contrast with thin-slice computed tomographic imaging with color contrast enabling clear definition of the cervical vasculature and its normal and abnormal structures.[160] The operating surgeon must be experienced and expert in parathyroid surgery. In adults and older adolescents, minimally invasive focused procedures to remove an anatomically identified parathyroid adenoma(s) may be employed utilizing ^{99m}Technetium-sestamibi administered 2 hours before surgery with dissection directed by insertion of a hand-held gamma probe until the adenoma is located and removed. Video assistance is an alternative method for localization of a parathyroid adenoma. A complementary technique for assessing the completeness of the removal of the adenoma intraoperatively is to measure peripheral levels of PTH by rapid immunoassay before and 10 minutes after removal of the adenoma; a 50% decline in PTH levels indicates complete excision; if the PTH concentration does not decline following removal of suspected abnormal parathyroid tissue, further exploration is undertaken and additional tissue removed as guided by the serum PTH level. These procedures are reported to be cost effective as they decrease operative time and patient morbidity; many patients return home within hours after leaving the operating suite. These techniques have been applied to the surgical management of the child and infant with a parathyroid adenoma or hyperplasia of multiple parathyroid glands. If there is parathyroid hyperplasia, total parathyroidectomy is performed and autotransplantation of small fragments of one gland to a forearm pocket undertaken. Following removal of a parathyroid adenoma, many patients develop transient hypocalcemia that may be managed by the administration of supplemental oral calcium. When there is severe osteitis fibrosa cystica and marked demineralization, substantial hypocalcemia may occur as a result of the "hungry bone" syndrome. Other complications of surgery include (transient or permanent) vocal cord dysfunction and the need for further operations because of the development of a second adenoma. If permanent hypoparathyroidism develops postoperatively, it is treated by the administration of calcitriol and supplemental calcium as needed.

Calcimimetic agents (phenylalkylamines; e.g., cinacalcet) bind to and activate the membrane CaSR on the parathyroid chief cell and thereby increase cytosolic levels of $Ca^{2+}{}_I$ and depress secretion of PTH in adults with mild primary hyperparathyroidism.[122] The use of these agents in children and adolescents with this disorder offers a potential treatment pathway for subjects with diffuse parathyroid hyperplasia. Indeed, this agent has been useful in the management of infants with milder forms of NSHPT. (However, in early 2013 the Food and Drug Administration suspended the use of cinacalcet in children and adolescents pending further evaluation of its safety in this population.) The secondary hyperparathyroidism of

chronic renal disease is best managed by lowering serum phosphate concentrations to the extent possible by limiting intake, administering oral phosphate binding agents, and maintaining serum Ca^{2+} levels within the low-normal range by the administration of calcitriol or analog; calcimimetic agents (e.g., cinacalcet) may also be useful in this situation with the caveat noted previously.[161] Parathyroidectomy may be necessary for effective management of refractory secondary and tertiary hyperparathyroidism as manifested by severe renal osteodystrophy, hypercalcemia, and systemic symptoms such as pruritus and bone pain.[156]

Hypercalcemia due to hypervitaminosis D or excessive production of calcitriol by granulomatous and chronic inflammatory tissues may be treated with glucocorticoids to suppress activity of $25OHD_3$-1α-hydroxylase. Ketoconazole (3 to 9 mg/kg/day in three divided doses) is an antifungal agent that also inhibits renal $25OHD_3$-1α-hydroxylase activity and promptly lowers calcitriol and calcium values in children and adults with similar disorders. Side effects of therapy with ketoconazole include nausea, vomiting, abdominal pain, depressed secretion of gonadal steroids, and adrenal production of cortisol. Therefore, careful monitoring of patients receiving ketoconazole is essential. Glucocorticoids ameliorate the hypercalcemia related to excessive interleukin-1β production in adolescents with juvenile rheumatoid arthritis. An attempt should be made to prevent hypercalcemia in the immobilized child or adolescent by ingestion of a low-calcium diet, avoidance of vitamin D, copious fluid intake, and early mobilization. Serum and urine calcium levels should be monitored frequently and fluids increased still further if hypercalciuria occurs. Once present, hypercalcemia is best treated by mobilization; saline diuresis or bisphosphonate administration may be necessary until eucalcemia is restored. Restriction of dietary calcium and limitation of exposure to sunlight may be appropriate in the long-term management of some patients with hypercalcemia not amenable to more specific treatment. Antiprostaglandin agents may be useful in the child with hypercalcemia associated with excessive production of these compounds. Specific treatment of diseases accompanied by hypercalcemia (thyrotoxicosis, hypoadrenocorticism) restores the eucalcemic state.

DISORDERS OF MAGNESIUM METABOLISM

In serum, magnesium is presented complexed to proteins and ionized or free. Approximately 50% of body magnesium stores are deposited within bone adsorbed to the surface of hydroxyapatite. The CaSR recognizes and responds to magnesium (Mg^{2+}) as well as calcium. In the kidney, 10% to 20% of filtered Mg^{2+} is reabsorbed in the proximal renal tubule and 65% to 70% of filtered Mg^{2+} is reabsorbed in the thick ascending limb of the loop of Henle by a passive paracellular process; 10% to 20% of filtered Mg^{2+} is reabsorbed in the distal convoluted tubule by an active transcellular system.[162] In the distal convoluted tubule, Mg^{2+} reabsorption occurs through a cation channel encoded by *TRPM6* and supporting components (discussed later). Mg^{2+} regulates the secretion but not the synthesis of PTH and the generation of calcitriol.[3]

Hypomagnesemia

Hypomagnesemia (serum total magnesium concentration < 1.5 mg/dL) leads to hypocalcemia by inhibiting the release of PTH and by interfering with its peripheral action. Hypomagnesemia may be congenital or acquired (Tables 18-7A and B). It occurs in infants born to mothers deficient in magnesium, those with preeclampsia or gestational or type 1 diabetes mellitus, and neonates with low birth weight due to prematurity or intrauterine growth restriction.[163] Prolonged nasogastric suctioning, malabsorptive disorders due to extensive intestinal resection ("short gut" syndrome), intestinal fistulas, or other

TABLE 18-7A **Disorders of Magnesium Homeostasis**

I Hypomagnesemia

A Congenital

1 Neonates of mothers with diabetes mellitus, eclampsia, magnesium deficiency
2 Intrauterine growth restriction, prematurity
3 Gene mutations (see Table 18-7B)

B Acquired

1 Drugs: loop and thiazide diuretics, antibiotics (amphotericin, gentamycin), sympathomimetic agents, proton pump inhibitors (omeprazole), digitalis, antineoplastic agents (cisplatin), cyclosporin
2 Nasogastric suction
3 Intestinal malabsorption (celiac disease, intestinal resection)
4 Secretory diarrhea (inflammatory bowel disease)
5 Acute pancreatitis
6 Hypermagnesuria due to diabetes mellitus or alcoholism or following renal tubular necrosis or renal transplantation
7 In association with hypokalemia

II Hypermagnesemia

A Acquired

1 Renal insufficiency
2 Excessive intake of magnesium salts or laxatives/antacids that contain magnesium
3 Diabetic ketoacidosis, adrenocortical insufficiency, hyperparathyroidism, lithium intoxication

TABLE 18-7B **Gene Mutations Associated with Hypomagnesemia**

Type MIM	Clinical	Gene	Chromosome	OMIM Gene	Inheritance
1 602014	Intestinal and renal malabsorption hypocalcemia	*TRPM6*	9q21.13	607009	AR
2 154020	Hypocalciuria	*FXYD2*	11q23.3	601814	AD
3 248250	Nephrocalcinosis	*CLDN16*	3q28	603959	AR
4 611718	Normocalciuria	*EGF*	4q25	131530	AR
5 248190	Hypercalciuria and nephrocalcinosis	*CLDN19*	1p34.2	610036	AR
6 613882	Normomagnesuria	*CNNM2*	10q24.32	607803	AD
Hypomagnesemia with myokymia 176260	Ataxia, myokymia	*KCNA1*	12p13.32	176260	AD
Hypomagnesemia with MODY5* 137920	MODY5 and renal cysts	*HNF1B*	17q12	189907	AD
SeSAME syndrome 612780	Seizures, sensorineural deafness, ataxia, mental retardation	*KCJN10*	1q23.2	602208	AR
Antenatal Bartter syndrome type 2 241200	Polyhydramnios, prematurity, failure to thrive	*KCNJ1*	11q24.3	600359	AR
Gitelman syndrome 263800	Muscle weakness, dermatitis	*SLC12A3*	16q13	600968	AR

*MODY, maturity onset diabetes of youth.
AR, autosomal recessive; AD, autosomal dominant.

diseases associated with chronic diarrhea and steatorrhea also lead to infantile hypomagnesemia. In renal tubular disorders such as Gitelman and Bartter syndromes (discussed later) as well as in subjects exposed to diuretics and nephrotoxic (cisplatin, cyclosporin, mercury, gentamycin) agents, hypermagnesuria leads to hypomagnesemia. In infants, children, and adolescents, hypomagnesemia may be clinically silent or it may be manifested by heightened neuromuscular irritability (carpal pedal spasm, tetany, seizures) and when prolonged and profound by muscle wasting, weakness, apathy, and tachycardia with prolonged PR and QT intervals by electrocardiography. In these age groups, hypomagnesemia may be primary and due to a specific defect in the intestinal absorption of Mg^{2+} or in the renal tubular resorption of filtered Mg^{2+} (discussed later) or secondary and due to gastrointestinal losses (chronic vomiting or diarrhea or malabsorptive states due to inflammatory bowel disease, bowel resection or fistulas, or pancreatitis), associated renal tubulopathies (Gitelman and Bartter syndromes), exposure to alcohol, diuretics, and chemotherapeutic agents, and specific endocrinopathies (diabetes mellitus, primary hyperparathyroidism, hyperaldosteronism).[2,164]

Gene mutations that lead to hypomagnesemia are listed in Table 18-7B.[162,165] Type 1 hypomagnesemia with hypocalcemia is an autosomal recessive disorder pathophysiologically due to a selective small intestinal defect in transcellular absorption of Mg^{2+}. The disease presents with hypocalcemic tetany or seizures in the neonatal period and can lead to myocardial, renal, and arterial calcinosis. Renal excretion of Mg^{2+} is normal in subjects with this disease. Hypocalcemia is attributable to decreased secretion of and peripheral insensitivity to PTH. This disorder is due to biallelic loss-of-function mutations in *TRPM6* (transient receptor potential cation channel, subfamily M, member 6), encoding a 2,022 amino acid protein with two functional domains: a Ca^{2+}- and Mg^{2+}-permeable ion channel domain and a protein tyrosine kinase domain that is expressed in the intestinal tract and kidney.[166] For full functional activity, TRPM6 must team with its homologue TRPM7 (MIM 605692) and form a functional TRPM6/TRPM7 complex at the surface of the cell.[167] Although most mutations of *TRPM6* associated with this disease (nonsense, deletion) have resulted in extensive loss of product, the naturally occurring missense mutation Ser141Leu specifically disrupts formation of the complex and hence the transcellular absorption of Mg^{2+}. Oral ingestion of large quantities of magnesium is effective therapy for this illness. Monoallelic inactivating mutations in *FXYD2* result in autosomal dominant hypomagnesemia type 2. *FXYD2* encodes a gamma subunit of a Na^+/K^+-ATPase expressed in the renal distal convoluted tubule. Loss-of-function mutations of *FXYD2* result in misrouting of its protein product to the basolateral membrane of the nephron's epithelial cells and leads to increased urinary

loss of Mg^{2+} but paradoxically to increased renal tubular reabsorption of Ca^{2+} in the loop of Henle. Type 3 primary hypomagnesemia is an autosomal recessive disorder due to decreased renal tubular paracellular reabsorption of filtered Mg^{2+} linked to biallelic loss-of-function mutations in *CLDN16*. This disease often presents in infancy and is associated with tetany, hypermagnesuria, hypercalciuria, mild hypocalcemia, nephrocalcinosis, impaired renal function, and secondary hyperparathyroidism. Claudin 16 (also termed paracellin 1) is a 305-amino-acid protein with four transmembrane domains and intracellular amino and carboxyl terminals that is expressed within the intercellular tight junctions of renal epithelial cells in the TALH and distal convoluted tubule where it facilitates paracellular transport and reabsorption of Mg^{2+} and Ca^{2+} from the renal tubule. The first extracellular loop of claudin 16 bridges the intercellular space and is the site of paracellular conductance of ions; a number of missense mutations in *CLDN16* have been identified, particularly at leucine 151 (Leu151Phe, Leu151Trp, Leu151Pro).[168] Most of the mutations in CLDN16 impair its normal movement to the renal epithelial cell's lateral surface; in other mutations (Ala62Val, His71Asp), products localize to the tight junctions but are functionally defective.[169] Oral administration of 20 times the normal daily requirement of magnesium has been successful therapy in these subjects. Biallelic inactivating mutations in *EGF* result in type 4 hypomagnesemia, a disorder associated with seizures and developmental delay but in which serum and urine levels of calcium are normal.[170] EGF is necessary for normal distal renal tubular Mg^{2+} resorption by TRPM6. The complex of hypomagnesemia, hypercalciuria, and visual impairment (macular colobomata, myopia, nystagmus) is designated type 5 hypomagnesemia and is due to biallelic loss-of-function mutations in *CLDN19*, a second renal epithelial tight junction protein that is localized to the distal renal tubule and eye and is necessary for paracellular transport of calcium and magnesium.[171] In addition to ocular anomalies (colobomata, nystagmus, severe myopia), mutations in *CLDN19* result in hypomagnesemia, hypercalciuria, nephrocalcinosis, and renal failure. Hypomagnesemia type 6 is an autosomal dominant disorder that presents in childhood or adolescence and is due to heterozygous loss-of-function mutations in *CNNM2*.[172] Despite hypomagnesemia, urine excretion of Mg^{2+} is inappropriately normal; serum and urine calcium values are normal in these subjects. *CNNM2* encodes a protein localized to the basolateral membrane of the renal thick ascending loop of Henle and the distal convoluted tubule where its expression is up regulated by Mg^{2+} deficiency. One function of CNMM2 may be to "sense" ambient Mg^{2+} concentrations.

Hypomagnesemia associated with myokymia (quivering of muscles) has been related to a heterozygous loss-of-function mutation (Asn255Asp) in *KCNA1* encoding a renal potassium channel that colocalizes with TRPM6 in the renal distal convoluted tubule.[173] Mutated KCNA1 may impair the ability of TRPM6 to reabsorb Mg^{2+} from the distal convoluted tubule leading to hypermagnesuria and hypomagnesemia. Hypomagnesemia has also been associated with mutations in *HNF1B* encoding hepatocyte nuclear factor 1 homeobox B, a transcription factor necessary for normal embryogenesis of the kidney and pancreas.[174] Inactivating mutations in HNF1B lead to malformations of the kidney (cysts, renal malformations) and early onset of diabetes mellitus. Hypomagnesemia is encountered in patients with hypokalemic metabolic acidosis in association with the SESAME syndrome (seizures, sensorineural deafness, ataxia, mental retardation, and electrolyte imbalance due to inactivation mutations in a gene—*KCNJ10*—encoding a potassium channel), antenatal Bartter syndrome type 2 (polyhydramnios, prematurity, poor postnatal growth, salt wasting, and hypercalciuria due to inactivating mutations in a gene encoding a second potassium channel—*KCNJ1*), and Gitelman syndrome, a variant of Bartter syndrome that presents in later childhood or adolescence or young adulthood (muscle weakness, potassium wasting, and hypocalciuria due to loss-of-function mutations in a gene encoding a thiazide-sensitive Na^+ Cl^- cotransporter—*SLC12A3*) (Table 18-7B).

The presence of hypomagnesemia is identified by measurement of serum Mg^{2+} concentrations, whereas its pathophysiologic etiology is determined by concurrent assay of calcium, phosphate, sodium, potassium, chloride, bicarbonate, creatinine, PTH, and vitamin D levels and assessment of its urinary loss and intestinal absorption.[163] Hypomagnesemic, hypocalcemic seizures are only transiently responsive and sometimes resistant to parenteral administration of elemental calcium alone. Intravenous or intramuscular administration of a 50% solution of magnesium sulfate ($MgSO_4.7H_20$, 0.05 to 0.1 mL/kg or 2.5 to 5 mg/kg elemental magnesium with cardiac monitoring) is often necessary to control convulsions in the hypomagnesemic neonate.[2,163] Oral magnesium supplements may also be helpful (50% $MgSO_4.7H_20$, 0.2 mL/kg/day). Chronic hypomagnesemic states are treated with oral magnesium supplements as tolerated, as excessively large doses may lead to diarrhea.

Hypermagnesemia

Hypermagnesemia (total magnesium > 2.5 mg/dL) is frequently recorded in the neonatal period after magnesium sulfate has been administered to the pregnant women with hypertension, preeclampsia, or toxemia of pregnancy. Most neonates with hypermagnesemia are asymptomatic; however, when serum Mg^{2+} concentrations are exceptionally high, hypotonia and depression of the central nervous system may be present and when extensive, metabolic bone disease may develop.[163] Thus, prolonged (9 to 10 weeks) administration of intravenous magnesium sulfate to women with multiple fetuses who have entered labor prematurely has been associated not only with hypermagnesemia but also with marked hypocalcemia in the offspring as well as significant osteopenia.[175] Hypermagnesemia may also result from its parenteral administration or oral ingestion of magnesium-containing antacids or enemas. In large amounts, magnesium sulfate suppresses secretion of PTH and decreases renal tubular reabsorption of calcium, factors contributing to hypocalcemia. The

hypermagnesemic neonate is most appropriately managed by adequate hydration to permit urinary excretion of the high magnesium load; if the newborn is also hypocalcemic and osteopenic administration of calcium and calcitriol is indicated. Hypermagnesemia may also develop in patients with renal insufficiency receiving magnesium-containing antacids. Mg^{2+} concentrations are modestly increased in patients with familial hypocalciuric hypercalcemia due to loss-of-function mutations in *CASR*.

DISORDERS OF SKELETAL MINERALIZATION

Disorders of Bone Mineralization in the Neonate and Infant

Low Bone Mass/Rickets

Rickets is a disorder of bone mineralization in the growing infant, child, and adolescent due to lack of vitamin D or its functional efficiency, calcium or phosphate, or decreased activity of alkaline phosphatase (Table 18-8).

TABLE 18-8 **Disorders of Bone Mineralization: Rickets**

I Vitamin D Deficiency

A Decreased Intake, Endogenous Synthesis, Retention, or Sequestration
1 Maternal vitamin D deficiency: breastfeeding
2 Reduced skin synthesis: sunlight deprivation, sunscreen use, increased skin pigmentation
3 Malabsorption: celiac disease, hepatobiliary dysfunction, short gut syndrome, cystic fibrosis, inflammatory bowel disease, gastric bypass surgery
4 Drugs: anticonvulsants, glucocorticoids, cholestyramine, antiretroviral agents
5 Nephrotic syndrome
6 Obesity

B Metabolic Errors
1 Deficiency of 25-hydroxylase
a Loss-of-function mutation of *CYP2R1*
b Hepatic dysfunction
2 Deficiency of 25OH-vitamin D_3-1-hydroxylase
a Loss-of-function mutation of *CYP27B1*
b Decreased renal mass: hypoplasia, chronic renal insufficiency
3 Impaired response to vitamin D
a Loss-of-function mutation of *VDR*
b Excessive heterogeneous nuclear ribonucleoprotein C1/C2 (*HNRNPC*)

II Calcium Deficiency, Primary

A Nutritional Deprivation
B Hypercalciuria: Hyperprostaglandin E2 Syndromes

III Phosphate Deficiency/Hypophosphatemia

A Transcellular Shifts
1 Glucose or insulin infusion
2 Refeeding
3 Respiratory alkalosis; hyperventilation
4 Salicylate poisoning
5 Catecholamines

B Decreased Intestinal Absorption
1 Nutritional deprivation
a Low-birth-weight infant
2 Decreased intestinal absorption
a Vitamin D deficiency: nutritional, synthetic error, insensitivity
b Aluminum, magnesium, or calcium-containing antacids
c Malabsorption due to chronic diarrhea, steatorrhea

C Hyperphosphaturia
1 X-linked dominant familial hypophosphatemic rickets (*PHEX*)*
2 X-linked recessive hypophosphatemic rickets (*CLCN5*)
3 Autosomal dominant hypophosphatemic rickets (*FGF23*)*
4 Autosomal dominant hypophosphatemia with urolithiasis 1 (*SLC34A1*)†
5 Autosomal dominant hypophosphatemia with urolithiasis 2 (*SLC9A3R1*)
6 Autosomal recessive hypophosphatemic rickets 1 (*DMP1*)*
7 Autosomal recessive hypophosphatemic rickets 2 (*ENPP1*)*
8 Autosomal recessive hypophosphatemic rickets with hypercalciuria (*SLC34A3*)
9 Hypophosphatemic rickets with hyperparathyroidism (*KL*)
10 Hypophosphatemic rickets with Fanconi renotubular syndrome (*SLC34A1*)‡
11 Oncogenic hypophosphatemic rickets/osteomalacia (*FGF23*, *sFRP4*, *MEPE*, *FGF7*)
12 FGF23 excess of uncertain pathogenesis—McCune-Albright syndrome (*GNAS*),* Jansen-type metaphyseal chondrodysplasia (*PTH1R*),* osteoglophonic dysplasia (*FGFR1*),* linear nevus sebaceous syndrome,* iron polymaltose*
13 Renal tubular acidosis/Fanconi syndrome
a Heritable: cystinosis, tyrosinemia, hereditary fructose intolerance, galactosemia, idiopathic
b Acquired: nephrotic syndrome, vitamin D deficiency, renal vein thrombosis, renal tubular insult due to cadmium, lead, bismuth, outdated tetracycline, 6-mercaptopurine, valproic acid, ifosfamide

D Other Causes
1 Hyperparathyroidism
2 Medications: diuretics (thiazides, acetazolamide, loop diuretics); antiretroviral agents (tenofovir, adefovir); antineoplastic drugs (ifosfamide, cisplatin); aminoglycosides, imatinib

IV Hypophosphatasia (*ALPL*)

A Perinatal, infantile, childhood, adult forms
B Odontohypophosphatasia
C Pseudohypophosphatasia

V Inhibitors of Mineralization

A Aluminum, Parenteral
B Bisphosphonates
C Fluoride

*Associated with increased expression of FGF23.
†Monoallelic.
‡Biallelic.

Adapted from Gattineni, J., & Baum, M. (2012). Genetic disorders of phosphate regulation. Pediatr Nephrol, 27, *1477-1487; Hori, M., Shimizu, Y., & Fukumoto, S. (2011). Minireview: fibroblast growth factor 23 in phosphate homeostasis and bone metabolism.* Endocrinology, 152, *4–10; Imel, E. A., & Econs, M. J. (2012). Approach to the hypophosphatemic patient.* J Clin Endocrinol Metab, 97, *696–706.*

Impaired deposition of hydroxyapatite onto collagen type I fibers in bone matrix leads to skeletal deformations (craniotabes, genu valgum, genu varum, metaphyseal flaring) and fractures. In the adult, deficiencies of vitamin D, calcium, or phosphate result in osteomalacia and increased fracture risk. Osteopenia may be defined as too little bone tissue with decreased thickness of bone cortex or diminished thickness or number of bone trabeculae that may be due either to suboptimal mineralization or to decreased quantity of bone matrix collagen type I. Osteoporosis is primarily a disorder of bone matrix formation with bone mass that is so low that fractures may occur after minor trauma.[176] Osteoporosis is the consequence of decreased synthesis or excessively rapid degradation of bone matrix proteins, particularly collagen type I that results in osteopenia because of a decreased collagen scaffold upon which to deposit hydroxyapatite.

Approximately 80% of total bone calcium in the full-term neonate is accrued in the last trimester of pregnancy as the in utero rate of calcium deposition increases more than twofold between 28 and 36 weeks, gestation. Infants with a low birth weight (LBW < 1500 g) or very low birth weight (VLBW < 1000 g) are particularly vulnerable to the development of osteopenia of prematurity (defined as postnatal bone mineral content that is less than the bone mineral content of the intrauterine fetus at the same postconceptual age), because they are unable to maintain the in utero rate of synthesis of organic bone matrix (osteoid) and the rate of calcium (third trimester, 100 to 130 mg/kg/day) and phosphate deposition into osteoid from the minerals provided via the gastrointestinal tract or by parenteral nutrition.[176-179] In this neonatal population, decreased calcification of bone matrix results in low bone mineral content, whereas depressed calcification of the cartilage growth plate can lead to rickets and its characteristics deformities. Postpartum, hypocalcemia and decrease in spontaneous movement against the force exerted by the muscular wall of the uterus also depress the rate of bone mineral acquisition, whereas an increased rate of bone resorption further decreases skeletal mass in premature infants.[179,180] Approximately 30% of preterm infants with birth weights of less than 1500 grams develop osteopenia.[181] Birth weight and rate of postnatal weight gain as well as umbilical cord concentrations of IGF-I are important determinants of bone mass in premature infants.[182] Necrotizing enterocolitis, a disorder that affects approximately 10% of LBW infants, increases the rate of bone resorption as assessed by measurement of serum (carboxyl telopeptide of collagen type I = ICTP) and urinary (deoxypyridinoline [Dpd]) markers of this process.[183] Malrotation of the intestinal tract or catastrophic necrotizing enterocolitis leading to intestinal infarction requiring extensive small bowel resection substantially increases the risk of malabsorption and subsequent low bone mass. Parenteral alimentation of LBW or VLBW neonates restricts administration of fluid, calcium, and phosphate (in part, because of the incompatibility of the coinfusion of these ions in high concentrations); excessive aluminum in parenteral fluids also adversely affects bone formation. Increased maternal parity, male gender, severe systemic disease (bronchopulmonary dysplasia), immobility, and pharmacologic agents (glucocorticoids, methylxanthines such as theophylline, diuretics such as furosemide) also adversely impact bone formation in these neonates.[180,184] Theophylline and furosemide increase urinary excretion of calcium.[185] Prenatal factors that contribute to low bone mass in VLBW and LBW neonates are intrauterine growth restriction (possibly by reducing placental transport of calcium and decreasing the rate of bone formation) and prenatal exposure to large amounts of magnesium sulfate, which when administered repeatedly to the mother in preterm labor leads to hypocalcemia and osteopenia by suppressing PTH secretion through its interaction with the CaSR and by competing with calcium for deposition at bone surfaces, respectively.[175]

In preterm neonates, serum levels of total and bone specific alkaline phosphatase, carboxyl terminal propeptide of collagen type I (PICP), and osteocalcin (markers of osteoblast activity and bone formation) are elevated relative to full-term neonates and older infants and continue to rise over the first 10 weeks of life.[186] Urinary hydroxyproline and Pyr/Dpd values (markers of osteoclast activity and bone resorption) are also increased, although serum concentration of ICTP, another marker of bone resorption, decline during the first 10 weeks after premature delivery. Overall, the data indicate that intrauterine and postnatal rates of bone turnover in preterm newborns are rapid and persistently elevated through 40 weeks postconceptual age, a conclusion confirmed by bone histomorphometry.[184] By photon absorptiometry and quantitative ultrasonography, bone mass of preterm infants appears to decline during the first several weeks after birth.[187] Serum levels of alkaline phosphatase and osteocalcin and urinary excretion of Pyr and calcium may remain elevated in LBW infants with osteopenia of prematurity relative to values in LBW infants without bone disease for the first year of life even though radiographic improvement in skeletal mineralization is usually evident by 6 months of age.[181]

Because standard roentgenograms may not detect low bone mineralization before deficits of 20% to 30% or greater have occurred in the second month of life, estimation of bone mineral content (BMC) by dual energy x-ray absorptiometry (DEXA) has become the preferred method to assess bone mineralization in infants because of its accuracy, reproducibility, rapidity of performance, and low radiation exposure (2 to 3 mrem).[179] Mean whole body BMC in the first 2 days of life ranges from 21.7 g in newborns with birth weights of 1001 to 1500 g to 78.8 g in neonates with birth weights of 3501 to 4000 g, whereas whole body bone mineral density (BMD) varies from 0.146 mg/cm^2 (1001 to 1500 g) to 0.234 g/cm^2 (3501 to 4000 g). In full-term healthy neonates, the mean whole body BMC measured by fan beam DEXA is 89.3 g (SD ± 14.1).[188] BMC and BMD increase through the first year of life and beyond as measured by DEXA. No racial, gender, or seasonal factors affect bone mineralization at this age; body weight is best correlated with bone mass. Quantitative ultrasonography (QUS) may also be employed to assess bone integrity and strength in preterm and other LBW infants; QUS may be performed at the crib side, there is no exposure to radiation, and it may be repeated as frequently as necessary.[189] QUS measures the

speed of sound (SOS) through a bone (humerus, tibia, radius, patella, os calcis, metacarpal, phalanx), a measurement that is correlated with the strength of the bone; mineral content is but one of several skeletal components (elasticity, cortical thickness, microstructure) that collectively contribute to bone strength. QUS also permits calculation of bone transmission time, a measurement that determines the difference in the velocity of sound as it travels through bone and surrounding soft tissue. There is considerable overlap of SOS values at various ages and somatic sizes; however, bone transmission times may discriminate to a greater extent between these parameters. Humeral and tibial QUS measurements are lower in preterm than term infants and correlate positively with gestational age, birth weight, postnatal length, and weight.[190] In neonates with intrauterine growth restriction, tibial SOS levels may be appropriate for gestational age or lower or even elevated.[189] The relationships between bone mineral density determined by DEXA and bone strength estimated by QUS in preterm and term newborns are weak but significant.[178]

With prolonged deprivation of calcium, phosphate, and vitamin D, not only does the LBW infant lag in accumulation of bone mass, but clinical and radiographic evidence of rickets also develops—usually between the sixth and twelfth postnatal weeks—and fractures may occur in as many as 24% of VLBW infants.[187] The LBW neonate at risk for low bone mass or rickets is best managed preventively by maintaining serum calcium concentrations between 8 and 11 mg/dL and serum phosphate values between 5.8 and 9 mg/dL and by providing by daily enteral administration of as much of the needed amounts of calcium (140 to 160 mg/100 kcal), phosphate (95 to 108 mg/100 kcal of formula), and vitamin D (400 U) as well as protein (for collagen synthesis) and energy (carbohydrates, lipids) as possible. When parenteral administration of nutrients is necessary in the LBW or VLBW neonate, an attempt should be made to administer the maximum amounts of calcium and phosphate safely attainable. The solubility of calcium and phosphate depends not only on their quantities but also on the forms selected for infusion (i.e., calcium chloride, gluconate, glycerophosphate, monobasic phosphate, or dibasic phosphate). Utilizing monobasic phosphate and glycerophosphate, it is possible to infuse as much as 86 mg of calcium/kg/dL and 46 mg/kg/dL of phosphate. However, these preparations increase the risk of metabolic acidosis and hypercalciuria.[179] Infusion of parenteral nutrition solutions containing 60 to 80 mg/kg/day of calcium and 40 to 50 mg/kg/day of phosphate provides only 60% to 70% of the estimated in utero bone mineral accretion rates.[179] Therefore, enteral feeding should begin as soon as possible in the VLBW infant by fortification of human breast milk with calcium 150 to 220 mg/kg/day and phosphate 75 to 140 mg/kg/day).[177,179] Prepared formulas for feeding of LBW neonates providing 200 mg/kg/day of calcium and 100 mg/kg/day of phosphate can result in up to 90 mg/kg/day of retained calcium and 40 mg/kg/day of retained phosphate.[177] The type of prepared formula (sources of protein, fat and carbohydrates) and its lipid and mineral additives determine the rate of intestinal calcium absorption and retention; therefore, the choice of formula must be carefully considered before it is selected. Nevertheless, parenteral nutrition, fortified human milk, and available preterm formulas are unable to provide the amounts of calcium and particularly phosphate that would normally accrue to the fetus in utero. Vitamin D 400 IU/day should also be provided to the preterm infant either enterally or parenterally. Monitoring of serum levels of calcium, phosphate, creatinine, and alkaline phosphatase and urinary excretion of calcium, phosphate, and creatinine is essential in order to prevent hypercalcemia and hypercalciuria and nephrocalcinosis. It is also important to avoid hypocalcemia because of the avidity of bone matrix for calcium once remineralization has commenced ("hungry bone syndrome"). Passive physical activity (daily range of motion with extension/flexion of all joints of each extremity in the supine infant for 4 weeks beginning after the neonate has been stabilized between 2 and 6 weeks of postnatal age) with or without gentle massage of the prone infant from head to toe increases serum levels of markers of bone formation as well as bone mineralization by DEXA and QUS changes consistent with augmented bone strength.[184,187,190] The efficacy and safety of bisphosphonates or of PTH[1-34] (discussed later) in the management of osteopenia of prematurity remain to be examined. Although in the prematurely born infant bone mass may remain low through infancy and early childhood, bone mineralization eventually catches up to the norms.[191,192]

Osteogenesis imperfecta congenita (OIC), or type II (MIM 166210), is a perinatal lethal disorder most commonly associated with heterozygous spontaneous loss-of-function mutations in the genes encoding collagen-α1(I) (*COL1A*) or collagen-α2(I) (*COL1A2*) (discussed later). The mutations may be partial gene deletions resulting in decreased synthesis of the triple helix of type I collagen or missense mutations that lead to amino acid substitutions (e.g., arginine, aspartic acid, cysteine) for the glycine residues that are essential for the normal three-dimensional conformation of collagen-α1(I)/collagen-α2(I) and synthesis of the triple helix and structural integrity of type I collagen in extracellular matrix of bone on which hydroxyapatite is deposited. Osteogenesis imperfecta type II may rarely be transmitted as an autosomal dominant trait by a parent who is mosaic for a heterozygous mutation in either collagen-α1(I) or -α2(I).[193] Clinical manifestations of OIC are variable and include fractures present at birth (which may also occur in newborns with osteogenesis imperfecta types I and III), deformities of the long bones, osteopenia of the skull with large fontanelles, intrauterine growth restriction, premature delivery, and death usually in infancy due to respiratory insufficiency. Radiologically, OIC has been categorized into three subgroups: A, characterized by short, broad, and "crumpled" long bones, angulation of the tibia, and continuous beading of the ribs; B, characterized by similar femoral and tibial configurations but incomplete beading of the ribs; and C, characterized by thin long bones with many fractures and thin beaded ribs. The diagnosis of OIC is most often made clinically and by its differentiation from achondrogenesis type I, thanatophoric dysplasia, and perinatal hypophosphatasia, and it is confirmed by quantitation of subnormal amounts of collagen

synthesized by fibroblasts in vitro and identification of the mutation in *COL1A1* or *COL1A2* by direct genotyping. The lethal mutations in collagen α1(I) are clustered at sites at which the collagen monomer binds integrins, matrix metalloproteinases, fibronectin, and cartilage oligomeric matrix protein; other mutations impair posttranslational processing of collagen α1(I) and thereby interfere with association of collagen chains or propagation of the triple helix configuration.[194] Mutations in *COL1A2* encoding collagen α2(I) coincide with binding sites for proteoglycans. Indeed, the lethal mutations in *COL1A1* leading to OIC are more functionally incapacitating to the protein than is complete absence of one COL1A1 allele that leads to the less clinically severe osteogenesis imperfecta type I.[195] Although administration of the bisphosphonate pamidronate has been helpful in infants with severe manifestations of osteogenesis imperfecta types III and IV, it has been ineffective in the lethal form of osteogenesis imperfecta type II (discussed later).[196,197] Lethal forms of osteogenesis imperfecta transmitted as autosomal recessive disorders are also attributable to inactivating mutations in *CRTAP* (MIM 605497), *PPIB* (MIM259440), or *LEPRE1* (MIM 610339) (discussed later)—three factors necessary for hydroxylation of proline-986 in COL1A1 and proline-707 in COL1A2, essential modifications of these proteins.[198,199]

Lysinuric protein intolerance (MIM 222700) is an autosomal recessive disorder of hepatic and renal tubular transport of dibasic amino acids (lysine, arginine, ornithine) that manifests itself in infancy and childhood by vomiting, diarrhea, failure to thrive, developmental delay, hepatomegaly, and cirrhosis. Affected infants and children have impaired urea synthesis due to decreased hepatic uptake of ornithine but are episodically hyperammonemic with increased urinary excretion of the dibasic amino acids; there is extremely low bone mass due to marked protein deprivation and perhaps due to increase in cytokine induced bone resorption. Administration of citrulline has been reported to increase growth and bone mass in some of these patients. Lysine protein intolerance is due to loss-of-function variants in *SLC7A7* encoding an amino acid transporter including deletion of multiple exons, duplications, missense, nonsense, and splice-site mutations dispersed throughout the gene; patients with the largest deletions had the most severe phenotypes.[200,201] Osteopenia may also be observed in infants with infantile hypophosphatasia (MIM 241500) and in those with muscular disorders restricting movement such as the Prader-Willi syndrome (MIM 176270), glycogen storage disease type II (Pompe disease, MIM 232300), and forms of arthrogryposis.

Increased Bone Mass

Increased bone mass may be generalized or localized; osteosclerosis refers to thickening of trabecular bone and hyperostosis to increase in cortical bone mass.[8] The infantile "malignant" form of osteopetrosis (MIM 259700) is one of several autosomal recessive disorders due to abnormal osteoclast differentiation or function resulting in defective reabsorption of the mineral phase of bone (discussed later). Infantile osteopetrosis is clinically manifested in affected infants by failure to thrive; delayed development; nasal obstruction; loss of sight, hearing, and other cranial nerve functions; and intense bone overgrowth leading to pancytopenia and increased susceptibility to infection, hepatosplenomegaly as sites of extramedullary hematopoiesis, increased susceptibility to fracture because of decreased bone strength despite high bone mass, mandibular osteomyelitis, and death often within the first several years of life due to sepsis, anemia, or hemorrhage. Physical examination reveals impaired linear growth, enlarged head circumference, frontal bossing, nystagmus, delayed eruption of primary teeth, ecchymoses, and hepatosplenomegaly. The radiographic hallmark of infantile osteopetrosis is relatively uniform increase in bone density of the skull, vertebrae, and axial skeleton with thickened cortical and trabecular bone; "Erlenmeyer flask" deformities at the distal ends of the long bones in older children; and alternating bands of sclerotic and lucent bone in the iliac wings and metaphyses.[8] Biomarkers for osteopetrosis are increased serum levels of acid phosphatase and the brain isoform of creatine kinase. Usually lethal infantile osteopetrosis may be due to homozygous or compound heterozygous loss-of-function mutations in one of three genes: *TCIRG1* encodes a subunit of the vacuolar proton pump within the osteoclast ruffled border through which hydrogen ions are transported from the cytosol into the subosteoclast resorption lacuna; *CLCN7* encodes a chloride channel required for movement of this cation into and acidification of the resorption lacuna; *OSTM1* encodes a subunit of CLCN7 necessary for its normal posttranslational processing (discussed later). In selected subjects bone marrow transplantation providing osteoclast precursor cells has been effective in arresting progression of this disorder, albeit often with substantial residual deficits. Transient but substantial hypercalcemia may occur after this procedure. Osteopetrosis due to deficiency of *CA2* encoding carbonic anhydrase II (osteopetrosis, autosomal recessive type 3, MIM 259730) may present in infancy with failure to thrive or fracture with insignificant trauma (discussed later). Disproportionate short stature is the cardinal manifestation of pycnodysostosis (MIM 265800) and is manifest during infancy or early childhood.[8] There is a relatively large head circumference with open fontanelles and cranial sutures, dysmorphic facial features (fronto-occipital prominence, proptosis, bluish sclerae, hypoplastic maxilla, micrognathia, highly arched palate, malocclusion, beaked nose), stubby and clubbed fingers with hypoplastic nails, narrow thorax, pectus excavatum, lumbar lordosis, kyphoscoliosis, and increased fracture risk. Radiographically, there is marked osteosclerosis that increases with age, open fontanelles and cranial sutures, thin clavicles with hypoplastic lateral ends, erosion and hypoplasia of the distal phalanges and ribs, and dense vertebrae yet normal transverse processes. Histologically, there is decreased osteoblastic and osteoclastic activity. Pycnodysostosis is due to homozygous or compound heterozygous loss-of-function mutations (stop, missense, nonsense) in *CTSK*, the gene encoding cathepsin K, a lysosomal cysteine protease expressed in osteoclasts; loss of cathepsin K activity impairs degradation of collagen and the resorption of organic matrix but not that of the mineral component of bone.

Disorders of Bone Mineralization and Formation in the Child and Adolescent

Bone formation may be impaired because of lack of minerals (calcium or phosphate) or because of deficient production of bone matrix. Bone mineralization may be excessive because of an increase in the rate of mineral deposition or a decrease in the rate of resorption of the mineral phase of bone. Ectopic calcification of extraskeletal tissues may occur when local calcium and phosphate levels are high, whereas extraskeletal ossification may ensue when the regulation of skeletal bone formation is deranged.

Rickets

Rickets and osteomalacia are disorders that result from decreased mineralization of the growth plate and bone matrix, respectively, and are due to deficiencies of calcium or phosphate[202,203] (Tables 18-8 and 18-9). During endochondral bone formation in children, cartilage matrix is elaborated and subsequently mineralized. When endochondral matrix is not fully mineralized, cartilage accumulates and there is thickening of the growth plate and disorganization of the chondrocytes; the ends of the long bones (particularly those that are weight bearing) distort, and rachitic deformities ensues.[203] During the

TABLE 18-9 **Disorders of Mineralization: Rickets and Osteomalacia**

Gene Chromosome MIM	Pathophysiology	Mutation: Clinical Manifestations
Disorders of Vitamin D Metabolism		
CYP2R1: cytochrome P450, subfamily IIR, polypeptide 1 11p15.2 608713	Hepatic 25-hydroxylase: enzyme that converts vitamin D3 to 25OHD3 (calcidiol)	Biallelic loss-of-function mutation leads to vitamin D hydroxylation-deficient rickets type 1B (also termed vitamin D–dependent rickets type 1B), AR
CYP27B1: cytochrome P450, subfamily XXVII, polypeptide 1 12q14.1 609506	25OHD3-1α hydroxylase: enzyme that converts 25OHD3 to 1,25(OH)2D3 (calcitriol)	Biallelic inactivating mutations result in vitamin D–dependent rickets type 1A, AR
VDR: vitamin D receptor 12q13.11 601769	Vitamin D receptor: transcription factor that transduces the effects of calcitriol on gene activation or repression	Biallelic loss-of-function mutations lead to resistance to calcitriol and vitamin D–dependent rickets type 2A, AR
HNRNPC: heterogeneous nuclear ribonucleoprotein C 14q11.2 164020	Encodes a ribonucleoprotein that regulates gene transcription by reciprocally and transiently occupying the VDRE in the upstream promoter region of vitamin D–responsive target genes	Overexpression in vitamin D–responsive tissues leads to prolonged occupancy of the VDRE that interferes with interaction of the VDR/RXR with the VDRE resulting in vitamin D–dependent rickets type 2B
Disorders of Phosphate Metabolism		
SLC34A1: solute carrier family 34 (sodium phosphate cotransporter), member 1 5q35.3 182309	Encodes NPT2a: sodium/phosphate cotransporter expressed on the apical membranes of epithelial cells of the proximal renal tubule; under the inhibitory control of PTH	Loss-of-function mutation results in hypophosphatemic rickets with nephrolithiasis, type 1, AD; Fanconi syndrome type 2, AR
SLC34A2: solute carrier family 34 (sodium phosphate cotransporter), member 2 4p15.2 604217	Encodes NPT2b: sodium/phosphate cotransporter expressed in the small intestine, lung, and testes	Loss-of-function mutations associated with pulmonary alveolar microlithiasis and testicular microlithiasis, AR
SLC34A3: solute carrier family 34 (sodium phosphate cotransporter), member 3 9q34 609826	Encodes NPT2c: sodium/phosphate cotransporter expressed on the apical membranes of epithelial cells of the proximal renal tubule	Loss-of-function mutation results in hereditary hypophosphatemic rickets with hypercalciuria, AR
SLC9A3R1: solute carrier family 9, member 3, regulator 1 17q25.1 604990	Encodes NHERF1: a renal tubular sodium/hydrogen exchange regulatory factor that binds NPT2a anchoring it to the luminal membrane of the proximal renal tubule; phosphorylation by PTH leads to its dissociation from and endocytosis of NPT2a	Loss-of-function mutations result in hypophosphatemic rickets with nephrolithiasis, type 2, AD
CLCN5: chloride channel 5 Xp11.23-p11.22 300008	Encodes a proximal renal tubular exchanger of chloride and hydrogen ions	Loss-of-function mutations result in X-linked recessive hypophosphatemic rickets, hypercalciuria, nephrocalcinosis, XLR

Continued

TABLE 18-9 **Disorders of Mineralization: Rickets and Osteomalacia—cont'd**

Gene Chromosome MIM	Pathophysiology	Mutation: Clinical Manifestations
PHEX: phosphate-regulating endopeptidase homologue, X-linked Xp22.1 300550	Ectoenzyme expressed on the cell membrane of osteoblasts; its physiologic substrate may be MEPE and pASARM, MEPE's phosphorylated product that coats hydroxyapatite and hinders mineral deposition	Loss-of-function mutation leads to X-linked hypophosphatemic rickets; associated with increased expression of FGF23; X-linked dominant
DMP1: dentin matrix acidic phosphoprotein 1 4q22.1 600980	Noncollagenous, serine-rich, bone matrix protein; a small integrin-binding ligand, N-linked glycoprotein (SIBLING) expressed in osteocytes	Loss-of-function mutations lead to increased osteocyte synthesis of FGF23, hyperphosphaturia, and autosomal recessive hypophosphatemic rickets, AR
ENPP1: ectonucleotide pyrophosphatase/phosphodiesterase 1 6q23.2 173335	Ectoenzyme expressed by chondrocytes, bone, and plasma cells that hydrolyzes ATP to pyrophosphate, an inhibitor of bone mineralization	Loss-of-function mutations result in autosomal recessive hypophosphatemic rickets with increased expression of FGF23, AR
ANKH: ANK, mouse, homologue of 5p15.2 605145	Transmembrane-spanning cell surface protein that regulates pyrophosphate secretion	Inactivating mutations result in mild hypophosphatemia and joint ankylosis, developmental delay, deafness, and dentinogenesis imperfecta; AR
FGF23: fibroblast growth factor 23 12p13.3 605380	Product of osteoblast and osteocyte that depresses renal tubular reabsorption of phosphate and inhibits synthesis of calcitriol	Gain-of-function mutation that decreases the rate of degradation of FGF23 results in autosomal dominant hypophosphatemic rickets, AD; excessive ectopic synthesis by neoplasms leads to hypophosphatemic rickets; loss-of-function mutation leading to decreased FGF23 synthesis results in autosomal recessive hyperphosphatemic familial tumoral calcinosis, AR
GALNT3: UDP-N-Acetyl-alpha-D-Galactosamine: polypeptide N-acetylglactosaminyl transferase 3 2q24.3 601756	Encodes an enzyme that is essential for O-glycosylation of Thr178 of FGF23 during posttranslational processing; failure of this step leads to degradation of FGF23 prior to its secretion	Loss-of-function mutation leads to autosomal recessive hyperphosphatemic familial tumoral calcinosis, AR
KL: α-Klotho 13q13.1 604824	Coreceptor with FGFR1(IIIc) for FGF23 that converts FGFR1(IIIc) into the specific FGF23 receptor enabling signal transduction	Loss-of-function mutation leads to autosomal recessive hyperphosphatemic familial tumoral calcinosis, AR; translocation t(9;13(q21.13;q13.1) results in excessive klotho formation leading to hypophosphatemic rickets and hyperparathyroidism
Hypophosphatasia		
ALPL: alkaline phosphatase, liver 1p36.12 171760	Tissue nonspecific alkaline phosphatase: ectoenzyme expressed on the cell membrane of osteoblasts, removes organically bound phosphate; major substrates are pyrophosphate, phosphoethanolamine, pyridoxal-5′-phosphate	Loss-of-function mutations lead to hypophosphatasia and rickets of variable severity and onset: perinatal, infancy, transitional, childhood, adult, odontohypophosphatasia, pseudohypophosphatasia; AR, AD

Adapted from Farrow, E. G., & White, K. E. (2010). Recent advances in renal phosphate handling. Nat Rev Nephrol*,* 6*, 207–217.*

processes of modeling and remodeling of trabecular bone and the periosteal and endosteal surfaces of cortical bone, osteoid is formed by osteoblasts; failure to mineralize bone matrix in these regions results in osteomalacia. During intervals of calcium or phosphate deprivation the actively growing, weight-bearing child with open cartilage growth plates develops rickets and osteomalacia, whereas adults develop only osteomalacia during remodeling as unmineralized bone matrix accumulates. Thus, rickets is the expression of defective endochondral mineralization at the growth plate and osteomalacia is the failure of mineralization of bone cortex and trabeculae.

Clinically, rickets is manifested by skeletal deformities such as delayed closure of the fontanelles, craniotabes (reversible compression of the skull's outer table), frontal bossing (expansion of cranial bones), and occasional craniosynostosis in infants; bowing of the forearms in the preambulatory infant and genu varum or valgum in the weight-bearing infant and child; delayed tooth eruption with poor enamel formation and propensity to caries; pectus carinatum, prominence of the costochondral junctions, flaring of the lower rib cage, scoliosis, and kyphosis; flaring of the metaphyses of the long bones; and tibial or femoral torsion (Figure 18-8).[204] Radiographically,

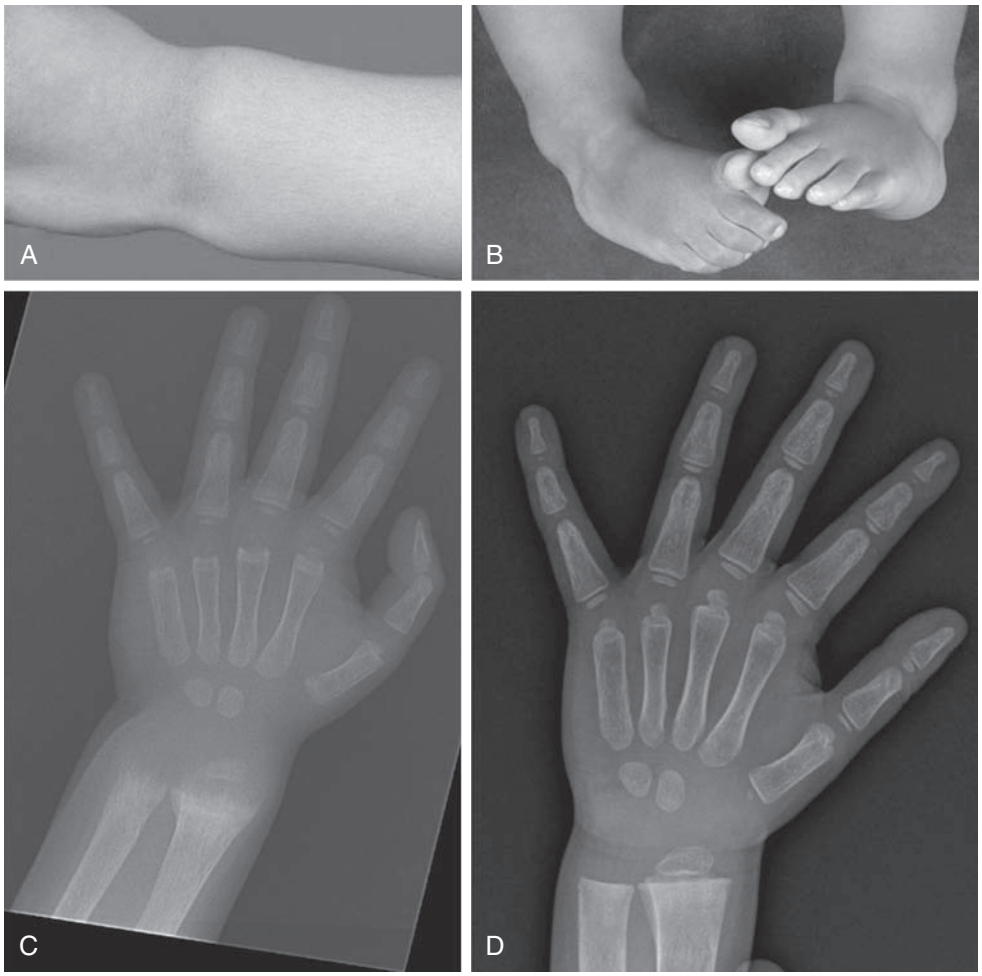


FIGURE 18-8 ■ Clinical and radiographic manifestations of vitamin D–deficient rickets. Note flaring of the distal metaphyses of the long bones and radiographic response to treatment with vitamin D. (From Girgis, C. M., Clifton-Bligh, R. J., Hamrick, M. W., et al. (2013). The roles of vitamin D in skeletal muscle: form, function, and metabolism. *Endocr Rev*, *34*, 33–83. Images are courtesy of Associate Professor Craig Munns, Children's Hospital Westmead, Sydney, Australia.)

rickets is characterized initially by epiphyseal widening and loss of the defined zone of provisional calcification followed by cupping, splaying, and fraying of the metaphyses of long bones (particularly the distal ulna, distal femur, and proximal tibia), demineralization, deformation of the long bones, and fractures.[203,204] Looser zones are radiolucent lines with dense borders that are pseudofractures located at the femoral neck and shaft. Histologically, as a consequence of impaired calcification within the cartilage growth plate, the pattern of chondrocyte differentiation and maturation is disrupted and disorganized, whereas osteoid seams widen at other sites of bone formation.[205] In subjects deprived of phosphate, it is the trabeculae that are primarily undermineralized.

Impaired mineralization of osteoid may be due to dietary deficiencies or depressed intestinal absorption of calcium, phosphate, or vitamin D; inadequate amounts of these nutrients in fluids utilized in total parenteral nutrition; inborn errors in the metabolism or action of vitamin D; defects in renal tubular conservation of phosphate or calcium; or abnormalities of alkaline phosphatase generation and function (see Table 18-8). Broadly, rickets may be considered to be *calciopenic* (usually related to nutritional deprivation of vitamin D or, rarely, to a defect in its metabolism to the active metabolite—calcitriol—or in its cellular action or to a decreased intake of calcium or its excessive loss in urine) or *phosphopenic* (related to renal phosphate wasting due to primary renal tubular defects in phosphate reabsorption or to the generation of excessive amounts of phosphatonins, compounds that inhibit renal tubular reabsorption of phosphate). Thus, nutritional rickets may be due to decreased intake of vitamin D (or inadequate exposure to sunlight) or calcium or to marginal intakes of both nutrients.[206] Dietary deficiency of phosphate is unusual given its wide availability, but this nutrient may be deficient in parenteral fluids. Bone mineralization may also be directly impaired by abnormalities of alkaline phosphatase generation or by agents such as aluminum or fluoride.[205]

Calciopenic Rickets. Calciopenic rickets is most often due to deficiency of vitamin D that depresses intestinal

absorption of dietary calcium and renal reabsorption of filtered calcium. As determined by the measurement of serum concentrations of calcidiol (25OHD), vitamin D deficiency is present when values are below 12 to 15 ng/mL.[207,208] Calcidiol concentrations between 15 and 19.5 ng/mL indicate vitamin D "insufficiency," whereas those ≥ 20 ng/mL are adequate or "sufficient."[208] However, these guidelines, based on the recommendations of the Institute of Medicine report, are not accepted by all authorities and remain the subject of ongoing investigation.[209] Alternate guidelines state that a normal 25OHD concentration be defined as greater than 30 ng/mL (> 75 nmol/L) with values of 20 to 30 ng/mL (50 to 75 nmol/L) used to define "insufficiency" and values of less than 20 ng/ml (50 nmol/L) considered to identify vitamin D "deficiency." Neonates born to severely vitamin D–deficient mothers may display signs of rickets at birth, including fractures and hypocalcemic seizures. Clinical manifestations of rickets in preambulatory infants include bowing of the forearms, craniotabes, frontal bossing, and delayed closure of the cranial fontanelles. In older infants and children, genu varum or valgum (bowed legs or "knock knees") or a "windswept" deformity involving both legs, flaring (widening) of the metaphyses of the long bones with markedly enlarged wrists, prominence of the costochondral junctions (rachitic rosary), and indentation of the lower anterior thoracic wall (Harrison's groove) are noted. Tooth eruption may be delayed and the enamel hypoplastic predisposing to dental caries. Short stature and suboptimal weight are also present frequently. Because vitamin D has many extraskeletal sites of action, a number of nonosseous, systemic symptoms are observed in children with vitamin D–deficiency rickets; these include muscular weakness manifested by hypotonia and delay in walking, anorexia, and increased susceptibility to infection particularly pneumonia (due both to the lack of the stimulatory effect of vitamin D on the immune system and weakness of the thoracic wall).[203] Hypocalcemia, tetany, and seizures may occur in a severely vitamin D–deficient infant without gross clinical or radiographic signs of rickets. Rarely, vitamin D deficiency in an adolescent may be associated with hypocalcemic seizures and fractures.[210] Deficiency of vitamin D is frequently observed in darkly skinned children and adolescents with limited exposure to sunlight, particularly during and at the end of the winter months, or in those ingesting vegetarian diets or who are being treated with anticonvulsant or antiretroviral drugs.[208] Radiographically, osteopenia with cortical thinning and thin stress fracture lines as well as cupping, widening, and irregularity (fraying) of the distal metaphyses of the long bones are observed in the rachitic subject.[9,10,202] Areas of osteitis fibrosa cystica associated with secondary hyperparathyroidism may sometimes develop.

After the introduction of cod liver oil as a dietary supplement in 1918 (and the later fortification of infant formulas and milk and other foods with irradiated ergosterol) and the discovery in 1919 that exposure to sunlight prevented development of rickets, nutritional deficiency of vitamin D became relatively unusual in North America only to reemerge several decades later. Because the fetus is supplied with vitamin D by the transplacental transfer of maternal calcidiol, deficiency of vitamin D occurs predominantly in neonates who are born to mothers with low stores of vitamin D (women of color or those who are vegetarian, poorly nourished, or have limited exposure to sunlight).[211] Rickets develops in infants and young children who ingest a diet low in vitamin D (little or no milk, meat, eggs, or fish) without supplemental vitamin D who have limited exposure to sunlight because they are confined indoors due to illness, the climate, or parental choice or because they wear clothing that protects the entire body from sunlight. Vitamin D deficiency is more common in breastfed black infants than in white infants due in large part to increased maternal skin pigmentation resulting in decreased endogenous synthesis of cholecalciferol that coupled with socioeconomic circumstances leads to lower levels of calcidiol in maternal serum and of vitamin D in breast milk.[205] Because normal human breast milk contains only 25 IU/L of vitamin D, it is recommended that all breastfed infants receive supplemental vitamin D 400 IU/day.[212] Inasmuch as most commercial infant formulas contain 400 IU/liter of vitamin D, it is also recommended that formula-fed infants who consume less than 1 liter of formula daily also receive a supplement of 400 IU/day of vitamin D. Infants older than 2 year of age, children, and adolescents should receive supplemental vitamin D 600 IU daily.

Although definitions of hypovitaminosis D vary between individual studies, subtle forms of vitamin D deficiency or "insufficiency" are likely prevalent throughout the North American population, particularly in the winter months when there is little sunlight exposure and the ultraviolet light requisite for endogenous synthesis of vitamin D is limited.[9,10,208,213] In a group of 307 healthy urban male and female adolescents 11 to 18 years of age in the northeastern United States, 42% had serum concentrations of 25OHD less than 20 ng/mL.[214] The prevalence of very low 25OHD values (< 15 ng/mL) was most common in black (36%) and Hispanic subjects (22%), whereas 6% of white students had such levels. There was an inverse relationship between serum levels of 25OHD and PTH with secondary hyperparathyroidism present in many subjects with low 25OHD concentrations, implying the possibility of incipient metabolic bone disease. Although clinically apparent vitamin D deficiency is unusual in U.S. youth, vitamin D insufficiency may be relatively frequent.[213,215] In addition to skin pigmentation and northern latitude, low serum calcidiol values have been attributed to meager consumption of milk and multivitamins, large intake of phosphate-containing soft drinks, and increase in fat mass into which vitamin D is deposited and thus not bioavailable. Similar findings have been recorded in apparently healthy children and adolescents in the United Kingdom and attributed to lower socioeconomic status, nonwhite ethnicity, decreased outdoor activities, older age, advanced pubertal development, and female gender.[216,217] Indeed, serum concentrations of calcidiol below 13 ng/mL have been recorded in up to 40% to 50% of both southern and northern European children and adolescents.[218] Assuming a reliable assay such as those employing liquid chromatography-tandem mass spectrometry that measure both $25OHD_2$ and $25OHD_3$, calcidiol concentrations

below 12 to 15 ng/mL in children clearly indicate vitamin D deficiency, whereas values between 12 to 20 ng/mL are consistent with vitamin D insufficiency.[207,208] Serum levels of 25OHD that exceed 60 to 100 ng/mL are indicative of vitamin D excess. Dietary deficiency of calcium may accentuate the adverse effects of borderline vitamin D stores.[206,219]

Vitamin D deficiency may also be the consequence of intestinal malabsorptive disorders such as celiac or inflammatory bowel diseases, biliary obstruction, gastric and intestinal resection, or pancreatic exocrine insufficiency (e.g., cystic fibrosis), or ingestion of calcium binding agents such as cholestyramine, or by increased hydroxylation of vitamin D to water soluble forms that increase its urinary loss by anticonvulsant drugs such as phenytoin.[10] Use of a therapeutic glucocorticoid is also associated with lowered serum levels of calcidiol.[220] In the majority of infants and children with rickets due to vitamin D deficiency, serum concentrations of total calcium are borderline-normal or low, phosphate levels low, and alkaline phosphatase activity and PTH concentrations increased (Table 18-10). Secondary hyperparathyroidism develops as the intestinal absorption of calcium is reduced and its serum levels reduced; PTH increases urinary phosphate loss and lowers serum phosphate concentrations while enhancing the rates of bone resorption and turnover. PTH also increases synthesis of calcitriol, which may increase the rate of calcidiol catabolism and further deplete the body's store of vitamin D.[205] Typically, in vitamin D deficiency serum concentrations of calcidiol are low, whereas calcitriol values may be normal, high, or low depending on whether vitamin D deficiency is modest, moderate, or severe. Serum concentrations of osteocalcin are low; serum levels of PICP (a marker of bone formation) and ICTP and urinary excretion of NTx (markers of bone resorption) are substantially increased in infants with vitamin D–deficiency rickets, indicating increased collagen turnover in this disorder.[221] With treatment these values increase transiently and then fall to age-appropriate norms before radiographic healing of the rachitic lesions is complete.

Prevention of vitamin D deficiency is its most effective management. Because the amount of vitamin D in human breast milk is approximately 20 to 25 IU/L and vitamin D supplementation of the breastfeeding mother may be inadequate to ensure normal calcidiol levels in her infant (unless she is receiving approximately 2000 IU/day), it is important that all breastfed infants receive a supplement of vitamin D daily (400 IU/day); by extension this recommendation is also appropriate for infants who are not receiving adequate amounts of vitamin D in their prepared formulas or diet or have suboptimal exposure to sunlight. In the active, lightly dressed white child with skin that normally tans with exposure to sunlight and who plays outdoors 30 minutes thrice weekly or 10 to 15 minutes daily between 1000 to 1500 hours during spring, summer, and fall, endogenous vitamin D synthesis is usually sufficient to achieve satisfactory serum levels of calcidiol.[205,208] A yellow- or brown-skinned child requires 30 to 45 minutes of outdoor play, whereas a black-skinned child requires 60 to 150 minutes of sunlight exposure to achieve serum calcidiol concentrations comparable to those of the white-skinned child. The latitude in which the child lives, season of the year, time of day, local environmental pollutants, amount of clothing, and use of sunscreen affect the time required for sunlight exposure to evoke adequate vitamin D synthesis in the individual child. Between November and February, little or no vitamin D can be synthesized in areas that are above 35 degrees latitude (such as Atlanta, Georgia), but vitamin D insufficiency is also prevalent in lower latitudes.[10,222] (It is somewhat ironic that an increase in the incidence of vitamin D deficiency in infants and children coincides with well-intentioned recommendations that exposure to sunlight be limited by sunscreen and protective clothing.) Obesity is another risk factor for hypovitaminosis D and decreased bone mineralization.[223,224] In obese adolescents, serum concentrations of calcidiol

TABLE 18-10 **Laboratory Data in Rickets of Varying Pathogenesis**

		Alkaline				
Type	**Calcium**	**Phosphate**	**Phosphatase**	**Calcidiol**	**Calcitriol**	**PTH**
Calcium deficiency	⇑⇓	⇓	⇑⇑	N	⇑	⇑
Phosphate deficiency	N,⇑	⇓⇓	⇑⇑	N	⇑	N,⇓
Vitamin D deficiency						
Mild	N,⇓	N,⇓	⇑	⇓	N	N
Moderate	N,⇓	⇓	⇑⇑	⇓	⇓,N,⇑	⇑
Severe	⇓	⇓	⇑⇑	⇓⇓	⇓	⇑⇑
Loss-of-function *CYP2R1* (25-hydroxylase)	⇓	⇓	⇑	⇓	⇓	⇑
Loss-of-function *CYP27B1* (25OHD-1α-hydroxylase)	⇓⇓	⇓⇓	⇑⇑⇑	N	⇓⇓⇓	⇑⇑⇑
Loss-of-function *VDR* (resistance to calcitriol)	⇓⇓	⇓⇓	⇑⇑⇑	N	⇑⇑⇑	⇑⇑⇑
Loss-of-function *PHEX* (X-linked hypophosphatemic rickets)	N	⇓⇓	⇑	N	N,⇓	N
Hypophosphatasia	N,⇑	N,⇑	⇓	N	N	N,⇓

N, normal; ⇓ low; ⇑ high.

are inversely and PTH values are directly related to fat mass.[223] After diet and exercise-induced weight loss, abnormalities in serum levels of calcidiol and PTH are reversed, indicating that the aberrations seen in obese children are the consequence and not the cause of obesity.[224] In a study of 236 adolescent candidates for bariatric surgery, more than 50% were vitamin D deficient as defined by low serum concentrations of calcidiol.[225] Although 43% of these subjects were Caucasian and 15% African American, 82% of the vitamin D–deficient patients were African American compared to 37% of the Caucasian adolescents.

Vitamin D deficiency in the infant may be managed by administration of vitamin D 1000 to 5000 IU/day for infants > 1 to 12 months of age.[177] For the child who is 1 year of age or older or an adolescent, vitamin-deficient rickets may be treated with 5000 to 10000 IU/day of vitamin D. At the beginning of treatment, elemental calcium (30 to 75 mg/kg/day in three divided doses per day) must also be administered to the vitamin D–deficient child receiving vitamin D in order to avoid the hypocalcemia that accompanies rapid remineralization of bone matrix (the "hungry bone syndrome").[177] When there is radiographic evidence of healing, the vitamin D dose is lowered to 400 to 600 IU/day. Alternative therapeutic regimens for treatment of rickets include 50,000 IU orally weekly for 8 weeks or the administration of a single oral (or intramuscular) dose of 150,000 to 600,000 units of vitamin D_3, depending on patient age and other individual circumstances.[10,205] Serial measurement of total or bone-specific alkaline phosphatase values is an effective tool for monitoring the efficacy of treatment as levels decline progressively in tandem with the roentgenographic healing of the rachitic lesions. To avoid hypocalcemia or hypercalcemia, hypercalciuria, and nephrocalcinosis, serial determinations of serum values of calcium, phosphate, alkaline phosphatase, and creatinine and urinary excretion of calcium and creatinine should be determined. Sequential radiographs document reversal of rachitic changes (see Figure 18-8).

Rickets due primarily to a low dietary intake of calcium has been observed in infants who ingest low-calcium containing formulas and in children from developing countries whose diets contain 200 mg (or less) of elemental calcium per day despite a normal intake of phosphate and adequate endogenous stores of vitamin D as determined by serum calcidiol levels.[205] Calcium intake in these infants and children is well below that recommended (400 to 600 mg/day in infants; 700 mg/day in children below 4 years of age; 1000 to 1300 mg/day in older children and adolescents). Histologically, bone biopsies from children with calcium-deficiency rickets reveal widened seams of unmineralized osteoid and low bone turnover rates, findings compatible with rickets. In Nigerian infants, rickets due to vitamin D deficiency is most prevalent between 4 to 12 months of age. In 123 older Nigerian children (34 to 63 months of age) with rickets, low serum concentrations of calcium, normal phosphate levels, normal to low calcidiol and elevated calcitriol concentrations, administration of calcium alone (1000 mg daily in divided doses orally over 24 weeks) resulted in more rapid decline in serum levels of alkaline phosphatase and in radiographic healing of rickets than did administration of vitamin D (600,000 IU intramuscularly at inception of the study and again 12 weeks later), data supportive of the concept that calcium deficiency alone was the cause of the rickets in this population.[226] Interestingly, calcidiol values rose and calcitriol levels declined with calcium supplementation alone, suggesting that a low-calcium diet and attendant calcium deficiency led to increased secretion of PTH and accelerated conversion of calcidiol to calcitriol. Calcium-deficiency rickets also occurs in the United States when infants and children are weaned to low-calcium–containing foods after completion of breastfeeding.[219] Calcium deficiency rickets may also develop as a consequence of impaired intestinal absorption of dietary calcium that has been bound by ingestion of high-fiber and phytate-containing cereals. In a study of 67 Indian children with nutritional rickets, the ingestion of a diet high in phytate was larger and the intake of calcium was 50% of that of an age- and gender-matched, clinically well control population.[206] There was an inverse correlation between dietary calcium intake and radiologic severity of rickets. However, in both populations serum concentrations of calcidiol were below 20 ng/mL and not significantly different from one another. These data led to the conclusion that clinical and radiologic manifestations of rickets in this population of Indian children were dependent on the co-occurrence of both calcium and vitamin D deficiencies. Calcium deficiency is best addressed by its prevention, ensuring adequate intake of this element according to established guidelines for growing children and adolescents. When present, calcium-deficiency rickets may be effectively treated by ensuring an intake of 1000 to 1500 mg of elemental calcium daily for 6 months with provision of normal amounts of vitamin D by sunlight exposure or supplementation.[227]

Dietary phosphate deficiency is unusual because phosphate is present in large amounts in most foods. Phosphate deficiency occurs in patients with depressed renal tubular reabsorption of phosphate (Tables 18-9 and 18-11A), in subjects with decreased intestinal phosphate absorption, in those receiving parenteral nutrition with fluids low in phosphate, in subjects ingesting large amounts of aluminum-containing antacids as aluminum and phosphate coprecipitate in the intestinal tract, and in premature infants drinking human breast milk without supplemental phosphate.[227,228] In very premature infants receiving long-term parenteral nutrition, development of metabolic bone disease is frequent and related not only to deficiencies of calcium, phosphate, and vitamin D but also to excess aluminum in the infusates. Infants receiving large amounts of aluminum containing antacids over prolonged periods for treatment of gastroesophageal reflux may also have substantially low bone mass. Aluminum lowers the rate of bone formation by several mechanisms: administered orally aluminum binds intestinal phosphate, thereby impeding its absorption leading to phosphate depletion; administered intravenously during total parenteral nutrition or during hemodialysis, aluminum inhibits osteoblastic function and prevents mineralization of osteoid; it also impairs the secretion of PTH and decreases 25OHD-1α hydroxylase activity.[227]

TABLE 18-11A **Disorders of Phosphate Homeostasis in Children**

I Hypophosphatemia

A Decreased Intestinal Absorption

1 Decreased intake/absorption: parenteral hyperalimentation, antacid abuse, starvation (anorexia nervosa), alcoholism
2 Malabsorption: vitamin D deficiency, metabolism, function

B Increased Urinary Excretion

1 Hypophosphatemic rickets: X-linked (*PHEX*), autosomal dominant (*FGF23*), autosomal recessive (*DMP1, ENPP1*), hereditary autosomal recessive with hypercalciuria (*SLC34A3*), X-linked recessive (*CLCN5*), tumor-induced osteomalacia, linear sebaceous nevus syndrome, fibrous dysplasia (*GNAS*)
2 Renal tubular defects: Dent and Fanconi syndromes, postrenal transplantation, hypomagnesemia, fructose intolerance, macrophage activation syndrome
3 Hyperparathyroidism, acidosis, respiratory alkalosis
4 Drugs: diuretics (thiazides, acetazolamide, loop diuretics), iron polymaltose infusions, bicarbonate, glucocorticoids, calcitonin, antineoplastic agents (ifosfamide, cisplatin), antiretroviral agents (tenofovir, adefovir), mannitol, bisphosphonates
5 Expansion of extracellular fluid volume

C Transcellular Shifts from Extracellular to Intracellular Space

1 Recovery from diabetic ketoacidosis/insulin administration, sepsis, salicylate intoxication
2 Administration of glucose, catecholamines; nutritional repletion, "hungry" bone syndrome
3 Respiratory alkalosis

II Hyperphosphatemia

A Increased Intake

1 Intravenous, oral, rectal

B Decreased Urinary Excretion

1 Renal insufficiency
2 Hypoparathyroidism, acromegaly
3 Pseudohypoparathyroidism (*GNAS*)
4 Familial tumoral calcinosis (*FGF23, GALNT3*)
a Hyperostosis hyperphosphatemia syndrome (*FGF23, GALNT3*)
5 Drugs: growth hormone, bisphosphonates

C Excess Bone Resorption

1 Severe illness: acidosis (metabolic or respiratory), hemolytic anemia, diabetic ketoacidosis, hepatitis, catabolic states, rhabdomyolysis, hyperthermia
2 Drugs: cytotoxic therapy

Modified from Ward, L. M. (2005). Renal phosphate-wasting disorders in childhood. Pediatr Endocrinol Rev, 2, *342–350; Imel, E. A., & Econs, M. J. (2012). Approach to the hypophosphatemic patient.* J Clin Endocrinol Metab, 97, *696-706.*

Patients requiring total parenteral nutrition should receive as much calcium and phosphate as can be administered safely as well as supplemental vitamin D 400 IU/day. Periodic measurements of serum levels of calcium, phosphate, alkaline phosphatase, PTH, and calcidiol, and serial skeletal radiographs and estimations of skeletal mineralization are recommended in patients receiving total parenteral nutrition; if metabolic bone disease develops in spite of these efforts, serum aluminum levels should be measured and if elevated (> 100 μg/L) a search for the source of the aluminum should be initiated and that product eliminated from the infusate if possible. Cadmium, fluoride, and saccharated ferric oxide are also able to impede normal bone mineralization.[227]

Metabolic and functional defects of vitamin D lead to rare forms of rickets.[208,229] Rickets due to a deficit in 25-hydroxylation (vitamin D hydroxylation-deficient rickets, type 1B [VDDR1B], MIM 600081) has been described in two brothers of Nigerian origin in whom a homozygous loss-of-function mutation (Leu99Pro) in *CYP2R1* eliminated hydroxylase activity of this 501 amino acid protein.[230] Hypocalcemia, hypophosphatemia, skeletal abnormalities of rickets, low plasma levels of calcidiol, and normal values of calcitriol were present in these siblings. 25-Hydroxylase deficiency transmitted as a dominant disorder not due to a mutation in the coding exons or intronic regions of *CYP2R1* has also been described.[231] Vitamin D hydroxylation-deficient rickets, type 1A (VDDR1A) (MIM 264700), or pseudovitamin D deficiency rickets (PDDR) type 1 is due to loss-of-function mutations in *CYP27B1*, the enzyme in the renal proximal tubule that catalyzes 1α-hydroxylation of 25OHD (calcidiol) to 1,25$(OH)_2$D (calcitriol), the biologically active metabolite of vitamin D.[232] VDDR1A is an autosomal recessive disease whose clinical manifestations—including bone deformities (bowing of the forearms), growth retardation, muscle weakness, delayed motor milestones (walking), or hypocalcemic seizures—appear between 6 and 30 months of age; hypoplasia of dental enamel may also occur; biochemically, hypocalcemia, hypophosphatemia, hyperphosphatasemia, and markedly elevated serum levels of PTH are typical; radiographs reveal rachitic deformities of the long bones.[233] The diagnosis of VDDR1A is established by finding normal serum concentrations of calcidiol while calcitriol values are extremely low and do not increase after administration of vitamin D or calcidiol, and the diagnosis is confirmed by identification of the mutation in *CYP27B1* (see Table 18-10).

The clinical, biochemical, and radiographic manifestations of VDDR1A resolve completely and reasonably rapidly following treatment with physiologic amounts of calcitriol (10 to 20 ng/kg/day). Treatment goals are restoration of eucalcemia without hypercalciuria, resulting in increased muscular strength and healing of the rachitic lesions permitting normal growth.[233] Lifelong therapy is necessary, but effective and compliant treatment leads to normalization of all biochemical parameters, normal linear growth and adult height, and normal bone mineralization. The dose of calcitriol often needs to be increased during pregnancy; however, gestation is uncomplicated and the offspring of affected mothers are normal.[233] VDDR1A is found with high frequency in a Quebec French-Canadian population but

TABLE 18-11B **Disorders of Phosphate Homeostasis**

Gene	MIM/Chromosome	Product	Disorder	MIM	Inherit
HYPOPHOSPHATEMIA					
DMP1	600980/4q22.1	Dentin matrix acidic phosphoprotein 1	Autosomal recessive HR1	241520	AR
ENPP1	173335/6q23.2	Ectonucleotide pyrophosphatase/Phosphodiesterase 1	Autosomal recessive HR2	613312	AR
SLC34A3	609826/9q34.3	Na^+/PO4 cotransporter IIC: NaPi2c (NPT2c)	Hereditary (H) HR c = with hypercalciuria	241530	AR
SLC34A1	182309/5q35.3	Na^+/PO4 cotransporter: NaPi2a (NPT2a)	HR c nephrolithiasis 1	612286	AD
			Fanconi syndrome 2	613388	AR
SLC9A3R1	604990/17q25.1	Na^+/H^+ exchange regulatory factor 1 (NHERF1)	HR c = with nephrolithiasis 2	612287	AD
FGF23	605380/12p13.32	FGF23	Autosomal dominant HR	193100	AD
PHEX	300550/Xp22.11	Phosphate regulating endopeptidase on the X chromosome	X-linked dominant HR	307800	X-link
CLCN5	300008/Xp11.23	Chloride channel 5	X-linked recessive HR	300554	"
			Dent disease 1	300009	"
OCRL	300535/Xq25-26	Phosphatidylinositol 4,5-bisphosphate-5-phosphatase	Dent disease 2	300555	"
KL	604824/13q13.1	Klotho (overexpression)	HR c = with hyperparathyroidism	612089	AD
GNAS	139320/20q13.32	Guanine nucleotide binding protein, alpha	Fibrous dysplasia	174800	
FGFR1	136350/8p11.23	FGF receptor 1	Osteoglophonic dysplasia	166250	AD
PTH1R	168468/3p21.31	PTH receptor 1	Jansen metaphyseal chondrodysplasia	156400	AD
Hyperphosphatemia					
FGF23	605380/12p13.32	FGF23	Tumoral calcinosis	211900	AR
KL	604/824/13q13.1	Klotho	"	"	"
GALNT3	601756/2q24.3	UDP-N-acetyl-alpha-D-galactosamine: polypeptide N-acetylgalactosaminyltransferase 3	"	"	"

HR, hypophosphatemic rickets.

Adapted from Gattineni, J., & Baum, M. (2012). Genetic disorders of phosphate regulation. Pediatr Nephrol, 27, *1477–1487; Hori, M., Shimizu, Y., & Fukumoto, S. (2011). Minireview: fibroblast growth factor 23 in phosphate homeostasis and bone metabolism.* Endocrinology, 152, *4–10.*

occurs in all races and in diverse geographic regions. *CYP27B1* encodes a 508-amino-acid mitochondrial cytochrome P450 hydroxylase with conserved sites that bind ferredoxin and heme and utilizes electrons from reduced nicotinamide adenine dinucleotide phosphate and oxygen to convert 25OHD to $1,25(OH)_2D$.[229] Many loss-of-function missense, nonsense, splicing, and duplication or deletion/frameshift mutations in *CYP27B1* lead to inactive or truncated protein products that are unable to bind either substrate (calcidiol) or heme, the latter defect preventing electron transfer and inhibiting catalysis.[229] The most common mutation in *CYP27B1* in the Quebec French-Canadian population at-risk for VDDR1A is deletion of guanine at nucleotide 958 (codon 88, exon 2) (958delG) that changes the reading frame resulting in premature termination of translation and an inactive product (the Charlevoix mutation). A second common mutation in this population is duplication of a 7-base pair sequence in exon 8; however, this mutation is also found in patients of other ethnicities (Asian, Hispanic).

Homozygous or compound heterozygous inactivating mutations of *VDR*, the gene encoding the vitamin D receptor, lead to resistance to the biologic effects of calcitriol (autosomal recessive vitamin D–dependent rickets, type 2A [VDDR2A] or hereditary vitamin D–resistant rickets [MIM 277440]).[229,232] Clinical and biochemical manifestations of resistance to calcitriol include severe infantile-onset bony deformities characteristic of rickets, growth retardation, varying degrees of alopecia, hypocalcemia, hypophosphatemia, hyperalkaline phosphatasia, and extraordinarily high serum concentrations of calcitriol (300 to 1000 pg/mL) and PTH; serum levels of 25-hydroxyvitamin D are normal, whereas those of 24,25-dihydroxyvitamin D are often low (see Table 18-10). Radiographic examination of the skeleton reveals rachitic

changes. The high calcitriol values reflect the combined stimulatory effects of hypocalcemia, hypophosphatemia, and secondary hyperparathyroidism on the activity of $25OHD_3$ 1α-hydroxylase together with a decrease in its rate of catabolism due to depressed activity of calcitriol-dependent 1,25α-dihydroxyvitamin D_3 24-hydroxylase. Alopecia is the result of impaired vitamin D function in epithelial nuclei and those of the outer root-sheath cells of the hair follicle. Loss-of-function mutations (particularly in the DNA-binding region) of *VDR* result in a phenocopy of the generalized alopecia associated with loss of *HR* function (hairless; MIM 602302, chromosome 8p21.2); interestingly, the role of the VDR in the maintenance of normal hair growth is not dependent on its binding to ligand.[232,234]

Although VDDR2A is primarily manifested during infancy and early childhood, clinical manifestations of this disorder may vary and patients with milder defects in *VDR* may not be identified until adolescence or adulthood. Spontaneous remission of the rachitic process may occur, most often between ages 7 and 15 or when the patient enters puberty.[235] Indeed, after puberty in many patients with VDDR2A, serum levels of calcium, phosphate, and alkaline phosphatase normalize and calcium supplementation is no longer needed.[235,236] Apparently, the skeletal requirement for calcium declines after completion of bone growth, and vitamin D–independent mechanisms of intestinal calcium absorption develop. In a study of 17 patients with VDDR2A between 1.5 and 37 years of age, biochemical parameters improved, the need for supplemental calcium declined, and BMD by DEXA normalized in the latter half of the second decade of life, whereas serum calcitriol values remained elevated.[236] Examination of intestinal calcium absorption revealed that the fractional absorption of calcium for patients who ingested a low-calcium diet was substantially lower than that for control subjects in young patients; in patients 18 to 26 years of age, the fractional absorption of calcium was significantly higher than control values; in adult patients, the fractional absorption of calcium was similar to control data. Nevertheless, the growth impairing effects of severe childhood rickets persisted into adulthood in the majority of patients.[236]

The diagnosis of VDDR2A is established by the presence of elevated serum concentrations of calcitriol in the rachitic patient (in the absence of administration of exogenous calcitriol) and confirmed by identification of the loss-of-function mutation in *VDR*. The VDR is composed of DNA-, ligand-, and retinoic X receptor-binding domains and a transactivation domain to which many comodulators of VDR function are recruited. The VDR and RXR bind as a heterodimeric transcription-activating complex. Loss-of-function mutations have been found in each VDR domain: thus, the mutated VDR may be unable to bind calcitriol because of decreased receptor number or affinity for ligand, incapable of forming heterodimers with the retinoic X receptor or of translocating to the targeted gene in the nucleus, or unable to bind to the vitamin D response element (VDRE) or to initiate gene transcription once bound to the VDRE (Figure 18-9). More than 30 distinct heterozygous mutations in *VDR* have been identified scattered throughout the VDR protein but with a large number of variants in the ligand binding domain.[229] In general, patients with vitamin D–resistant rickets without alopecia may be more responsive to treatment. Administration of high doses of calcitriol (1 to 6 μg/kg/day) and supplemental calcium (1 to 3 g of elemental calcium daily) has been effective in increasing serum calcium concentrations and healing rickets in patients with nonsense and missense mutations in *VDR* that lead to decreased affinity for ligand or alter nuclear targeting; it is appropriate to provide a trial of high-dose calcitriol/calcium therapy to each patient with vitamin D–resistant rickets regardless of the *VDR* mutation.[232] During treatment, serum values of calcium, phosphate, alkaline phosphatase, creatinine, and PTH, urinary calcium and creatinine excretion, skeletal radiographs, and renal ultrasounds for development of nephrocalcinosis are monitored serially. In patients refractory to oral therapy, continuous intravenous or intracaval administration of large amounts of calcium (0.4 to 1.4 grams of elemental calcium/m^2/day) normalizes calcium, phosphate, alkaline phosphatase, and PTH values; heals rickets; and increases the growth rate in selected children. If this mode of therapy is employed, careful monitoring for catheter sepsis and cardiac arrhythmia as well as hypercalcemia, hypercalciuria, and nephrocalcinosis is mandatory. After healing of the rickets by parenteral calcium, maintenance therapy with large doses of oral calcium (3.5 to 9 grams of elemental calcium/m^2/day) is appropriate. In younger infants with vitamin D–resistant rickets prior to the development of florid rickets, high doses of oral calcium may ameliorate the rachitic process. These clinical observations indicate that the major defect in patients with vitamin D–resistant rickets is lack of calcium. With treatment, growth may normalize in patients with vitamin D resistance, but alopecia, if present, is unlikely to resolve.[235] As already noted, spontaneous improvement may occur in some patients with VDDR2A during and after puberty.[236]

Vitamin D–dependent rickets, type 2B (VDDR2B, MIM 600785) is a second form of resistance to vitamin D with a phenotype similar to that of VDDR2A (except that alopecia is unusual) but with an intact vitamin D nuclear receptor; VDDR2B is due to inhibition of binding of the liganded VDR-retinoic X receptor heterodimer to the VDRE by members of a family of heterogeneous nuclear ribonucleoproteins.[237,238] *HNRNPC* encodes heterogeneous nuclear ribonucleoprotein C, an RNA binding protein; it is one of several ribonucleoproteins that are able to bind the VDRE and thereby to inhibit its transactivation by the VDR-retinoic X receptor heterodimer.[238] The mechanism by which overexpression of these otherwise normal ribonucleoproteins occurs and the relationship of this observation to VDDR2B are unknown at present.

Phosphopenic Rickets. Hypophosphatemia in childhood may be due to hereditary or acquired disorders (Tables 18-8, 18-9, 18-11A, and 18-11B). Acute hypophosphatemia is accompanied by irritability, paresthesias, confusion, muscle weakness, and ileus.[239] Chronic hypophosphatemia in children is often manifested by rickets. Hypophosphatemia may be the consequence of the movement of extracellular phosphate into cells (during treatment of diabetic ketoacidosis with insulin as the intracellular phosphorylation of carbohydrates increases, acute respiratory alkalosis due to

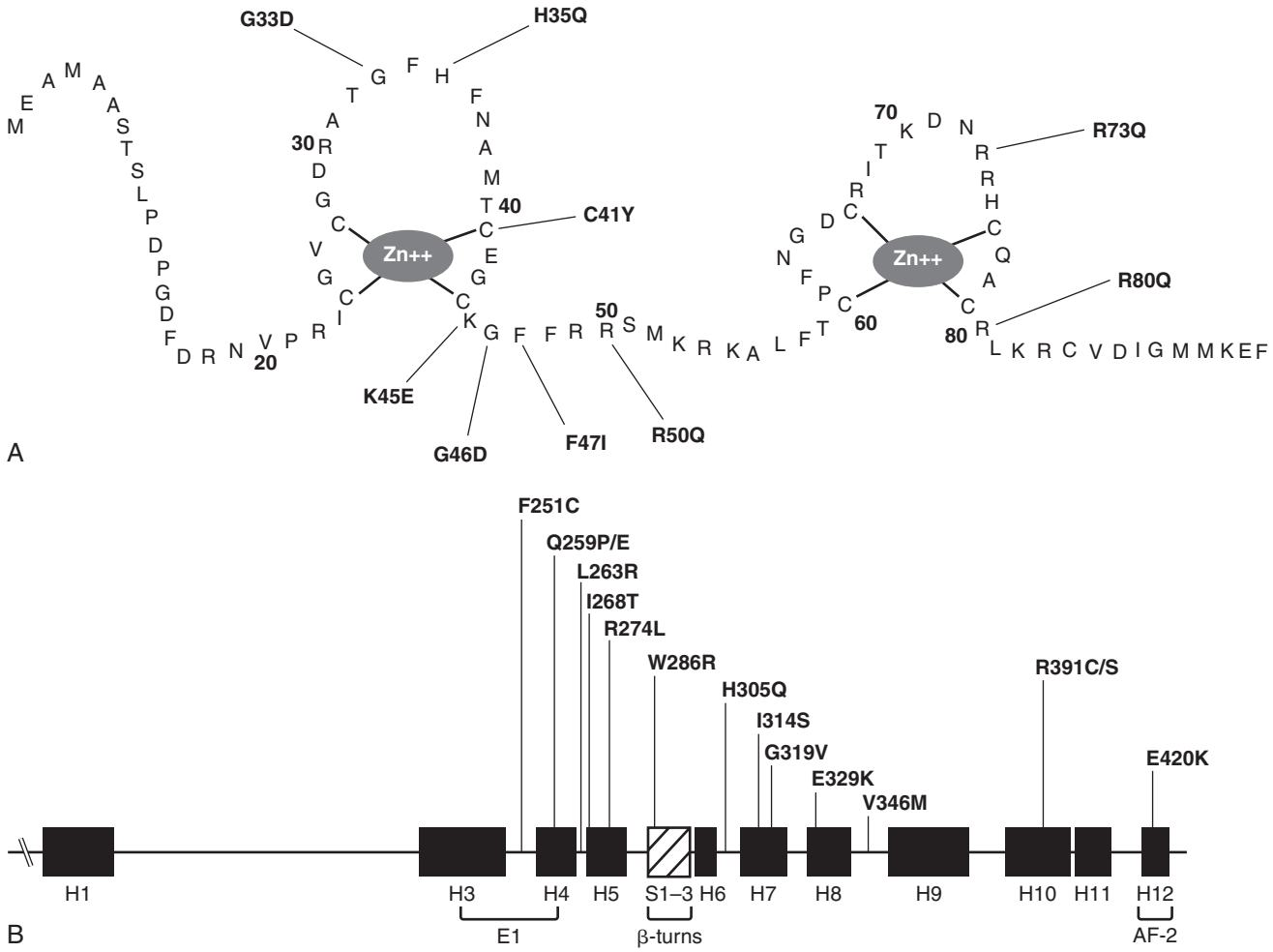


FIGURE 18-9 ■ Mutations in the vitamin D receptor (VDR) in patients with end-organ insensitivity to vitamin D. (From Malloy, P. J., & Feldman, D. (2010). Genetic disorders and defects in vitamin D actions. *Endocrinol Metab Clin North Am, 39,* 333–346.)

hyperventilation, sepsis), impaired intestinal absorption of phosphate (vitamin D deficiency, chronic ingestion of magnesium or aluminum containing antacids, chronic diarrhea or steatorrhea), or increased renal phosphate wasting (secondary to increased secretion of PTH or primary due to intrinsic abnormalities in the mechanisms that control renal tubular reabsorption of this anion). Hypophosphatemia is observed in nutritionally deprived subjects such as the LBW neonate or older patient who is dependent on parenteral alimentation, those with severe anorexia, or those who are addicted to alcohol. Hypophosphatemia occurs in many critically ill patients, especially those with sepsis. Rapid remineralization of osteopenic bone is accompanied by hypophosphatemia and hypocalcemia (the "hungry bone syndrome"). Most frequently, chronic hypophosphatemia is the result of hyperphosphaturia, due to either a primary or a secondary defect in the regulation of renal tubular phosphate reabsorption in the proximal (where 60% to 70% of the filtered load is reabsorbed) or distal (where 10% to 15% of filtered phosphate is reabsorbed) renal tubule.[228,239] Thus, increased secretion of PTH due to primary hyperparathyroidism or secondary to vitamin D deficiency or some other cause can lead to hyperphosphaturia and hypophosphatemia. The Fanconi syndromes are characterized by multiple functional renal tubular defects including phosphate reabsorption. Renal tubular defects that specifically impede the processes through which phosphate is reabsorbed by the renal tubules include abnormalities of transcellular transport of phosphate and excessive production of phosphaturic substances other than PTH such as fibroblast growth factor 23 (FGF23) and other phosphatonins (minhibins) and their cofactors.

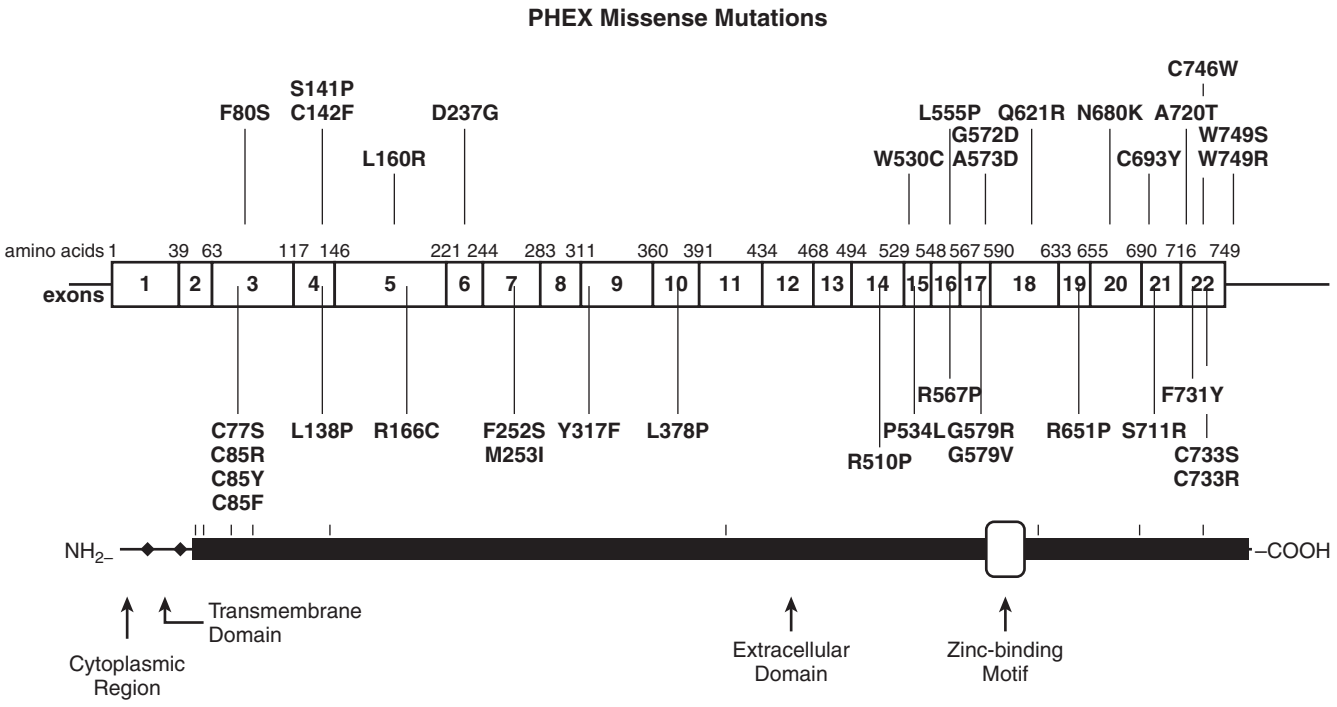
In developed countries where severe vitamin D–deficiency rickets is not a common problem, X-linked dominant hypophosphatemic rickets (XLHR) is the most frequent form of rickets encountered (1:20,000 births). XLHR is due to loss-of-function mutations in *PHEX* (PHosphate-regulating Endopeptidase homologue, X-linked) encoding a zinc metallopeptidase expressed by bone cells (discussed later).[240] XLHR is manifested in both affected hemizygous males and heterozygous females, albeit with substantial inter- and intrafamilial variability in its clinical expression. Physical findings in children with XLHR include short stature; genu varum or valgum that develops when the infant begins to walk;

flaring of the metaphyses; rachitic rosary; frontal bossing; increased frequency of dental decay or periradicular abscesses in teeth free of caries; and bone, muscle, and joint aching and stiffness. Craniotabes, tetany, and muscular weakness are not found in patients with XLHR as they are in those with vitamin D deficiency. Because they are no longer growing, adults with XLHR have osteomalacia, bone pain, an increased fracture rate, and frequent dental abscesses; they may also develop stenosis of the spinal canal and progressive ankylosis of the vertebrae and large joints. Enthesopathy—the calcification of tendons, ligaments, and joint capsules—is common in adults with XLHR and may also occur in children with XLHR. Although completely penetrant, the clinical expression of XLHR may vary widely among affected families and among affected children in the same family.[241] Although serum levels of total calcium and Ca^{2+} are normal, hypophosphatemia is marked due to its urinary wastage because of substantially decreased renal tubular reabsorption of filtered phosphate and limited intestinal absorption of this anion. Serum concentrations of PTH and calcidiol are normal, but calcitriol values are inappropriately low for the degree of hypophosphatemia; serum alkaline phosphatase activity is increased.[242] Many of these biochemical abnormalities are present in the affected patient within the first several months after birth. Because the serum Ca^{2+} concentration is normal, secondary hyperparathyroidism does not occur unless the patient with XLHR receives excessive amounts of supplemental phosphate (discussed later). Normally, phosphate induces differentiation and death of hypertrophic chondrocytes in the cartilage growth plate by activating the mitochondrial apoptotic pathway that is caspase 9 dependent.[232,243] Thus, hypophosphatemia leads to a delayed loss and increased numbers of hypertrophic chondrocytes and expansion of the growth plate characteristic of rickets. Histomorphometrically in XLHR, unmineralized osteoid accumulates along the trabeculae within cancellous bone.

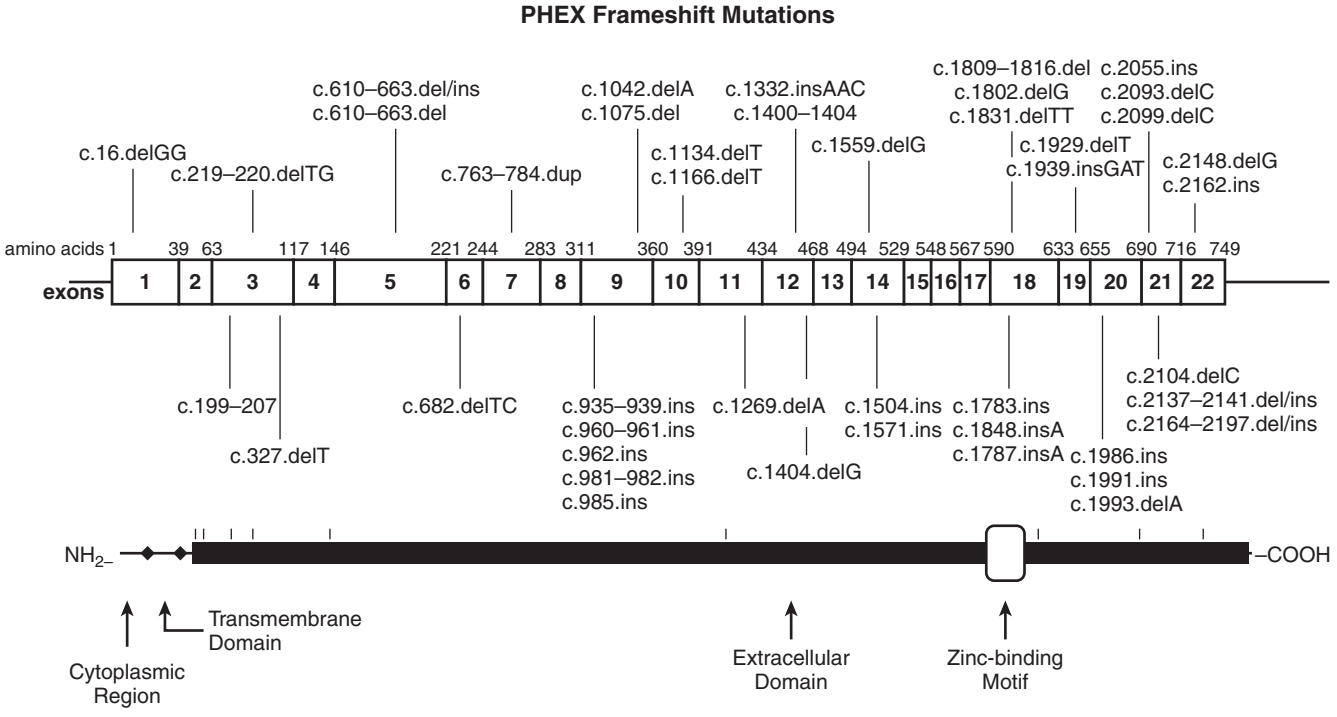
Classical XLHR is caused by loss-of-function mutations in *PHEX*, a 22 exon gene that encodes a 749-amino-acid integral membrane protein with very long extracellular (702 amino acids), transmembrane (27 amino acids), and short intracellular (20 amino acids) domains that structurally resembles several neutral endopeptidases (e.g., endothelin-converting enzyme-1, Kell antigen). The extracellular domain has 10 conserved cysteine residues and a pentapeptide motif (His-Glu-Phe-Thr-His) characteristic of zinc metallopeptidases that may either convert propeptides to active forms or degrade and inactivate their substrates. *PHEX* is expressed on the surface of osteoblasts and osteocytes as well as by teeth, muscle, lung, liver, testis, and ovary; *PHEX* transcription by osteoblasts is down-regulated by calcitriol.[241,244] In most patients with XLHR, inactivating mutations of *PHEX* have been found primarily in the extracellular domain and include frameshift deletions (16%), duplications, insertions (8%), deletional-insertional, splice site (15%), nonsense (27%), and missense (34%) mutations, many in exons 15 and 17; a mutation has also been identified in the 5′-untranslated region (A ⇒ G transversion 429 bp upstream of the ATG initiation site) (Figures 18-10A, 10B).[240,245] Because PHEX is a glycosylated protein, failure of glycosylation leads to its sequestration within the endoplasmic reticulum, thus impeding its movement (trafficking) to the cell membrane; other mutations interfere with the catalytic function of the protein or its three-dimensional conformation. Mutations in *PHEX* arise spontaneously in more than 20% of patients with XLHR. There is no correlation between the location or type of mutation in *PHEX* and the clinical manifestations or severity of the disease.[241,245,246]

Fibroblast growth factor (FGF)-23 is a substance synthesized by osteoblasts and osteocytes that inhibits phosphate reabsorption by the renal tubule and when secreted in excess leads to hyperphosphaturia and consequent hypophosphatemia.[247] Serum concentrations of FGF23 are high in patients with XLHR and are inversely correlated with serum phosphate values. FGF23 is synthesized as a 251-amino-acid protein; after removal of the 24-amino-acid signal sequence, mature FGF23 circulates as both 227- and 154-amino-acid glycosylated peptides, the latter isoform lacking the 73-amino-acid carboxyl terminal sequence.[247] Acting through its receptor (FGFR1) and coreceptor (α-klotho), FGF23 inhibits sodium-dependent phosphate uptake by renal proximal tubular cells and depresses 25OHD-1α hydroxylase activity and thus synthesis of calcitriol. These two bioactivities define a "phosphatonin," a family of phosphaturic agents that includes, in addition to FGF23, matrix extracellular phosphoglycoprotein (MEPE) and serum frizzled related protein-4 (sFRP4).[239,248] FGF23 inhibits phosphate reabsorption by down-regulating expression of *SLC34A1* and *SLC34A3*, respectively, encoding type II (a and c) sodium-phosphate cotransporters (NPT2a, NPT2c) in the apical membranes of the proximal renal tubule by facilitating their internalization and removal from the renal tubule brush border and thereby depressing reabsorption of filtered phosphate.[239] FGF23 also down-regulates expression of *CYP27B1* encoding 25-hydroxyvitamin D-1α hydroxylase, thus decreasing the synthesis of calcitriol. FGF23 is expressed and secreted primarily by osteoblasts and osteocytes; FGF23 is coexpressed with PHEX in these cells. Although FGF23 is inactivated by cleavage between amino acids Arg179 and Ser180, it is not a biologic substrate for PHEX.[249] Rather, FGF23 is cleaved by subtilisin-like proprotein and furin-like convertases. Disruption of the regulatory mechanism(s) that result in increased FGF23 synthesis in patients with XLHR is unknown but may involve products of *PHEX*, *DMP1*, and *ENPP1*.[247,250]

Although FGF23 is not a physiologic substrate of PHEX, a phosphorylated peptide derived from another phosphatonin (MEPE) may well be the primary PHEX substrate.[250-253] MEPE, a 525-amino-acid noncollagenous protein secreted by osteoblasts, is found in extracellular bone matrix, teeth, and brain. Because MEPE inhibits renal tubular uptake of phosphate and depresses synthesis of calcitriol, it is a phosphatonin. MEPE is also one of the SIBLING (short integrin-binding ligand-interacting glycoprotein) group of noncollagenous matrix proteins whose members include, in addition to MEPE, osteopontin, bone sialoprotein, dentin matrix protein 1, and dentin sialophosphoprotein 1. MEPE contains an acidic serine aspartate-rich MEPE-associated motif



A



B

FIGURE 18-10 ■ Missense (**A**) and frameshift (**B**) mutations in PHEX resulting in X-linked recessive hypophosphatemic rickets. (From PHEXdb, McGill University, Montreal, Quebec, www.phexdb.mcgill.ca/Missense.html and www.phexdb.mcgill.ca/Frameshift.html.)

(ASARM) in its carboxyl terminal region that can be released by cathepsin B; phosphorylated (p)ASARM binds avidly to hydroxyapatite. A noncatalytic property of PHEX is its ability to bind to MEPE, thereby preventing proteolytic degradation of MEPE by cathepsin B and thus preventing the release of ASARM. In the absence of normal PHEX-MEPE binding, ASARM is released from MEPE by cathepsin B, and then its serine residue is phosphorylated. In patients with XLHR, serum ASARM levels are increased. Serine-phosphorylated pASARM coats the surface of hydroxyapatite by interacting with its calcium atoms; it thus impairs further deposition of calcium and phosphate and thereby inhibits mineralization. pASARM is also a substrate for the enzymatic activity of PHEX, which proteolytically cleaves the peptide between serine-glutamate and serine-aspartate residues; by doing so, PHEX destroys pASARM and prevents pASARM-mediated inhibition of bone mineralization. Although nonphosphorylated ASARM also binds (weakly) to hydroxyapatite, inhibits mineralization, and can be enzymatically cleaved by PHEX, the role of nonphosphorylated ASARM in the mineralization process is as yet incompletely understood. Accordingly, in XLHR the primary pathophysiologic abnormalities appear to be the failure of mutated, bioinactivated PHEX (1) to bind to MEPE and thereby to inhibit the cathepsin B–mediated proteolytic cleavage of MEPE and release of ASARM and (2) to destroy enzymatically the pASARM derived from MEPE and thereby permit pASARM to coat hydroxyapatite and inhibit mineralization. In addition to the role of PHEX-pASARM in the pathogenesis of XLHR, the two phosphatonins—FGF23 and MEPE—present in excess exacerbate the mineralization defect by impairing renal tubular reabsorption of phosphate thus increasing renal phosphate excretion and decreasing the supply of phosphate ions necessary for the construction of hydroxyapatite and by decreasing the synthesis of calcitriol, thus depressing the intestinal absorption of calcium. There is a feedback relationship between pASARM and FGF23 as pASARM increases expression of *FGF23* in bone cells.[253] Inasmuch as ASARM motifs are present in all SIBLING proteins, pASARM is also derived from osteopontin, providing another source of a peptide that is able to bind to and inhibit mineralization of hydroxyapatite; osteopontin-derived pASARM is also a PHEX substrate.[250,252] The pathophysiologic roles of osteopontin and its pASARM moiety in XLHR are uncertain at present.

Normally, FGF23 values are highest in young infants and decline during child and adolescence to adult values (approximately 30 pg/mL utilizing an immunometric assay that detects intact FGF23). FGF23 levels increase with phosphate loading and decline in response to phosphate deprivation.[254] Elevated levels of FGF23 are present in patients with many forms of hypophosphatemic rickets including XLHR, autosomal dominant and recessive hypophosphatemic rickets, the disorders of McCune-Albright, osteoglophonic dysplasia, and Jansen-type metaphyseal chondrodysplasia, the linear nevus sebaceous syndrome, and tumor induced rickets/osteomalacia.[255] Serum PTH concentrations are normal in subjects with primary hypophosphatemia due to an abnormality in renal tubular resorption of phosphate—either intrinsic to the renal cell or mediated by a circulating phosphatonin.[240] Secondary hyperparathyroidism as a cause of hypophosphatemia may be suspected by the presence of elevated serum concentrations of PTH (e.g., vitamin D deficiency).

The diagnosis of XLHR is established when the typical family history (if the patient does not carry a de novo mutation), clinical findings (deformities of the lower extremities, flaring of the metaphyses), roentgenographic (rachitic changes) and laboratory data (hypophosphatemia, hyperphosphaturia, inappropriately low serum level of calcitriol, normal serum concentration of PTH, calcium, creatinine, and 25OHD) are present and when other causes of hypophosphatemia and hyperphosphaturia have been excluded (see Tables 18-8, 18-9, 18-10, and 18-11). The diagnosis of XLHR may be further confirmed by identification of the *PHEX* mutation, although no mutation in *PHEX* is detected by current methods in perhaps half of patients with XLHR.[246] Rarely, somatic and germline mosaicism for a mutation in *PHEX* may mimic autosomal dominant transmission of hypophosphatemic rickets.[256] The primary therapeutic agents employed in the treatment of XLHR are calcitriol (25 to 70 ng/kg/day) administered in two daily doses with the larger dose given at night when PTH secretion tends to increase and elemental phosphorus 0.25 to 3 grams daily (beginning at a dose of 30 mg/kg/day and increasing to 70 mg/kg/day administered in four to six divided daily doses to a maximum of 3500 mg/day) depending on age, size, compliance, and response to therapy.[240,241] Table 18-3 lists the preparations of oral phosphate; infants and young children may tolerate a phospha-soda solution more readily than other preparations; when able, most older children prefer a chewable phosphate tablet to the powder form that is dissolved in water or juice. Acidic potassium phosphate products are preferred, as they do not increase intravascular volume and phosphate excretion and do acidify the urine, thereby increasing the solubility of calcium phosphate. Calcitriol (Rocaltrol; Roche Pharmaceuticals) is available as an oral solution at a concentration of 1 μg/mL and as either 0.25- or 0.5-μg capsules. If hypercalcemia or hypercalciuria (urine calcium excretion more than 4 mg/kg/day) occurs during treatment, the dose of calcitriol should be lowered; if that exacerbates the rachitic process, an agent (e.g., amiloride) that increases renal tubular resorption of calcium may be added cautiously to the therapeutic program. Frequent (every 3 months) clinical evaluation and measurement of serum and urine calcium, phosphate, alkaline phosphatase, creatinine levels, and serum intact PTH values are essential to avoid hypercalcemia, hypercalciuria and nephrocalcinosis, and secondary hyperparathyroidism, as high doses of phosphate may lead to counterproductive secondary (and sometimes tertiary) hyperparathyroidism. Two goals of therapy of XLHR are to maintain serum phosphate concentrations determined before a daytime dose of phosphate in the low normal range and alkaline phosphatase values within the high normal range. Renal sonograms prior to treatment and at 12-month intervals during therapy to identify an early

stage of nephrocalcinosis and yearly skeletal radiographs to assess the degree of healing of the rickets are recommended. Complete radiologic healing of XLHR is often difficult to attain. Development of nephrocalcinosis (and compromise of renal function in some patients) is directly related to the amount of phosphate the patient receives; hyperoxaluria has also been implicated in the pathogenesis of nephrocalcinosis. Co-management with an experienced orthopedist is important, as the orthopedist may prescribe braces or, in patients with extreme and progressive deformities, perform corrective surgery. Femoral and tibial hemiepiphysiodeses are surgical procedures for the correction of limb deformities in children with XLHR who are younger than 10 years of age.[257] Osteotomies may be required by older subjects.

The development of drugs that suppress FGF23 secretion or interfere with its function would address one of the fundamental pathogenic mechanisms of XLHR. Calcitonin decreases serum FGF23 concentrations in patients with XLHR.[258] In one study, after a single injection of 200 IU of salmon calcitonin to seven patients with XLHR, serum levels of FGF23 declined 23% in 4 hours and remained 12% below baseline values 16 hours after calcitonin administration. Serum levels of phosphate and calcitriol increased after calcitonin administration, whereas urinary phosphate excretion was not altered.[258] Whether calcitonin will prove to be an agent that can improve treatment of patients with XLHR remains to be explored. Very short-term administration of the calcimimetic cinacalcet to children with XLHR led to a suppression of phosphate-induced secretion of PTH, suggesting that this drug might be useful for preventing the secondary hyperparathyroidism that often develops when XLHR patients are treated with phosphate and calcitriol, but further data on the efficacy and safety of this agent in children with XLHR are required.

Although the birth length of children with XLHR is normal, growth rate is slow during the first several years of life, leading to progressive shortening of height. Many children with XLHR are significantly short by 5 years of age, although some patients (particularly girls) with minimal involvement may grow normally. Treatment of the older child with XLHR with calcitriol and phosphate may improve growth rate, in part due to correction of the deformities of the lower extremities. During puberty, gain in height is normal in boys (+28.2 cm) and girls (+24.2 cm) with XLHR; thus, the compromised adult stature in XLHR is related to impaired early childhood and preadolescent growth. In a series of 19 closely monitored children with XLHR, initiation of treatment with calcitriol and phosphate at a mean chronologic age of 4.2 months (range 7 weeks to 6 months, N = 8) resulted in greater adult stature (−0.2 versus −1.2 SDS) than when treatment began at a mean age of 2.1 years (range 1.3 to 8 years, N = 11) with no difference in complication rate (secondary hyperparathyroidism, nephrocalcinosis, craniosynostosis) between the two groups.[259] The enhanced growth response to early treatment was likely related to the normal length and mild skeletal and biochemical signs of rickets in early infancy and the prevention of more clinically significant bone disease as the child aged. Despite close adherence to treatment, many children with XLHR remain stunted in height due primarily to short lower limbs and retain mild to moderate radiographic signs of rickets and mildly elevated serum alkaline phosphatase activities, although the extent of lower limb deformities is partially ameliorated by good compliance.[260] Human growth hormone increases glomerular filtration rate, renal tubular resorption and serum concentrations of phosphate, and the rate of accrual of bone mineral; it accelerates height velocity and the rate of increase in limb length of children with XLHR and may modestly increase adult stature in some but not very many subjects.[261-263]

Many untreated adults with XLHR are hypophosphatemic; serum alkaline phosphatase activity is increased and osteomalacia is present on bone biopsy; nevertheless, these patients are often clinically asymptomatic except for frequent dental abscesses, degenerative hip disease due to deformities of the lower limbs, and hearing impairment.[240] Occasionally, a female with XLHR may be clinically well despite isolated hypophosphatemia. Roentgenographic manifestations of XLHR in adults include thickening of the spinous processes and fusion of the vertebrae and stenosis of the spinal canal. BMD determined by single and dual photon absorptiometry tends to be normal in adults with XLHR (despite the histomorphologic abnormalities), suggesting that most of these subjects are not at increased risk for osteoporotic fractures. However, in approximately 25% of adults with XLHR there is clinical evidence of osteomalacia such as progressive lower limb deformities, bone pain, fractures, and pseudofractures. Treatment of selected adult XLHR patients with calcitriol and phosphate may be beneficial.[241]

Inactivating mutations in *CLCN5* encoding a voltage-gated proximal renal tubular chloride channel lead to X-linked recessive hypophosphatemic rickets with hypercalciuria, nephrocalcinosis, and renal failure (XLRH, MIM 300554) as well as X-linked nephrocalcinosis, nephrolithiasis, and renal failure (MIM 310468), and Dent disease (aminoaciduria, proteinuria, glycosuria, hypercalciuria, nephrocalcinosis, nephrolithiasis; MIM 300009).[264] Mutations in different domains of this 12 exon, 746-amino-acid transmembrane chloride channel result in varying clinical and biochemical manifestations; the Ser244Leu mutation in *CLCN5* has been associated with X-linked recessive hypophosphatemic rickets.

Autosomal dominant hypophosphatemic rickets (ADHR, MIM 193100) is a partial phenocopy of XLHR with hyperphosphaturia and inappropriately normal or low serum levels of calcitriol that is due to mutations (Arg176Gln, Arg179Trp) in *FGF23* that render the product less susceptible to cleavage between amino acid residues 179 and 180 by subtilisin-like proteases and thus extend the biologic life and effects of this peptide.[239,242,255] ADHR may be incompletely penetrant, variable in age of onset (childhood to adult), and rarely self-limiting. The clinical, biochemical, and radiographic findings in ADHR are similar to those of XLHR except that ADHR subjects manifest muscle weakness as a consequence of hypophosphatemia. ADHR may be identified by its pattern of transmission and detection of a mutation in *FGF23*. Treatment involves administration of calcitriol and

phosphate with close serial monitoring both for safety and because hyperphosphaturia may occasionally resolve spontaneously.

Hypophosphatemic rickets with hyperparathyroidism (MIM 612089) is a disorder characterized by the onset of hypophosphatemia and diffuse parathyroid hyperplasia in infancy.[265] Pathogenetically, it is considered to be the result of excessive production of α-klotho, the coreceptor with FGFR1(IIIc) for FGF23 that converts FGFR1(IIIc) into the specific FGF23 receptor that transduces the FGF23 intracellular signaling pathway and its bioactivity. α-Klotho excess appears to enhance the production and bioactivity of FGF23 resulting in hyperphosphaturia and hypophosphatemia and also to mediate parathyroid gland hyperplasia leading to excessive synthesis of PTH.[247] In one patient with this disorder, excessive production of α-klotho was attributed to a spontaneously occurring balanced translocation between the long arms of chromosomes 9 and 13 [−t(9;13)(q21.13;q13.1)] (near the site of *KL*) possibly involving a promoter region that permitted excessive expression of *KL* and synthesis of α-klotho.[265]

Autosomal dominant hypophosphatemia with urolithiasis type 1 (MIM 612286) is due to *monoallelic* inactivating mutations in *SLC34A1* encoding the sodium-phosphate cotransporter NPT2a (also designated NaPi-IIa). Haploinsufficiency of NPT2a in the brush border membrane of proximal renal tubular cells leads to diminished phosphate reabsorption, hyperphosphaturia, hypophosphatemia, increased synthesis of calcitriol with augmented absorption of intestinal calcium, hypercalciuria, formation of renal calculi, and osteopenia. Autosomal recessive Fanconi syndrome with hypophosphatemic rickets (MIM 613388) (also designated Fanconi renotubular syndrome 2) is due to *biallelic* inactivating mutations in *SLC34A1* encoding the sodium-phosphate cotransporter NPT2a.[266,267]

Autosomal dominant hypophosphatemia with urolithiasis type 2 (MIM 612287) is attributable to heterozygous loss-of-function mutations in *SLC9A3R1* encoding the sodium hydrogen exchanger regulatory factor 1 (NHERF1).[242,268,269] NHERF1 is an adaptor protein; one of its several functions is to anchor the sodium-phosphate cotransporter NPT2a to the brush border of the renal proximal tubule; another is to modulate PTH1R signaling in response to PTH. Loss of NHERF1 function results in decreased expression of NPT2a at the apex of proximal renal tubular cells and thus in diminished renal tubular reabsorption of filtered phosphate, hyperphosphaturia, and hypophosphatemia. Hypophosphatemia leads to increased renal tubular synthesis of calcitriol and intestinal absorption of calcium resulting in mild hypercalcemia, hypercalciuria, and nephrolithiasis.[270] Patients with mutations in *SCL9A3R1* are also osteopenic. In these subjects, serum levels of PTH and FGF23 are normal.[268]

Hereditary hypophosphatemic rickets with hypercalciuria (MIM 241530) is due to biallelic inactivating mutations in *SLC34A3* encoding the sodium-phosphate cotransporter NPT2c.[266] This 599-amino-acid protein with eight transmembrane domains is located in the brush border of juxtamedullary proximal renal tubular cells. Loss of activity of NPT2c leads to hyperphosphaturia, hypophosphatemia, increased synthesis of calcitriol with augmented absorption of intestinal calcium, hypercalciuria, the formation of renal calculi, and rickets. Inasmuch as the regulation of calcitriol synthesis is normal in these subjects, its production is substantially elevated in response to hypophosphatemia; thus, intestinal absorption of calcium and its urinary excretion are increased. Heterozygotic carriers of inactivating mutations in *SLC34A3* also have moderately increased serum concentrations of calcitriol, hyperphosphaturia, and hypercalciuria but do not have identified metabolic bone disease. Loss-of-function missense and nonsense mutations have been found throughout the coding region of *SLC34A3* as well as deletions in introns 9 and 10. This disorder may be treated with phosphate salts in conjunction with hydration and avoidance of a high-sodium diet; supplemental vitamin D is not needed and may even be detrimental.

Excessive production of FGF23 with hypophosphatemic rickets and inappropriately low calcitriol values has also been documented in patients with autosomal recessive hypophosphatemic rickets (discussed later), the McCune-Albright syndrome of fibrous dysplasia (MIM 1740800) due to a gain-of-function mutations in *GNAS*; the linear nevus sebaceous syndrome (MIM 163200) related to postzygotic somatic gain-of-function mutations in *HRAS* or *KRAS*; osteoglophonic dysplasia (MIM 166250; craniosynostosis, rhizomelic shortening of the limbs, noncalcifying bone lesions) associated with activating mutations in *FGFR1*, opsismodysplasia (MIM 258480), a spondylo(epi)metaphyseal dysplasia with delayed ossification, micromelia, platyspondyly, vertebral hypoplasia) attributed to biallelic inactivating mutations in *INPPL1* encoding an inositol-1,4,5-trisphosphatase that hydrolyzes inositol-1,4,5-trisphosphate to inositol-4,5-bisphosphate, an important transducer of intracellular signaling; Jansen type metaphyseal chrondrodysplasia (MIM 156400) due to constitutively activating mutations in *PTH1R*.[248,255,271-273] In most of these disorders, there is excessive skeletal production of FGF23 of unknown pathogenesis, although it has been suggested that in situations in which the rate of bone remodeling is high, the production of FGF23 is also increased.[247] Tumor-induced osteomalacia/rickets is an acquired disorder due to excessive synthesis of one of several phosphatonins by a tumor of mesodermal origin. The majority of such tumors have secreted FGF23, but these neoplasms have also synthesized produced frizzled related protein-4, matrix extracellular phosphoglycoprotein, and FGF7 all of which increase urinary phosphate excretion and suppress renal synthesis of calcitriol, albeit perhaps not to the same extent as FGF23.[248,274] The identification of an FGF23 secreting tumor is dependent on the sensitivity of the FGF23 assay employed.[275] Although unusual in children, tumor-induced osteomalacia/rickets has been described in this age group. For example, an 11-year-old girl had significant bone pain and functional limitation associated with biopsy-proven hypophosphatemic osteomalacia/rickets and markedly elevated serum levels of FGF23.[276] Following removal of a benign fibro-osseous tumor from a small exostosis on a distal ulnar metaphysis, serum FGF23 concentrations normalized within 7 hours

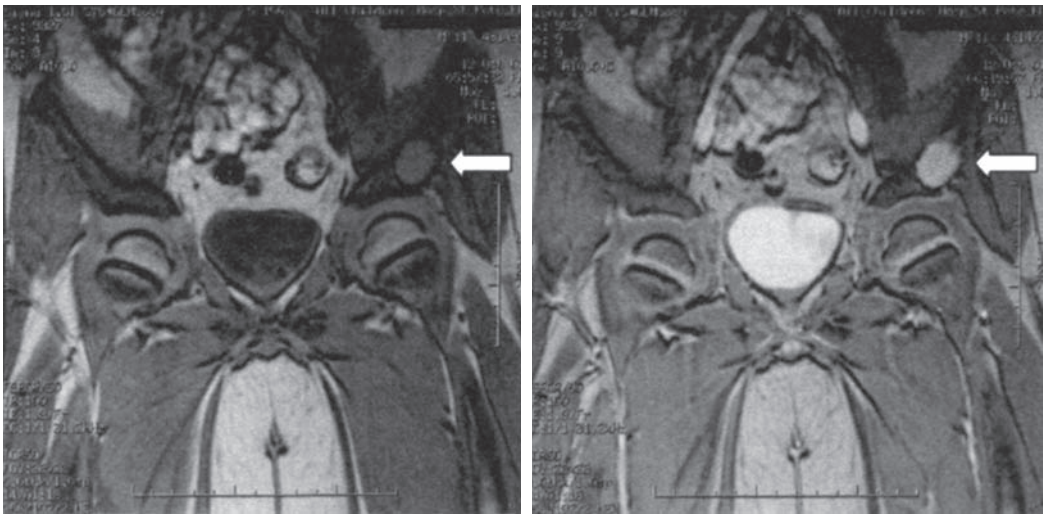


FIGURE 18-11 ■ Demonstration by gradient recall echo magnetic resonance imaging of a FGF23 producing hemangiopericytoma in the left iliac wing of an 11-year-old boy with tumor-induced rickets. (From Shulman, D. I., Hahn, G., Benator, R., et al. (2004). Tumor-induced rickets: usefulness of MR gradient echo recall imaging for tumor localization. *J Pediatr, 144*, 381–385.)

postoperatively and phosphate levels were normal 2 weeks later. Clinical symptoms abated and radiographic and histomorphometric abnormalities resolved within 1 year after surgery. In an 11-year-old boy with severe bone pain, weakness progressing to confinement to a wheelchair, hyperphosphaturia and hypophosphatemia, elevated levels of FGF23 (1874 RU/mL) declined to normal values (43 RU/mL) within 48 hours after removal of a FGF23-containing hemangiopericytoma from his left iliac wing.[277] This lesion had not been identified by routine roentgenograms, computed tomographic or magnetic resonance imaging, or technetium bone scan; the tumor was demonstrated only by gradient recall echo magnetic resonance imaging (Figure 18-11). Within 2 weeks after surgery the lad was walking without assistance, and several weeks thereafter he resumed normal activity.

Mutations in two genes result in autosomal recessive hypophosphatemic rickets (ARHR) that is clinically, biochemically, pathogenetically, and histomorphometrically similar to XLHR and ADHR but distinguished by its mode of transmission and development of osteosclerosis at the base of the skull and in calvarial bones. ARHR type 1 (ARHR1, MIM 241520) is due to biallelic loss-of-function mutations in the gene encoding dentin matrix acidic phosphoprotein 1 (*DMP1*).[255,278,279] DMP1 is a noncollagenous, serine-rich, bone matrix calcium-binding protein that is a secreted small integrin-binding ligand, N-linked glycoprotein (SIBLING) expressed by osteocytes; DMP1 is important for bone mineralization and may also play a role in the regulation of FGF23 synthesis.[250] ARHR type 2 (ARHR2, MIM 613312) is due to biallelic loss-of-function mutations in the gene encoding ectonucleotide pyrophosphatase/phosphodiesterase (*ENPP1*), a pyrophosphohydrolase expressed by chondrocytes and bone and plasma cells that hydrolyzes ATP to pyrophosphate, an inhibitor of bone mineralization.[242,255] The pathophysiology of bone disease due to ENPP1 deficiency resembles that of hypophosphatasia due to a deficiency of alkaline phosphatase (discussed later). Mutations in ENPP1 have also been associated with generalized arterial calcification of infancy; indeed, the same genetic mutation in *ENPP1* may be clinically expressed as either disease in the same family.[280,281,282] Serum concentrations of FGF23 are elevated in these subjects, and calcitriol values are inappropriately normal; urinary calcium excretion is normal. The mechanism or mechanisms by which loss of ENPP1 activity increases FGF23 synthesis are unclear.

Increased urinary phosphate excretion due to acquired and heritable disorders of the proximal renal tubule is characteristic of the metabolic bone disease that accompanies various forms of the Fanconi syndrome of renal tubular acidosis, glucosuria, and amino aciduria (heritable: cystinosis, tyrosinemia, galactosemia, oculo-cerebral-renal syndrome, fructose intolerance, Wilson disease; acquired: renal transplantation, nephrotic syndrome, renal vein thrombosis, mercury, lead and copper poisoning, outdated tetracycline).[283] In addition to hypophosphatemia, acidosis contributes to the pathogenesis of bone disease in Fanconi syndrome by increasing the solubility of the mineral phase of bone and increasing urinary loss of calcium and by impairing conversion of calcidiol to calcitriol.

Hyperphosphatemia may be due to intrinsic gene variants or to exogenous causes such as excessive parenteral administration of phosphate or to the administration of phosphate containing enemas, an acutely increased rate of cellular destruction (crush injuries, rhabdomyolysis, during chemotherapy for malignancy), a decreased rate of renal phosphate excretion (renal failure), impaired secretion or action of PTH, and during administration of some bisphosphonates (see Tables 18-11A and B).[239] Hyperphosphatemic familial tumoral calcinosis (MIM 211900) is an autosomal recessive disorder characterized by progressive deposition of calcium phosphate in soft

tissues and in periarticular regions of the long bones, manifested clinically by periarticular masses of ectopically deposited calcium and at times by complaints of bone pain.[284] Cortical bone hyperostosis and periosteal elevation are common radiologic findings. The disorder is due to the decreased availability of biofunctional FGF23 due to loss-of-function mutations in *FGF23* itself, the rapid degradation of FGF23 because it has not been glycosylated due to an inactivating mutation in *GALNT3* encoding UDP-N-acetyl-alpha-D-galactosamine: polypeptide N-acetylglactosaminyl transferase 3, or tissue nonresponsiveness to FGF23 because of an inactivating mutation in KL, encoding its coreceptor α-klotho. Surgical excision of tumoral masses that impair function, administration of intestinal phosphate binders, agents that increase renal phosphate excretion (acetazolamide), and diets low in calcium and phosphate have been the major therapeutic strategies employed in the treatment of these patients.[239]

Disorders of Alkaline Phosphatase Activity. In its most severe forms, hypophosphatasia (MIM 241500) is an autosomal recessive disorder due to loss-of-function mutations in *ALPL*, the gene encoding tissue nonspecific (bone/liver/kidney) alkaline phosphatase (TNSALP); less damaging forms of this disease are transmitted as autosomal dominant traits (MIM 241510, 146300).[108-110] Alkaline phosphatases are zinc and magnesium-dependent alkaline optimum orthophosphoric monoester phosphohydrolases. Bone, liver, and kidney alkaline phosphatase isoforms differ not by their amino acid structure but by their patterns of posttranslational glycosylation.[109] Homotetrameric bone TNSALP is anchored through its carboxyl terminal to the exterior cell membrane of hypertrophic chondrocytes and osteoblasts (i.e., it is an ectophosphatase) by a phosphatidylinositol-glycan moiety. Pathophysiologically, decreased TNSALP activity leads to accumulation of its endogenous substrates: inorganic pyrophosphate, pyridoxal 5′-phosphate, and phosphoethanolamine. Pyrophosphate is an inhibitor of osteoid mineralization and when present in excess leads to the inadequate formation of hydroxyapatite resulting in rickets or osteomalacia. Calcification of bone matrix is impaired by pyrophosphate coating of the surface of hydroxyapatite crystals and restricting their growth; in addition, the inability to raise bone matrix inorganic phosphate levels to values sufficient to permit the deposition of hydroxyapatite may contribute to decreased bone mineralization in this disorder. Thus, the pathophysiology of bone disease due to TNSALP deficiency resembles that due to the deficiency of ENPP1 in which disorder pyrophosphate also accumulates but by a distinctively different mechanism (as discussed previously). Decreased pyridoxal 5′-phosphate, the major circulating form of vitamin B_6, leads to pyridoxine-responsive seizures.

The incidence of severe hypophosphatasia ranges from 1/2500 newborns in Canadian Mennonite families to 1/300,000 in European populations.[109] Clinical manifestations of inactivating mutations of *ALPL* reflect primarily the extent of loss-of-function of mutant *ALPL*; the younger the age of onset, the more severe is the disease likely to be. Currently, there are seven recognized forms or types of hypophosphatasia: six classical forms (numbers 1 to 6) and one transitional type (here designated type 2A):

1. The *perinatal* form is evident in utero and is most often lethal (at times even before birth) due to marked osteopenia leading to malformation of the skull, intracranial hemorrhage, pyridoxine-dependent seizures, fractures of the ribs, and deformities of the chest wall resulting in postnatal respiratory insufficiency and apnea; the extremities are short, bowed, and fractured; radiologic findings include absent or extremely poor bone mineralization with rachitic deformities, irregular radiolucent extensions into metaphyses, apparently "missing" vertebrae, and protruding midshaft fibular and ulnar bone spurs.
2. The *infantile* form (MIM 241500) becomes clinically apparent in the first 6 months of life and is characterized by anorexia, impaired linear growth and weight gain, deformities of the long bones and rib cage, and widely "open" fontanelles and sutures (calvarial hypomineralization) with functional craniosynostosis with increased intracranial pressure manifested by prominence of the anterior fontanelle, proptosis, and papilledema, weakness, developmental delay, pyridoxine-dependent seizures, hypotonia, constipation, hypercalcemia, hypercalciuria, nephrocalcinosis, early shedding of deciduous teeth, and radiographic evidence of rickets with marked skeletal hypomineralization; the neonatal and infantile forms of hypophosphatasia are transmitted as autosomal recessive disorders; until recently, the majority of patients with perinatal hypophosphatasia and 50% of those with infantile hypophosphatasia died of respiratory failure before their first birthday (discussed later).

2A. In the *benign prenatal* or *transitional* form of hypophosphatasia, the fetus and neonate manifest skeletal deformities but not radiographic signs of rickets; shortly after birth there is spontaneous and substantial improvement; findings that distinguish this less severe type of hypophosphatasia are fetal crowding, normal fetal bone mineralization, and appropriate chest volume; this form of hypophosphatasia may be associated with either monoallelic or biallelic mutations in *TNSALP*.[285,286]

3. In the *childhood* form of hypophosphatasia (MIM 241510), clinical findings of hypophosphatasia develop after 6 months of age and range from isolated premature shedding of deciduous teeth to physical and radiographic evidence of low bone mass and rickets and musculoskeletal and joint pain; radiography may demonstrate radiolucent projections from the growth plate into metaphyses; spontaneous clinical improvement may occur during puberty and this form may evolve into type 4.
4. The *adult* form (MIM 146300) may be subclinical and identified only during study of a family with an offspring with a more significant form of hypophosphatasia; the parent may have a history of premature loss of teeth, increased susceptibility to recurrent fractures predominantly of the metatarsal bones, pseudofractures of the proximal femur,

chondrocalcinosis, or pseudogout due to articular deposition of calcium pyrophosphate dihydrate crystals; subtrochanteric femoral pseudofractures may be demonstrated radiographically.

5. *Odontohypophosphatasia* (MIM 146300) is manifested only by premature shedding of primary teeth without radiologic abnormalities; periodontitis may also develop; the childhood and adult forms of hypophosphatasia and odontohypophosphatasia are usually (but not invariably) transmitted as monoallelic, autosomal dominant disorders.
6. *Pseudohypophosphatasia* is phenotypically and biochemically similar to classic infantile hypophosphatasia, but serum alkaline phosphatase activity in vitro is normal, indicating that in this disorder enzyme activity toward artificial substrates in high pH solutions is preserved but alkaline phosphatase activity toward endogenous substrates at pH 7.4 is abnormal.[109]

In addition to the site(s) of mutation of *ALPL*, the clinical severity of hypophosphatasia is related inversely to the age at which skeletal disease is evident and to individual biologic (possibly epigenetic) factors that affect the expression of the disease with wide individual variability even in family members with the identical *ALPL* mutation(s). In the lethal perinatal form of hypophosphatasia, missense, nonsense, and donor splice site mutations and frameshift deletions in *TNSALP* cluster in crucial segments of the protein within (Ala94Thr, Arg167Trp) or near (Gly103Arg, Gly317Asp) its catalytically active enzymatically functional site or at the dimeric interface (Ala23Val, Arg374Cys) or they may impair movement of TNSALP to its active transmembrane site.[109] Mutations may interrupt binding of TNSALP to the phosphorylated ligand or destabilize attachment of necessary cofactors such as zinc or magnesium. *ALPL* mutations associated with the childhood form of hypophosphatasia (Arg119His, Asp361Val) tend to cluster on the three-dimensional surface of the enzyme molecule at sites that relate to its tethering to the cell surface or its formation of tetramers; such variants may retain substantial residual bioactivity. In some patients with moderately severe hypophosphatasia, heterozygotic mutations in *ALPL* (Gly46Val, Arg167Trp, Asn461Ile) exert a dominant-negative effect on the intact TNSALP dimer; these mutations have been clustered at the enzyme active site or at the domain(s) involved with dimerization, tetramerization, or membrane anchoring.[287] Odontohypophosphatasia has been associated with heterozygotic mutations in *ALPL* (Pro91Leu, Ala99Thr).

Diagnostically, in addition to the clinical findings and radiographic features of rickets, patients with hypophosphatasia have low serum levels of total and bone-specific alkaline phosphatase activity in contrast to other forms of rickets in which alkaline phosphatase values are increased (see Table 18-10). Additionally, there are increased serum concentrations of pyrophosphate (controls < 200 μmol/g of creatinine) and pyridoxal-5′-phosphate (~5000 nM) and elevated urinary excretion of phosphoethanolamine (controls < 15 years of age 83 to 222 μmol/g of creatinine). Serum concentrations of calcium and phosphate are often elevated in patients with the perinatal and infantile forms of the disease, because intestinal absorption of calcium and renal tubular reabsorption of phosphate are at least normal, whereas the bone formation rate is depressed; calcium values are usually normal in the childhood form of hypophosphatasia.[108,109] Serum levels of PTH are normal or low; there is no evidence of secondary hyperparathyroidism. Although serum alkaline phosphatase activity in vitro is normal in patients with pseudohypophosphatasia, it is functionally inadequate in vivo—perhaps because the TNSALP protein is sequestered within the cell due to a defect in its transport to the cell surface or because the protein is active with artificial substrate but not with natural substrates at physiologic pH. The prenatal diagnosis of hypophosphatasia may be considered in a fetus in whom ultrasonography has demonstrated short ribs and long bones, cupping of the metaphyses, osseous spurs, patchy ossification, and demineralization of the skull and thoracic spine.[288] To be included in the differential diagnosis of a fetal sonogram with these findings are achondrogenesis and osteogenesis imperfecta type II. Other causes of hypophosphatasemia include hypothyroidism; vitamin C deficiency; starvation; magnesium, zinc, or copper deficiency; celiac disease; profound anemia; and massive transfusion.[109]

Until recently, treatment of the perinatal and infantile forms of hypophosphatasia has been unsatisfactory. Attempts to administer alkaline phosphatase by infusion of serum from patients with Paget disease and high alkaline phosphatase activity have been of only occasional and limited benefit. Bone marrow transplantation has been beneficial in some subjects, whereas transplantation of bone fragments and osteoblasts has effectively restored alkaline phosphatase activity in a few patients with infantile hypophosphatasia.[108,289] A biosynthetic human recombinant ectodomain of tissue nonspecific alkaline phosphatase fused to the human IgG1 Fc domain and a terminal deca-aspartate motif targeting bone (asfotase alfa; Enobia Pharma) has been developed that has proven extraordinarily effective in the treatment of patients with the perinatal and infantile forms of hypophosphatasia.[111] Administration of this compound to nine such patients was followed by radiographic healing of rickets after 24 weeks of treatment; in addition motor development and pulmonary function improved. No serious adverse effects were recorded in this study. A clinical trial (NCT00739505) of the efficacy and safety of biosynthetic human recombinant tissue nonspecific alkaline phosphatase fusion protein in patients with the adult form of hypophosphatasia has been completed as well; data analysis of this cohort is anticipated.[290] Administration of even small doses of vitamin D or its metabolites should be avoided, as these patients easily develop hypercalcemia. Hypercalcemia in infants with hypophosphatasia responds to bisphosphonates and transiently to calcitonin. Seizures may be responsive to pyridoxine administration. Milder adult forms of hypophosphatasia may be responsive to teriparatide (rhPTH$^{1\text{-}34}$).[291]

Serum bone-specific alkaline phosphatase activity is normally increased during puberty and in response to skeletal injuries and reflects increased osteoblastic activity. Transient increase in serum alkaline phosphatase activity may also be observed in children with viral infections (including human immunodeficiency virus), after liver or kidney transplantation, or during treatment of

leukemia/lymphoma.[292] Transient hyperphosphatasemia of infancy and early childhood is encountered in clinically well infants and children younger than 5 years of age (median age: 16 months); serum activities of both bone and liver alkaline phosphatase isoforms are increased (despite absence of liver or bone disease); it is a self-limited process with alkaline phosphatase values returning to normal within several months.[292] Transient hyperphosphatasemia (alkaline phosphatase values > 1000 U/L) has been reported to occur in approximately 2% to 3% of children younger than 2 years of age.[293] It has been suggested that benign and transient hyperphosphatasemia are the consequence of excessive sialylation of alkaline phosphatase that delays its degradation and extends its half-life, but the reason for the excessive sialic acid content of the molecule is uncertain.[292] Rarely, hyperphosphatasemia may be familial and transmitted as a benign autosomal dominant trait or associated with mental retardation (MIM 239300). Occasionally, an adolescent with persistent, nonfamilial, benign hyperphosphatasemia may be encountered. Hyperphosphatasemia is also present in patients with juvenile Paget disease/hyperostosis corticalis deformans juvenilis (MIM 239000), familial expansile osteolysis (MIM 174810), and osteosclerotic hyperostosis corticalis generalisata/van Buchem disease (MIM 239100) (discussed later).

Chronic Kidney Disease-Mineral and Bone Disorder

Chronic kidney disease-mineral and bone disorder (CKD-MBD) is the term presently employed to characterize the aberrations in calcium and phosphate metabolism, PTH and vitamin D synthesis and secretion, skeletal homeostasis, and ectopic vascular, extravascular, and cardiac calcifications that are encountered in patients with impaired renal function.[293a,294] CKD-MBD replaces the designation of renal osteodystrophy that pertains solely to the pathologic changes present in the bones of patients with chronic renal failure. Renal osteodystrophy is most commonly associated with rapid bone remodeling and increased rates of both bone resorption and formation that are due to secondary hyperparathyroidism ("high-turnover" lesions of osteitis fibrosa). Adynamic ("low-turnover") bone disease with relatively low PTH secretion and osteomalacia due to accumulation of aluminum may also occur. Generally, areas of both low and high bone turnover, termed *mixed renal osteodystrophy*, are detected by histomorphometry.[157,295]

As renal function declines, the secretion of both FGF23 and PTH increases. It is likely that FGF23 secretion increases relatively early in patients with CKD when phosphate retention first occurs and even when the glomerular filtration rate exceeds 80 mL/min/1.73 m^2.[296] The secretion of PTH increases when the glomerular filtration rate declines to values less than 70 mL/min/1.73 m^2.[294] Inasmuch as serum concentrations of FGF23 rise before renal function declines in patients with CKD-MBD, urinary phosphate excretion increases—initially maintaining normal serum phosphate values; thus, FGF23 levels increase before serum levels of phosphate rise.[296] Furthermore, increasing production of FGF23 as renal failure progresses contributes to declining renal tubular synthesis of calcitriol and lower intestinal calcium absorption. As the glomerular filtration rate progressively declines, urinary phosphate excretion is impaired, leading to its intracellular and extracellular accumulation, hyperphosphatemia (when the glomerular filtration rate falls below 20 mL/min/1.73 m^2), and modest hypocalcemia.[294] Increasing serum concentrations of phosphate, falling levels of calcium (due both to decreased intestinal absorption of this cation attributable to calcitriol deficiency and the need to maintain a constant calcium x phosphate product), and rising levels of FGF23 that exert a direct stimulatory effect on *PTH* expression lead to a secondary increase in PTH generation and when uncontrolled to tertiary hyperparathyroidism. Other factors that contribute to the development of parathyroid chief cell hyperplasia and secondary hyperparathyroidism in CKD-MBD include down-regulation of the expression of *CASR* in uremic parathyroid glands (raising of the set point concentration at which calcium depresses *PTH* expression) and skeletal insensitivity to PTH. Phosphate may also slow the rate of degradation of PTH mRNA within the parathyroid gland and exert a direct enhancing effect on the growth of parathyroid glands as well. Because calcitriol inhibits parathyroid gland growth and function, a decrease in its synthesis also results in increased proliferation of parathyroid chief cells and synthesis of PTH. In the presence of elevated PTH secretion and various cytokines (IL-1, -6, -11, TNF, macrophage-colony stimulating factor [M-CSF]), osteoclastogenesis, and the rates of bone resorption and formation are increased. Acidosis contributes to the dissolution of the mineral phase of bone directly and by impairing osteoprotegerin-mediated inhibition of osteoclast generation.

Chronic renal disease and osteodystrophy in children may be clinically silent except for the failure of linear growth; as the disease progresses deformities of the extremities, slipped epiphyses, fractures, bone and joint pain, muscular weakness, and lassitude develop. In patients in whom the rate of bone formation is diminished and the volume of unmineralized bone (i.e., osteomalacia) increased, the process has been due to an accumulation of aluminum at the mineralization front, but with discontinuation of aluminum-containing phosphate binders osteomalacia in renal failure is now unusual.[157] In the absence of osteomalacia, adynamic bone disease in chronic renal failure is the result of decreased PTH generation due to improved control of serum phosphate levels, increased calcium stores, higher levels of PTH$^{7\text{-}84}$ and other carboxyl terminal fragments of PTH that inhibit bone resorption, and other factors that affect tissue response to PTH. The cardiovascular complications (hypertension, ectopic vascular calcifications, uremic vasculopathy, cardiomyopathy) that develop in children with CKD-MBD begin prior to the need for and progress during dialysis and are the primary cause of death in children and adolescents with CKD.[293a] Hyperphosphatemia, secondary hyperparathyroidism, and elevated levels of FGF23 adversely affect the cardiovascular system.

Biochemically, CKD-MBD is marked primarily by hyperphosphatemia, low-normal serum calcium levels, and increased serum PTH and FGF23 concentrations and alkaline phosphatase activity. Radiographic signs of rickets, low bone mass, and pseudofractures are often

present in children with renal osteodystrophy. Therapeutically, the goals in treating a child with chronic renal failure in an effort to minimize the adverse systemic effects of CKD-MBD are to maintain (near) normal serum calcium, phosphate, and alkaline phosphatase values and to prevent the development or progression of secondary hyperparathyroidism. To do so, minimal amounts of supplemental vitamin D and calcium may be needed and dietary phosphate restriction imposed. Calcitriol is able to decrease the rate of bone formation in patients with chronic renal insufficiency by inhibiting osteoblast differentiation or function, decreasing PTH synthesis, altering degradation of PTH within the parathyroid glands, and decreasing expression of *PTH1R*. Calcium-containing oral phosphate binders may also be useful. Dialysis fluids must be prepared with aluminum-free water. When indicated, suppression of PTH secretion may be further achieved with the use of calcitriol analogs such as paricalcitol or of a synthetic ligand of the CaSR—cinacalcet hydrochloride (with caveats previously mentioned).[157,294] Experimentally, although immunoneutralization of FGF23 in a rat model of CKD-MBD led to a decrease in PTH secretion, increased calcitriol and calcium concentrations, and improved bone formation, there was an increase in aortic calcification and mortality rate in the anti-FGF23 treated animals.[297] Thus, "control" of FGF23 activity may have paradoxically unwanted consequences. Even after successful renal transplantation, secondary hyperparathyroidism may persist for months or years—its extent and intensity reflecting the severity and duration of chronic renal failure before renal transplantation, development of nodular or monoclonal hyperplasia of the parathyroid glands, and the 20-year life span of the parathyroid chief cell.[298] Five years after renal transplantation during childhood, serum concentrations of PICP, osteocalcin, and ICTP remain significantly increased, indicating an accelerated bone turnover rate, whereas areal and volumetric bone mineral densities at the distal third of the nondominant radius are normal for height but subnormal for age.[299] Hypercalcemia and persistent secondary or tertiary hyperparathyroidism requiring parathyroidectomy may become apparent in children after renal transplantation. The osteopenic effects of glucocorticoids and immune suppressive agents such as cyclosporin are observed in postrenal transplantation patients as well.

Disorders of Bone Mineralization

Bone mineralization may be depressed or exaggerated by dysfunction of either osteoblasts or osteoclasts. Abnormalities of osteoblast function result in either impaired (e.g., osteogenesis imperfecta) or exaggerated bone mineralization (e.g., osteosclerosis). Thus, in patients with the osteosclerotic disorder hyperostosis corticalis generalisata/van Buchem disease type 1 (MIM 239100), decreased expression of *SOST* encoding sclerostin, an inhibitor of WNT signaling by its interaction with the WNT coreceptors low-density lipoprotein receptor-related proteins (LRP) 5 and 6, is associated with enhanced osteoblast function and increased bone mineralization. Although derived from a common hematopoietic stem cell, there are a number of osteoclast subtypes with unique physiologic profiles that reflect the site at which they act (intramembranous or endochondral bone, cartilage); the remodeling process in which they participate (targeted—to sites of bone replacement, stochastic—hormonally [PTH, calcitonin, calcitriol] mediated for maintenance of eucalcemia); the type of bone (cortical, trabecular) upon which they act; and the time of day (diurnal variation) at which the osteoclast is most active.[300] Decreased osteoclast formation or function results in increased bone mass and various forms of osteopetrosis, whereas augmented osteoclast activity leads to decreased bone mass due to osteolysis. In a number of bone disorders, skeletal areas of both increased and decreased bone mineralization may be present. Juvenile Paget disease/hyperostosis corticalis deformans juvenilis (MIM 239000) is a hyperphosphatasemic disorder that often begins in early childhood and is characterized clinically by expanded and bowed extremities, nontraumatic fractures of the long bones, kyphosis, macrocephaly, and muscular weakness; this disorder may progress to wheelchair dependence.[301] Serum levels of alkaline phosphatase are markedly increased, a coupled response to increased osteoclastic action. Radiographic skeletal abnormalities include cortical thickening, both osteosclerosis and osteopenia, coarse trabeculations, and progressive skeletal deformities. The disorder is due to biallelic loss-of-function mutations of *TNFRSF11B* (MIM 602643) encoding osteoprotegerin, a member of the tumor necrosis factor (TNF) receptor superfamily that functions as a decoy acceptor for and consequently an inhibitor of *R*eceptor *A*ctivator of *N*uclear factor κB-*L*igand (RANKL), the stromal cell-osteoblast derived osteoclastogenic factor. Normally, osteoprotegerin inhibits osteoclastogenesis; hence, inactivating mutations of *TNFRSF11B* are associated with enhanced osteoclastic activity. The severity of juvenile Paget disease depends on the site of mutation in *TNFRSF11B*—those that result in deletion of the entire gene or those in the ligand binding domain that involve loss of cysteine residues result in marked clinical disease.[302] Treatment with recombinant osteoprotegerin has resulted in clinical and radiologic improvement as has the administration of bisphosphonates, but the latter agents are associated with the risk of substantial hypocalcemia.[303,304] Familial expansile osteolysis (MIM 174810), early onset familial Paget disease of bone, and expansile skeletal hyperplasia are autosomal dominant disorders that are pathophysiologically similar to juvenile Paget disease although clinically and etiologically distinct. These three disorders are due to monoallelic gain-of-function mutations of the gene (*TNFRSF11A*, MIM 603499) encoding RANK that result in an increase in NFκB signaling as a consequence of which there is augmented osteoclastogenesis.[305] In adolescents with familial expansile osteolysis, focal areas of increased bone turnover in the appendicular skeleton appear in the second decade of life followed by medullary expansion, pathologic fractures, and skeletal deformities; deafness and premature loss of dentition may occur in these subjects. Patients with either expansile skeletal hyperplasia or early onset Paget disease of bone demonstrate focal areas of osteolytic and hyperostotic bone, premature loss

of teeth, and deafness. Increased activity of mutant RANK is thought due to tandem duplications of 18 or 27 bases in the signal peptide region of exon 1 of *TNFRSF11A*. However, because the mutant forms of RANK are trapped in the endoplasmic reticulum and unable to interact with RANKL directly, the mechanisms through which these heterozygous mutations increase NFκB-mediated osteoclastogenesis are uncertain.[305,306] It has been hypothesized that mutant RANK may be able to interact with residual intact RANK and either slow its rate of degradation or prolong its interaction with RANKL.[305] Conversely, loss-of-function mutations of *TNFRSF11A* are associated with autosomal recessive, osteoclast-poor osteopetrosis type 7, and hypogammaglobulinemia (discussed later).[305,307] Inactivating mutations in *TNFSF11* encoding RANKL are associated with osteoclast-poor osteopetrosis type 2 (discussed later).

Low Bone Mass

Low bone mass is often associated with increased fracture risk, although in some disorders with high bone mass the fracture rate is increased because abnormal bone microarchitecture leads to decreased bone strength (e.g., osteopetrosis, picnodysostosis, discussed later).[308] Fifty percent of boys and 40% of girls will sustain a traumatic fracture during childhood or adolescence (peak incidence between 11 and 12 years in girls and 13 and 14 years in boys), most often at the distal radius because of a transient decline in cortical strength at this site.[308,309] It is important to distinguish a high-impact traumatic fracture from one that is the result of increased bone fragility such as compression vertebral fractures and "spontaneous" femoral fractures due to a primary or secondary disorder of bone mineralization (Table 18-12A and B).

TABLE 18-12A Disorders of Bone Mineralization: Low Bone Mass

I Primary/Genetic

A Osteogenesis Imperfecta (types I - >XIII; see Table 18-12B)
B Osteoporosis-Pseudoglioma Syndrome (LRP5)
C Idiopathic Juvenile Osteoporosis (LRP5)
D Marfan Syndrome (FBN1)
E Ehlers-Danlos Syndrome (COL1A1)
F Homocystinuria (CBS)
G Fibrous Dysplasia (GNAS)
H Glycogen Storage Disease Type I (G6PC)
I Galactosemia (GALT)
J Menkes: Kinky Hair Syndrome (ATP7A)
K Hypophosphatasia (ALPL)
L Rickets (genetic forms; see Tables 18-9 and 18-11)

II Secondary

A Suboptimal Nutrition
1 Socioeconomic
2 Cultural
3 Excessive exercise (athletic amenorrhea)
4 Anorexia nervosa
5 Vitamin D deficiency
6 Malabsorption: cystic fibrosis, celiac disease, biliary atresia, "short gut" syndrome, post gastric bypass surgery
7 Idiopathic hypercalciuria

B Endocrinopathies/Metabolic Diseases
1 Constitutional delay in growth and sexual development
2 Hypogonadism
  a Hypergonadotropic: gonadal dysgenesis (Turner, Klinefelter syndromes), aromatase deficiency, estrogen receptor deficiency
  b Hypogonadotropic: Kallmann syndrome, excessive physical activity, hyperprolactinemia
3 Diabetes mellitus
4 Hyperglucocorticoidism
5 Hyperthyroidism, excessive exogenous thyroxine
6 Hyperparathyroidism
7 Panhypopituitarism
8 Inborn errors of metabolism: homocystinuria, lysinuric protein intolerance, propionic aciduria, methylmalonic aciduria, glycogen storage disease, galactosemia

C Disuse/Immobilization
1 Fracture
2 Cerebral palsy
3 Duchenne muscular dystrophy
4 Quadriplegia/paraplegia
5 Spina bifida
6 Weightlessness

D Inflammatory Diseases
1 Juvenile arthritis
2 Systemic lupus erythematosus
3 Dermatomyositis
4 Inflammatory bowel disease

E Drugs
1 Glucocorticoids, immune suppressants, anticonvulsant drugs, antiretroviral therapy, warfarin, lithium, methotrexate, cyclosporine
2 Alcohol, tobacco
3 Aromatase inhibitors, medroxyprogesterone
4 Protein pump inhibitors

F Chronic Illness
1 Hemoglobinopathies: thalassemia, sickle cell disease
2 Hemophilia
3 Cranial radiation
4 Renal failure, postrenal transplantation, nephrotic syndrome
5 Malignancy: leukemia, lymphoma, cytotoxic chemotherapy
6 Human immunodeficiency virus infection, highly active antiretroviral therapy
7 Severe burns

Adapted from Boyce AM, Gafni RI (2011). Approach to the child with fractures. J Clin Endocrinol Metab 96:*1943-1952; Ferrari S, Bianchi ML, Eisman JA, et al. (2012). Osteoporosis in young adults: pathophysiology, diagnosis, and management. Osteoporos Int 23:2735-2748; Rauch F, Bishop, N. (2008). Juvenile osteoporosis. In C. J. Rosen (Ed.),* Primer on the metabolic bone diseases and disorders of mineral metabolism, *(7th ed.) Washington, DC: American Society of Bone and Mineral Metabolism, 264-267; Bachrach LK, Ward LM (2009). Clinical review: bisphosphonate use in childhood osteoporosis.* J Clin Endocrinol Metab *94:400-409.*

TABLE 18-12B **Disorders of Mineralization: Osteogenesis Imperfecta**

Type: MIM Gene Chromosome	Severity	Clinical Features	Growth Impairment	Blue Sclera	Inheritance	Functional Defect
I: 166200: *COL1A1*: collagen type I, alpha-1 17q21.33 1120150 or *COL1A2*: collagen type I, alpha-2 7q22.13 120160	Mild	Few (often vertebral) fractures, little deformity, hearing loss in 50%; rarely dentinogenesis imperfecta	Minimal	Present: intense	AD	Nonsense and frameshift mutations result in haploinsufficiency and decreased production of collagen type I
II: 166210: *COL1A1* or *COL1A2*	Perinatal lethal: congenital (see also types VII, VIII, IX, X)	Many rib and long bone fractures in utero and at birth, severe long bone deformities, "beaded" ribs, unmineralized calvarium	Severe	Present	AD, parental mosaicism	
III: 259420: *COL1A1* or *COL1A2*	Severe, progressive, deforming	Moderate to severe bowing, multiple long bone and vertebral fractures, dentinogenesis imperfecta, hearing loss	Severe	Present but lighten with age	AD	Glycine substitutions in *COL1A1* or *COL1A2* result in structurally abnormal collagen type I
IV: 166220: *COL1A2* or *COL1A1*	Moderately deforming	Mild to moderate bowing, fractures, dentinogenesis imperfecta	Moderate, variable	Grayish or absent	AD	Glycine substitutions in *COL1A1* or *COL1A2* result in structurally abnormal collagen type I
V: 610967: *IFITM5*: interferon-induced transmembrane protein-5 11p15.5 614757	Moderately deforming, clinically similar to type IV	Mild to moderate bone fragility, ossification of interosseous membranes of forearm, hyperplastic callus formation at fracture sites; "meshlike" bone lamellation pattern	Mild to moderate	Absent	AD	Osteoblast specific membrane protein essential for osteoblast differentiation; functional consequence of the constant mutation in *IFITM5* is uncertain
VI: 613982: *SERPINF1*: serpin peptidase inhibitor, clade F, member 1 17p13.3 172860	Moderately to severely deforming, clinically similar to type IV	Onset of fractures of long bones and vertebrae in infancy, increased alkaline phosphatase activity and osteoid, "fish-scale" pattern of lamellation	Moderate to severe	Grayish	AR	Loss-of-function mutations in pigment epithelium-derived factor (PEDF): regulator of bone mineralization and osteoclast differentiation
VII: 610682 **CRTAP*: cartilage associated protein 3p22.3 605497	Moderately deforming to severely deforming or lethal (see type II)	Fractures may be present at birth, rhizomelia, limb deformities	Moderate	Absent or faint	AR	Inactivating mutations (duplication) of *CRTAP* impair 3-hydroxylation of prolyl-986 of procollagen α1(I)
VIII: 610915 1p34.2 610339	Severely deforming to lethal, overlaps types II and III	Markedly decreased bone mineralization, scoliosis, platyspondyly, prominent metaphyses, long phalanges	Severe	Absent	AR	Inactivating mutations of *LEPRE* encoding prolyl-3-hydroxylase (leprecan) impair 3-hydroxylation of prolyl-986 of procollagen α1(I)

IX: 259440: *_PPIB_: peptidyl-prolyl isomerase B 15q22.31 123841	Lethal to severely deforming (see type II)	Shortened, bowed, and fractured long bones in midgestation	Severe	Gray	AR	Inactivating mutations of *PPIB* encoding peptidyl-prolyl isomerase B impair 3-hydroxylation of prolyl-986 of procollagen α1(I)
X: 613848: *SERPINH1*: serpin peptidase inhibitor, clade H, member 1 11q13.5 600943	Severe	Short bowed femora in utero, multiple fractures in first month of life, dentinogenesis imperfecta	Severe	Present	AR	Loss-of-function mutations in CBP2 (HSP47): chaperone, essential for maintenance of integrity of the triple helical structure of collagen type 1 and its resistance to proteolytic degradation
XI: 610968 17q21.2 607063	Severe and progressive deformities, overlaps type III	Limbs are short and bowed, joint contractures; dentinogenesis imperfecta; "fish-scale" pattern of bone lamellation (see type VI)	Moderate	Gray	AR	Loss-of-function mutations in a chaperone protein, FKBP65: essential for posttranslational processing of procollagen type I; also mutated in Bruck syndrome type 1
XII: 613849: *SP7*: transcription factor specificity factor (Sp)7 12q13.13 606633	Moderate to severe, resembles OI type IV	Multiple fractures in early infancy; short, bowed long bones, highly arched palate	Severe	Absent	AR	Inactivating mutations in Osterix, a transcription factor essential for osteoblast differentiation and bone mineralization
XIII: 614856: *BMP1*: bone morphogenetic protein 1 8p21.3 112264	Moderate to severe; resembles type III	Generalized deformities of all bones, multiple fractures despite *increased* bone mineral density	Severe	Faint blue	AR	Inactivating mutations of procollagen type I C-terminal propeptide endoproteinase impair collagen formation
XIV: 615066: *TMEM38B*: transmembrane protein 38B 9q31.1 611236	Moderate, similar to type IV	Variable severity; fractures occur in utero or during early childhood; moderate bowing of the lower limbs	Moderate	Absent	AR	Inactivating mutations of a component of an intracellular monovalent cation channel necessary for Ca^{2+} release from storage sites
Undesignated						
PLOD2: procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2 3q24 601865	Severe; osseous fragility with congenital joint contractures	Fractures in infancy, pterygia, scoliosis	Severe	Absent	AR	Encodes a procollagen lysyl hydroxylase necessary for formation of collagen type 1 telopeptide; Bruck syndrome 2

*Form a complex essential for hydroxylation of carbon 3 of proline at position 986 of procollagen type α1(I).

Adapted from Marini, J. C. (2008). Osteogenesis imperfecta. In C. J. Rosen (Ed.), Primer on the metabolic bone diseases and disorders of mineral metabolism *(7th ed.) (pp. 446-450). Washington, DC: American Society of Bone and Mineral Metabolism; Barnes, A. M., Chang, W., Morello, R., et al. (2006). Deficiency of cartilage-associated protein in lethal osteogenesis imperfecta.* N Engl J Med, 355, *2757–2764; Bishop, N. (2010). Characterising and treating osteogenesis imperfecta.* Early Human Devel, 86, *743-746; Rauch, F., Lalic, L., Roughley, P., & Glorieux, F. H, (2010). Relationship between genotype and skeletal phenotype in children and adolescents with osteogenesis imperfecta.* J Bone Miner Res, 25, *1367–1374.*

In adults, *osteopenia* and *osteoporosis* ("porous bone") are terms that designate states of reduced bone mass and matrix, abnormal bone microarchitecture, and decreased bone strength that increase the risk for fracture; osteomalacia refers to a decrease in the mineral phase of bone.[310,311,312] Osteoporosis in older adults (primarily postmenopausal women) is defined by the World Health Organization (WHO) as an areal bone mineral density (aBMD) at a specific bone site that is −2.5 or more standard deviations (SD) below the mean peak young adult value for gender (T score). Postmenopausal osteoporosis is usually the consequence of an increase in the rate of bone resorption relative to that of bone formation and is due to estrogen deficiency. In older adults, osteopenia is present when the aBMD lies between −1.1 and −2.4 SD below the mean peak young adult value for gender (a T score of −1.1 to −2.4), and a normal aBMD is one that is not more than one SD below or above the mean peak young adult value (a T score of −1.0 to +1.0). When the aBMD is more than +2 SD above the mean for age and gender (a T score of +2), bone mass is considered high. Because in premenopausal women aBMD alone is not a good assessor of fracture risk, the International Society for Clinical Densitometry (ISCD) recommends that use of the term *osteoporosis* be restricted to those patients with both low aBMD (as defined by a BMD Z score of −2—i.e., one that is below −2 SDs for age- and gender-matched control subjects) and history of low-impact (fragility) fractures.[313] Women with aBMDs that are below −2 SDs but have no history of fracture are designated as having an aBMD that is "below expected range for age"; the use of the term *osteopenia* is eschewed in this population. The International Osteoporosis Foundation (IOF) has defined osteoporosis in young adults as a vertebral or hip aBMD T score that is below −2.5 and is associated with a disorder that adversely affects bone mineralization.[312] Both groups agree that in young adults, the presence of osteoporosis is not defined by low BMD alone. Many of the diseases that lead to osteoporosis in young adults begin during childhood and adolescence (see Table 18-12A).

The WHO and IOF-designated criteria for low or high bone mass do not apply to children and adolescents in whom variability in height, weight, and stage of sexual maturation as well as gender affect bone mineralization. Use of the terms *osteopenia* and *osteoporosis* had been discouraged when describing decreased bone mass in children and adolescents in favor of the designation of increased bone fragility as evidenced by fractures caused by low impact trauma in association with low bone mass (−2 SD) for chronologic age, height, or stage of sexual maturation for gender.[314] However, the term *osteoporosis* has been reintroduced and by definition is applied to a child/adolescent with both low bone mass for gender, age, height, and sexual maturation *and* one who has sustained a low impact fracture.[314,315,316] A fracture due to bone fragility/decreased bone strength is defined as one that has occurred from a vertical distance that is equal to or less than standing height.[315] *Osteopenia* is a term that is not employed in the pediatric population. In children and adolescents, bone mass may be quantitated by DEXA determination of aBMD of "whole body" or "whole body minus head."[315,317,318] However, inasmuch as the skull does not respond directly to environmental factors such as exercise, aBMD of whole body minus head/skull is generally considered a more appropriate method of measurement of bone mineral content or density.[316] Regional (lumbar spine, hip, distal one-third radius) aBMD standard data are also available.[317] (DEXA analyses of hip bone mass are variable in children and adolescents and not usually employed for analysis of BMC or BMD.) DEXA measurements of the nondominant forearm may be employed to determine aBMD of the radius and ulna in the ultra distal, middistal, and one-third distal regions.[319] A portable DEXA-like x-ray device that measures aBMD in the one-third distal region of the nondominant forearm provides data comparable to those recorded by a fixed DEXA unit and thus may enable expanded use of this technology.[319] The method employed to determine bone mineralization (DEXA, quantitative computed tomography [QCT], peripheral QCT [pQCT], quantitative ultrasonography [QUS]) and the specific instrument, the software version utilized for analysis, and the ethic mix of the reference population should be stated in the report of bone mineralization.[317,318,320] Low bone mass is defined as a BMC or BMD that is −2 SD (a Z score of −2) for gender, age, height, and stage of sexual maturation by the method employed for its measurement.[317,318] Despite its limitations (provision of areal rather than volumetric BMD data and failure to distinguish between trabecular and cortical bone), DEXA is the most widely employed bone densitometric method in children at this time, although pQCT may become the method of choice in the future because it quantitates bone volume and mass as well as other dimensions of cortical and trabecular bone. Relative to bone strength determined by calculation of the stress-strain index of a long bone with data garnered by pQCT, DEXA whole body aBMC (minus the skull) for height also appears to afford a reliable measurement for determining cortical bone strength and hence fracture risk.[321] Relative to healthy prepubertal and pubertal boys without a history of fractures, those in whom a traumatic fracture has occurred tend to have as a group lower (although still within the broad range of normal) regional BMDs, aberrant microstructure, and decreased bone strength, abnormalities present even in the prepubertal state that may reflect an intrinsic bone defect.[309]

Although heritable factors account for 60% to 80% of optimal bone mineralization, modifiable factors that contribute to the development of osteopenia and osteoporosis in adulthood (weight-bearing exercise, nutrition, body mass, hormonal milieu) have their genesis in utero, infancy, childhood, and adolescence.[310,311,322] In children as in adults, bone mass, composition, microarchitecture, and size determine bone strength. Because many youths consume excessive amounts of carbonated beverages and diluted fruit juices, thus limiting their intake of milk, children and adolescents ingest only 55% to 70% of the recommended daily calcium allowance (1300 mg/day), although late pubertal males tend to consume more than do pubertal females.[323] In adolescent and adult females, an excessive intake of cola drinks with high phosphoric acid levels lowers body calcium content by sequestering dietary calcium in the intestinal tract and increasing

dissolution of bone mineral to neutralize acid with the consequent development of mild secondary hyperparathyroidism.[324] Sedentary, non-weight-bearing activities encouraged by television, videogames, and computer games also impair bone mineralization.[325] Although body weight and fat mass often correlate with bone mass, overall fat has a negative effect on the accrual of bone mass.[325,326] In a study of 300 male and female adolescents and young adults (13 to 21 years of age) employing both the DEXA assessment of body composition and the QCT measurement of the axial and appendicular skeletons, a positive correlation between lean mass and all bone measurements has been demonstrated in both genders, whereas fat mass has had either an inverse or no relationship to bone mass. These observations indicate that bone mass and strength are determined by dynamic muscular force and not by static load.[326] Further evidence of the adverse effects of increased fat mass on bone strength during childhood is the observation that the odds ratios for fractures of the foot, ankle, leg, and knee increase as the body mass index rises, particularly in 6- to 11-year-old children.[327] An obesity-induced decrease in bone mass may be due, in part, to marginal stores of vitamin D, because at similar intakes of vitamin D obese subjects have lower serum levels of 25-hydroxyvitamin D than do normal-weight subjects, to the diversion of progenitor cells from the pathway of osteoblastogenesis to that of adipogenesis, and to products of white fat cells (leptin, adiponectin) that may exert either inhibitory and stimulatory effects on bone mass.

In children and adolescents, the longer and more intense the weekly sporting activity is (soccer, basketball, gymnastics, tennis), the greater are the vertebral and femoral aBMDs independently of calcium intake.[328] In pre- and peripubertal children, simple school physical education programs utilizing jumping, hopping, and skipping exercises two to three times weekly significantly increase areal BMD at the femoral trochanter in as little as 8 months relative to children engaged in a standard physical education curriculum.[329] Thus, suboptimal nutrition and sedentary activities during childhood and adolescence, as well as the consumption of colas and alcohol and the smoking of cigarettes, prevent optimal bone mineralization and increase the likelihood of later development of osteoporosis and its complications.[323] Because the risk of developing an osteoporotic fracture as an adult declines by 40% for every 5% increase in peak bone mineral mass, the foundation for the prevention of osteoporosis in the adult must be constructed in the child and adolescent by maintaining adequate calcium intake, vitamin D stores (serum concentrations of 250HD $>$ 20 ng/mL), and complementary weight-bearing activity during these formative years. Although calcium and vitamin D supplementation for 1 year can increase bone mass in premenarchal girls, combined quantitative analyses of multiple trials of calcium supplementation in children have revealed little effect on BMD or reduction of the risk of fracture.[330,331] Nevertheless, the effects of sustained calcium supplementation over the many years of childhood and adolescence on future fracture risk merits systematic examination. Other nutrients (e.g., magnesium, vitamins C and K, and copper) must also be consumed for optimal bone matrix synthesis.[332] It may be possible to identify the child or early pubertal subject at (genetic) risk for the accrual of low peak bone mass and thus for later development of osteopenia/osteoporosis (e.g., offspring of a mother with osteopenia or osteoporosis, survey of children utilizing portable DEXA devices). Axial and appendicular BMD and bone size determined by central or peripheral QCT in the normal early pubertal boy and girl may accurately predict these measurements at sexual maturity.[333] If so, children at risk for low peak bone mass might benefit from a targeted diet and exercise program during puberty that increases these values.

Nutritional deprivation depresses the rate of bone accrual, a process observed most dramatically in subjects with anorexia nervosa. The majority of postmenarchal, late adolescent females with anorexia nervosa have significantly decreased total body, vertebral, and femoral neck aBMDs, although volumetric BMDs may be normal for their small bone size.[334] In adolescent females with anorexia nervosa, decreased bone mineralization is associated with a slow rate of bone turnover as demonstrated by lower serum concentrations of osteocalcin, estradiol, free testosterone, IGF-I, leptin, and bone-specific alkaline phosphatase and depressed urinary excretion of deoxypyridinoline relative to normal-weight subjects. In these patients, serum levels of osteoprotegerin correlate negatively with fat mass and leptin values and with lumbar spine areal (by DEXA) and apparent BMDs.[335] The decline in bone mass in adolescents with anorexia nervosa may be attributed to nutritional deprivation, chronic acidemia, and functional hypogonadism. Pathophysiologically, the osteopenia encountered in patients with anorexia nervosa is the result of generalized nutritional deprivation with a suboptimal intake of protein, calcium, and vitamin D, hypercortisolemia, and lowered IGF-I generation leading to decreased osteoblast-mediated bone formation coupled with hypoestrogenically mediated enhanced osteoclast-stimulated bone resorption.[336] The bone loss of the patient with anorexia nervosa does not fully recover even after a return to normal weight, resulting in a several-fold increase in fracture risk for these women. In adolescents with anorexia nervosa, the administration of estrogen/progestin does not increase bone mass or prevent its loss. Bisphosphonates, IGF-I, and dehydroepiandrosterone have been reported to increase or maintain BMD in small series of patients with anorexia nervosa, but their use should be limited.[336] The "athletic triad" of suboptimal body fat mass, amenorrhea in women, and decreased bone mass attributed to low sex hormone production is encountered in the highly trained female athlete and in elite male long-distance runners. Additionally, the adolescent amenorrheic athlete has an abnormality of bone microarchitecture.[337] There is a population of children, adolescents, and young adults with "constitutional thinness" that is characterized by long-standing underweight for height, normal growth, sexual maturation and fertility, and the absence of systemic disease or psychologic dysfunction in whom aBMD is low but fracture risk is apparently not increased.[338] Young adults who experienced delay in onset and

completion of sexual development as adolescents may also have low bone mass.[312]

Acute immobilization of the healthy, active child and adolescent leads to a sudden reduction in weight bearing and a consequent decrease in the mechanical load on bones and thus a lowered rate of bone formation; in the presence of continued bone resorption, hypercalciuria and later hypercalcemia and lowered bone mass develop.[339] In the chronically partially or fully immobilized child or adolescent (due to cerebral palsy, spastic quadriplegia, muscular dystrophy), the fracture rate (primarily of the femur) is high during such simple maneuvers as turning, dressing, or feeding. In this group of subjects, not only lack of weight bearing but also the severity of the primary illness, body size and pubertal status, state of general nutrition, vitamin D and calcium intake, coexisting inflammatory states, and medications (e.g., anticonvulsants, glucocorticoids) as well as indoor confinement adversely impact bone mass and fracture risk. In a study group of 117 patients (2 to 19 years of age) with moderate to severe cerebral palsy, distal femoral aBMD Z scores were below −2.0 in 77%, and the incidence of low femoral and vertebral aBMDs as well as fractures increased with advancing age.[340] Distal femoral and lumbar vertebral aBMDs increase at slower than normal rates as the child with spastic cerebral palsy ages resulting in diminution of aBMD Z score in the older subject.[341] Both intravenous administration of pamidronate and subcutaneous injections of recombinant human GH have increased aBMD in small selected groups of children with quadriplegic and spastic cerebral palsy.[342,343] Areal BMD increased substantially in 26 immobile patients with quadriplegic cerebral palsy (3 to 17 years of age) during the administration of the bisphosphonate alendronate (1 mg/kg/week orally), calcium (600 mg/day), and vitamin D (400 IU/day) over 1 year of treatment.[344] Assisted standing by itself increases BMD in children with severe cerebral palsy.[345]

The fundamental importance of normal gonadal sex steroid secretion during age-appropriate sexual maturation is emphasized by the observation that in adult males with delayed sexual development, radial, vertebral, and femoral areal BMDs are lower than in males with normal timing of pubertal maturation.[312] Volumetric BMD may be either normal or subnormal in young adult men with a history of delayed adolescence. Reduced bone mass and abnormal microarchitecture result in decreased skeletal strength and an increased risk of fracture; histomorphometrically, in adult sex-steroid deprived osteoporotic bone there is decrease in cortical width, trabecular number, osteoid, and mineralization activity.[346] As a consequence of estrogen (and androgen) deficiency, there is an increase in the production but a decline in the life span of osteoblasts and osteocytes, whereas osteoclastogenesis is stimulated and the life span of osteoclasts prolonged; these events reflect the sum of the activities of multiple pro-osteoclastogenic cytokines whose synthesis is regulated by estrogen including M-CSF, IL-1, IL-6, TNFα, RANKL, and osteoprotegerin. Although osteopenia responsive to estrogen has been recorded in adult males with aromatase deficiency, mature women with complete androgen insensitivity are also osteopenic despite normal to increased estrogen production, clearly indicating that androgens, too, are important for normal bone mineralization.[346]

There is an increased risk of fractures in prepubertal and adolescent girls and adult women with Turner syndrome that is attributable to both intrinsically abnormal bones (as manifested by Madelung deformity of the wrist, scoliosis, cubitus valgus and shortened metacarpal and metatarsal bones) and osteopenia related to chronic estrogen deprivation.[347] Although decreased relative to chronologic age, areal and volumetric BMDs may be normal relative to height or bone age in girls with Turner syndrome. In patients with Turner syndrome, there is selective deficiency of cortical bone, whereas trabecular bone density is normal. The frequency of wrist fractures is increased during childhood, as may be the general risk for fractures in adults with Turner syndrome. Estrogens, growth hormone, and particularly the administration of both agents increase bone calcium deposition and aBMD in adolescents with Turner syndrome.[348] If estrogen replacement therapy is begun in the early adolescent years, cortical and trabecular bone mass increase substantially, but if estrogen replacement is delayed decreased cortical bone mass persists into adulthood, although trabecular bone mass normalizes.[347] Although recombinant human growth hormone increases growth rate and adult stature in many patients with Turner syndrome, its effects on bone density and fracture risk in these subjects is uncertain. As with all individuals, an appropriate intake of calcium and vitamin D and weight-bearing exercises should be strongly endorsed. However, the use of bisphosphonates is not recommended in patients with Turner syndrome, because these drugs act primarily upon trabecular bone that responds well to estrogens alone.[347]

Pubertal subjects with primary (Klinefelter syndrome, galactosemia, post radiation or chemotherapy) or secondary hypogonadism (anorexia nervosa, excessive physical training, hypogonadotropism) also have decreased bone mineralization. By decreasing estrogen production, even short-term (6 months) use of the intramuscular contraceptive depot medroxyprogesterone acetate (MPA) results in significant loss in bone mass in adolescent females and young women (18 to 21 years of age) when BMD would ordinarily be increasing; however, bone mass increases over time after this agent is discontinued.[336] The adverse impact of depot MPA on BMD may be prevented by concomitant administration of estrogen. After treatment of the child with central precocious puberty for 1 to 2 years with a gonadotropin releasing hormone analog that suppresses pituitary-gonadal function, there may be arrest or even decline in bone mineral accumulation in the peripheral and axial skeletons, a process that can be prevented or reversed by the co-provision of 1 gram of calcium per day during analogue therapy.[349]

The low bone mass of glucocorticoid excess is the result of (1) inhibition of osteoblastogenesis; (2) an increase in the rate of apoptosis of the osteoblast and osteocyte, leading to decrease in the rate of bone matrix formation and microfracture repair; (3) enhanced osteoclastogenesis; and (4) a decrease in the rate of apoptosis of the osteoclast—permitting prolonged and excessive bone resorption.[350,351]

Because of decreased osteocyte regulation of bone remodeling, during each remodeling cycle the amount of bone replaced is far less than the amount removed and skeletal microarchitecture degraded, resulting in declining bone mass and strength. At the molecular level, glucocorticoids suppress expression and synthesis of RUNX2 and BMP-2, factors essential for prenatal and postnatal osteoblast differentiation, respectively, and increase osteoblast expression of RANKL and decrease expression of osteoprotegerin, changes that favor osteoclastogenesis.[350,352] Glucocorticoids interfere with WNT/β-catenin signaling and consequently inhibit synthesis of collagen type I as well as increase its rate of degradation; they impair IGF-I formation and function. These compounds direct the mesenchymal osteoblast precursor into the pathway of adipocyte differentiation. To a limited extent, glucocorticoids inhibit normal vitamin D metabolism and thereby vitamin D–dependent intestinal absorption of calcium. They increase renal loss of calcium by a direct effect on the renal tubule leading to secondary hyperparathyroidism.[350,352] Glucocorticoids also reduce production of sex hormones in the adolescent and adult. The muscle weakness of chronic glucocorticoid exposure reduces the impact of mechanical forces on bone formation. Finally, the disease (e.g., asthma, nephrotic syndrome, chronic inflammatory disorders such as juvenile arthritis) for which pharmacologic doses of glucocorticoids have been prescribed may contribute to decreased bone mass by impairing mobility and by elaboration of osteoclastogenic cytokines. Glucocorticoids adversely affect trabecular bone formation initially and later impair cortical bone accumulation.[351] Although glucocorticoid-reduced bone mass accumulation is greater with frequent intermittent bursts of oral glucocorticoids than with inhaled glucocorticoids in boys with asthma, in high doses over prolonged periods inhaled glucocorticoids can also lead to decreased bone mass.[353-355] In young adults with asthma there is an inverse relationship between vertebral and femoral aBMDs and the cumulative dose of inhaled glucocorticoid with increasing fracture risk as the dose and duration of glucocorticoid administration increases; a cumulative dose of 5000 mg leads to a 1 SD decline in vertebral BMD.[356] In adult women with 21-hydroxylase–deficient congenital adrenal hyperplasia treated with glucocorticoids, bone mineralization is modestly reduced, in part related to the extent to which adrenal androgen production is suppressed.[357] It is suggested that when children begin to receive either oral or inhaled glucocorticoids that they also be instructed to ingest age-appropriate amounts of calcium and vitamin D and to engage in weight-bearing exercises. In children experiencing adverse effects of glucocorticoids on growth and bone mineralization, it is important to lower their steroid dose to the greatest extent possible and to withdraw them if at all feasible. In 7 of 10 children with juvenile rheumatoid arthritis and other rheumatic disorders and glucocorticoid/illness-mediated low bone mass, pamidronate at a dose of 2 to 4 mg/kg per infusion administered at 6-month intervals was followed by decline in bone pain, improved ambulation, and a progressive increase in BMD of the lumbar spine.[358] Although generally recommended for adults being treated with glucocorticoids, routine administration of a bisphosphonate to children receiving these agents is not recommended unless there is steady decline in bone mass or the patient has had a low-impact or fragility fracture attributable to low bone mass.[351] Teriparatide ($PTH^{1\text{-}34}$) increases bone mass in adults with glucocorticoid-induced osteoporosis, but its efficacy and safety in children with this problem have not yet been evaluated. Denosumab, a human monoclonal, neutralizing antibody against RANKL (thus mimicking the antiosteoclastogenic effects of osteoprotegerin) may also prove useful in the treatment of glucocorticoid-mediated deficits in bone mass.[351]

Growth hormone (GH) exerts direct and indirect actions on bone formation. In growth plate cartilage, GH stimulates proliferation of resting chondrocytes and promotes local generation of IGF-I; in bone, it enhances osteoblast differentiation, proliferation, and function resulting in increased osteoblast synthesis of IGF-I, IGFBP-3, osteocalcin, bone-specific alkaline phosphatase, and procollagen type I; it also stimulates osteoclastogenesis and bone resorption.[359,360] IGF-I regulates differentiation of chondrocytes and maintains their rate of proliferation.[360] Osteoblast and osteocyte-synthesized IGF-I affects osteoblast maturation and the rates of matrix mineralization and bone turnover and mediates the response of bone to a mechanical load and to parathyroid hormone.[360] Administration of GH to GH-deficient children and adults increases the rates of both bone formation and destruction, the latter predominating initially; over long periods of treatment (12 to 18 months) GH increases aBMD in these patients; to achieve peak bone mass, however, GH therapy must be continued into adulthood. Nevertheless, in many untreated adults with congenital GH deficiency due to an inactivating mutation in hypothalamic *GHRH*, volumetric BMD is often normal despite low aBMD values reflecting the smaller size of their bones.[361] Thus, although lack of GH has long been considered a cause of low bone mass in patients with GH deficiency of either childhood- or adult-onset, critical analysis of data concerning bone mass and fracture risk in children and adults with GH deficiency has questioned the validity of this relationship.[362] GH primarily affects formation of cortical bone (an observation consistent with the known clinical effects of GH on exuberant growth of membrane bone). In part, low bone mass in the GH-deficient child may be attributed to the small bone size of the short child compared to age peers when examined as aBMD by DEXA. However, measurement of bone mass by either size-corrected aBMD or volumetric methods (e.g., pQCT) indicates that volumetric BMD is normal in the majority of GH-deficient subjects. Thus, neither isolated GH deficiency or resistance to the effects of GH due to loss-of-function mutations of the GH receptor is associated with increase in fracture risk in children or adults.[362] In patients with hypopituitarism of adult onset associated with multiple pituitary hormone deficiencies, fracture risk is increased, but this may be attributable to a loss of gonadotropin stimulated sex hormone secretion and to the decreased mechanostat effects of reduced muscle mass on bone accretion in these fully grown subjects.

Thyroid hormone, through direct action on the osteoblast, increases synthesis of osteocalcin, alkaline

phosphatase, and IGF-I; it also enhances osteoclastogenesis and, thus, the rate of bone resorption, the latter effect predominating in patients with excess thyroid hormone in whom there is increase in the rate of bone turnover but decrease in the length of the bone remodeling cycle (primarily due to shortening of the bone formation phase) resulting in a net loss of mineralized bone.[363] As in adults with thyrotoxicosis, whole body, vertebral, and femoral BMDs are low in children and adolescents with hyperthyroidism, but they substantially increase within the first 12 to 24 months after restoration of the euthyroid state. Administration of physiologic replacement doses of thyroxine to children with acquired or congenital hypothyroidism does not adversely affect bone mineralization during childhood, although adults with congenital hypothyroidism have a 10% reduction in radial bone mass. In adolescents with type 1 diabetes mellitus, whole body, axial, and appendicular bone mass assessed by DEXA is decreased relative to control subjects and inversely related to hemoglobin A1c values reflecting the adverse effects of chronic hyperglycemia and insulin deficiency upon bone formation.[364,365] Utilizing pQCT in young, prepubertal subjects with type 1 diabetes mellitus, cortical bone cross-sectional area and BMD were found to be decreased, implying an increased risk for fracture.[366]

Bone mass is decreased in 80% of children with acute lymphoblastic leukemia, and 40% sustain a fracture within the first 2 years of treatment. Pathogenetic factors involved in the suppression of bone remodeling and the development of low bone mass in these subjects include adverse effects of the disease itself directly on bone; radiation injury of bone; inhibitory effects of glucocorticoid, chemotherapeutic, antibiotic, and immunosuppressive agents on bone formation; diminished physical activity; decreased caloric, protein, and vitamin D intake; sex hormone deficiency due to delayed or arrested adolescent development; and GH deficiency in children who have received cranial radiation.[367] Cyclosporine A induces bone loss in organ transplant recipients by increasing osteoblast expression of RANKL and decreasing production of osteoprotegerin thereby augmenting osteoclastogenesis. Methotrexate and intrathecal chemotherapy exert significant inhibitory effects on bone mineralization in children with malignancies who should receive appropriate calcium and vitamin D supplements and weight-bearing exercises to the extent possible.[367] Those who develop GH deficiency following cranial radiation may be treated with rhGH if they remain GH deficient after the primary illness has been in prolonged remission. Decreased bone mineralization is common in the post bone marrow or solid organ transplant subject; its diverse pathogenesis includes the primary disease itself and the chronic illness that may accompany it, the use of high-dose glucocorticoids and antirejection medications, as well as altered intestinal, renal, hepatic, and gonadal function.[368] In addition to the provision of adequate nutrition, calcium, and vitamin D, in adults the effects of transplantation may be partially ameliorated by the administration of bisphosphonates.

In severely burned patients and children with hemophilia, sickle cell disease, central diabetes insipidus, Marfan syndrome, homocystinuria, lysinuric protein intolerance, and propionic and methylmalonic aciduria, aBMD is also decreased and fracture risk increased. Children with cystic fibrosis may have low vertebral and femoral neck aBMD Z scores as a consequence of suboptimal nutrition, vitamin D deficiency, chronic inflammation, concomitant diabetes mellitus, pubertal delay, and drug therapy. However, approximately one third of optimally managed cystic fibrosis patients with good clinical control may nevertheless have subnormal BMDs (a Z score below −1 but seldom below −2.5), although this is not necessarily translated into increased fracture risk.[369] Low bone mass and vertebral collapse may be early manifestations of chronic inflammatory bowel disease. Vitamin D deficiency and secondary hyperparathyroidism as well as the chronic inflammatory state and therapeutic agents such as glucocorticoids likely contribute to decreased bone formation and increased bone resorption in this illness. That whole body aBMC in children, adolescents, and young adults with chronic inflammatory bowel disease may be normal relative to lean body mass (although reduced relative to racial, age, and height norms) does not necessarily imply that bone strength in these patients is normal as evidenced by the increased fracture risk of adults with this disorder.[370] Low bone mass is common in children and adults with celiac disease.[371] Pathogenetic factors associated with low bone mass in children with celiac disease include malabsorption of vitamin D, calcium, protein, and other nutrients, synthesis of osteoclast activating and proinflammatory cytokines such as interferon γ and interleukins −15, −18, and −21, and secondary hyperparathyroidism. In children and adolescents infected with the human immunodeficiency virus, whole body BMD is decreased as a consequence of the infective agent itself, the chronic inflammatory state it induces, suboptimal nutrition, and the administration of highly active antiretroviral therapy that may have direct effects on osteoblast and osteoclast generation and function.[372,373] Despite clinical well-being and normal linear growth, the rate of accrual of bone mass is decreased in these subjects, whereas the rate of bone resorption is increased. Bone mass is reduced in children with a variety of connective tissue diseases (juvenile idiopathic arthritis, systemic lupus erythematosus, juvenile dermatomyositis) due to the chronic inflammatory state and production of pro-osteoclastic cytokines and to therapy with glucocorticoids.[374]

Idiopathic juvenile osteoporosis (MIM 259750) is an unusual disorder of generalized low bone mass of unknown pathogenesis that appears in both boys and girls between 8 and 12 years of age and often resolves as sexual maturity is achieved.[375] In affected subjects, roentgenograms obtained for evaluation of joint, muscle, or back pain, difficulty walking, foreshortening of the trunk, or the presence of kyphosis reveal biconcave vertebrae or vertebral compression fractures, radiolucent areas in long bones, and metaphyseal fractures. DEXA and QCT analyses demonstrate decreased bone mineralization. Chemical studies are normal. Dynamic histomorphometric studies reveal low rates of bone formation and turnover with a reduction in cancellous bone volume and trabecular thickness due primarily to decreased osteoblast activity on the endosteal but not the periosteal bone

surface; there is no evidence of increased bone resorption. Idiopathic juvenile osteoporosis is quite likely to be genetically heterogenous in origin. Mutation analyses of *COL1A1* and *COL1A2* have been normal in these subjects. In 15% of patients with juvenile osteoporosis, a familial heterozygous loss-of-function mutation in the gene encoding LDL receptor-related protein 5 (*LRP5*) has been detected.[376] Homozygous loss of *LRP5* results in the osteoporosis-pseudoglioma syndrome (discussed later). One of the most difficult diagnostic challenges is the clinical distinction between idiopathic juvenile osteoporosis and osteogenesis imperfecta type I (discussed later). The latter disease is characterized clinically by a positive family history, onset in early infancy, lifelong persistence, diaphyseal fractures, wormian bones, lax ligaments and decreased muscular strength, blue sclerae, abnormal dentition, hearing loss, and a high rate of bone turnover.[196,375] Additionally, there are some children with low bone mass and recurrent fractures whose clinical picture is not as severe as those with idiopathic juvenile osteoporosis, suggesting that there is a wide spectrum of clinical findings in children and adolescents with marginal bone mineralization.[375] In children with classical idiopathic juvenile osteoporosis, symptomatic treatment is offered; in some patients calcitriol or supplemental sodium fluoride has been of benefit. Although the disorder ameliorates and even disappears at puberty, treatment of the prepubertal patient with sex steroids does not seem to accelerate the healing process. Administration of the bisphosphonate pamidronate has been helpful in reducing bone pain and increasing vertebral BMD in small groups of children with idiopathic juvenile osteoporosis.[375]

Low density lipoprotein receptor-related protein 5 (*LRP5*, MIM 603506) is a cell membrane protein expressed by osteoblasts that serves as coreceptor for signal transduction through the WNT-frizzled-β-catenin pathway leading to differentiation and function of osteoblasts. WNT signaling enhances differentiation of pluripotential mesenchymal precursor cells into the pathway of chondrogenesis and osteogenesis and impedes its differentiation into the pathway of adipogenesis.[377] By stimulating RUNX2, β-catenin further directs the osteochondroprogenitor cell into the osteoblastic track, and in the mature osteoblast, β-catenin enhances expression of osteoprotegerin and thus depresses osteoclastogenesis.[378] Sclerostin (encoded by *SOST*, MIM 605740) binds to the extracellular domain of LRP5 and thereby inhibits WNT signaling.[379] Intrinsic abnormalities of *LRP5* expression have been associated clinically with disorders of both impaired and excessive bone mineralization. The osteoporosis-pseudoglioma syndrome (MIM 259770) is characterized clinically by congenital or early infantile onset of severe visual impairment due to micro-ophthalmia and hyperplasia of the vitreous (pseudoglioma that may be erroneously identified as retinoblastoma) leading to retinal detachment, glaucoma, and blindness; marked osseous fragility with craniotabes and fractures during late infancy, childhood, or adolescence; and variable cognitive impairment, ligamentous laxity, and hypotonia.[380] The disorder is transmitted as an autosomal recessive trait and is due to biallelic (homozygous or compound heterozygous) inactivating (missense [Val336Met, Arg494Gln], nonsense [Arg428Ter, Arg1002Ter], frameshift, splice-site) mutations in *LRP5*, primarily located in the extracellular domain of LRP5.[381,382] Missense mutations likely prevent normal binding of LRP5 to the product of the mesoderm development gene (MIM 607783, chromosome 15), a chaperone protein that directs LRP5 to the cell membrane. Although often asymptomatic, heterozygous carriers are usually osteopenic; however, vision is not impaired. Intravenous administration of pamidronate over several years can increase bone mass in children with this disorder.[382] Loss-of-function mutations in *LRP5* have also been associated with familial exudative vitreoretinopathy (MIM 133780), a developmental disorder of retinal vasculature that may be transmitted as either an autosomal dominant (Leu145Phe) or an autosomal recessive (Arg570Gly, Arg752Gly) trait; these patients also have reduced bone mass. LRP5 transduces not only the WNT signal but also that of Norrin (MIM 310600, chromosome Xp11.4). Norrin signaling modulates ocular vitreoretinal formation. Gain-of-function mutations in *LRP5* are associated with increased bone mass (discussed later).

Evaluation and Management of Low Bone Mass

Evaluation of the child or adolescent with a possible low-impact fracture due to decreased bone strength (previously defined as a fracture sustained by a fall from a distance that is less than the standing height of the patient) begins with historical review and physical examination directed to the identification of factors that might adversely affect skeletal formation and bone mineralization.[308,315] In addition to genetic and hormonal influences, the most important elements necessary for the accrual and maintenance of bone mass that need be inquired about are those that relate to diet (sufficient intake of calcium and protein, absence of anorexia nervosa), sustained normal vitamin D stores either by exposure to sunlight or ingestion of supplements, consistent but not excessive weight-bearing exercise, and the presence or absence of diseases (e.g., asthma) or therapeutic agents (e.g., glucocorticoids) that might impair bone development. It is important to examine the patient's growth pattern to determine whether statural growth has been normal and normal weight for stature and gender has been attained and maintained; an assessment by physical examination of stages of primary and secondary sexual maturation is also essential. Determination of skeletal maturation (bone age) is useful to establish if the child is growing in accord with his or her genetic potential. If pertinent, systemic illnesses such as chronic renal disease, celiac disease, inflammatory bowel disease, and endocrinopathies should be eliminated. Assessment of areal bone mineralization is most often accomplished by DEXA in children with referral to age and gender-specific reference data.[315,318] Interpretation of a DEXA BMD relative to height and to bone age is often useful.[383] In a series of 304 children and adolescents undergoing DEXA bone mineralization studies at a children's hospital, 36% were doing so because of a history of fractures, 27% because of hypogonadism, and 22% because of

gastrointestinal (celiac, inflammatory bowel) disease.[384] Low body mass index and low vitamin D stores were the most significant predictors of subnormal BMD (below −2 SDs for age and gender). Interestingly, for subjects with a history of fractures, there was no difference in the number of fractures in those with a BMD below or above −2 SDs for age and gender. Depending on the individual patient, measurements of serum calcium, phosphate, alkaline phosphatase, creatinine, iPTH, 25OHD, and reproductive hormones, as well as disease-specific analytes (e.g., for inflammatory bowel or celiac diseases), may be indicated. Bone turnover markers are of marginal diagnostic utility in children, although they are perhaps useful as indices of therapeutic response.[385,386] After assessment and when necessary and appropriate, intervention may include elimination of or reduction in glucocorticoid dose in an asthmatic child or treatment of an accompanying underlying systemic disease or endocrinopathy that may be of pathogenetic significance in the development of low bone mass. When attempting to prevent bone loss or restore lost bone, initial efforts are directed to the assurance that these basic approaches are being utilized to the fullest extent possible for the specific patient.

Therapeutic agents increase skeletal mass either by inhibiting bone resorption (antiresorptive or anti-remodeling drugs) or by stimulating bone formation (anabolic agents).[387,388] The most widely employed antiresorptive medications are sex hormones, selective estrogen receptor modulators, teriparatide ($PTH^{1\text{-}34}$), denosumab, and bisphosphonates. Selective estrogen receptor modulators (SERMS) are triphenylethylene, benzothiophene, or naphthalene-related compounds (e.g., raloxifene) that bind with high affinity to estrogen receptor α in specific tissues where they alter the three-dimensional configuration of the receptor and recruit tissue-selective cohorts of various cofactors, thus either reducing (breast, brain) or inducing (bone) receptor function in targeted sites.[389] SERMs decrease osteoclast formation primarily at trabecular bone sites, but their efficacy in increasing BMD is less than that of estrogens themselves.[390]

Denosumab is a human monoclonal antibody to RANKL that binds tightly to its ligand and prevents its interaction with RANK, thereby inhibiting osteoclastogenesis; denosumab thus acts as a "pseudo-osteoprotegerin." In postmenopausal women with decreased bone mineralization, denosumab decreases bone resorption and increases lumbar spine BMD.[391] Experience with its use in children is limited. Strontium renalate is an agent that has been considered for treatment of low bone mass in adults, because it is incorporated into the structure of bone mineral.[352] Although nasal salmon calcitonin inhibits osteoclast function directly and has modest bone restorative effects, it is seldom utilized for the treatment of osteoporosis in adults or children.[392]

Bisphosphonates are analogs of pyrophosphate with carbon substituted for the oxygen bridge between two phosphate groups; also attached to the carbon atom are two side chains: R1 is usually a hydroxyl group or chloride atom that together with phosphate residues binds tightly to hydroxyapatite and coats bone surface; the R2 side chain may be "simple" and contain chloride or sulfur atoms, or it may be more complex and heavier with nitrogen atoms and ring structures composed of carbon and oxygen (Figure 18-12). Bisphosphonates that contain nitrogen as a constituent in one of the side chains are substantially more potent than are the "simple" bisphosphonates.[393] Bisphosphonates chelate the calcium ions of hydroxyapatite and thus are targeted to bone; within the resorption lacuna beneath an osteoclast, bisphosphonates dissociate from hydroxyapatite as the pH is lowered by osteoclast secretion of H^+ and are then endocytosed into the interior of the osteoclast. Once within the osteoclast, bisphosphonates impede osteoclast function by hastening their death by two distinct mechanisms: (1) "simple" bisphosphonates such as medronate, etidronate, and clodronate are metabolized into analogs of adenosine triphosphate (ATP) that interfere with release of phosphate and hence with energy generation; additionally, "simple" bisphosphonates prevent movement of ATP into the osteoclast's mitochondria as a consequence of which mitochondria disintegrate and osteoclast apoptosis is initiated—thereby further interfering with bone resorption; (2) large, nitrogen-containing bisphosphonates (pamidronate, zoledronate) act by impairing cholesterol synthesis by inhibiting farnesyl pyrophosphate synthase activity in osteoclasts. This enzyme is essential for synthesis of cholesterol through the mevalonate pathway; its inhibition prevents prenylation of proteins, a posttranslational modification that enables prenylated proteins to interact with proteins and to bind to cell membranes.[393] Inhibition of this pathway in osteoclasts impairs formation of the ruffled border and actin rings, movement of osteoclast products into the resorption lacuna, and resorption of degraded bone products—all metabolic functions of osteoclasts essential for bone resorption. The biologic activity of a bisphosphonate on osteoclast

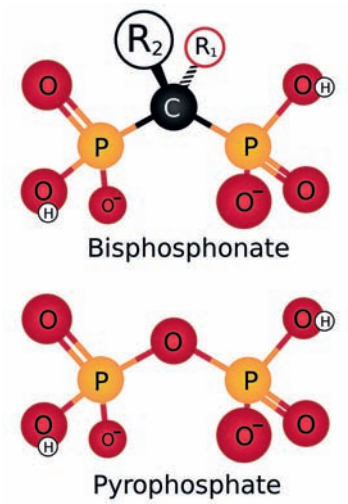


FIGURE 18-12 ■ Bisphosphonates are analogues of pyrophosphate in which carbon has been substituted for the oxygen bridge between two phosphate groups; two side chains (R1, R2) are attached to the carbon atom: R1 may be a hydroxyl group or chloride atom that together with phosphate residues binds tightly to hydroxyapatite and coats bone surface; R2 may contain chloride or sulfur atoms or it may be composed of ring structures containing carbon, oxygen, and nitrogen. (Courtesy of Wikimedia Commons User Adenosine.) *This image can be viewed in full color online at ExpertConsult.*

function is observed immediately after its administration as serum calcium concentrations decline rapidly; indeed, this rapid effect has been utilized in the treatment of hypercalcemic infants and children. In adults, the effects of bisphosphonates on bone mass last long after the agent has been discontinued (the "residence time"), enabling some compounds to be given as infrequently as once yearly (zoledronate). Indeed, the bisphosphonates remain in bone for extremely long intervals and their long-term effects are cumulative. Histomorphometric analysis has revealed that bisphosphonate-mediated inhibition of osteoclast stimulated bone resorption increase bone mineralization by decreasing the number of resorption cavities and thus the remodeling space, preserving cancellous (trabecular) bone architecture, and decreasing porosity of cortical bone. Bisphosphonates have been useful in improving mineralization in children with osteogenesis imperfecta (discussed later) as well as in those with glucocorticoid induced osteoporosis, osteoporosis-pseudoglioma syndrome, Menkes disease, and cerebral palsy (as discussed previously). In most infants and children, intravenous pamidronate (0.5 to 1 mg/kg/dose on 3 consecutive days every 2 to 4 months up to 2 to 15 mg/kg/yr administered once every 3 to 6 months) has been utilized, although a number of different regimens have been employed with reasonably similar increases in BMD, decline in fracture incidence, and improved well being.[308] Limited data indicate that oral bisphosphonates (pamidronate, alendronate, olpadronate) administered daily also increase BMD in children with osteogenesis imperfecta but with lower efficacy than intravenous administration and, therefore, are not generally utilized in these patients. At present, the Food and Drug Administration has not approved bisphosphonates for use in children.

Side effects of bisphosphonates have been both acute (fever, myalgia, abdominal pain, vomiting, hypocalcemia) and chronic (inflammatory disorders of the eye, osteonecrosis of the jaw in the elderly, and induced "osteopetrosis") (discussed later).[394] Experimentally and in adults receiving long-term therapy, bisphosphonates can suppress bone turnover and contribute to hypermineralization, the latter leading paradoxically to reduced mechanical strength and increased fracture risk.[395] Therefore, when considering a child for treatment with bisphosphonates one must carefully evaluate the primary diagnosis and whether the patient's low bone mass and fracture frequency merit therapy in view of the potential side effects of bisphosphonates. In addition, there are many unanswered questions concerning which bisphosphonate to use, its route of administration, the dose of the drug, the duration of therapy, and the method of outcome analysis. The use of bisphosphonates should be confined to centers with experience in the care and management of children with bone disease.[308] Treatment with bisphosphonates several years prior to conception does not appear to have an adverse effect on fetal outcome, but treatment during pregnancy is contraindicated because of possible toxicity.[396]

$PTH^{1\text{-}34}$ (teriparatide) is the most widely employed bone anabolic agent. Administered continuously, $PTH^{1\text{-}84}$ and its analog $PTH^{1\text{-}34}$ increase osteoclast-mediated dissolution of bone, but when administered intermittently these agents exert anabolic effects on osteoblast function and bone formation. This property is employed clinically through the use of $PTH^{1\text{-}34}$ in the treatment of postmenopausal women with osteoporosis. When administered intermittently in small amounts, $PTH^{1\text{-}34}$ preferentially accelerates the rates of bone remodeling and of bone formation relative to that of bone resorption by direct effects on osteoblast differentiation, maturation, and longevity.[388] $PTH^{1\text{-}34}$ also acts on the osteocyte to decrease the production of sclerostin, an inhibitor of bone synthesis that acts by repressing WNT- and BMP-mediated bone formation (discussed later).[397] The quantity of bone formed in each remodeling unit is increased with consequent augmentation of trabecular thickness and interconnectivity and periosteal new bone formation and cortical thickness resulting in increased bone size, mass, and strength. Side effects of $PTH^{1\text{-}34}$ administration include transient hypercalcemia, hypercalciuria, and the development of antibodies to the peptide—all rather unusual events. Although osteosarcoma has been observed in mice receiving very high doses of PTH and $PTH^{1\text{-}34}$, no malignant disorders have been recorded in adults receiving either agent. The pediatric use of $PTH^{1\text{-}34}$ has been primarily limited to children with hypocalcemia due to a gain-of-function mutation in *CASR* and consequent hypercalciuric hypocalcemia, which is one form of familial isolated hypoparathyroidism.[24] An analog of PTHrP ($PTHrP^{1\text{-}36}$) that increases lumbar spine BMD in postmenopausal women with osteoporosis has been described but not further explored.[388,398] Future potential therapeutic agents for the management of low bone mass are those that would enhance the WNT/β-catenin signaling pathway of osteoblast differentiation and function such as substances that increase LRP5 activity, inhibit sclerostin or Dickkopf function, or enhance BMP, TGFβ, or vascular endothelial growth factor A (VEGF) signaling within mesenchymal stem cells directing their differentiation into osteoblasts.[388,399]

Osteogenesis Imperfecta

Osteogenesis imperfecta or "brittle bone disease" is a disorder of increased bone fragility due to low bone mass that varies in clinical severity from lethality in utero or perinatally to mildly increased susceptibility to fractures in later life and that may be transmitted as either autosomal dominant or autosomal recessive traits.[196,199] The original Sillence classification of four types of osteogenesis imperfecta based on clinical characteristics and disease course has been expanded to include at least 10 additional numbered types of this disorder and the identification of additional mutant genes to which the illnesses can be attributed (see Table 18-12B).[193,196,199,400,401] (The types of osteogenesis imperfecta are numbered in the order of their clinical description.) The hallmark of each type is increased bone fragility, but severity varies with type II being lethal in the perinatal period due to respiratory insufficiency as a consequence of multiple rib fractures that reduce chest volume and type I being a relatively benign form (discussed later); in decreasing order of severity, the categories are type II > type VIII ≥ type III > types IV, V, VI, VII > type I. In osteogenesis

imperfecta types I through IV, heterozygous loss-of-function mutations have been identified in one or the other of the two genes (*COL1A1*, *COL1A2*) encoding procollagen subunits α1(I) and α2(I), respectively, that intertwine to form collagen type I in bone, skin, ligaments, tendons, sclerae, and teeth.[193,196] (The triple helical structure of type I collagen is comprised of two collagen α1[I] [COL1A1] peptide and one collagen α2[I] [COL1A2] peptide, each of which is composed of triple repeats of glycine and two additional amino acids—often proline, hydroxyproline, or lysine.) Transmitted as an autosomal dominant trait, insertions, duplications, frameshift or point mutations within *COL1A1* or *COL1A2* reduce the amount of collagen synthesized or alter its structure and properties interfering with the development of a normal three-dimensional configuration, thus leading to decreased bone formation, low bone mass, and increased fracture risk (Figure 18-13). To a limited extent, the site of the mutations identified within *COL1A1* or *COL1A2* is related to the clinical phenotype of osteogenesis imperfecta types I through IV. Mutations that lead to a stop codon result in a truncated procollagen product that is rapidly degraded; thus, only normal collagen type I is produced but in reduced amounts. Lethal mutations in *COL1A1* are those that alter an amino acid with a branched or charged side chain and those within the binding sites of the collagen monomer for integrins, matrix metalloproteins, fibronectin, and cartilage oligomeric matrix protein and those that result in binding to and degradation of intact procollagen subunits.[193] Lethal mutations in *COL1A2* are those that interfere with its binding to proteoglycans. Mutations (Arg134Cys) in *COL1A1* may also be found in patients with classical Ehlers-Danlos syndrome (MIM 130000) of hyperextensible skin and laxity of ligaments of the spine and large and small joints. Children with clinical features of both osteogenesis imperfecta (osseous fragility) and Ehlers-Danlos syndrome have been described.[402] In these patients, the mutations have been concentrated within the first 90 amino acids of the helical region of collagen-α1(I) and prevent normal posttranslational removal of the procollagen amino-propeptide; although the mutant protein can be incorporated into collagen, the structural integrity of the product is impaired, as its fibrils are thin and weak.[403] Osteogenesis imperfecta types I-IV describe clinical forms of this disease associated with loss-of-function mutations in *COL1A1* or *COL1A2* that are transmitted in an autosomal dominant manner.

Osteogenesis imperfecta type I (MIM 166200) is an autosomal dominant disorder (new mutation in 33% of patients) due primarily to "functional null alleles"—the result of splicing defects or point mutations leading to insertion errors or truncation (*COL1A1*: Gly178Cys, Arg963Ter, IVS26DS), mutations that result in decreased transport of procollagen-α1(I) into the cytoplasm or its release into matrix—thereby modestly decreasing production of intact procollagen type I. Its clinical manifestations are relatively benign: intensely blue sclerae that are present at birth and persist throughout adulthood, modestly low bone mass, infrequent fractures with little deformity (however, 15% of affected children develop deformities and 24% kyphoscoliosis before 10 years of age), low normal adult stature, hearing loss in 50%, mitral valve prolapse in 18%, and rarely dentinogenesis imperfecta (osteogenesis imperfecta type IB). (Paradoxically, patients with mild osteogenesis imperfecta type I due to mutations in the procollagen type I carboxyl terminal propeptide cleavage sites [*COL1A1*, p.Asp1219Asn; *COL1A2*, p.Ala1119Thr] may have normal or increased lumbar spine BMD by both DEXA and QCT examinations

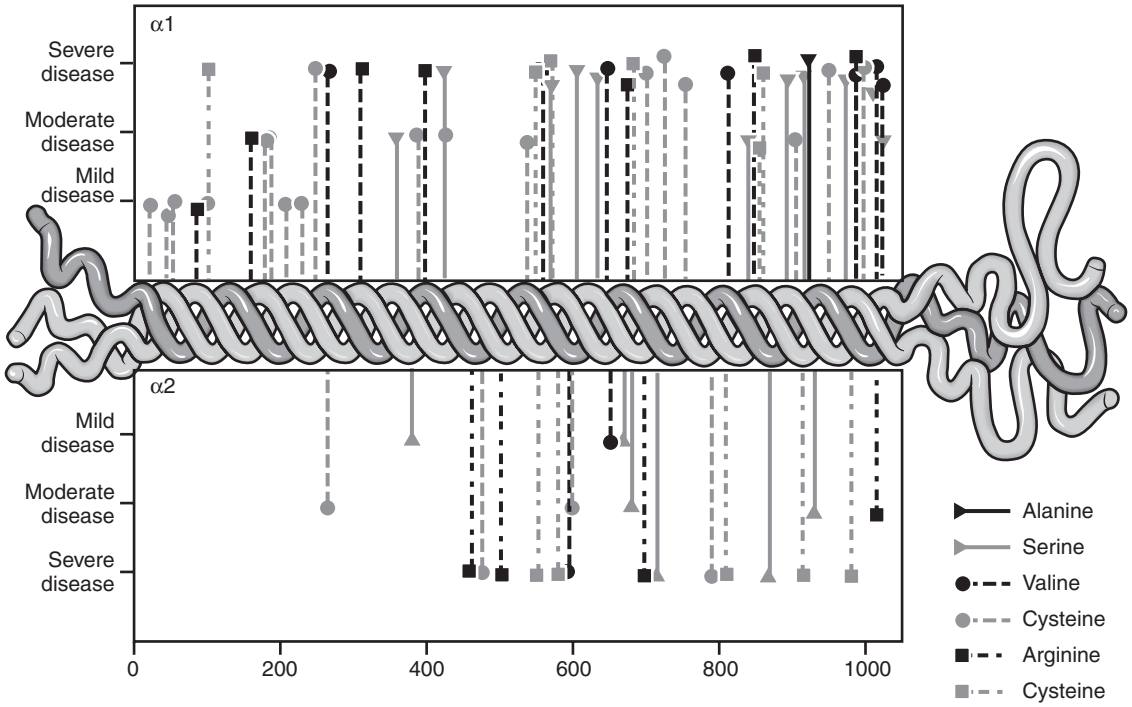


FIGURE 18-13 ■ Mutations in *COL1A1* and *COL2A1* that result in glycine substitutions associated with osteogenesis imperfecta of variable clinical severity. (From Prockop, D. J. (2005). Type II collagen and avascular necrosis of the femoral head. *N Engl J Med, 352,* 2268–2270.)

despite increased bone fragility.[404] Please see the discussion of osteogenesis imperfecta type XIII, presented later.) Mutations in *COL1A2* less frequently lead to the phenotype of osteogenesis imperfecta type I. Subjects with mutations in *COL1A1* more often have blue sclerae and taller stature than those with mutations in *COL1A2*. Osteogenesis imperfecta type II (MIM 166210) is a disorder that is lethal in the perinatal period or in early infancy. It is usually the result of de novo heterozygous mutations in *COL1A1* or *COL1A2* with alternative amino acid being substituted for glycine in the triple helical domains of the procollagen α1(I)/α2(I) chains (*COL1A1*: Gly94Cys, Gly391Arg, Gly1003Ser; *COL1A2*: Gly547Asp, Gly865Ser, Gly976Asp); these mutations lead to the synthesis of abnormal procollagen chains that bind to and thereby inactivate intact procollagen peptides in a dominant-negative manner severely curtailing the synthesis of intact collagen type I. Clinically, it is manifested by in utero fractures, long bone deformities, very little calvarial mineralization, and death due to respiratory insufficiency. Lethal phenotypes of osteogenesis imperfecta are also associated with homozygous loss-of-function mutations in *LEPRE*, the gene encoding prolyl 3-hydroxylase 1, *CRTAP*, encoding cartilage-associated protein, *PPIB*, encoding peptidyl-prolyl isomerase B, and *SERPINH1*, encoding serpin peptidase inhibitor (discussed later) (see Table 18-12B).[194] Osteogenesis imperfecta type III (MIM 259420) is an autosomal dominant trait due to point or frameshift mutations in *COL1A1* (Gly154Arg, Gly844Ser) or *COL1A2* (Gly526Cys). It is characterized by recurrent fractures leading to progressive bone deformities that are often apparent at birth, kyphoscoliosis, extreme short stature, blue sclerae that lighten with age, abnormal dentition (in 80% of children younger than 10 years of age), and hearing loss. Osteogenesis imperfecta type IV (MIM 166220) is an autosomal dominant disease usually associated with point mutations or small deletions in *COL1A2* (Gly586Val, Gly646Cys, Gly1012Arg) and occasionally in *COL1A1* (Gly175Cys, Gly832Ser). It is of variable severity with prolonged survival, mild to moderate bone deformities, short stature, normal sclerae, dentinogenesis imperfecta, and hearing loss.

Among the most important posttranslational modification of type I procollagen (a triple helix comprised of two pro-COL1A1 and one pro-COL1A2 chains) is hydroxylation of proline and lysine residues by prolyl and lysyl hydroxylases. Hydroxylation of proline residues at carbon 4 affords thermal stability, whereas hydroxylation of lysyl residues permits binding of carbohydrates (galactose or glucosyl-galactose) and the formation of cross-links within or between procollagen chains.[199] Hydroxylation of proline at carbon 3 in position 986 of COL1A1 and at position 707 of COL1A2 are especially critical for stability of the three-dimensional configuration of type I procollagen and its subsequent maturation to collagen type I.[199] Prolyl 3-hydroxylase 1 (P3H1, also termed *leprecan* and encoded by *LEPRE1*, MIM 610339) specifically hydroxylates carbon 3 of the proline residue at codon 986 in COL1A1 and at codon 707 in COL1A2, reactions that require interaction of P3H1 with cartilage-associated protein (CRTAP; MIM 605497) and peptidyl-proline (cis-trans) isomerase B (PPIB also termed cyclophilin B, MIM 123841); these proteins that also serve as chaperones for the movement of collagen from the endoplasmic reticulum.[199] *CRTAP* is expressed in the proliferative zone of developing cartilage and at the chondro-osseous junction. *Crtap* "knockout" mice develop an osteochondrodysplasia (kyphoscoliosis, rhizomelic shortening of the proximal segment of the limbs) and severe osteopenia, the latter due to reduced production and alteration in the quality of osteoid and consequent decreased rate of mineral deposition.[405] Mice deficient in *Crtap* are unable to 3-hydroxylate the proline residue near the carboxyl terminus of bone COL1A1 leading to increased hydroxylation of lysine residues and resultant abnormal structure of the collagen fibril—changes that result in defective mineralization of bone collagen type I. Nonhydroxylated proline at codon 986 in bone COL1A1 was demonstrated in 3 of 11 patients with a recessively transmitted lethal or severe osteogenesis imperfecta characterized by multiple fractures of the long bones resulting in rhizomelic shortening of the limbs with externally rotated and abducted legs, poorly mineralized calvaria and ribs, proptotic eyes, and white or light blue sclerae.[406] This proved due to homozygous or compound heterozygous loss-of-function mutations in *CRTAP*—frameshift (c.879delT), 16 bp duplication in exon 1, nonsense (Gly276Ter), missense (Met1Ile), splice donor site of exon 1 at the first intronic nucleotide (IVS1+1G ⇒ C)—that interfered with effective hydroxylation of the proline residue at codon 986 of bone procollagen α1(I). This disorder has been designated osteogenesis imperfecta type VII (MIM 610682). In other patients with osteogenesis imperfecta type VII, a homozygous mutation in *CRTAP* has been identified in which alteration of one nucleotide (c.472 - 1021C ⇒ G) generates a cryptic splice donor site that leads to inclusion of 73 bp of intron 1 into the genome of *CRTAP*, thus extending exon 2.[405] This mutation results in more rapid degradation of CRTAP and consequently leads to decreased 3-hydroxylation of proline 986 in bone COL1A1. Osteogenesis imperfecta type VII has been identified in a Native American population in northern Quebec. Clinically, it is an autosomal recessive disorder in which fractures are present at birth; often the frequency of fractures declines with advancing age, particularly after adolescence; the sclerae are slightly bluish; there is progressive skeletal deformation, which leads to rhizomelic shortening of the limbs and restricted ambulation.[407] Inactivating mutations in *CRTAP* can also result in a lethal form of osteogenesis imperfecta resembling that of type II.

The phenotype of osteogenesis imperfecta type VIII (MIM 610915) overlaps with those of types II/III; in addition to osseous fragility, it is associated with substantial growth retardation, white sclerae, and bulbous metaphyses. It is due to loss-of-function mutations in *LEPRE1* encoding P3H1.[408] Osteogenesis imperfecta type VIII occurs primarily in African-American and Middle Eastern subjects.[408-410] CRTAP and P3H1 mutually stabilize one another in the collagen prolyl 3-hydroxylation complex.[411] Loss-of-function mutations in *PPIB*, the gene encoding cyclophilin B, has been designated osteogenesis imperfecta type IX, an often lethal disorder in which osseous fragility is evident in utero (see Table 18-12B).[412-414]

Heterozygous mutations in *IFITM5* (encoding interferon-induced transmembrane protein-5 or bone-restricted ITIFM5-like protein, MIM 614757) result in osteogenesis imperfecta type V (MIM 610967), a fragile bone syndrome that is phenotypically similar to osteogenesis imperfecta type IV but whose phenotype is markedly variable (see Table 18-12B).[415-417] Distinguishing features of osteogenesis imperfecta type V are calcification of the interosseous membranes between radius and ulna and exuberant callous formation at fracture sites. Other clinical characteristics of type V osteogenesis imperfecta are autosomal dominant transmission, moderate to severe bone fragility (lumbar spine areal BMD Z scores range between −7.7 and −0.7), moderate to mild growth retardation (adult height Z scores vary from −8.7 to −0.1), dislocation of the radial head, white sclerae, and normal dental development. Histologically, there is irregular arrangement of lamellae. After excluding mutations in *COL1A1* and *COL1A2* in patients with autosomal dominantly transmitted osteogenesis imperfecta, several groups of investigators identified the common mutation in *IFITM5* by whole exome sequencing—a heterozygous c.-14C > T transition within its 5′ untranslated region.[415-417] This mutation is 14 bp upstream of the reference initiation codon and creates a new initiation signal that adds 5 amino acids (Met-Ala-Leu-Glu-Pro) to the amino terminus of IFITM5 increasing its length from 132 to 137 amino acids. IFITM5 is a protein that is abundantly expressed within osteoblast membranes during embryonic and postnatal development of the skeleton; it has amino and carboxyl extracellular termini, two transmembrane domains, and an intracellular helical domain. IFITM5 is an osteoblast differentiation factor that is involved in protein trafficking and folding; experimentally, loss of *IFITM5* results in decreased bone formation, particularly in utero.[418] The functional consequence (whether it is activating or inactivating) of the identified consistent mutation in *IFITM5* is uncertain at present.

Osteogenesis imperfecta type VI (MIM 610968) is also phenotypically similar to type IV. It is an autosomal recessive disorder due to loss-of-function mutations in *SERPINF1* (serpin peptidase inhibitor, clade F, member 1; MIM 172860) that is characterized clinically by severe bone fragility that first manifests after 6 months of age, excess unmineralized osteoid, and a "fish-scale" pattern of lamellation of bone matrix on microscopic examination consistent with a defect in bone mineralization.[199,419-421] Serpins are a family of serine protease inhibitors (first identified in retinal pigment epithelial cells). *SERPINF1* encodes pigment epithelium derived factor (PEDF), a peptide synthesized by chondrocytes, osteoblasts and osteoclasts that interferes with function of vascular endothelial growth factor (VEGF), a protein that enhances migration of osteoblasts and osteoclasts into cartilage; PEDF may regulate bone mineralization and increase synthesis of osteoprotegerin. Thus, loss-of-function mutations in *SERPINF1* may interfere with bone formation while increasing osteoclast generation and bone dissolution. Serum concentrations of PEDF are undetectable in patients with osteogenesis imperfecta type VI, providing a diagnostic tool for this illness.[422] Interestingly, patients with osteogenesis imperfecta type VI do not respond to treatment with bisphosphonates as well as do patients with other forms of this heterogeneous disease.

Osteogenesis imperfecta type X is an autosomal recessive disorder due to biallelic inactivating mutations in *SERPINH1* (serpin peptidase inhibitor, clade H, member 1; MIM 600943) encoding a multifunctional 418-amino-acid peptide designated collagen type 1 binding protein (CBP) 2 that is also termed heat shock protein (HSP) 47. Type X osteogenesis imperfecta is a severe disorder with fractures occurring throughout the skeleton that begins in utero.[199,423] CBP2/HSP47 is essential for cartilage organization, the formation of endochondral bone, and the fabrication and maintenance of the integrity of the triple helical structure of procollagen type I, its transcellular movement and secretion, and its resistance to proteolytic degradation.[423-425] Type XI osteogenesis imperfecta is transmitted as a severe autosomal recessive disorder that begins in infancy; affected patients have repeated long bone fractures and progress to wheelchair dependency in early childhood; they may or may not develop joint contractures but do not display dentinogenesis imperfecta.[426-428] It is due to inactivating mutations in *FKBP10* (FK506-binding protein 10; MIM 607063) encoding an endoplasmic reticular protein (FKBP65) that is also a chaperone for procollagen type I —one that is essential for hydroxylation of lysine in its telopeptide, a modification necessary for cross-linking, as well as its transcellular movement and secretion.[429] In the absence of functional FKBP65, procollagen type I accumulates in the endoplasmic reticulum of the osteoblast. Mutations in *FKB506* have also been recorded in patients with Bruck syndrome 1 (MIM 259450; pterygia, congenital contractures, fractures in infancy resulting in limb deformities, growth retardation, scoliosis), suggesting that osteogenesis imperfecta type XI and Bruck syndrome 1 are likely allelic disorders whose clinical manifestations depend on the mutation site and other modifying factors.[426,430] Osteogenesis imperfecta type XII is a moderately severe form of this disease characterized by multiple fractures in early infancy with short, bowed long bones and highly arched palate but normal teeth, hearing, and sclerae.[431] It is transmitted as an autosomal recessive disorder and is due to inactivating mutations in *SP7* (transcription factor specificity factor 7 or Osterix; MIM 606633). Osterix is a 431-amino-acid essential transcription factor for osteoblast differentiation and function and hence for both endochondral and intramembranous bone development; its expression is regulated by *RUNX2*.[432] Type XIII osteogenesis imperfecta is a disorder of moderate severity with multiple fractures annually, either borderline low or *elevated* bone mass by DEXA, substantial growth retardation, faintly blue sclerae, and normal teeth.[433,434] It is the result of biallelic inactivating mutations (Phe249Leu; c.34G>C) in *BMP1* (bone morphogenetic protein 1; MIM 112264). BMP1 is a multifunctional protein; one of its most important activities is as a procollagen type I C-terminal propeptide endoproteinase. Inactivation of *BMP1* impairs proteolytic removal of the carboxyl terminal propeptide from procollagen type I and the normal assembly of mature collagen type I fibrils. Interestingly,

this form of osteogenesis imperfecta is associated with either borderline low or even increased lumbar spine aBMD by DEXA despite which treatment with bisphosphonates has been clinically and radiographically useful.[433] In this regard, it should be noted that in patients with mild osteogenesis imperfecta type I due to mutations in the procollagen type I carboxyl terminal propeptide cleavage sites also have normal or increased lumbar spine BMD by both DEXA and pQCT examinations.[404] Type XIV osteogenesis imperfecta is a disorder of variable severity with fractures occurring in utero or during early childhood but with normal dentition, hearing, and sclerae that has been identified in Middle Eastern families.[435,436] It is an autosomal recessive disorder due to a homozygous inactivating mutation (deletion of exon 4) in *TMEM38B* (transmembrane protein 38B; MIM 611236) encoding the 291-amino-acid trimeric intracellular cation channel type B (TRICB). TRICB is one component of TRIC, a monovalent cation channel that is critical for the release of Ca^{2+} from intracellular storage sites such as the sarcolemma and endoplasmic reticulum and thus maintenance of cytosolic Ca^{2+} concentrations, a process necessary for normal cell differentiation, division, and function (including the osteoblast). Bruck syndromes type 1 (MIM 259450) and 2 (MIM 609220) are skeletal disorders marked by increased bone fragility and congenital joint contractures, the latter due to restricted fetal movements. Bruck syndrome type 2 is due to biallelic loss-of-function mutations in *PLOD2* (procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2; MIM 601865), a lysyl hydroxylase.[437] Hydroxylation of collagen type I lysine residues is essential for the integrity of cross-linked collagen telopeptides and for the posttranslational attachment of galactose or glucosyl-galactose to collagen. A pathophysiologic classification of the various types of osteogenesis imperfecta is presented in Table 18-12C.

The clinical manifestations of osteogenesis imperfecta vary from mild (e.g., type I) to moderate (e.g., types IV, V, VI, VII, XIII, XIV), severe (e.g., types III, VII, VIII, IX, X, XI, XII), or lethal (e.g., types II, VII, VIII, IX, X).[199,438] Radiographic findings in subjects with osteogenesis imperfecta include, in addition to diffusely low bone mass, thin cortices, metaphyseal flaring, fractures and bone deformities resulting there from, wormian skull bones (frequent but not pathognomonic of osteogenesis imperfecta), platybasia that may compress overlying hindbrain, vertebral compression, and a triradiate pelvis.[193] Bone densitometry reveals decreased mineralization, the extent of which correlates to a degree with clinical manifestations. The diagnosis of osteogenesis imperfecta is established by clinical criteria and confirmed by genotyping of *COL1A1* or *COL1A2* or other pertinent gene(s), although failure to detect a genetic mutation does not necessarily rule out this disorder. Undetectable serum levels of PEDF are indicative of osteogenesis imperfecta due to inactivation of *SERPINF1*.[422] Occasionally, biopsy of the iliac crest and histologic examination of the bone may be necessary for subclassification of the disorder. Determination of the rate of synthesis and forms of procollagen secreted by dermal fibroblasts in vitro permits identification of the forms and relative amounts of collagen subunits and intact proteins being synthesized, but in general gene analyses have superceded this procedure.[439,440] Osteogenesis imperfecta type II can be identified prenatally by fetal ultrasonography; types I, III, and IV can be determined prenatally by analysis of collagen synthesized by cells cultured from chorionic villus biopsies and by analysis of the *COLA1A*, *COLA12*, *CRTAP*, or other genes. Included in the differential diagnosis of recurrent fractures in children are child abuse, various forms of rickets including hypophosphatasia, the McCune-Albright syndrome of fibrous dysplasia (MIM 174800), juvenile Paget disease, and juvenile osteoporosis.

TABLE 18-12C **Pathophysiologic Classification of Disorders of Collagen Formation Associated with Osteogenesis Imperfecta**

Site	Gene(s)	OI Types
Osteoblast and bone formation	*IFITM5*	V, IV
	SERPINF1	VI, IV
	SP7	XII, IV
Synthesis of collagen type I subunits	*COL1A1*	I, II, III, IV
	COL1A2	
Post-translational processing of collagen type I		
Chaperoned movement	*SERPINH1*	X, III
	FKBP10	XI, III, Bruck syndrome 1
Prolyl-3 hydroxylation	*LEPRE1*	VIII, II
	CRTAP	VII, II
	PPIB	IX, II
Lysyl hydroxylation	*PLOD2*	Bruck syndrome 2
Procollagen type I C-terminal propeptide endoproteinase	*BMP1*	XIII, III
Uncertain	*TMEM38B*	XIV

Modified from Rohrbach M, Giunta, C. (2012). Recessive osteogenesis imperfecta: clinical, radiological, and molecular findings. Am J Med Genet Part C 160C:175-189.

The basic management of patients with osteogenesis imperfecta is directed to prevention of fractures to the extent possible and to the treatment of fractures that do occur by sound orthopedic procedures and by orthopedists familiar with this disorder. Expert orthopedic management is essential for the child with osteogenesis imperfecta, as the use of intramedullary rods for correction of long bone deformities and for their linear growth and the proper correction of scoliosis require substantial experience.[439] Rehabilitative services and physical therapy to improve muscle strength and mobility within the constraints of bone fragility are to be encouraged as are protected ambulation and exercises such as swimming.[193] The use of bisphosphonates for the management of infants, children and adolescents with osteogenesis imperfecta types I, III, and IV has been of substantial benefit. Patients with these disorders have often responded symptomatically (decreased musculoskeletal pain, increased mobility) to the intermittent intravenous administration of a bisphosphonate (e.g., pamidronate) and bone mass has increased, but the effect of bisphosphonates on the fracture rate is uncertain.[439,441] It has been recommended that bisphosphonates be employed in infants with osteogenesis imperfecta with congenital and recurrent fractures, deformities of the long bones, and decreased bone mass. Infants as young as 2 months of age have safely tolerated 4-hour intravenous infusions of pamidronate (0.5 mg/kg/day for 3 consecutive days every 6 to 8 weeks) realizing clinical improvement such as decline in bone pain (perhaps a placebo effect), an increase in lumbar vertebral BMD of 86% to 227%, and a decrease in fracture rate after 1 year of therapy.[193] Bisphosphonate administration is also recommended for children with OI and recurrent fractures of the extremities or vertebral collapse that is symptomatic in concert with demonstrated decreased bone mineralization.[442] In children (3 to 16 years of age), pamidronate administered as a 4-hour infusion (1.5 to 3 mg/kg/day) for 3 consecutive days every 4 months resulted in increases in lumbar spine BMD of 42% per year, metacarpal cortical width of 27% per year, and vertebral size as well as a decline in fracture rate, and symptomatic improvement. During 2 to 4 years of intravenous pamidronate administration, increase in vertebral (trabecular) bone mass and size are accompanied by a decline in the extent of vertebral compression and fewer compressed vertebrae than in untreated patients.[443] In the iliac crest, bisphosphonates increase cortical bone thickness and trabecular number but not trabecular thickness; in metacarpals bisphosphonates enhance cortical thickness. However, in treated subjects the relative risk of long bone fracture after treatment with bisphosphonates is modest at best.[193] In older children and adolescents with osteogenesis imperfecta, bisphosphonates are employed if the patient has more than two fractures in 1 year and bone mass is low.[439] Among the complications of bisphosphonate therapy are transient hypocalcemia and a "flulike" reaction of fever, vomiting, and rash after the first exposure managed symptomatically. Administration of bisphosphonates is also accompanied by transient hypocalcemia, increased levels of PTH and calcitriol, and decreased levels of markers of bone turnover.[441] Near maximal benefits of bisphosphonates on lumbar vertebral aBMD (DEXA) and on mean cortical width, cancellous bone volume, and trabecular bone formation rate by histomorphometric analysis of iliac crest bone biopsies are achieved within the first 2 to 4 years of treatment with little further change with more prolonged therapy.[444] Because of the accumulation and persistence of bisphosphonates in bone and their long-term effects, it is recommended that these agents be administered to patients with osteogenesis imperfecta for no more than 2 to 4 years.[445] In addition to pamidronate, children with osteogenesis imperfecta have been treated with intravenous infusions of zolendronate every 4 to 6 months or with oral residronate or alendronate.[441] Transplantation of mesenchymal stem cells or bone marrow stromal cells have been undertaken in patients with osteogenesis imperfecta, but this is an experimental therapy, as are specific-gene replacement and mutation silencing strategies.[446]

Fibrous Dysplasia

Fibrous dysplasia is a usually benign fibro-osseous lesion that involves long bones, ribs, and skull; it may be monostotic, polyostotic, or panostotic.[447,448] It may be an isolated abnormality but characteristically occurs in patients with the McCune-Albright syndrome (MIM 174800) in association with very large, irregularly edged café-au-lait pigmentations and various endocrinopathies including isosexual precocious puberty, hypersomatotropism, thyrotoxicosis, and hyperadrenocorticism, as well as dysfunction in many other tissues (heart, liver, pancreas). Bone and skin lesions are often on the same side of the body.[448] Fibrous dysplasia is due to mosaicism for postzygotic, early embryonic, somatic, gain-of-function missense mutations (Arg201 to Cys, His, Ser, Gly; Gln227Leu) in *GNAS*, the gene encoding the α subunit of the Gs-protein that render Gsα constitutively active by prolonging its biologic life.[449] The extent and severity of disease are determined by the point in fetal development at which the mutation occurs and its tissue distribution. The mutations result in loss of intrinsic guanosine triphosphatase activity within the Gsα subunit; thus, the stimulatory effect of Gsα on adenylyl cyclase is extended thereby increasing the generation of cyclic AMP. Among the targeted signaling pathways of cyclic AMP is that involving WNT/β-catenin in osteoblast progenitor cells.[448] In response to excess cyclic AMP, clones of mutated mesenchymal preosteoblasts proliferate, but their differentiation to mature osteoblasts is incomplete and their secreted matrix is abnormal; continued expansion of osteoprogenitor cells in bone marrow leads to local fibrosis. As the osteogenic cells increase in number, they steadily erode contiguous bone. These lesions can also synthesize FGF23 and thus lead to hyperphosphaturia, hypophosphatemia, and excess unmineralized osteoid and a rickets-like clinical state. Fibrodysplastic lesions are initially silent, whereas osteoclasts at the periphery of the lesions actively compress and thin bone cortices, ultimately resulting in bone pain and pathologic fractures of the long bones, particularly the proximal femoral metaphyses. Children between 6 and 10 years of age have the highest fracture rate (0.4 fractures per year). Within the skull base and facial bones, expansion of fibrous dysplastic lesions leads to disfiguration and compression of cranial

nerves. Radiographically, the fibrodysplastic lesion is viewed as a "cystlike" medullary structure with a "ground-glass" consistency without a trabecular pattern.[448] Histologically, there are abundant immature bone marrow stromal cells, incompletely differentiated osteoblasts, irregularly formed bone trabeculae that may resemble Chinese characters, many undermineralized osteoid seams characteristic of osteomalacia, and islands of cartilage. The clinical manifestations of fibrous dysplasia depend on the sites and extent of bone involvement and associated endocrinopathies. Diagnosis of fibrous dysplasia is based on clinical characteristics and confirmation of the genetic mutation in *GNAS*. In addition to managing the multiple endocrinopathies and organ defects, attention must be paid to the osseous lesions. Fractures are repaired by standard techniques including intramedullary nailing when indicated; occasionally it may be feasible to evacuate a fibrodysplastic lesion surgically and to fill the cavity with bone grafts. In children with fibrous dysplasia, the bisphosphonate pamidronate has been useful in ameliorating bone pain but not the skeletal lesions. In a 9-year-old boy with a rapidly growing femoral mass due to fibrous dysplasia (and a somatic mutation in *GNAS*), treatment with denosumab led to a rapid decline in the rate of tumor growth and lowered the levels of bone turnover markers.[450] However, the child developed hypocalcemia and secondary hyperparathyroidism, requiring supplemental calcium, phosphate, and calcitriol. Cessation of denosumab was marked by a rebound increase in values of bone turnover markers and hypercalcemia.

High Bone Mass

Abnormally increased bone mass is the consequence of disruption of the normal equilibrium between the processes of bone formation and resorption. Thus, it may be due to a decrease in the rate of bone resorption as a result of a paucity or abnormal function of osteoclasts or to an increase in the rate of bone formation due to excessive osteoblast activity. An increase in cortical bone width is termed *hyperostosis*; thickening of trabecular bone is termed *osteosclerosis*.[8] Tables 18-13A and B list selected dysplastic, metabolic, and other diseases associated with increased bone mass and ectopic bone formation in children and adolescents. Failure of osteoclast-mediated bone resorption leads to osteopetrosis that may be associated with a large number of poorly functioning osteoclasts ("osteoclast-rich" osteopetrosis) or with a normal number or paucity of osteoclasts ("osteoclast-poor" osteopetrosis).[300] Impaired development of osteoclasts results in "osteoclast-poor" osteopetrosis and is attributable to mutations in genes encoding RANKL (*TNFSF11*) and RANK (*TNFRSF11A*). *IKBKG* is an X-linked gene that encodes a subunit of the inhibitor of kappa B (IκB)

TABLE 18-13A **Disorders of Bone Mineralization: High Bone Mass**

I Decreased Bone Resorption

A Osteopetrosis

1 Autosomal recessive (infantile/intermediate)
  a Osteoclast-rich/normal (*TCIRG1, CLCN7, OSTM1, CA2, PLEKHM1, SNX10*)
  b Osteoclast poor (*TNFSF11, TNFRSF11A*)
2 Autosomal dominant (adult) (*LRP5, CLCN7*)
3 X-linked (*IKBKG*)
4 Pycnodysostosis (*CTSK*)
5 Osteopetrosis with neuroaxonal dystrophy
6 Drug-induced: bisphosphonates

II Increased Bone Formation

A Activating Mutations of LRP

1 Autosomal dominant high bone mass (*LRP5*)
2 Endosteal osteosclerosis (van Buchem disease type 2) (*LRP5*)
3 Sclerosteosis type 2 (*LRP4*)

B Inactivating Mutations of SOST

1 Sclerosteosis type 1
2 Van Buchem disease type 1

C Dysplasias

1 Dysosteosclerosis
2 Infantile cortical hyperostosis (Caffey disease) (*COL1A1*)
3 Juvenile Paget disease (*TNFRSF11B*)
4 Metaphyseal dysplasia (Pyle disease)
5 Osteopoikilosis (*LEMD3*)
6 Progressive diaphyseal dysplasia (Camurati-Engelmann disease) (*TGFB1*)
7 Tubular stenosis, type 1 (Kenny-Caffey syndrome) (*TBCE*)

D Metabolic Disorders

1 Fluorosis
2 Heavy metal poisoning
3 Hypervitaminosis A, D
4 Hypoparathyroidism, pseudohypoparathyroidism
5 Milk-alkali syndrome
6 Renal osteodystrophy

E Ectopic Calcification/Bone Formation

1 Tumoral calcinosis, normophosphatemic, familial (*SAMD3*)
2 Tumoral calcinosis, hyperphosphatemic, familial (*GALNT3, FGF23, KL*)
3 Fibrodysplasia ossificans progressiva (*ACVR1*)
4 Progressive osseous heteroplasia (*GNAS*)

F Other

1 Hepatitis C–associated osteosclerosis
2 Ionizing radiation
3 Sarcoidosis
4 Sickle cell disease (*HBB*)
5 Tuberous sclerosis (*TSC1*)
6 Sickle cell disease
7 Leukemia

Modified from Whyte, M. P. (2008). Sclerosing bone disorders. In C. J. Rosen (Ed.), Primer on the metabolic bone diseases and disorders of mineral metabolism *(7th ed.) (pp. 412–423). Washington, DC: American Society of Bone and Mineral Metabolism.*

TABLE 18-13B **Gene Mutations Associated with Osteopetrosis and Increased Bone Mass**

Disease Type MIM	Gene Chromosome	Clinical Manifestations	Pathophysiology
Osteoblast Mediated			
Autosomal dominant osteopetrosis type I 607634	*LRP5*: Low-density lipoprotein receptor-related protein 5 11q13.2 603506	Endosteal hyperostosis, osteosclerosis, van Buchem disease type 2; not associated with increased fracture risk	Monoallelic gain-of-function mutations in a transmembrane WNT coreceptor expressed on plasma membrane of osteoblasts and osteocytes enhance WNT/β-catenin mediated osteoblast proliferation and function
Osteoclast Mediated			
Autosomal dominant osteopetrosis type II 166600	*CLCN7*: Chloride channel 7 16p13.3 602727	Osteopetrosis: intermediate severity: sclerosis of skull, "rugger jersey" spine, pelvis; fractures; dental abscesses	Monoallelic inactivating mutations of the α3 subunit of a channel protein that transports Cl^- into subosteoclastic lacunae impede hydroxyapatite dissolution (see autosomal recessive osteopetrosis types IV,V,VIII)
Autosomal recessive osteopetrosis type I 259700	*TCIRG1*: T cell immune regulator 1 11q13.2 604592	Osteopetrosis: severe infantile malignant form; hepatosplenomegaly, hydrocephalus, hypocalcemia	Inactivating mutations of a subunit of vacuolar proton pump that transports H^+ into the subosteoclastic lacunae disrupt dissolution of hydroxyapatite
Autosomal recessive osteopetrosis type II 259710	*TNFSF11*: Tumor necrosis factor ligand superfamily, member 11 13q14.11 602642	Osteopetrosis: moderate severity	Loss-of-function mutations in RANKL decrease RANK-mediated osteoclastogenesis
Autosomal recessive osteopetrosis type III 259730	*CA2*: Carbonic anhydrase II 8q21.2 611492	Osteopetrosis: moderate severity; renal tubular acidosis, intracranial calcification	Loss-of-function mutations of an osteoclast enzyme that generates protons (H^+) from carbonic acid that are secreted into subosteoclast lacunae impair dissolution of hydroxyapatite
Autosomal recessive type IV 611490	*CLCN7*: Chloride channel 7 16p13.3 602727	Osteopetrosis: severe, infantile malignant form; neurodegeneration and retinal atrophy	Biallelic inactivating mutations in the α3 subunit of a channel protein that transports Cl^- into the subosteoclastic lacunae severely impair dissolution of hydroxyapatite (see autosomal dominant type II and autosomal recessive type V)
Autosomal recessive osteopetrosis type V 259720	*OSTM1*: Osteopetrosis-associated transmembrane protein 1 6q21 607649	Osteopetrosis: severe infantile malignant form; retinal and neural dysplasia	Inactivating mutations in OSTM1, a cofactor with CLCN7 in a molecular complex that cooperatively enables transport of Cl^- from the osteoclast into the subosteoclastic lacuna, impair dissolution of hydroxyapatite (see autosomal recessive type IV)
Autosomal recessive osteopetrosis type VI 611497	*PLEKHM1*: Pleckstrin homology domain-containing protein, family M, member 1 17q21.3 611466	Osteopetrosis: intermediate severity; Erlenmeyer flask deformities of long bones	Inactivating mutations of a protein that may affect vesicular transport within the osteoclast and the attachment of osteoclast to bone
Autosomal recessive osteopetrosis type VII 612301	*TNFRSF11A*: Tumor necrosis factor receptor superfamily, member 11A 18q21.33 603499	Osteopetrosis: severe; with hypogammaglobulinemia in some patients	Inactivating mutations of RANK decrease NFκB-mediated osteoclastogenesis and osteoclast function

Autosomal recessive osteopetrosis type VIII 615085	*SNX10*: Sorting nexin 10 7p15.2 614780	Osteopetrosis: severe; with macrocranium, optic atrophy	Inactivating mutations may impair movement of TCIRG1 to the osteoclast's ruffled border and secretion of H^+ into the subosteoclastic lacuna
X-linked osteopetrosis 300301	*IKBKG*: Inhibitor of kappa light polypeptide gene enhancer in B cells, kinase of, gamma Xq28 300248	Osteopetrosis: with immunodeficiency, lymphedema, anhidrotic ectodermal dysplasia	Also termed NEMO (NFκB essential modulator): inactivating mutations of this component of IκB kinase complex decrease NFκB-mediated osteoclastogenesis and osteoclast function
Pycnodysostosis 265800	*CTSK*: Cathepsin K 1q21.3 601105	Osteosclerosis: skull, maxilla, phalanges, bone fragility	Biallelic loss-of-function mutations in an osteoclast lysosomal cysteine proteinase secreted into subosteoclast lacunae impair degradation of bone matrix
Osteoblast Mediated			
van Buchem disease 239100	*SOST*: Sclerostin 17q21.31 605740	Osteosclerosis: increased thickness of skull, mandible, ribs, long bones; leads to cranial nerve insults: impaired vision, hearing, facial movement	Biallelic loss of a specific downstream 3′ regulatory element of *SOST* transcription decreases inhibition of WNT/β-catenin mediated osteoblast differentiation and function
Sclerosteosis 1 269500	*SOST:* Sclerostin 17q21.31 605740	Severe, progressive generalized skeletal osteosclerosis and hyperostosis; increased intracranial pressure; loss of vision, hearing, facial movement; syndactyly of second and third fingers	Biallelic loss-of-function mutations in *SOST* decrease inhibition of WNT/β-catenin mediated osteoblast differentiation and function
Sclerosteosis 2 614305	*LRP4*: Low density lipoprotein receptor-related protein 4 11p11.2 604270	Osteosclerosis and hyperostosis of skull and long bones; impaired hearing, facial movement; syndactyly, dysplastic nails	Mono- or biallelic loss-of-function mutations decrease sclerostin-mediated inhibition of WNT/β-catenin stimulated osteoblast differentiation and function
Progressive diaphyseal dysplasia (Camurati-Engelmann disease) 131300	*TGFB1*: Transforming growth factor, beta-1 19q13.2 190180	Osteosclerosis of diaphyses of long bones; limb pain; disturbed gait	Monoallelic mutations lead to constitutive activation of TGFβ1, stimulation of bone formation, and repression of bone resorption
Osteopoikilosis 166700	*LEMD3*: LEM domain-containing protein 3 12q14.3 607844	Osteosclerotic foci in metaphyses and epiphyses of long bones, pelvis, scapula that are symmetrical but unequal; subcutaneous collagen containing nodules	Monoallelic loss-of-function mutations in a nuclear membrane protein that modulates signal transduction by both BMPs and TGFβ
Osteopathia striata 300373	*AMER1/FAM123B*: Family with sequence similarity 123, member B Xq11.2 300647	Dense linear striations that course vertically within the long bones, fan-shaped striations in the iliac bones, sclerosis of skull with compromise of cranial nerve function	Inactivation of an X-linked protein that enhances degradation of β-catenin, its loss extends WNT/β-catenin stimulated osteoblast maturation and function,

Modified in part from Stark, Z., & Savarirayan, R. (2009). Osteopetrosis. Orphanet J Rare Dis, *4, 4, doi: 10.1186/1750-1172-4-5; Ihde, L. L., Forrester, D. M., Gottsegen, C. J., et al. (2011). Sclerosing bone dysplasias: review and differentiation from other causes of osteosclerosis.* RadioGraphics, 31, *1865–1882.*

kinase complex that is also essential for activation of NFκB and osteoclastogenesis. Despite being present in normal number or in abundance ("osteoclast-rich" osteopetrosis), osteoclast activity may be reduced because of decreased synthesis of acid (due to loss-of-function mutations in *CA*, the gene encoding carbonic anhydrase), impaired production of ion channels that transport H^+ and Cl^- into the subosteocytic lacunae (channel proteins encoded by *TCIRG1* or its cofactor *OSTM1* and *CLCN7*, respectively), or subnormal generation of proteinases such as cathepsin K (due to inactivating mutations in *CTSK*). Excessive deposition of bone mineral due to increased osteoblast function may be a consequence of decreased inhibition of the WNT/β-catenin signal transduction pathway due to loss-of-function mutations in *LRP4* or *LRP5* (encoding low-density lipoprotein receptor-related proteins 4 and 5, respectively) or *SOST* (encoding sclerostin). Interestingly, in patients with inactivating mutations in *LRP5* and autosomal dominant osteopetrosis type I (see Table 18-13B), there is also a paucity of osteoclasts.[300]

Osteopetrosis or "marble bone disease" is a group of dominant or recessive heritable disorders manifested by increased bone mineralization with heterogeneous clinical manifestations including increase osseous fragility that vary in severity from lethality to radiographic skeletal changes primarily.[8,451] Histopathologically, bone in the majority of forms of osteopetrosis is characterized by quiescent osteoclasts—either increased, normal, or few in number—and retained "islands" of calcified cartilage formed during endochondral ossification (primary spongiosa) due to failure of reabsorption of immature bone.[8] Although densely packed with mineral, osteopetrotic bone is actually quite fragile because the abnormality in bone remodeling due to decreased osteoclastic bone resorption leads to the incorporation of weak calcified growth plate cartilage into bone and delays the repair of microfractures. Radiographically, osteopetrosis is characterized by a diffuse increase in bone mass involving both cortical and trabecular bone, diaphyseal/metaphyseal widening with an "Erlenmeyer flask" appearance, alternating bands of sclerotic and lucent bone at the ends of the long bones, iliac crest, and vertebrae, sclerotic changes at the base of the skull, narrow medullary cavities, and pathologic fractures.[8] Cranial computed tomography often reveals narrowing of the bony canals through which cranial nerves (II, III, IV, VII, VIII) pass. Although, classically, three clinical forms of osteopetrosis were identified—infantile malignant, intermediate, and adult—as knowledge of the genetic mutations responsible for the disease have been delineated this classification has been replaced by one that identifies its mode of transmission (autosomal dominant or recessive) and in accord with the responsible mutated gene (see Tables 18-13A and B). Thus, the infantile/malignant form of osteopetrosis (MIM 259700) may be due to biallelic mutations in *TCIRG1*, *CLCN7*, or *OSTM1*—genes encoding osteoclast H^+ and Cl^- transport channel proteins that enable the osteoclast to secrete acid into the lacunae to dissolve hydroxyapatite, the mineral phase of bone. A severe form of osteopetrosis associated with hypogammaglobulinemia is attributable to deleterious variations in *TNFRSF11A*. Forms of osteopetrosis of intermediate severity are the result of mutations in *TNFSF11*, *CA*, *PLEKHM1*—genes encoding RANKL, carbonic anhydrase, and a protein that is involved with vesicular transport within the osteoclast and the attachment of osteoclast to bone, respectively. Activating mutations in *LRP5* and heterozygous inactivating variations in *CLCN7* are associated with osteopetrosis of mild to intermediate severity that are transmitted as autosomal dominant characteristics.

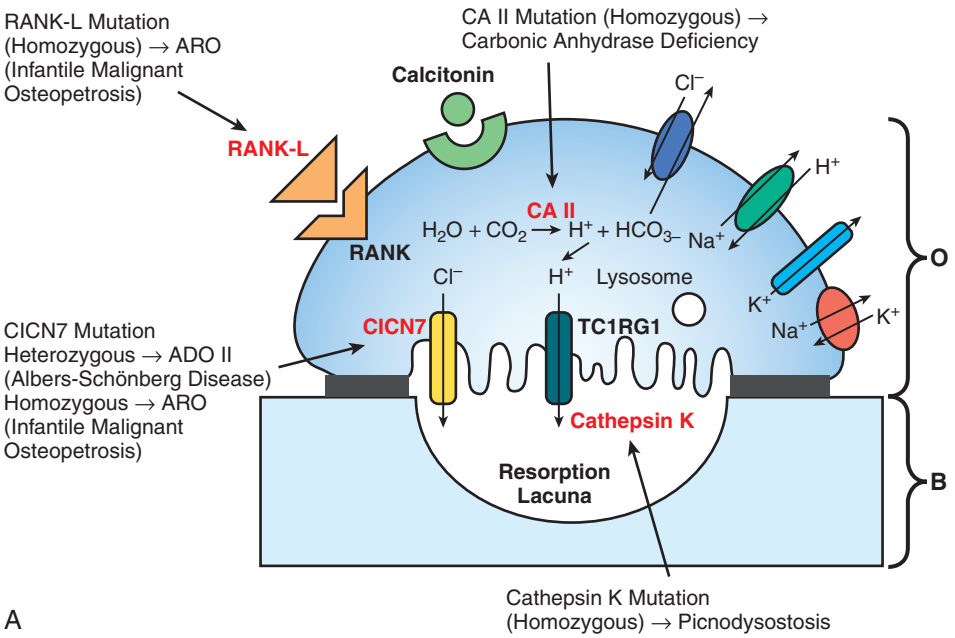
The infantile/malignant form of osteopetrosis is an autosomal recessive disorder (Albers-Schonberg disease) with attenuated growth particularly of the limbs, delayed development, increased fracture rate, and failure of tooth eruption. Bony overgrowth leads to macrocephaly, maldevelopment of the paranasal sinuses, and symptomatic nasal "stuffiness." Narrowing of cranial foramina compromises cranial nerve function (II, III, VII, VIII) with consequent blindness and deafness. Decrease in bone marrow volume leads to depressed intramedullary hematopoiesis, anemia, and leukopenia partially compensated by extramedullary hematopoiesis and ensuing hepatosplenomegaly with consequent increased susceptibility to infection and hemorrhage. Retention of teeth within the sclerotic jaw leads to recurrent and persistent mandibular and maxillary osteomyelitis. Physical examination reveals short stature, macrocephaly, frontal bossing, and small facial features. Osteopetrotic neonates and infants are often hypocalcemic (because of inability to reabsorb calcium deposited in bone) with secondary hyperparathyroidism and elevated calcitriol values; levels of serum acid phosphatase and the brain isoenzyme of creatine kinase are also increased.[7,8] Death usually occurs, often within the first decade of life, due to sepsis, anemia, or hemorrhage. Autosomal recessive osteopetrosis may be due to biallelic mutations in the α3 subunit of the osteoclast's vacuolar proton pump (*TCIRG1*), its chloride channel (*CLCN7*), the osteopetrosis-associated transmembrane protein-1 (*OSTM1*), or a protein that guides TCIRG1 to the osteoclast's ruffled membrane (*SNX10*) (discussed later). Biallelic mutations in *TNFRSF11A* encoding RANK give rise to a severe form of osteopetrosis often in association with hypogammaglobulinemia.[452]

The intermediate clinical form of osteopetrosis is also transmitted as an autosomal recessive trait and is associated with short stature, macrocephaly, recurrent fractures, variable compromise of cranial nerve function, abnormal dental development predisposing to osteomyelitis of the mandible or maxilla, and anemia. Pathogenetically, it represents the variable penetrance of one of the genetic mutations that may be associated with the infantile form of osteopetrosis, predominantly of *CLCN7*, but also of *CA*, *TNFSF11*, and *PLEKHM1*. Mutations in *TNFSF11* encoding RANKL also result in an osteopetrosis of moderate severity. There are two clinical and radiographic forms of autosomal dominant osteopetrosis: type I (MIM 607634) is characterized by an enlarged and dense cranial vault and diffuse vertebral sclerosis and is related to activating mutations of *LRP5*; it is not associated with an increase in fracture rate as bone strength is actually increased; type II (MIM 166600) is typified by thickening of the vertebral end plates resembling "bone within bone" and resulting in a "rugger jersey spine" and sclerotic

bands of bone in the pelvis and base of the skull. It is a variant of Albers-Schonberg disease that results from heterozygotic loss-of-function mutations in *CLCN7*.[8] Affected subjects manifest cranial nerve compromise (16%), mandibular and nonmandibular osteomyelitis (19%), osteoarthritis of the hip (27%), and fractures (78%). Clinical evidence of the disease tends to worsen over time. However, the expression of the trait is variable. Thus, one third of carriers of an inactivating mutation in *CLCN7* have no radiographic or clinical manifestations, although they do have significantly higher BMD than do subjects with the wild-type gene.[453] In one quarter of clinically apparent patients with a heterozygous loss-of-function mutation in *CLCN7*, the expression of illness (fractures, osteomyelitis, compromised vision) is identifiable at birth or early in infancy or childhood. Patients with radiologic/clinical manifestations of this disorder have elevated serum concentrations of tartrate-resistant acid phosphatase and the BB isoform of creatine kinase elaborated by osteoclasts; these values are normal in unaffected carriers.[8]

Genetic mutations that impede resorption of bone in osteopetrotic subjects are related to abnormalities in osteoclastogenesis or osteoclast function—particularly the efficiency of acidification of the resorption lacuna beneath the osteoclast's ruffled membrane and mineral dissolution or enzymatic degradation of organic bone matrix. *TNFSF11* encodes RANK-ligand (RANKL), a 317-amino-acid transmembrane protein expressed on the surface of stromal cells and osteoblasts that interacts as a trimer with its receptor (RANK encoded by *TNFRSF11A*), a plasma membrane protein expressed by osteoclast precursor cells to form a heterohexameric complex that stimulates NFκB and thereby induces osteoclastogenesis.[454] Inactivating mutations in *TNFSF11* and *TNFRSF11A* result in impaired osteoclast formation and thus "osteoclast-poor" osteopetrosis. Loss-of-function variations in the structure of RANKL associated with osteopetrosis include a missense mutation (Met199Lys) and 2-bp (828delCG) and 5-bp (intron 7 -532+4_532+8) deletions of *TNFSF11* (Figures 18-14A, 14B).[455] Patients with osteopetrosis due to inactivating mutations in *TNFSF11* do not respond to hematopoietic stem cell transplantation because the abnormality in osteoclastogenesis is extrinsic to the osteoclast cell line; because recombinant RANKL protein can transform the monocytes of affected patients into functioning osteoclasts in vitro, a potentially alternative therapeutic avenue may be available.[455] Biallelic missense (Gly53Arg, Arg 170Cys), nonsense (Trp244Stop, Gly280Stop), and insertion loss-of-function mutations in the extracellular domain of RANK also impair binding to RANKL and consequent stimulation of NFκB and osteoclastogenesis.[305,452] Many patients with inactivating mutations in TNFRSF11A also develop impaired B lymphocyte function and hypogammaglobulinemia.[307] Hematopoietic stem cell transplantation is curative in osteopetrotic subjects with deleterious mutations in *TNFRSF11A*; as anticipated, monocytes from these subjects are functionally unresponsive to recombinant RANKL and to macrophage-colony stimulating (M-CSF) proteins in vitro.[452] Inactivating mutations in *IKBKG*, encoding the inhibitor of the kinase of kappa light polypeptide gene enhancer in B cells (gamma subunit), alternatively termed NFκB essential modulator (NEMO), also impair NFκB generation and osteoclastogenesis resulting in the syndrome of osteopetrosis, lymphedema, anhidrotic ectodermal dysplasia, and immunodeficiency (OL-EDA-ID, MIM 300301).[456] *IKBKG* is located on the long arm of the X chromosome, and thus its loss is transmitted as an X-linked trait. IKBKG is a 419-amino-acid component of the IκB kinase complex that activates NFκB. The co-association of osteopetrosis and juvenile xanthogranuloma has also been recorded in a neonate with a mutation in *PLEKHM1*.[457]

Carbonic anhydrase II (one of several zinc metalloisoenzymes) is a protein that is expressed in osteoclasts, erythrocytes, brain, and kidney; it regulates the formation of carbonic acid from water and carbon dioxide ($CO_2 + H_2O \Rightarrow H_2CO_3$) that then dissociates to form proton/hydrogen (H^+) and bicarbonate (HCO_3^-) ions. Loss-of function homozygous or compound heterozygous (Lys17Glu, Tyr40Ter, His107Tyr, Asn252Asp) mutations in *CA2* lead to autosomal recessive osteopetrosis that presents in childhood with failure to thrive, short stature, visual impairment, and developmental delay in association with mild proximal and severe distal renal tubular acidosis, cerebral calcifications within the cortex and basal ganglia, and osteopetrosis with increased fracture risk.[8] Osteopetrosis is of modest severity and usually nonprogressive; it may even improve at puberty. Bicarbonate may be employed to normalize acid-base balance. After generation by carbonic anhydrase II, H^+ is extruded from the osteoclast into the subobsteoclastic resorption lacuna through transporters and proton pumps. *TCIRG1* (T cell immune regulator 1) encodes an 822-amino-acid, 116 kDa protein that is a subunit of the osteoclast's vacuolar proton pump (H^+-ATPase). (By alternative splicing, this gene also encodes a 614 amino acid protein—TIRC7 —that is essential for activation of T lymphocytes.) Biallelic inactivating (missense, nonsense, deletion, insertion, splice site) mutations in *TCIRG1* whose loss impairs transport of H^+ and thus decreases bone mineral resorption have been found in 50% of subjects with the neonatal/infantile form of lethal osteopetrosis (MIM 259700).[458,459] TCIRG1 may be guided to the osteoclast's ruffled membrane above the subosteoclastic lacuna by the product of *SNX10*; a biallelic mutation (Arg51Gln) of *SNX10* also results in infantile malignant osteopetrosis.[460] Osteoclasts in this disorder are distinguished by the large number of cytoplasmic vesicles they contain. Loss-of-function mutations in *CLCN7*, a chloride channel expressed in the ruffled membrane of the activated osteoclast and its lysosomes, also impair acidification of the subosteoclastic resorption space and hence mineral dissolution.[461] Heterozygotic inactivating mutations (Arg767Trp; 2 bp deletion, 1423AG) of *CLCN7* lead to an autosomal dominant form of osteopetrosis (MIM 166600), whereas biallelic loss-of-function mutations (Ile261Phe, Arg762Gln, Leu766Pro) are found in infants with the lethal, autosomal recessive, and intermediate forms of this disease (MIM 611490).[462,463] Mutations in *CLCN7* account for 15% of subjects with severe



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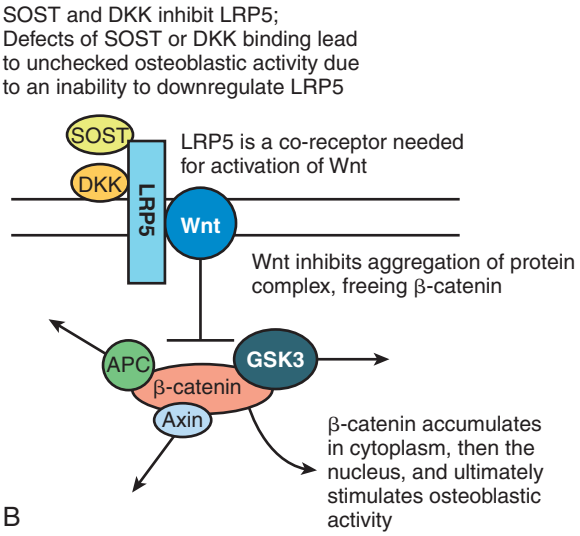


B

FIGURE 18-14 ■ Biochemical defects in osteoclasts (**A**) and osteoblasts (**B**) associated with osteopetrosis (see text for details). (From Ihde, L. L., Forrester, D. M., Gottsegen, C. J., et al. (2011). Sclerosing bone dysplasias: review and differentiation from other causes of osteosclerosis. *RadioGraphics, 31*, 1865–1882.) *This image can be viewed in full color online at ExpertConsult.*

and 40% of patients with intermediate autosomal recessive osteopetrosis and 75% of adults with autosomal dominant osteopetrosis type II.[462] *CLCN7* is coexpressed with and complexed to *OS*teopetrosis-associated *Trans**m**embrane protein 1 (*OSTM1*) in endosomes and lysosomes and in the ruffled membrane of activated osteoclasts.[464] By decreasing posttranslational stability of CLCN7, homozygous loss-of-function (nonsense, deletion) mutations in *OSTM1* have been pathogenetically related to autosomal recessive lethal osteopetrosis in a subset of patients.[465,466] The product of *PLEKHM1* is a protein that may be essential for vesicular transport within the osteoclast and for formation of its supralacunar ruffled borders; biallelic inactivating mutations of *PLEKHM1* result in osteopetrosis of intermediate severity.[467,468] Monoallelic gain-of-function mutations (Gly171Arg/Val, Ala242Thr, del Gly171/Glu172) in *LRP5* augment WNT/β-catenin signal transduction leading to increased osteoblast differentiation and function and asymptomatic high bone mass or autosomal dominant osteopetrosis type I, a disorder characterized by extremely dense skulls (with endosteal hyperostosis), vertebrae, and long bones, large mandible, and torus palatinus.[469]

Related to the long-acting inhibitory effects of bisphosphonate on bone modeling and remodeling, the administration of high doses of intravenous pamidronate (2800 mg) over a 3-year period resulted in an acquired "osteopetrosis-like" disorder in a 12-year-old boy with unexplained hyperalkaline phosphatasemia that persisted for at least several years after this agent was discontinued.[394] The metaphyses were extremely dense and club shaped, the base of the skull sclerotic, and the vertebral endplates thickened. Histologically, iliac crest biopsy revealed bars of calcified cartilage and quiescent osteoclasts. Despite the severity of the radiographic and microscopic findings, the patient was clinically well with normal growth and without evidence of bone marrow suppression or extramedullary hematopoiesis, although the risk for future fractures may have been increased.

A multidisciplinary team skilled in the management of patients with osteopetrosis is essential for the optimal care of infants and children with this disorder. Hypocalcemia and rickets occurring in neonates and infants with osteopetrosis may be managed with supplemental calcium and vitamin D.[8,470] In addition to appropriate orthopedic, neurosurgical, and anesthesiology input, medical therapy of osteopetrotic patients may at times be helpful. Nonspecific treatment with interferon-γ and high doses of calcitriol (with limited calcium intake) has arrested progression of the disease and even led to its regression in some osteopetrotic children.[8] In this setting, calcitriol acts as an osteoclast activating factor, whereas interferon γ indirectly stimulates osteoclast formation and increases the generation of superoxide in osteoclasts, an important factor for osteoclast-mediated bone resorption.[471] Depending on the cause of osteopetrosis, most patients (but not those with abnormalities of RANKL) improve after bone marrow or hematopoietic stem cell transplantation from human leukocyte antigen-identical donors with replacement of defective osteoclasts by normal osteoclast progenitor cells.[8,472,473] Hypercalcemia often complicates the posttransplantation period as osteoclast function resumes, particularly in patients with inactivating mutations in *TNFRSF11A*.[452] Hypercalcemia has been managed with dietary restriction, calcitonin, and bisphosphonate administration or by use of an inhibitor of RANKL such as denosumab, a monoclonal antibody raised against RANKL that binds to and depresses RANKL activity, that has been employed successfully to manage posttransplant hypercalcemia in children with this cause of osteopetrosis.[474] With improved treatment, there has been increased life span and improved developmental progress. Osteopetrosis due to deficiency of carbonic anhydrase II is not corrected by restoration of normal systemic acid-base balance, but bone marrow transplantation may be useful.[8] Identification and correction of the specific underlying gene defect may ultimately be feasible in patients with osteopetrosis.[475,476]

Pycnodysostosis (MIM 265800) is clinically manifested by disproportionate short stature during infancy and childhood with macrocranium and open cranial sutures, high forehead, small facial features, proptosis, bluish sclerae, beaked and pointed nose, micrognathia, highly arched palate, retained primary teeth, short fingers with hypoplastic nails, narrow thorax, pectus excavatum, and kyphoscoliosis with lumbar lordosis.[8] Radiologically, there is increased bone density that becomes progressively worse with age despite which susceptibility to fractures is increased; other characteristics include open fontanelles, impaction of permanent and supernumerary teeth, clavicles that are slim and hypoplastic laterally, partial or total absence of the ribs and hyoid bone, and acro-osteolysis of the distal phalanges (a pathognomic characteristic of pycnodysostosis).[477] Laboratory data and bone histology are basically normal, although there is biochemical evidence of decreased osteoblastic and osteoclastic activity. The microscopic observation that an abundance of osteoclasts with ruffled borders surrounded by enlarged clear zones suggested that dissolution of bone mineral was normal but that degradation of matrix was abnormal in these patients. Indeed, there are large amounts of decalcified matrix within both the subosteoclastic lacunae and the osteoclasts.[300] Pycnodysostosis is due to biallelic loss-of-function mutations in *CTSK* encoding cathepsin K, the osteoclast's lysosomal cysteine protease that degrades organic matrix after the mineral phase of bone has been reabsorbed. Among the variations in *CTSK* identified in patients with pycnodysostosis have been unipaternal isodisomy for chromosome 1 with paternal *CTSK* harboring an inactivating Ala277Val mutation, Leu9Pro substitution in the signal peptide of the precursor form of the enzyme protein preventing completion of its posttranslational processing, and Ter330Trp substitution permitting the addition of 19 amino acids to the carboxyl terminus of this enzymatic protein.

In contrast to diseases that increase bone mass by decreasing bone resorption are those disorders that primarily increase bone formation (see Figure 18-14B). An example of the latter is the familial, relatively benign form of autosomal dominant high bone mass (MIM 601884) that is associated with a heterozygous gain-of-function mutation (Gly171Val) in *LRP5*.[478] Loss-of-function mutations in the same gene are associated with idiopathic juvenile osteoporosis, the osteoporosis-pseudoglioma syndrome, and familial exudative vitreoretinopathy. LRP5 (and LRP6) is a coreceptor for WNT proteins whose primary receptor is Frizzled. After binding to LRP5 and Frizzled, WNT glycoprotein activates a canonical pathway that involves repression of glycogen synthase kinase 3 and leads to dephosphorylation of β-catenin permitting its translocation to the nucleus where it interacts with T-cell factor/lymphoid enhancing factor to control target genes that divert the mesenchymal stem cell into the track leading to osteoblastogenesis. In the mature osteoblast, WNT/β-catenin stimulates expression of osteoprotegerin, thus impairing osteoclastogenesis. Dickkopf and sclerostin (discussed later) are proteins that bind to the extracellular domain of LRP5 and internalize the receptor complex thereby blocking WNT signaling.[379] Mutations in LRP5 associated with high bone mass are clustered near the amino terminal of its extracellular domain at the sites of binding to Dickkopf and sclerostin.[381] The Gly171Val mutation in *LRP5* interferes with the binding of LRP5 to Dickkopf and thus prolongs the interaction of LRP5 with Frizzled, thereby augmenting the WNT/β-catenin signal and increasing bone formation.[479] Although generally benign, activating mutations of *LRP5* may also be associated with neurologic complications such as hearing loss, headaches, and pain in the extremities.[381] In some families, heterozygous activating mutations (Ala242Thr) of *LRP5* and exuberant bone formation have been associated with autosomal dominant generalized endosteal osteosclerosis (van Buchem disease, type 2—MIM 607636) or autosomal dominant osteopetrosis type I (Gly171Arg, Thr253Ile) (as discussed previously). However, the dense bones encountered in these subjects are not prone to fracture as in classic forms of osteopetrosis.

Sclerosteosis type 1 is an autosomal recessive disorder that is first manifested in childhood and is characterized

by very thick peripheral and cranial bones with calvarial overgrowth leading to facial disfigurement; entrapment of cranial nerves II, VII, and VIII; increased intracranial pressure; and brainstem compression.[8,379,480] Affected patients also have variable asymmetric cutaneous or bony syndactyly of the index and middle fingers and excessive somatic growth; they are extraordinarily resistant to fractures. Serum levels of alkaline phosphatase and procollagen type 1 N-terminal propeptide (P1NP) are elevated in these subjects, whereas concentrations of sclerostin are low or unmeasurable.[480] Sclerosteosis type 1 is due to biallelic loss-of-function mutations in *SOST* encoding sclerostin, a 213-amino-acid peptide secreted primarily by osteocytes embedded within bone. Physiologically, sclerostin inhibits WNT-mediated bone formation by binding to and internalizing LRP5, the coreceptor for WNT.[379,480] When sclerostin activity is decreased, increased bone formation ensues. Sclerostin also increases osteoclastogenesis by increasing osteocytic synthesis of RANKL.[480] In heterozygous carriers of inactivating mutations in *SOST*, bone mass is increased but not to pathologic levels.[481] Hyperostosis corticalis generalisata/van Buchem disease type 1 is an autosomal recessive disorder characterized by thickening and enlargement of the bones of the skull, mandible, ribs, and diaphyses of the long bones that result in increased bone density and encroachment upon cranial nerve passageways resulting in abnormalities of vision, hearing, and facial movement.[8,480] However, patients with van Buchem disease type 1 do not have syndactyly or tall stature, thus distinguishing it from sclerosteosis type 1. This disorder is also due to biallelic loss of expression of *SOST* but as a result of deletion of a regulatory element that is 35 kb downstream (5′) of *SOST* rather than by a mutation within the exonic/intronic structure of *SOST* itself.[482] Thus, this disease is allelic to sclerosteosis; it differs clinically in that gigantism and hand abnormalities are present in patients with sclerosteosis but not in those with van Buchem disease type 1. The clinical manifestations of sclerosteosis type 2 are similar to those of type 1 but are pathogenetically related to biallelic loss-of-function mutations in *LRP4*.[483] The inhibitory effect on bone formation of the interaction of sclerostin and LRP5/LRP6 is facilitated by the binding of sclerostin to LRP4. Thus, inactivating mutations (Arg1170Trp, Trp1186Ser) in *LRP4* impede the interaction of sclerostin with LRP5/6 and thus the inhibitory effect of sclerostin on osteogenesis. Treatment of these disorders is primarily symptomatic.

Progressive diaphyseal dysplasia (Camurati-Engelmann disease) is an autosomal dominant, cranial-peripheral hyperostotic disorder with variable expressivity that presents in children with problems such as limping, waddling gait, or leg pain, fatigue, and nonprogressive muscular weakness. Radiographically, there is symmetrical cortical thickening (hyperostosis) due to increased periosteal and endosteal bone formation in the diaphyses of the long bones, axial skeletons, and skull.[484,485] Pathogenetically, this disorder is primarily due to missense mutations within the "latency associated peptide" domain of the precursor propeptide of TGFβ1 (*TGFB1*). Normally, after posttranslational processing, two latency associated peptides are noncovalently linked to two mature TGFβ1 peptides to form a "latency complex." Mutations within the latency associated peptide domain of *TGFB1* (particularly at codon 218, a mutational "hot spot") impair this association, resulting in premature activation of TGFβ1 and consequent stimulation of bone formation and repression of bone resorption.[486] TGFβ1 also inhibits myogenesis and adipogenesis. Because of the inhibitory effects of glucocorticoids on bone formation and its stimulatory effects on bone resorption, short courses of these agents have been useful in alleviating many of the clinical symptoms and radiologic abnormalities in patients with progressive diaphyseal dysplasia.[484] Other hereditary sclerosing bone dysplasias include osteopoikilosis (MIM 166700) due to heterozygous inactivating mutations in *LEMD3* encoding a nuclear membrane protein that modulates signal transduction by BMPs and TGFβ, osteopathia striata (MIM 300373) the result of loss-of-function mutations in *AMER1/FAM123B* encoding a protein that promotes the proteasomal degradation of β-catenin (its loss thus extending the biologic activity of the WNT/β-catenin signal transduction system that promotes osteoblast maturation and function), and several forms of endosteal hyperostosis.[485] The hereditary and nonhereditary (e.g., intramedullary osteosclerosis) sclerosing bone dysplasias must be differentiated from acquired sclerosing diseases such as sickle cell anemia and myelofibrosis.[485]

Heterotopic Bone Formation/Ectopic Calcification

Disorders of heterotopic ossification are those in which bone develops outside of the skeleton and within soft tissues (Table 18-13C).[53] Dysregulation of the processes of differentiation and maturation enable precursor cells to develop as osteoblasts that then produce normal endochondral or membrane bone but in abnormal, extraskeletal sites. Sporadic heterotopic bone formation occurs in sites of severe wounds and burns, after spinal cord injuries, and in areas of pressure ulcers. Fibrodysplasia ossificans progressiva (FOP, MIM 135100) is a disabling disorder of ectopic bone formation that may develop spontaneously or at sites of injury and leads to ankylosis of all major joints that severely limits mobility.[53,487] It is characterized by progressive ectopic ossification of skeletal muscle and connective tissue (fascia, tendons, ligaments) leading to immobility and fusion of the mandible, neck, spine, hips, and other joints and the development of a "second skeleton" that encases and imprisons the body. The disorder may be present at birth and is often manifest by 5 years of age. FOP is also associated with congenital abnormalities of the great toes (hallux valgus, malformed first metatarsal bones, monophalangism), characteristic facial features (long narrow face, small mandible, low set ears), deafness, scalp baldness, and mild developmental delay.[488] Microscopically, there is normal but ectopic endochondral osteogenesis that occurs after a preceding inflammatory phase that develops either in the absence of trauma or following minor injury such as an immunization; the pathologic course proceeds through the phases of monocytic infiltration, muscle fiber degeneration, fibrous proliferation, angiogenesis, chondrogenesis, and osteogenesis.[53] Although usually sporadic because affected subjects rarely

TABLE 18-13C **Genetic Causes of Heterotopic Bone Formation and Ectopic Calcification**

Disease Type MIM	Gene Chromosome	Clinical Manifestations	Pathophysiology
Heterotopic Bone Formation			
Fibrodysplasia ossificans progressiva 135100	*ACVR1*: Activin A receptor, type I 2q24.1 102576	Progressive ossification of skeletal muscles, fascia, tendons, and ligaments	Specific monoallelic activating mutation (Arg206His) in the cytoplasmic domain of a type 1 BMP receptor increases extraskeletal chondrogenesis and osteogenesis
Progressive osseous heteroplasia 166350	*GNAS*: GNAS complex locus 20q13.32 139320	Ossification of dermis beginning in infancy followed by membranous bone formation within deep muscle and fascia in childhood	Monoallelic inactivating mutations in the paternally encoded $G_s\alpha$ subunit of G-protein enable subcutaneous bone formation; related to pseudopseudohypoparathyroidism
Ectopic Calcification			
Tumoral calcinosis, hyperphosphatemic, familial 211900	*GALNT3*: UDP-N-acetyl-alpha-D-galactosamine: polypeptide N-acetylgalactosaminyl-transferase 3 2q24.3 601756	Progressive deposition of calcium phosphate crystals in periarticular region, soft tissue, and bone	Biallelic mutations in a cofactor required for synthesis of bioactive FGF23 and renal tubular excretion of phosphate
Tumoral calcinosis, hyperphosphatemic, familial 211900	*FGF23*: Fibroblast growth factor 23 12p13.32 605380	Progressive deposition of calcium phosphate crystals in periarticular region, soft tissue, and bone	Biallelic inactivating mutations in a factor that inhibits renal tubular resorption of phosphate
Tumoral calcinosis, hyperphosphatemic, familial 211900	*KL*: Klotho 13q13.1 604824	Progressive deposition of calcium phosphate crystals in periarticular region, soft tissue, and bone	Biallelic inactivating mutation in a cofactor enabling FGF23 to inhibit renal tubular resorption of phosphate
Tumoral calcinosis, normophosphatemic, familial 610455	*SAMD9*: Sterile alpha motif domain-containing protein 9 7q21.2 610456	Postinflammatory periarticular and peripheral calcified masses	Biallelic inactivating mutations in a protein that regulates cell division, motility, longevity, and inflammation

reproduce, FOP can be transmitted as an autosomal dominant trait. This disease is primarily due to a highly specific (c.617G > A transition leading to Arg206His) mutation in *ACVR1* (encoding activin A receptor, type-1); another described *ACVR1* mutation in patients with classical FOP is c.744G > C transversion leading to Arg258Ser.[53,489] Activins are members of the TGFβ superfamily that includes the bone morphogenetic proteins (BMPs) together with the inhibins and Mullerian duct inhibiting factor. *ACVR1* encodes a type I BMP receptor that is expressed in chondrocytes and osteoblasts.[53] The Arg206His mutation in *ACVR1* resides at the junction of the receptor's cytoplasmic glycine-serine activation and tyrosine kinase domains and results in a constitutively active BMP type I receptor product that signals through the SMAD and mitogen-activated protein kinase transduction pathways to direct the pluripotent mesenchymal stem into the chondrogenic pathway leading to (ectopic) endochondral new bone formation. BMPs alone are able to stimulate complete endochondral osteogenesis in ectopic sites.[490] Patients with clinical variants of FOP and *ACVR1* mutations other than Arg206His and Arg258Ser have been described; they have been classified as those with FOP plus abnormalities of the brain, eye, or bone marrow, or those with FOP variants—either without abnormalities of the great toes or with less severe heterotopic osteogenesis; patients with mutations in codon 328 have varied in severity from classical to late-onset FOP; patients with an Arg201Ile mutation in *ACVR1* have adult onset of extraskeletal ossification and normal toes.[53,491] Management of these patients is primarily symptomatic and palliative to the extent possible, although immunosuppression may diminish the intensity of extraskeletal ossification.[53,492]

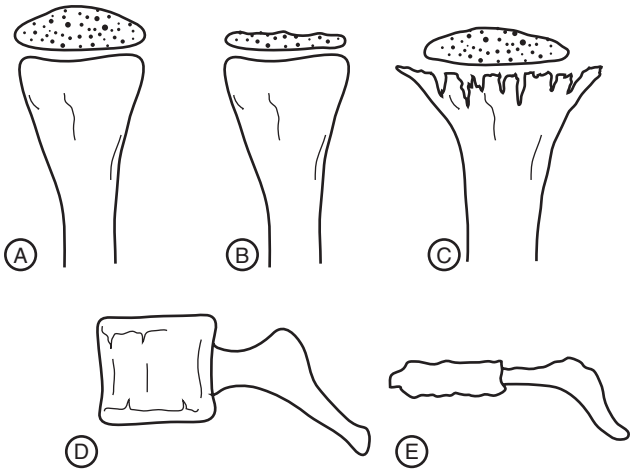
Progressive osseous heteroplasia (POH, MIM 166350) is characterized by multiple foci (trunk, extremities, or digits) of adipose tissue-associated dermal intramembranous bone formation (osteoma cutis) beginning in infancy in the absence of any local injury or inflammatory insult.[53] Lesions may be asymptomatic or painful. Over time, heterotopic ossification progresses into skeletal muscle and deep connective tissue and may be incorporated into skeletal bone. POH is transmitted as an autosomal dominant trait and occurs in both boys and girls; it is due to inactivating mutations of the *GNAS* allele usually inherited from the father.[493,494] Paternal transmission of mutated *GNAS* is associated with significant intrauterine growth restriction and more severe clinical manifestations of POH than when the *GNAS* mutation is transmitted by the mother.[494] Identical mutations in *GNAS* may be clinically manifested as either POH, pseudohypoparathyroidism (PHP), or pseudopseudohypoparathyroidism (PPHP) in different members of the same family (e.g., 1 bp del, 725C); all of these disorders

are associated with subcutaneous (dermal) ossification (as discussed previously). However, patients with POH do not have physical features of Albright's hereditary osteodystrophy nor are they hormone resistant. Only symptomatic treatment of patients with POH is currently available.

Extraskeletal calcification/ossification may occur sporadically in a number of hypercalcemic, hyperphosphatemic, or dystrophic states (renal failure, hypo- and hyperparathyroidism, sarcoidosis, after cell lysis induced by cancer chemotherapy, subcutaneous fat necrosis, dermatomyositis, atherosclerosis) as well as in specific diseases (e.g., pseudohypoparathyroidism type IA, McCune-Albright syndrome).[495] Familial tumoral calcinosis is a disorder characterized by deposition of crystals of basic calcium phosphate in soft tissues, periarticular spaces, and at times bone. Hyperphosphatemic and normophosphatemic forms of familial tumoral calcinosis have been described. Hyperphosphatemic familial tumoral calcinosis presents in childhood with recurrent bone pain, extensive and large cutaneous, periarticular, and vascular deposits of calcium phosphate; in some patients the ectopic calcifications may be confined to the eyelids; it is characterized radiographically by cortical hyperostosis, periosteal reaction, and mineral deposits around large joints, particularly the hips and shoulders.[496,497] Microscopically, there is a histiocytic response with formation of calcified bursa-like structures.[498] Laboratory studies reveal marked hyperphosphatemia and relative hypophosphaturia due to increased renal tubular reabsorption of phosphate and inappropriately normal or elevated serum calcitriol levels, because, despite hyperphosphatemia, PTH secretion is not increased and synthesis of calcitriol and intestinal calcium absorption persist. The disorder is due to a functional loss of FGF23 action and consequently unhindered renal tubular reabsorption of phosphate. The pathophysiology of this disorder is, thus, the mirror image of that associated with X-linked and autosomal dominant forms of hypophosphatemic rickets and tumor-induced osteomalacia in which there is exaggerated FGF23 production and activity leading to hyperphosphaturia and consequent hypophosphatemia, rickets, and osteomalacia. Hyperphosphatemic familial tumoral calcinosis is genetically heterogeneous. Homozygous inactivating mutations (Ser71Gly; Met96Thr, Ser129Phe) in *FGF23* have been identified in patients with this disorder; in the absence of FGF23, renal tubular resorption of filtered phosphate is unopposed.[496] More commonly detected in patients with hyperphosphatemic familial tumor calcinosis are biallelic loss-of-function microdeletions, splice site, and missense or nonsense mutations (Arg162Stop, Thr272Lys, Cys574Gly, Gln592Stop) in *GALNT3* (UDP-*N*-acetyl-alpha-D-galactosamine: polypeptide *N*-acetylgalactosaminyltransferase 3).[499] The product of *GALNT3* is a glycosyl transferase that initiates *O*-glycosylation in which *N*-acetylgalactosamine is the first sugar in the side chain, a step that is essential for secretion of intact and functional FGF23. Failure to *O*-glycosylate FGF23 at Thr178 in the Golgi apparatus permits its rapid intracellular cleavage between Arg179 and Ser180 to biologically inactive amino and carboxyl terminal fragments.[500] Serum concentrations of intact FGF23 are low or nondetectable, whereas carboxyl terminal FGF23 levels are elevated in patients with familial tumoral calcinosis due to either gene mutation. Therapy with an oral phosphate binder and the carbonic anhydrase inhibitor acetazolamide has resulted in hyperphosphaturia and reabsorption of ectopic calcifications without change in serum phosphate or calcium concentrations.[501] The hyperostosis hyperphosphatemia syndrome (MIM 610233) is a clinical variant of familial tumoral calcinosis and is also due to mutations in either *FGF23* or *GALNT3*; symptoms and signs may precede development of the more typical phenotype of familial tumoral calcinosis.[499,500] Hyperphosphatemic familial tumoral calcinosis has also been attributed to a homozygous loss-of-function mutation (His193Arg) in *KL* encoding α-klotho, a cofactor necessary for the interaction of FGF23 with its receptor in the renal tubule.[502] Normophosphatemic familial tumor calcinosis is a form of dystrophic calcification, as inflammatory lesions always precede ectopic calcification, that is due to inactivating mutations (Arg344Stop, Lys1495Glu) in *SAMD9* (encoding sterile alpha motif domain-containing protein 9), a 1589-amino-acid protein that regulates cell division, motility, and longevity.[503] SAMD9 generation is responsive to tumor necrosis factor-alpha (TNFα) and interferon-gamma (IFNγ) and regulates expression of EGR1 (MIM 128990), a transcription factor that controls expression of *TGFB1* and is involved with cell migration, inflammation, and tissue calcification.[504]

OSTEOCHONDRODYSPLASIAS

The osteochondrodysplasias are composed of a heterogeneous group of malformations of cartilage and bone that initially were grouped according to clinical and radiologic characteristics into those involving long bone growth alone (epiphyseal, metaphyseal, or diaphyseal dysplasias) or those involving the long bones and vertebrae (spondyloepiphyseal or spondyloepimetaphyseal dysplasias) and variants thereof (Figure 18-15).[505] More recently, disorders of skeletal morphology or bone cell function have been classified according to known genetic variations (e.g., *FGF23*, *COLA1A*) that adversely influence bone development or mineralization and within which mutations in one gene may give rise to several clinically defined disorders together with disorders characterized by clinical and radiographic findings that may be attributable to variations in many different genes (e.g., acromesomelic dysplasias). Thus, abnormal skeletal phenotypes have been classified into 40 groups based on the underlying genetic mutations or clinical and radiographic manifestations of the disorders.[322,506] Groups 1 through 8 are multiple disorders attributed to variations in a single gene (e.g., group 1 *FGFR3*, group 2 *COL1A1*, group 3 *COL11A1*, group 4 *DTDST*, group 5 *PLC*, group 6 *AGC1*, group 7 *FLNA*, group 8 *TRPV4*). Groups 9 through 40 rely on clinical and radiographic findings for classification; within each group, variations in different genes may give rise to the same observed abnormality(ies) (e.g., group 10 multiple epiphyseal dysplasias: *COMP*, *COL9A2*, *MATN3*; group



Involvement	Disease Category
A+D	Normal
B+D	Epiphyseal dysplasia
C+D	Metaphyseal dysplasia
B+E	Spondyloepiphyseal dysplasia
C+E	Spondylometaphyseal dysplasia
B+C+E	Spondyloepimetaphyseal dysplasia

FIGURE 18-15 ■ Anatomic characterization of osteochondrodysplasias. (From Alanay, Y., & Rimoin, D. L. (2008). Chondrodysplasias. In C. J. Rosen (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (7th ed.) (pp. 428-429). Washington, DC: American Society of Bone and Mineral Metabolism.)

13 spondylo-epi-[meta]-physeal dysplasias: *MATN3*, *SEDL*, *DYM*; group 17 mesomelic/rhizo-mesomelic dysplasias: *SHOX*, *GPC6*, *RDR2*). Group 23 encompasses variants of osteopetrosis, group 25 variants of osteogenesis imperfecta, group 27 the lysosomal disorders, and group 30 the overgrowth syndromes.[506] Disorders of skeletal development and function are of interest not only because of the diagnostic and therapeutic clinical challenges they present but also because they have identified many basic physiologic factors that normally regulate cartilage and bone development and function. The application of genome wide association studies (GWAS) to the classification of skeletal disorders has added significant insight into the plethora of genes and intracellular signaling pathways that influence skeletal differentiation, morphology, and function.[322]

Table 18-14 describes some of the more commonly encountered genetic variations associated with the osteochondrodystrophies. Achondroplasia (MIM 100800), the most common of the human chondrodystrophies (1/15,000 to 1/40,000 live births), and its related osteochondrodystrophies (hypochondroplasia, thanatophoric dwarfism, SADDAN syndrome) are due to gain-of-function mutations in *FGFR3*, the gene encoding fibroblast growth receptor 3. FGFR3 is a transmembrane protein with three immunoglobulin domains in the extracellular region of the receptor and two tyrosine kinase domains in its intracellular portion (Figure 18-16). The four disorders are transmitted as autosomal dominant traits but with a high rate of spontaneous mutations (primarily of the paternal *FGFR3* allele). Clinically, achondroplasia is manifested by short limbs but normal trunk length, large head, frontal bossing, and depressed nasal bridge, and complicated by increased risk for cervical cord compression and spinal stenosis.[507] Thanatophoric dysplasia is characterized by severe bony malformations, particularly of the skull, long bones, and ribs, the latter leading to respiratory insufficiency and early death; there are two radiographic forms of this disorder (I and II). The syndrome of severe achondroplasia-developmental delay-acanthosis nigricans (SADDAN syndrome) is a clinical phenotype intermediate in severity between those of thanatophoric dysplasia and achondroplasia. It is associated with significant rhizomelic short stature, acanthosis nigricans, and mild developmental challenges; acanthosis nigricans may also develop in patients with classical achondroplasia and in those with mutations in FGFR2.[508] Hypochondroplasia is a clinically less severe manifestation of mild short limbed (rhizomelic) short stature that presents in mid-childhood. Mutations in *FGFR3* correlate with the clinical phenotype. Although achondroplasia is most often (98%) associated with a monoallelic missense mutation (Gly380Arg) in the transmembrane domain of FGFR3, Gly375Cys and other variants in *FGFR3* have been recorded in patients with achondroplasia. The birth to normal parents of a second child with achondroplasia may reflect paternal germinal mosaicism for the Gly380Arg mutation in *FGFR3*.[509] Thanatophoric dysplasia type I is related to mutations (Arg248Cys, Gly370Cys) in the extracellular ligand-binding region next to the transmembrane domain of the receptor and to substitutions at the normally terminal codon 807—Ter807Gly, Ter807Cys, and Ter807Arg—all of which lead to the addition of 141 amino acids to the carboxyl terminus of the FGFR3 protein. Thanatophoric dysplasia type II is associated with a mutation (Lys650Glu) in the distal tyrosine kinase domain. Dual mutations in the same *FGFR3* allele have also been associated with thanatophoric dysplasia.[510] SADDAN syndrome is also the result of a Lys650Met mutation. Hypochondroplasia has been associated with mutations within the proximal (Asn540Lys in 60%) and distal (Lys650Gln) tyrosine kinase domains, respectively, as well in the immunoglobulin domains of the extracellular region (Ser84Leu, Arg200Cys) and transmembrane domain (Val381Glu) of *FGFR3*.[507,511] It is noteworthy that different mutations at Lys650 result in three distinct phenotypes: hypochondroplasia, thanatophoric dysplasia type II, and SADDAN syndrome. *FGFR3* activating mutations have also been encountered in patients with Muenke nonsyndromic coronal craniosynostosis and Crouzon syndrome—evidence of the genetic heterogeneity of these clinical syndromes. A loss-of-function mutation (Arg621His within the distal tyrosine kinase domain) in *FGFR3* has been identified in a family whose members display the phenotype of tall stature, camptodactyly, and hearing loss.[512]

The Gly380Arg variant of *FGFR3* alters the conformation of the gene product enabling ligand-independent autophosphorylation of tyrosine residues 647 and 648 within its cytoplasmic tyrosine domain that then is able to propagate signals through the MAPK and signal the transducer and activator of transcription (STAT) pathways resulting in inhibition of mitosis, matrix synthesis, and terminal (hypertrophic) differentiation.[513] Experimentally, in

TABLE 18-14 **Genetic Variants Associated with Osteochondrodystrophies (Selected)**

Gene Chromosome MIM	Pathophysiology	Clinical Disorder (MIM)
TRIP11: Thyroid hormone interactor 11 14q31-q32 604505	Coactivator of the nuclear triiodothyronine receptor; interacts with microtubules and Golgi apparatus	Achondrogenesis, type IA (200600), AR
Collagenopathies		
COL2A1: Collagen, type II, alpha 1 12q13.11 120140	Subunit of collagen type II, the major collagen of cartilage comprised of three 3-alpha 1(II) chains	Achondrogenesis, type II/ Hypochondrogenesis (200610), AD Spondyloepiphyseal dysplasia congenita (183900), AD Spondyloepimetaphyseal dysplasia (184250), AD
COL9A1: Collagen, type IX, alpha 1 6q13 120210	Encodes a collagen that is a component of hyaline cartilage	Multiple epiphyseal dysplasia, type 6 (614135), AD Stickler syndrome type 4 (614134), AR
COL10A1: Collagen, type X, alpha 1 6p22.1 120110	Component of cartilage expressed at late stages of endochondral bone formation	Metaphyseal chondrodysplasia, Schmid type (156500), AD
COL11A1: Collagen, type XI, alpha 1 1p21.1 120280	Subunit of collage type XI composed of two alpha 1 subunits and a modified COL2A1 subunit; important for fibrillogenesis	Stickler syndrome type 2 (604841), AD Marshall syndrome (154780), AD
FLNB: Filamin B 3p14.3 603381	Cytoplasmic actin-binding protein enabling formation of and communication with the cytoskeleton; influences vertebral segmentation, endochondral ossification, joint formation	Atelosteogenesis I (108720), AD Atelosteogenesis III (108721), AD
Fibroblast Growth Factor Receptors		
FGFR1: Fibroblast growth factor receptor 1 8p11.22 136350	Transmembrane tyrosine kinase receptor for FGFs	Pfeiffer (101600), AD
FGFR2: Fibroblast growth factor receptor 2 10q26.13 176943	Transmembrane tyrosine kinase receptor for FGFs	Apert (101200), AD Crouzon (123500), AD Jackson-Weiss (123150), AD Antley-Bixler with normal steroidogenesis (207410), AD
FGFR3: Fibroblast growth factor receptor 3 4p16.3 134934	Transmembrane tyrosine kinase receptor for FGFs	Achondroplasia (100800), AD Hypochondroplasia (146100), AD Thanatophoric dysplasia types I (187600) and II (187601), AD
Sulfation Disorders		
SLC26A2- Solute carrier family 26 (sulfate transporter) 5q32 606718	Also termed DTDST; encodes sulfate transporter essential for normal collagen synthesis	Achondrogenesis, type IB (600972), AR; Atelosteogenesis II (256050), AR Diastrophic dysplasia (222600), AR Multiple epiphyseal dysplasia, type 4 (226900), AR
HSPG2: Heparan sulfate proteoglycan of basement membrane 1p36.12 142461	Also termed perlecan, a heparan sulfate proteoglycan; coreceptor for FGFR2; stabilizes basement membranes and regulates their permeability	Schwartz-Jampel type 1 myotonic chondrodystrophy (255800), AR
PAPSS2: 3-Prime-phosphoadenosine 5-prime-phosphosulfate synthase 2 10q23.2-23.3 603005	Enzyme that synthesizes the sulfate donor (3′-phosphoadenosine 5′-phosphosulfate) from ATP and sulfate; cofactor for adrenocortical sulfotransferase	Spondyloepimetaphyseal dysplasia (Brachyolmia type 4) (612847), AR
ARSE: Aryl sulfatase E Xp22.33 300180	Enzyme that removes a sulfate group from its protein	Chondrodysplasia punctata type 1 (302950), X-linked recessive
PTPN11: Protein tyrosine phosphatase, nonreceptor, type 11 12q24.1 176876	Encodes SHP2: a nonreceptor protein tyrosine phosphatase that acts upstream of RAS	Monoallelic loss-of-function mutations lead to metachondromatosis (156250), AD Noonan syndrome with multiple lentigenes: monoallelic gain-of-function mutations result in Noonan syndrome (163950), AD

TABLE 18-14 **Genetic Variants Associated with Osteochondrodystrophies (Selected)—cont'd**

Gene Chromosome MIM	Pathophysiology	Clinical Disorder (MIM)
Others		
COMP: Cartilage oligomeric matrix protein 19p13.1 600310	Chondrocyte protein that binds calcium and collagen types I, II, and IX	Pseudoachondroplasia (177170), AD Multiple epiphyseal dysplasia (132400), AD
TRPV4: Transient receptor potential cation channel, subfamily V, member 4 12q24.11 605427	Cation channel that mediates calcium influx	Metatropic dysplasia (156530), AD Spondyloepimetaphyseal dysplasia: Maroteaux (184095), AD
SHOX: Short stature homeobox Xp22.33 312865	Homeobox gene transcription factor located also on the pseudoautosomal region of Yp	Leri-Weill dyschondrosteosis (127300), X-linked dominant Langer dysplasia (249700), biallelic/X and Y
SOX9: SRY-box 9 17q24.3 608160	Transcription factor essential for chondrogenesis and testicular differentiation	Campomelic dysplasia (114290), Monoallelic due to haploinsufficiency
RUNX2: Runt-related transcription factor 2 6p21 600211	Osteoblast specific transcription factor	Cleidocranial dysostosis (119600), AD
PTH1R: PTH receptor 1 Xp22.33 168468	GPCR recognizing PTH and PTHrP with equal affinity	Inactivating mutations lead to hypocalcemia and Blomstrand osteochondrodysplasia (215045), AR; Eiken syndrome (600002), AR; activating mutations result in hypercalcemia and Murk-Jansen metaphyseal chondrodysplasia (156400), AD
PRKAR1A: Protein kinase, cAMP-dependent regulatory, type 1, alpha 17q24.2 188830	Component of PKA response to cyclic AMP that leads to cascade of intracellular signal transduction signals in response to Gαs that regulate cell division, differentiation, metabolism, apoptosis	Gain-of-function mutation leads to acrodysostosis (101800) and peripheral resistance to the biologic effects of PTHrP, PTH, and TSH; (de novo)
POR: Cytochrome P450 oxidoreductase 7q11.2 124015	Flavoprotein cofactor that donates electrons to microsomal 17α-hydroxylase, 21-hydroxylase, and aromatase	Inactivating mutations lead to congenital adrenal hyperplasia usually with genital ambiguity in both 46XX and 46XY subjects (613571), AR; at times in association with skeletal abnormalities (Antley-Bixler syndrome: craniosynostosis, midface hypoplasia, choanal stenosis, femoral bowing, radioulnar synostosis, 201750, AR)
RMRP: Mitochondrial RNA-processing endoribonuclease, RNA component of 9p13.3 157660	Untranslated RNA subunit of the mitochondrial RNA-processing endoribonuclease: RNase MRP	Dependent on the severity of the biallelic loss-of-function mutation(s) the spectrum of anauxetic dysplasia (607095), cartilage hair hypoplasia (250250) emerges
INPPL1: Inositol polyphosphate phosphatase like-1 11q13.4 600829	Enzyme that hydrolyzes inositol-1,4,5-trisphosphate to inositol-4,5-bisphosphate: transducer of intracellular signaling	Inactivating mutations result in opsismodysplasia (258480); associated with increased skeletal synthesis of FGF23, AR

Adapted from Warman, M. L., Cormier-Daire, V., Hall, C., et al. (2011). Nosology and classification of genetic skeletal disorders: 2010 revision. Am J Med Genet *(Part A),* 155A, *943–968.*

mice expressing the gain-of-function mutation in *Fgfr3* associated with achondroplasia, there is enhanced bone development but decreased bone mass and increased osteoclastic activity.[514] Thus, FGFR3 normally functions as a negative regulator of cartilage and bone formation. As a single gene disorder, it is possible to identify a mutation in *FGFR3* in an affected fetus by analysis of fetal cell-free DNA in maternal plasma, if the mother is unaffected.[515] Activating mutations in *FGFR1* and *FGFR2* have been associated with chondrodysplasias complicated by premature craniosynostosis (Pfeiffer, Apert, Crouzon, Jackson-White, Antley-Bixler, and Beare-Stevenson cutis gyrata syndrome). Interestingly, loss-of-function mutation in *FGFR1* have also been associated with hypogonadotropic hypogonadism (MIM 147950), as FGFR1 is an essential neuronal migration factor.[516] Mutations in *FGFR4* have not been associated with osteochondrodysplasias to date.

Defective formation of several types of collagen due to mutations in *COL2A1*, *COL9A1*, *COL9A2*, *COL10A1*, *COL11A1*, and *COL11A2* result in a large number of skeletal malformations depending on the site and developmental timing of the synthetic error (see Table 18-14). Mutations in several different collagen-encoding genes (*COL2A1*, *COL9A1*, *COL9A2*, *COL11A1*, *COL11A2*)

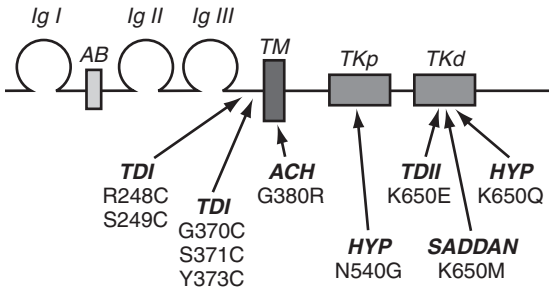


FIGURE 18-16 ■ Mutations in fibroblast growth factor receptor-3 leading to achondroplasia (ACH), hypochondroplasia (HYP), thanatophoric dysplasia (TD) types I and II, and severe achondroplasia-developmental delay-acanthosis nigricans (SADDAN). (From Horton, W. A. (2006). Molecular pathogenesis of achondroplasia. *Growth Genet Horm, 22,* 49–54.)

result in variants of Stickler syndrome characterized by abnormalities of the skeleton (epiphyseal dysplasia), face (micrognathia, cleft palate), and vision (myopia, retinal detachment), as well as impaired hearing that may be transmitted as autosomal dominant or recessive traits. Atelosteogenesis is a skeletal disorder characterized by absent, short, or distally tapered femora and humeri, short bowed tibiae and ulnae, absent fibulae, vertebral hypoplasia, and subnormal metacarpal ossification; this disorder has several variants; it is the result of monoallelic loss-of-function mutations in *FLNB* encoding a cytoplasmic protein that binds actin, thereby enabling actin to form the cytoskeleton, and it also facilitates intracellular communication between the cell membrane and its cytoskeleton. Abnormalities of sulfate transport, collagen matrix protein sulfation, and sulfatase activity result in several chondrodysplasias. *SLC26A2* encodes a sulfate transporter that is mutated in patients with diastrophic dysplasia (DTD) and achondrogenesis type IB. Spondylepimetaphyseal dysplasia/brachyolmia type 4 is due to biallelic loss-of-function mutations (Thr48Arg, Ser438Ter) in the gene (*PAPSS2*) encoding 3′-phosphoadenosine-5′-phosphosulfate synthase 2, an enzyme with dual activities; it catalyzes both the synthesis of adenosine 5′-phosphosulfate and its phosphorylation to 3′-phosphoadenosine 5′-phosphosulfate, the universal sulfate donor necessary for sulfation of cartilage and bone matrix proteins. Clinical manifestations of this disorder include short limbs, kyphoscoliosis, brachydactyly, and enlarged knee joints. Inactivating mutations in *ARSE*, encoding a member of the sulfatase enzyme family, lead to X-linked recessive brachytelephalangic chondrodysplasia punctata type 1.[517] This disorder is marked clinically by compromised stature attributable to rhizomelic shortening of the limbs, stippling of the epiphyses, craniofacial defects, atrophic and pigmented ichthyosiform skin lesions, alopecia, cataracts, and developmental delay. Loss-of-function mutations (insertions, missense, nonsense) in *SOX9*—a transcription factor that is expressed in both developing chondrocytes where it is coexpressed with *COL2A1* and in the genital ridges during gonadal differentiation—cause both campomelic dysplasia (MIM 114290) and male-to-female sex reversal in 75% of affected 46XY subjects. Inasmuch as SOX9 is essential for normal expression and function of *COL2A1* during chondrogenesis, its absence leads to impaired cartilage formation and malformation of bones embryologically derived from endochondral bone. In the developing bipotential gonad of the 46XY fetus, the expression of *SOX9* is governed by SRY; *SOX9* expression is essential for normal differentiation and development of Sertoli cells and other components of the testis. Clinically, campomelic dysplasia is characterized by prenatal onset of bowing of tubular bones, hypoplastic scapulae, 11 ribs, cleft palate, and micrognathia leading often to neonatal death as well as failure of masculinization of the 46XY conceptus.

The X- and Y-linked pseudoautosomal gene *SHOX* (*S*hort stature *HO*meobo*X*-containing gene) encodes two nuclear proteins: SHOXa with 292 amino acids and SHOXb with 225 amino acids. SHOX is a transcription factor expressed in late proliferative, prehypertrophic, and hypertrophic chondrocytes one of whose target genes is *NPPB* encoding natriuretic peptide precursor B.[518] Absence of one *SHOX* functional gene is the proximate cause of the short stature of girls with Turner syndrome and some children with "idiopathic" short stature. Heterozygous microdeletions and intragenic inactivating mutations (Leu 132Val, Ala170Pro, Arg195Stop) of *SHOX* resulting in haploinsufficiency are present in patients with Leri-Weill dyschondrosteosis typified by mesomelic limb shortening and growth retardation, Madelung deformity of the wrist, bowing of the radius, and ulnar dislocation. The Leri-Weill phenotype has been recorded in patients with intact *SHOX* but with microdeletions of downstream segments of the X chromosome pseudoautosomal region, implying the presence of modifying genes in this region.[519] Biallelic loss (X and Y chromosomes) of *SHOX* leads to Langer mesomelic dysplasia typified by severe hypoplasia of the ulna and fibula and a thickened, curved radius and tibia.

Both gain- and loss-of-function mutations of *PTH1R* lead to abnormalities of bone formation and growth. Blomstrand chondrodysplasia is an autosomal recessive disorder lethal in utero that may be identified in the fetus with short, extremely dense long bones and markedly advanced skeletal maturation as well as somatic anomalies such as aortic coarctation and facial anomalies. Pathologically, epiphyseal cartilage is reduced, and there are irregular columns and erratic distribution of chondrocytes within matrix. The abnormality is the result of inactivating mutations (deletions, missense mutations—Pro132Leu, Arg383Gln) of the gene encoding the PTH/PTHrP receptor. The Eiken syndrome of severely delayed ossification of the epiphyses and pelvis is also due to a biallelic loss-of-function mutation (Arg485Stop) in *PTH1R*. Patients with Murk-Jansen metaphyseal chondrodysplasia have short limbs and fingers, micrognathia, and deformities of the spine and pelvis, but they survive to adulthood where the average adult height is 125 cm and childbearing is possible. Characteristically, these patients have hypercalcemia, hypophosphatemia, and low or undetectable serum levels of both PTH or PTHrP. The disorder is due to monoallelic activating mutations (His223Arg, Thr410Pro) of *PTH1R* and is associated with extraordinary delay in chondrocyte differentiation and decreased mineralization due to excessive bone resorption. Both disorders reflect the altered functional effects of PTHrP acting through PTH1R in developing cartilage where it normally acts to slow differentiation and decrease

the rate of chondrocyte apoptosis, thus prolonging chondrocyte proliferation and enhancing long bone growth.

Anauxetic dysplasia (MIM 607095) is a spondylometaepiphyseal dysplasia transmitted as an autosomal recessive disorder characterized by intrauterine growth retardation (birth length < 40 cm), severely compromised adult height (< 85 cm), hypodontia, and mild mental retardation. All bones are malformed; there are few chondrocytes in the cartilage growth plates. It is due to loss-of-function mutations (insertions) in *RMRP* (*R*NA component of *M*itochondrial *R*NA-*P*rocessing endoribonuclease), a gene that encodes the untranslated RNA subunit of the mitochondrial RNA-processing endoribonuclease, RNase MRP.[520,521] This enzyme is involved in (1) the assembly of ribosomes (the structural units in which translation and protein synthesis take place), (2) the generation of RNA primers for replication of mitochondrial DNA, and (3) the regulation of the cyclin-dependent cell cycle. The mutations that result in anauxetic dysplasia impair ribosome assembly and protein synthesis exclusively. Inactivating mutations (duplications, insertions) in *RMRP* that modestly impair both ribosomal assembly and regulation of the cell cycle are present in patients with cartilage hair hypoplasia (MIM 250250) and metaphyseal dysplasia without hypotrichosis (MIM 250460). Thus, cartilage hair hypoplasia and anauxetic dysplasia represent different manifestations and the functional severity of inactivating mutations of *RMRP*.

Errors in the biosynthesis of cholesterol have been associated with a number of disorders that adversely affect bone development as well as many other systems (Table 18-15) (Figure 18-17).[522] Cholesterol is a constituent of the cell's plasma membrane as well as the membranes of intracellular organelles; it covalently binds to the amino terminal and is essential for the function of Indian hedgehog, Sonic hedgehog, and Desert hedgehog, inductive factors necessary for the normal development of cartilage and bone, brain, and testes, respectively. It is a precursor of steroids and bile acids. It is likely that lack of cholesterol exerts its teratologic effects through many pathways—by impairing membrane function or the intracellular signaling response(s) to normal stimuli. Additionally, accumulation of precursors of cholesterol may be toxic to the developing fetus. In patients with the Smith-Lemli-Opitz syndrome, inactivating mutations of the microsomal enzyme Δ^7-dehydrocholesterol reductase (*DHCR7*) impair the final step in the synthetic pathway of cholesterol from 7-dehydrocholesterol and lead to intrauterine and postnatal growth retardation, short limbs, syndactyly and polydactyly, characteristic facial features (blepharoptosis, anteverted nares, broad alveolar ridges, cleft palate), congenital malformations of the heart and central nervous system, microcephaly, incomplete virilization of male external genitalia, hypoplastic thumbs, developmental delay, autism, and compromised adrenocortical function, a malformation syndrome with an incidence of 1/15,000 to 1/40,000 births.[523]

TABLE 18-15 **Osteochondrodystrophies Attributable to Genetic Variants of Proteins Essential for Cholesterol Synthesis**

Gene Chromosome MIM	Pathophysiology	Clinical Disorder (MIM)
DHCR7: 7-Dehydrocholesterol reductase 11q12-q13 602858	3β-Hydroxysteroid Δ^7 reductase converts 7-dehydrocholesterol to cholesterol	Smith-Lemli-Opitz syndrome (270400), AR
DHCR24: 24-Dehydrocholesterol reductase 1p32.3 606418	3β-Hydroxysterol Δ^{24} reductase converts desmosterol to cholesterol	Desmosterolosis (602398), AR
SC5DL: Sterol C5-desaturase-like 11q23.3 602286	Microsomal enzyme (3β-hydroxysteroid-Δ5-desaturase) that converts lathosterol to 7-dehydrocholesterol, precursor of cholesterol and cholecalciferol (vitamin D)	Lathosterolosis (607330), AR
EBP: Emopamil-binding protein Xp11.23 300205	Dual function protein: binding protein; 3β-Hydroxysteroid Δ^7,Δ^8 isomerase converts cholest-8(9)-en-3β-ol to lathosterol	Chondrodysplasia punctata type 2 (302960), X-linked dominant
NSDHL: NAD(P)H steroid dehydrogenase-like protein Xq28 300275	3β-Hydroxysteroid C4 demethylase complex converts 4,4-dimethylcholest-8(9)-en-3β-ol to cholest 8(9)-en-3β-ol; other members of complex are *SC4MOL* (607545) and *HSD17B7* (606756)	CHILD (congenital hemidysplasia, ichthyosiform erythroderma, limbs defects) syndrome (308050), X-linked dominant
LBR: Lamin B receptor 1q42.12 600024	Dual function protein: promotes heterochromatin binding to inner nuclear membrane; 3β-Hydroxysteroid Δ^{14} reductase important for synthesis of cholesterol	HEM (hydrops-ectopic calcification-"moth-eaten" or Greenberg) dysplasia (215140), AR
POR: Cytochrome P450 oxidoreductase 1q11.2 124015	Flavoprotein electron donor to all P450 enzymes including P450c17, P450c21, P450arom	Antley-Bixler syndrome with genital anomalies and disordered steroidogenesis, (201750), AR (ABS with exclusively skeletal anomalies [207410] due to mutation in *FGFR2*, AD)

Adapted from Forbes, F. D., & Herman, G. E. (2011). Malformation syndromes caused by disorders of cholesterol synthesis. J Lipid Res, 52, *6–34.*

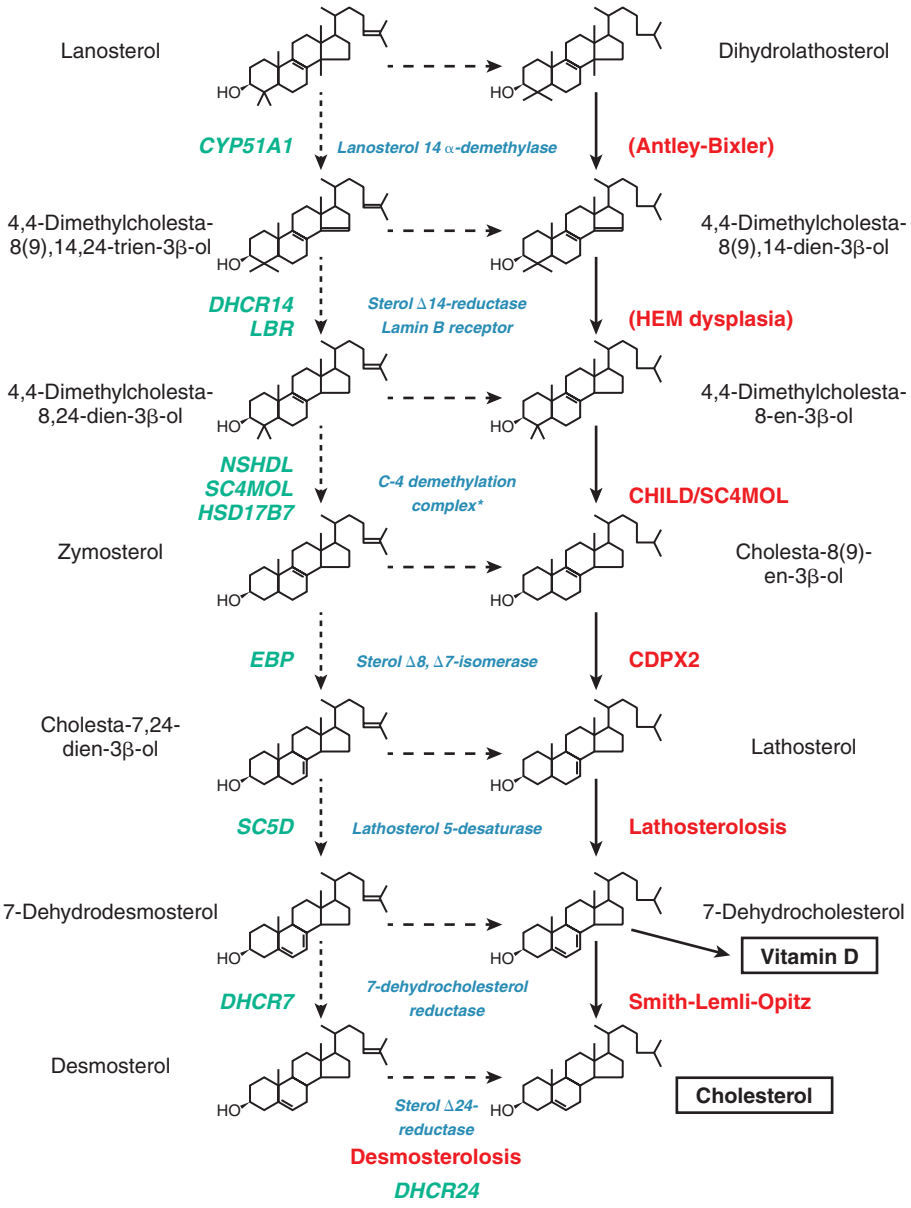


FIGURE 18-17 ■ Biosynthesis of cholesterol depicting the sites of enzyme activity lost by inactivating mutations that lead to skeletal, genital, and systemic malformations. The conversion of lanosterol to cholesterol may proceed through two pathways as depicted (see text for details). (From Forbes, F. D., & Herman, G. E. (2011). Malformation syndromes caused by disorders of cholesterol synthesis. *J Lipid Res, 52*, 6–34.) *This image can be viewed in full color online at ExpertConsult.*

Stippled epiphyses are detected by radiologic examination. Serum concentrations of cholesterol are depressed, whereas those of 7-dehydrocholesterol are elevated. The 7-dehydrocholesterol/cholesterol ratio is related to the clinical severity of the Smith-Lemli-Opitz phenotype under usual circumstances.[524] Anecdotal reports suggest that cholesterol supplementation may improve the health, behavior, and growth of children with the Smith-Lemli-Opitz syndrome.

Desmosterolosis is an autosomal recessive disorder with diverse clinical manifestations that include impaired growth, osteosclerosis, shortened limbs, macro- or microcephaly, cleft palate, micrognathia, thick alveolar ridges, developmental delay, and spasticity. Cranial magnetic resonance imaging reveals little white matter and a thinned to almost complete agenesis of the corpus callosum. Desmosterolosis is due to biallelic inactivating mutations of the gene (*DHCR24*) encoding 3β-hydroxysterol-Δ^{24} reductase, an enzyme that converts desmosterol to cholesterol.[522,525,526] Lathosterolosis is characterized by microcephaly, bitemporal narrowing of the skull, cataracts, anteverted nostrils, and other physical anomalies recorded in patients with the Smith-Lemi-Opitz syndrome.[522] It is the result of loss-of-function mutations in the gene (*SC5DL*) encoding 3β-hydroxysteroid-Δ5-desaturase, an enzyme that converts lathosterol to 7-dehydrocholesterol. Chondrodysplasia punctata 2 is an X-linked dominant disorder (Conradi-Hünermann-Happle syndrome) that is ordinarily (but not always) lethal in the affected male. It is characterized clinically

in the affected female by short stature with asymmetric short proximal limbs (rhizomelic dwarfism), frontal bossing, scaly and erythematous skin lesions that resemble ichthyosis in children and atrophic pigmentary lesions in adults, coarse hair with alopecia, and cataracts; reflecting functional X-chromosomal mosaicism in females, the phenotype may vary from stillborn to mildly affected.[522] Radiographic examination reveals generalized osteosclerosis, irregular punctate calcification (stippling) of epiphyses of the long bones, vertebrae, and tracheal cartilage in children. This disorder is due to loss-of-function mutations in *EBP* (*E*mopamil-*B*inding *P*rotein), the gene encoding 3β-hydroxysteroid-Δ^8,Δ^7isomerase, an enzyme that converts cholesta-8(9)-en-3β-ol to lathosterol; in the serum of subjects with chondrodysplasia punctata 2 plasma concentrations of 8-dehydrocholesterol cholest-8(9)-en-3β-ol are elevated. This protein also binds many unrelated molecules; its genetic designation derives from its ability to bind emopamil (i.e., EBP), a calcium ion antagonist. X-linked recessive and autosomal recessive forms of the Conradi-Hünermann-Happle syndrome have also been reported, suggesting genetic heterogeneity for this phenotype. The CHILD syndrome of congenital hemidysplasia with ichthyosiform erythroderma or nevus and limb defects is remarkable for its unilateral distribution of anomalies that are confined to half of the body.[522] It is an X-linked disorder due to loss-of-function mutations in *NSDHL* (*N*ADPH *s*teroid *deh*ydrogenase-*l*ike) encoding a sterol dehydrogenase or decarboxylase that is part of the 3β-hydroxysteroid C-4 sterol demethylase complex that also includes the products of *SC4MOL* and *HSD17B7*. This complex converts 4,4-dimethylcholesta-8-en-3β.ol to cholesta-8(9)-en-3β-ol.

The often-lethal Greenberg dysplasia of hydrops-ectopic calcification-moth-eaten (HEM) skeletal dysplasia is transmitted as an autosomal recessive trait associated with short-limbed dwarfism, polydactyly, and irregularly decreased calcification of the long bones together with calcification of the larynx and trachea. It is due to a loss-of-function mutation in the gene (*LBR*) encoding the lamin B receptor, a nuclear envelope inner membrane protein that not only binds lamin B but also has 3β-hydroxysteroid-Δ^{14} reductase activity, an enzyme that converts 4,4-dimethylcholesta-8(9)-dien-3β-ol to 4,4-dimethylcholesta-8-en-3β-ol, a necessary step for normal cholesterol biosynthesis. 3β-hydroxysteroid-Δ^{14} reductase activity is also encoded by *DHCR14*, an endoplasmic reticular enzyme that too catalyzes reduction of unsaturated C14-C15 intermediate sterols.[522,527] The HEM dysplasia is considered to be a laminopathy rather than an inborn error in cholesterol biosynthesis. The Antley-Bixler syndrome (MIM 207410) of craniosynostosis, humeroradial synostosis, femoral bowing midface hypoplasia, choanal stenosis/atresia, and joint contractures has been associated with heterozygous mutations in *FGFR2* (MIM 176943). When these skeletal anomalies coexist with genital malformations and defects in steroidogenesis (MIM 201750), biallelic loss-of-function mutations have been identified in *POR* (MIM 124015), encoding P450 oxidoreductase, a donor of electrons for the P450 enzymes required for synthesis of adrenocortical and gonadal steroids. Thus, when POR activity is decreased, function of the enzyme encoded by *CYP51A1* MIM601637)) that converts dihydrolathosterol to 4, 4-dimethylcholesta-8(9).14-dien-3β-ol is depressed.

CONCLUDING REMARKS

Deciphering of the complex mechanisms that underlie the pathophysiology of illnesses that adversely affect the regulation of calcium, phosphate, and magnesium metabolism; chondrocyte differentiation and growth, and bone formation, mineralization, and strength has been further informed by the insights that advances in genetics, epigenetics, and proteomics have brought to these clinical problems. It may be anticipated that further clarification of the basic mechanisms that underlie these disorders will enable translation of these findings into the care of many disorders that affect young patients.

ADDENDUM

Loss-of-function mutations in *GNA11* encoding guanine nucleotide-binding protein alpha 11 (chromosome 19p13.3, MIM 139313) have been identified in patients with autosomal dominant familial hypocalciuric hypercalcemia type 2 (MIM 145981).[534] Gain-of-function mutations in GNA11 have been found in patients with autosomal dominant hypoparathyroidism.[534,535]

Inactivating mutations in *AP2S1* encoding adaptor protein-2α subunit (chromosome 19q13, MIM 602242) involving solely the Arg15 residue of AP2S1 have been identified in patients with autosomal dominant familial hypocalciuric hypercalcemia type 3 (MIM 600740) clinically associated with hypophosphatemia, elevated serum levels of PTH, and decreased bone mineralization.[536] AP2S1 is one subunit of a heterotetrameric protein critical for clathrin-related endocytosis of G protein-coupled receptors. Inactivating variants of AP2S1 decrease sensitivity of CASR to Ca^{2+}_0, reduce its rate of endocytosis, and decrease intracellular signal transduction.

Kenny-Cafey syndrome 2 (MIM 127000) has been attributed to monoallelic loss-of-function mutations in *FAM111A* (MIM 615292, chromosome 11q12.1), a protein with sequences common to trypsin like proteinases. [537,538]

Osteogenesis imperfecta type XV (MIM 615220) has been assigned to mutations in *WNT1*[539,540]. *WNT1* encodes the ligand of the osteoblast membrane receptor Frizzled; binding of *WNT1* to Frizzled and its co-receptors LRP 5/6 regulates intracellular signaling through the β-Catenin, $G_{\alpha q}$-protein, and PLC signal transduction pathways resulting in both osteoblastogenesis and osteoblast function. Biallelic loss-of-function in *WNT1* have been identified in several families whose members have had fractures at a young age, bone deformities, decreased bone mineralization, growth retardation, and occasionally blue sclerae. Dental development and hearing have been normal in these patients but some affected subjects have had developmental delay and brain malformations.[541]

Abbreviations

ADHR	Autosomal dominant hypophosphatemic rickets
AHO	Albright hereditary osteodystrophy
AMP	Adenosine monophosphate
APECED	Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy
APS	Autoimmune polyendocrine syndrome
ARHR	Autosomal recessive hypophosphatemic rickets
ASARM	Acidic serine aspartate-rich MEPE-associated motif
ATP	Adenosine triphosphate
BCE	Bovine cartilage equivalent
BMC	Bone mineral content
BMD	Bone mineral density
BMP	Bone morphogenetic protein
Ca^{2+}_e	Calcium-ionized, extracellular
CaSR	Calcium sensing receptor
CBP	Collagen binding protein (HSP)
CKD-MBD	Chronic kidney disease-mineral and bone disorder
COL1A1	Bone collagen subunit α1 (I)
CRTAP	Cartilage-associated protein
CTx	Carboxyl terminal cross-link telopeptide of collagen type I
DEXA	Dual-energy x-ray absorptiometry
DGCR	DiGeorge syndrome critical region
DGS	DiGeorge syndrome
DMR	Differentially methylated region
Dpd	Deoxypyridinoline
FGF	Fibroblast growth factor
FGFR	FGF receptor
FIHP	Familial isolated primary hyperparathyroidism
FISH	Fluorescent in situ hybridization
FOP	Fibrodysplasia ossificans progressiva
GDNF	Glial-derived neurotrophic factor
GDP	Guanosine diphosphate
GH	Growth hormone
GLP-2	Glucagon-like peptide 2
GPCR	G-protein coupled receptor
$G_s\alpha$	α Subunit of stimulatory G-protein (Gsα)
GTP	Guanosine triphosphate
H^+	Hydrogen ion
HDR	Hypoparathyroidism, deafness, renal disease syndrome (Barakat disease)
HHC	Hereditary hypocalciuric hypercalcemia
HRD	Hypoparathyroidism, retardation, dysmorphism syndrome
HSP	Heat shock protein (CBP)
ICTP	Carboxyl telopeptide of collagen type I
IFITM5	Interferon-induced transmembrane protein-5 or bone-restricted ITIFM5-like protein
IGF	Insulin-like growth factor
IL	Interleukin
IOF	International Osteoporosis Foundation
ISCD	International Society for Clinical Densitometry
KCS	Kenny-Caffey syndrome
LBW	Low birth weight
MAPK	Mitogen-activated protein kinase
M-CSF	Macrophage-colony stimulating factor
MCT	Medullary carcinoma of thyroid
MEN	Multiple endocrine neoplasia
MEPE	Matrix extracellular phosphoglycoprotein
Mg^{2+}	Magnesium
MIM	Mendelian Inheritance in Man*
MPA	Medroxyprogesterone acetate
NFκB	Nuclear factor κB
NHERF1	Sodium hydrogen exchanger regulatory factor 1
NSHPT	Neonatal severe hyperparathyroidism
NTx	Amino terminal telopeptide of collagen type I
OIC	Osteogenesis imperfecta congenita
OSTM1	Osteopetrosis-associated transmembrane protein-1
P3H1	Prolyl 3-hydroxylase 1
PDDR	Pseudovitamin D–deficiency rickets
PEDF	Pigment epithelium derived factor
PHEX	Phosphate-regulating endopeptidase homologue, X-linked
PHP	Pseudohypoparathyroidism
PICP	Carboxyl terminal propeptide of collagen type I
PIIINP	Amino terminal propeptide of collagen type III
POH	Progressive osseous heteroplasia
PPHP	Pseudopseudohypoparathyroidism
PTG	Parathyroid gland
PTH	Parathyroid hormone
PTHrP	PTH related protein
PTHR1	PTH/PTHrP receptor 1
Pyr	Pyridinoline
QCT	Quantitative computed tomography
QUS	Quantitative ultrasound
RANK	Receptor activator of nuclear factor κB
RANKL	RANK-ligand
SDS	Standard deviation score
SERM	Selective estrogen receptor modulator
sFRP4	Serum frizzled related protein-4
SIBLING	Short integrin-binding ligand-interacting glycoprotein
SOS	Speed of sound
TALH	Thick ascending limb of the loop of Henle
TGFβ	Transforming growth factor β
TNF	Tumor necrosis factor
TNSALP	Tissue nonspecific alkaline phosphatase
TRAP	Tartrate resistant acid phosphatase
TSH	Thyroid-stimulating hormone
VDDR1A	Vitamin D–dependent rickets type 1A
VDR	Vitamin D receptor
VDRE	Vitamin D response element
VEGF	Vascular endothelial growth factor
VLBW	Very low birth weight
WBS	Williams-Beuren syndrome
WINAC	WSTF including nucleotide assembly complex
WHO	World Health Organization
WSTF	Williams syndrome transcription factor
XLHR	X-linked dominant hypophosphatemic rickets
XLRH	X-linked recessive hypophosphatemic rickets
$1,25(OH)_2D_3$	1,25-Dihydroxyvitamin D_3 (calcitriol)
$25OHD_3$	25-Hydroxyvitamin D_3 (calcidiol)

*www3.ncbi.nlm.nih.gov/Omim.
A, adenine; C, cytidine; G, guanine; T, thymine.

REFERENCES

1. Abrams, S. A. (2012). Neonatal hypocalcemia. *UpToDate*. Retrieved from http://www.uptodate.com/contents/neonatal-hypocalcemia
2. Carpenter, T. O. (2008). Disorders of mineral metabolism in childhood. In C. J. Rosen (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (7th ed.) (pp. 349–353). Washington, DC: American Society of Bone and Mineral Metabolism.
3. Carpenter, T. O. (2006). Neonatal hypocalcemia. In M. J. Favus (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (6th ed.) (pp. 224–224). Washington, DC: American Society for Bone and Mineral Research.
4. Allgrove, J. (2001). Parathyroid disorders. *Curr Paediatr*, *11*, 357–363.
5. Robertson, W. C. (2002). Calcium carbonate consumption during pregnancy: an unusual cause of neonatal hypocalcemia . *J Child Neurol*, *17*, 853–855.
6. Kurt, A., Sen, Y., Elkiran, O., et al. (2006). Malignant infantile osteopetrosis: a rare cause of neonatal hypocalcemia. *J Pediatr Endocrinol Metab*, *19*, 1459–1462.
7. Engiz, O., Kara, S., Bagrut, D., et al. (2012). Infantile malignant osteopetrosis: a rare cause of neonatal hypocalcemia. *J Pediatr Endocrinol Metab*, *25*, 1205–1207
8. Whyte, M. P. (2008). Sclerosing bone disorders. In C. J. Rosen (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (7th ed.) (pp. 412-423). Washington, DC: American Society of Bone and Mineral Metabolism.
9. Holick, M. F. (2006). Resurrection of vitamin D deficiency and rickets. *J Clin Invest*, *116*, 2062–2072.
10. Holick, M. F. (2007). Vitamin D deficiency. *N Engl J Med*, *457*, 266–281.
11. Hollis, B. W., & Wagner, C. L. (2004). Vitamin D requirements during lactation: high dose maternal supplementation as therapy to prevent hypovitaminosis D for the mother and nursing infant. *Am J Clin Nutr*, *80*, 1752S–1758S.
12. Hollis, B. W., & Wagner, C. L. (2006). Nutritional vitamin D status during pregnancy: reasons for concern. *CMAJ*, *174*, 1287–1290.
13. Baumber, L., Tufarelli, C., Patel, S., et al. (2005). Identification of a novel mutation disrupting the DNA binding activity of GCM2 in autosomal recessive familial isolated hypoparathyroidism. *J Med Genet*, *42*, 443–448.
14. Thomee, C., Schubert, S. W., Parma, J., et al. (2005). GCMB mutation in familial isolated hypoparathyroidism with residual secretion of parathyroid hormone. *J Clin Endocrinol Metab*, *90*, 2487–2492.
15. Shaw, N. (2009). A practical approach to hypocalcaemia in children. In J. Allgrove, & N. Shaw (Eds.), *Calcium and bone disorders in children and adolescents* (Vol. 16) (pp. 73–92). Basel: Karger.
16. Mirczuk, S. M., Bowl, M. R., Nesbit, M. A., et al. (2010). A missense glial cells missing homolog B (GCMB) mutations, Asn502His, causes autosomal dominant hypoparathyroidism. *J Clin Endocrinol Metab*, *95*, 3512–3516.
17. Doyle, D., Kirwin, S. M., Sol-Church, K., & Levine, M. A. (2012). A novel mutation in the GCM2 gene causing severe congenital hypoparathyroidism. *J Pediatr Endocrinol Metab*, *25*, 741–746.
18. Grigorieva, I. V., Mirczuk, C., Gaynor, K. U., et al. (2010). Gata3-deficient mice develop parathyroid abnormalities due to dysregulation of the parathyroid-specific transcription factor Gcm2. *J Clin Invest*, *120*, 2144–2155.
19. Mittleman, S. D., Hendy, G. N., Fefferman, R. A., et al. (2006). A hypocalcemic child with a novel activating mutation of the calcium-sensing receptor gene: successful treatment with recombinant human parathyroid hormone. *J Clin Endocrinol Metab*, *91*, 2474–2479.
20. Bowl, M. R., Nesbit, M. A., Harding, B., et al. (2005). An interstitial deletion-insertion involving chromosomes 2p25. 3 and Xq27. 1, near SOX3, causes X-linked recessive hypoparathyroidism. *J Clin Invest*, *115*, 2822–2831.
21. Shiohara, M., Mori, T., Mei, B., et al. (2004). A novel gain-of-function mutation (F821L) in the transmembrane domain of calcium-sensing receptor is a cause of severe sporadic hypoparathyroidism. *Eur J Pediatr*, *163*, 94–98.
22. Watanabe, S., Fukumoto, S., Chang, H., et al. (2002). Association between activating mutations of calcium-sensing receptor and Bartter's syndrome. *Lancet*, *360*, 692–694.
23. Theman, T. A., Collins, M. T., Dempster, D. W., et al. (2009). PTH(1–34) replacement therapy in a child with hypoparathyroidism caused by a sporadic calcium receptor mutation. *J Bone Miner Res*, *24*, 964–973.
24. Winer, K. K., Sinali, N., Reynolds, J., et al. (2010). Long-term treatment of 12 children with chronic hypoparathyroidism: a random trial comparing synthetic human parathyroid hormone 1–34 versus calcitriol and calcium. *J Clin Endocrinol Metab*, *95*, 2680–2688.
25. Yagi, H., Furutani, Y., Hamada, H., et al. (2003). Role of TBX1 in human del22q11.2 syndrome. *Lancet*, *362*, 1366–1373.
26. McDonald-McGinn, D. M., & Sullivan, K. E. (2011). Chromosome 22q11.2 deletion syndrome (DiGeorge syndrome/velocardiofacial syndrome). *Medicine*, *90*, 1–18.
27. Brauner, R., Le Harivel de Gonneville, A., Kindermans, C., et al. (2003). Parathyroid function and growth in 22q11 deletion syndrome. *J Pediatr*, *142*, 504–508.
28. Sobin, C., Kiley-Brabeck, K., Dale, K., et al. (2006). Olfactory disorder in children with 22q11 deletion syndrome. *Pediatrics*, *118*, 697–703.
29. Robin, N. H., & Shprintzen, R. J. (2005). Defining the clinical spectrum of deletion 22q11.2. *J Pediatr*, *147*, 90–96.
30. Shooner, K. A., Rope, A. F., Hopkin, R. J., et al. (2005). Genetic analyses in two extended families with deletion 22q11 syndrome: importance of extracardiac manifestations. *J Pediatr*, *146*, 382–387.
31. Paylor, R., Glaser, B., Mupo, A., et al. (2006). Tbx1 haploinsufficiency is linked to behavioral disorders in mice and humans: implications for 22q11 deletion syndrome. *Proc Natl Acad Sci U S A*, *103*, 7729–7734.
32. Moon, A. M. (2006). Mouse models for investigating the developmental basis of human birth defects. *Pediatr Res*, *59*, 749–755.
33. Sahoo, T., Theisen, A., Rosenfeld, J. A., et al. (2011). Copy number variants of schizophrenia susceptibility loci are associated with a spectrum of speech and developmental delays and behavior problems. *Genet Med*, *13*, 777–784.
34. Wentzel, C., Fernstrom, M., Ohrner, Y., et al. (2008). Clinical variability of the 22q11.2 duplication syndrome. *Europ J Med Genet*, *51*, 501–510.
35. Carelle-Calmels, N., Saugier-Verber, P., Girard-Lemaire, F., et al. (2009). Genetic compensation in a human genomic disorder. *N Engl J Med*, *360*, 1211–1216.
36. Van Esch, H., Groenen, P., Nesbit, M. A., et al. (2000). GATA3 haplo-insufficiency causes human HDR syndrome. *Nature*, *406*, 419–422.
37. Moldovan, D., Carvahlo, R., Jorge, Z., & Medeira, A. (2011). A new case of HDR syndrome with severe female genital tract malformation [Letter]. *Am J Med Genet*, *155A*, 2329–2330.
38. Chiu, W.-Y., Chen, H.-W., Chao, H.-W., et al. (2006). Identification of three novel mutations in the GATA3 gene responsible for familial hypoparathyroidism and deafness in Chinese families. *J Clin Endocrinol Metab*, *91*, 4587–4592.
39. Nesbit, M. A., Bowl, M. R., Harding, B., et al. (2004). Characterization of GATA3 mutations in the hypoparathyroidism, deafness, and renal dysplasia (HDR) syndrome. *J Biol Chem*, *279*, 22624–22634.
40. Hershkovitz, E., Parvari, R., Diaz, G. A., & Gorodischer, R. (2004). Hypoparathyroidism-retardation-dysmorphism (HRD) syndrome: a review. *J Pediatr Endocrinol Metab*, *17*, 1583–1590.
41. Parvari, R., Hershkovitz, E., Grossman, N., et al. (2002). Mutation of TBCE causes hypoparathyroidism-retardation-dysmorphism and autosomal recessive Kenney-Caffey syndrome. *Nature Genet*, *32*, 448–452.
42. Courtens, W., Wuyts, W., Poot, M., et al. (2006). Hypoparathyroidism-retardation-dysmorphism syndrome in a girl: a new variant not caused by a TBCE mutation—clinical report and review. *Am J Med Genet* (Part A), *140*, 611–617.
43. Thakker, R. V. (2004). Genetics of endocrine and metabolic disorders: parathyroid. *Rev Endocrinol Metab Dis*, *5*, 37–51.
44. Hoogendam, J., Farih-Sips, H., Wynaendts, C. W., et al. (2007). Novel mutations in the parathyroid hormone (PTH)/PTH-related peptide receptor type 1 causing Blomstrand osteochondrodysplasia type I and II. *J Clin Endocrinol Metab*, *92*, 1088–1095.

45. Decker, E., Stellzig-Eisenhauer, A., Fiebig, B. S., et al. (2008). PTHR1 loss-of-function mutations in familial, nonsyndromic primary failure of tooth eruption. *Am J Hum Genet*, *83*, 781–786.
46. Duchetelat, S., Ostergaard, E., Cortes, D., et al. (2005). Recessive mutations in PTHR1 causing contrasting skeletal dysplasias in Eiken and Blomstrand syndromes. *Hum Molec Genet*, *14*, 1–5.
47. Richard, N., Abeguile, G., Coudray, N., et al. (2012). A new deletion ablating NESP55 causes loss of maternal imprint of A/B GNAS and autosomal dominant pseudohypoparathyroidism type 1b. *J Clin Endocrinol Metab*, *97*, E863–E867.
48. Pinsker, J. E., Rogers, W., McLean, S., et al. (2006). Pseudohypoparathyroidism type 1a with congenital hypothyroidism. *J Pediatr Endocrinol Metab*, *19*, 1049–1052.
49. Mantovani, G. (2011). Pseudohypoparathyroidism: diagnosis and treatment. *J Clin Endocrinol Metab*, *96*, 3020–3030.
50. Mantovani, G., de Sanctis, L., Barbieri, A. M., et al. (2010). Pseudohypoparathyroidism and GNAS epigenetic defects: clinical evaluation of Albright hereditary osteodystrophy and molecular analysis in 40 patients. *J Clin Endocrinol Metab*, *95*, 651–658.
51. Long, D. N., Levine, M. A., & Germain-Lee, E. L. (2010). Bone mineral density in pseudohypoparathyroidism type 1a. *J Clin Endocrinol Metab*, *95*, 4465–4475.
52. Levine, M. A. (2006). Parathyroid hormone resistance syndromes. In M. J. Favus (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (6th ed.) (pp. 220–224). Washington, DC: American Society for Bone and Mineral Research.
53. Shore, E. M., & Kaplan, F. S. (2010). Inherited human diseases of heterotopic bone formation. *Nat Rev Rheumatol*, *6*, 518–527.
54. Wan, M., Li, J., Herbst, K., et al. (2011). LRP6 mediates cAMP generation by G-protein coupled receptors through regulating the membrane targeting of $G\alpha_s$. *Sci Signal*, *4*, ra15.
55. Linglart, A., Menguy, C., Couvineau, A., et al. (2011). Recurrent PRKAR1A mutation in acrodysostosis with hormone resistance. *N Engl J Med*, *364*, 2218-2226.
56. Thakker, R. V. (2006). Hypocalcemia: pathogenesis, differential diagnosis, and management. In M. J. Favus (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (6th ed.) (213–215). Washington, DC: American Society for Bone and Mineral Research.
57. Oskarsdottir, S., Persson, C., Eriksson, B. O., & Fasth, A. (2005). Presenting phenotype in 100 children with the 22q11 deletion syndrome. *Eur J Pediatr*, *164*, 146–153.
58. Hsu, S. C., & Levine, M. A. (2004). Perinatal calcium metabolism: physiology and pathophysiology. *Semin Neonatol*, *9*, 23–36.
59. Levine, M. A. (2010). Investigation & management of hypocalcemia. Endocrine Society Annual Meeting, Boston, Meet-the-Professor, 81–86.
60. Newfield, R. S. (2007). Recombinant PTH for initial management of neonatal hypocalcemia. *N Engl J Med*, *356*, 1687–1688.
61. Domico, M. B., Huynh, V., Anand, S. K., & Mink, R. (2006). Severe hyperphosphatemia and hypocalcemic tetany after oral laxative administration in a 3-month-old infant. *Pediatrics*, *118*, 1580–1583.
62. Shoback, D. (2008). Hypoparathyroidism. *N Engl J Med*, *359*, 391–403.
63. Arnold, A., Horst, S. A., Gardella, T. J., et al. (1990). Mutation of the signal peptide-encoding region of the preproparathyroid hormone gene in familial isolated hypoparathyroidism. *J Clin Invest*, *86*, 1084–1087.
64. Goswami, R., Mohapatra, T., Gupta, N., et al. (2004). Parathyroid hormone gene polymorphism and sporadic idiopathic hypoparathyroidism. *J Clin Endocrinol Metab*, *89*, 4840–4845.
65. Tashjian, A. H., Jr., & Chabner, B. A. (2002). Commentary on clinical safety of recombinant human parathyroid hormone 1–34 in the treatment of osteoporosis in men and postmenopausal women. *J Bone Miner Res*, *17*, 1151–1161.
66. Mayer, A., Ploix, C., Orgiazzi, J., et al. (2006). Calcium-sensing receptor antibodies are relevant markers of acquired hypoparathyroidism. *J Clin Endocrinol Metab*, *89*, 4484–4488.
67. Goltzman, D., & Cole, D. E. C. (2006). Hypoparathyroidism. In M. J. Favus (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (6th ed.) (pp. 216–219). Washington, DC: American Society for Bone and Mineral Research.
68. Eisenbarth, G. S., & Gottlieb, P. A. (2004). Autoimmune polyendocrine syndromes. *N Engl J Med*, *350*, 2068–2079.
69. Husebye, E. S., & Anderson, M. S. (2010). Autoimmune polyendocrine syndromes: clues to type 1 diabetes pathogenesis. *Immunity*, *32*, 479–487.
70. Perheentupa, J. (2006). Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. *J Clin Endocrinol Metab*, *91*, 2843–2850.
71. Wolff, A. S. B., Erichsen, M. M., Meager, A., et al. (2007). Autoimmune polyendocrine syndrome type 1 in Norway: phenotypic variation, autoantibodies, and novel mutations in the autoimmune regulator gene. *J Clin Endocrinol Metab*, *92*, 595–603.
72. Orlova, E. M., Bukina, A. M., Kuznetsova, E. S., et al. (2010). Autoimmune polyglandular syndrome type 1 in Russian patients: clinical variants and autoimmune regulator mutations. *Horm Res Paediatr*, *73*, 449–457.
73. Brown, E. M. (2009). Anti-parathyroid and anti-calcium sensing receptor antibodies in autoimmune hypoparathyroidism. *Endocrinol Metab Clin NA*, *38*, 437–446.
74. Alimohammadi, M., Bjorklund, P., Hallgren, A., et al. (2080). Autoimmune polyendocrine syndrome type 1 and NALP5, a parathyroid autoantigen. *N Engl J Med*, *358*, 1018–1028.
75. Uchida, D., Hatakeyama, S., Matsushima, A., et al. (2004). AIRE functions as an E3 ubiquitin ligase. *J Exp Med*, *199*, 167–172.
76. Abramson, J., Giraud, M., Benoist, C., & Mathis, D. (2010). Aire's partners in the molecular control of immunological tolerance. *Cell*, *140*, 123–135.
77. Bjorses, P., Halonen, M., Palvimo, J. J., et al. (2000). Mutations in the AIRE gene: effects on subcellular location and transactivation function of the autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy protein. *Am J Hum Genet*, *66*, 378–392.
78. Downs, R. W., Jr. (2006). Miscellaneous causes of hypocalcemia. In M. J. Favus (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism*, (6th ed.) (pp. 227–229) Washington, DC: American Society for Bone and Mineral Research.
79. Bradley, T. J., Metzger, D. L., & Sanatani, S. (2004). Long on QT and low on calcium. *Cardiol Young*, *14*, 667–670.
80. Gavalas, N. G., Kemp, E. H., Krohn, K. J. E., et al. (2007). The calcium-sensing receptor is a target of autoantibodies in patients with autoimmune polyendocrine syndrome type 1. *J Clin Endocrinol Metab*, *92*, 2107–2114.
81. Meloni, A., Furcas, M., Cetani, F., et al. (2008). Autoantibodies against type 1 interferons as an additional diagnostic criterion for autoimmune polyendocrinopathy syndrome type I. *J Clin Endocrinol Metab*, *93*, 4389–4397.
82. Winer, K. K., Zhang, B., Shrader, J. A., et al. (2012). Synthetic human parathyroid hormone 1–34 replacement therapy: a randomized crossover trial comparing pump versus injections in the treatment of chronic hypoparathyroidism. *J Clin Endocrinol Metab*, *97*, 391–399.
83. Cole, D. E. C., Kooh, S. W., & Vieth, R. (2000). Primary infantile hypomagnesemia: outcome after 21 years and treatment with continuous nocturnal nasogastric magnesium infusion. *Eur J Pediatr*, *159*, 38–43.
84. Langman, C. B. (2006). Hypercalcemic syndromes in infants and children. In M. J. Favus (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (6th ed.) (pp. 209–212). Washington, DC: American Society for Bone and Mineral Research.
85. Lietman, S. A., Germain-Lee, E. L., & Levine, M. A. (2010). Hypercalcemia in children and adolescents. *Curr Opin Pediatr*, *22*, 508–515.
86. Fridriksson, J. H., Helmrath, M. A., Wessel, J. J., & Warner, B. W. (2001). Hypercalcemia associated with extracorporeal life support in neonates. *J Pediatr Surg*, *36*, 493–497.
87. Hak, E. B., Crill, C. M., Bugnitz, M. C., et al. (2005). Increased parathyroid hormone and decreased calcitriol during neonatal extracorporeal membrane oxygenation. *Intensive Care Med*, *31*, 264–270.
88. Obermannova, B., Banghova, K., Sumnik, Z., et al. (2009). Unusually severe phenotype of neonatal primary hyperparathyroidism due to a heterozygous inactivating mutation in the CASR gene. *Eur J Pediatr*, *168*, 569–573.
89. Diaz-Thomas, A., Iyer, P., Root, A., & Cannon, J. (2011). Functional characterization of calcium sensing receptors (CaR) bearing loss-of-function or gain-of-function missense mutations. Abstract presented at the annual meeting of the Endocrine Society, Boston, June.

90. Lietman, S. A., Tenenbaum-Rakover, Y., Jap, T. S., et al. (2009). A novel loss-of-function mutation, Gln459Arg, of the calcium-sensing receptor gene associated with apparent autosomal recessive inheritance of familial hypocalciuric hypercalcemia. *J Clin Endocrinol Metab*, *94*, 4372–4379.
91. Myashiro, T., Kunii, I., Manna, T. D., et al. (2004). Severe hypercalcemia in a 9-year-old Brazilian girl due to a novel inactivating mutation of the calcium-sensing receptor. *J Clin Endocrinol Metab*, *89*, 5936–5941.
92. Brown, E. M. (2005). Editorial: mutant extracellular calcium-sensing receptors and severity of disease. *J Clin Endocrinol Metab*, *90*, 1246–1248.
93. Mallet, E. (2004). Primary hyperparathyroidism in neonates and childhood: the French experience (1984–2004). *Horm Res*, *69*, 180–188.
94. Levine, M. A. (2006). Spectrum of hyperparathyroidism in children. Endocrine Society 88th Annual Meeting, Boston, June 24–27, Meet-the-Professor, 390–396.
95. Sathasivam, A., Garibaldi, L., & Murphy, J. (2006). Transient neonatal hyperparathyroidism: a presenting feature of mucolipidosis type II. J Pediatr Endocrinol Metab, 19, 859–862.
96. Coutinho, M. F., Encarncao, M., Gomes, R., et al. (2011). Origin and spread of a common deletion causing mucolipidosis type II: insights from patterns of haplotypic diversity. *Clin Genet*, *80*, 273–280.
97. Antonell, A., Del Campo, M., Magano, L. F., et al. (2010). Partial 7q11.23 deletions further implicate GTF2I and GTF2IRD1 as the main genes responsible for the Williams-Beuren syndrome neurocognitive profile. *J Med Gen*, *47*, 312–320.
98. Pober, B. R. (2010). Williams-Beuren syndrome. *N Engl J Med*, *362*, 239–252.
99. Barnett, C., & Krebs, J. E. (2011). WSTF does it all: a multifunctional protein in transcription, repair, and replication. *Biochem Cell Biol*, *89*, 12–23.
100. Selicorni, A., Fratoni, A., Pavesi, M. A., et al. (2006). Thyroid anomalies in Williams syndrome: investigation of 95 patients. *Am J Med Genet*, *140A*, 1098–1101.
101. Van der Aa, N., Rooms, L., Vandeweyer, G., et al. (2009). Fourteen new cases contribute to the characterization of the 7q11.23 microduplication syndrome. *Europ J Med Genet*, *52*, 94–100.
102. Mervis, C. B., Dida, J., Lam, E., et al. (2012). Duplication of GTF2I results in separation anxiety in mice and humans. *Am J Hum Genet*, *90*, 1064–1070.
103. Schlingmann, K. P., Kaufmann, M., Weber, S., et al. (2011). Mutations in CYP24A1 and idiopathic infantile hypercalcemia. *N Engl J Med*, *365*, 410–425.
104. Streeten, E. A., Zarbalian, K., & Damcott, C. M. (2012). CYP24A1 mutations in idiopathic infantile hypercalcemia [Letter]. *N Engl J Med*, *365*, 1741–1742.
105. Tebben, P. J., Milliner, D. S., Horst, R. L., et al. (2012). Hypercalcemia, hypercalciuria, and elevated calcitriol concentrations with autosomal dominant transmission due to CYP24A1 mutations: effects of ketoconazole therapy. *J Clin Endocrinol Metab*, *97*, E423–E427.
106. Ji, H.-F., & Shen, L. (2012). CYP24A1 mutations in idiopathic infantile hypercalcemia [Letter]. *N Engl J Med*, *365*, 1741.
107. Nozu, K., Iijima, K., Kaway, K., et al. (2009). In vivo and in vitro splicing assay of SLC12A1 in an ante-natal salt-losing tubulopathy patient with an intronic mutation. *Hum Genet*, *126*, 533–538.
108. Whyte, M. P. (2006). Hypophosphatasia. In M. J. Favus (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (6th ed.) (pp. 351–353). Washington, DC: American Society for Bone and Mineral Research.
109. Whyte, M. P. (2012). Hypophosphatasia. In F. H. Glorieux, H. Jueppner, & J. Pettifor (Eds.), *Pediatric bone: biology and diseases* (3rd ed.) (pp. 771–794). San Diego, CA: Academic Press.
110. Whyte, M. P. (2008). Enzyme defects and the skeleton. In C. J. Rosen (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (7th ed.) (pp. 454–458). Washington, DC: American Society of Bone and Mineral Metabolism.
111. Whyte, M. P., Greenberg, C. R., Salman, N. J., et al. (2012). Enzyme-replacement therapy in life-threatening hypophosphatasia. *N Engl J Med*, *366*, 904–913.
112. Benjamin, R. W., Moats-Staats, B. M., Calikoglu, A., et al. (2008). Hypercalcemia in children. *Pediatr Endocrinol Rev*, *5*, 778–784.
113. Ezgu, F. S., Buyan, N., Gunduz, M., et al. (2004). Vitamin D intoxication and hypercalcaemia in an infant treated with pamidronate infusions. *Eur J Pediatr*, *163*, 163–165.
114. Davies, J. H. (2009). A practical approach to the problems of hypercalcaemia. In J. Allgrove, & N. Shaw (Eds.), *Calcium and bone disorders in children and adolescents.* (Vol. 16) (pp. 93–114). Basel: Karger, Endocr Dev.
115. White, E., McKenna, J., Cavanaugh, A., & Breitwieser, G. E. (2009). Pharmacochaperone-mediated rescue of calcium sensing receptor loss of function mutants. *Molec Endocrinol*, *23*, 1115–1123.
116. Shane, E., & Irani, D. (2006). Hypercalcemia: pathogenesis, clinical manifestations, differential diagnosis, and management. In M. J. Favus (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (6th ed.) (pp. 176–180). Washington, DC: American Society for Bone and Mineral Research, 176–180.
117. Marx, S. J. (2006). Familial hypocalcemic hypercalciuria. In M. J. Favus (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (6th ed.) (pp. 188–190). Washington, DC: American Society for Bone and Mineral Research.
118. Egbuna, O. I., & Brown, E. M. (2008). Hypercalcaemic and hypocalcaemic conditions due to calcium sensing receptor mutations. *Best Pract Res Clin Rheumatol*, *22*, 129–148.
119. Magno, A. L., Ward, B. K., & Ratajczak, T. (2011). The calcium-sensing receptor: a molecular perspective. *Endocr Rev*, *32*, 3–30.
120. Pidasheva, S., Grant, M., Canaff, L., et al. (2006). Calcium-sensing receptor dimerizes in the endoplasmic reticulum: biochemical and biophysical characterization of the CASR mutants retained intracellularly. *Hum Molec Genet*, *15*, 2200–2209.
121. Pallais, J. C., Kifor, O., Chen, Y.-B., et al. (2004). Acquired hypocalciuric hypercalcemia due to autoantibodies against the calcium-sensing receptor. *N Engl J Med*, *351*, 362–369.
122. Bilezikian, J. P., & Silverberg, S. J. (2006). Primary hyperparathyroidism. In M. J. Favus (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (6th ed.) (pp. 181–185). Washington, DC: American Society for Bone and Mineral Research.
123. Kollars, J., Zarroug, A. E., van Heerden, J., et al. (2005). Primary hyperparathyroidism in pediatric patients. *Pediatrics*, *115*, 974–980.
124. Marx, S. J., & Simonds, W. F. (2005). Hereditary hormone excess: genes, molecular pathways, and syndromes. *Endocr Rev*, *26*, 615–661.
125. Thakker, R. V., Newey, P. J., Walls, G. V., et al. (2012). Clinical Practice Guidelines for Multiple Endocrine Neoplasia type 1 (MEN1). *J Clin Endocrinol Metab*, *97*, 2990–3011.
126. Newey, P. J., Bowl, M. R., Cranston, T., & Thakker, R. V. (2010). Cell division cycle protein 73 homolog (CDC73) mutations in hyperparathyroidism-jaw tumor syndrome (HPT-JT) and parathyroid tumors. *Hum Mutat*, *31*, 295–307.
127. Newey, P. J., Nesbit, M. A., Rimmer, A. J., et al. (2012). Whole-exome sequencing studies of nonhereditary (sporadic) parathyroid adenomas. *J Clin Endocrinol Metab*, *97*, E1995–E2005.
128. Warner, J., Epstein, M., Sweet, A., et al. (2004). Genetic testing in familial isolated hyperparathyroidism: unexpected results and their implications. *J Med Genet*, *41*, 155–160.
129. Carpten, J. D., Robbins, C. M., Villablanca, A., et al. (2002). HRPT2, encoding parafibromin, is mutated in hyperparathyroidism-jaw tumor syndrome. *Nature Genet*, *32*, 676–680.
130. Simonds, W. F., James-Newton, L. A., Agarwal, S. K., et al. (2002). Familial isolated hyperparathyroidism: clinical and genetic characteristics of 36 kindreds. *Medicine*, *81*, 1–26.
131. Juhlin, C. C., & Hoog, A. (2010). Parafibromin as a diagnostic instrument for parathyroid carcinoma-Lone ranger or part of the posse? *Intern J Endocrinol*. doi:10. 1155/2010/324964.
132. Yang, Y.-J., Han, J.-W., Youn, H.-D., & Cho, E.-J. (2010). The tumor suppressor, parafibromin, mediates histone H3 K9 methylation for cyclin D1 repression. *Nucl Acids Res*, *38*, 382–390.
133. Mossiman, C., Hausmann, G., & Basler, K. (2006). Parafibromin/Hyrax activates Wnt/Wg target gene transcription by direct association with beta-catenin/Armadillo. *Cell*, *125*, 327–341.

134. Rubin, M. R., & Silverberg, S. J. (2005). Editorial: HRPT2 in parathyroid cancer: a piece of the puzzle. *J Clin Endocrinol Metab*, *90*, 5505–5507.
135. Guarnieri, V., Scillitani, A., Muscarella, L. A., et al. (2006). Diagnosis of parathyroid tumors in familial isolated hyperparathyroidism with HRPT2 mutation: implication for cancer surveillance. *J Clin Endocrinol Metab*, *91*, 2827–2832.
136. Krebs, L. J., Shattuck, T. M., & Arnold, A. (2005). HRPT2 mutational analysis of typical sporadic parathyroid adenomas. *J Clin Endocrinol Metab*, *90*, 5015–5017.
137. Asgharian, B., Turner, M. L., Gibril, F., et al. (2004). Cutaneous tumors in patients with multiple endocrine neoplasm type 1 (MEN1) and gastrinomas: prospective study of frequency and development of criteria with high sensitivity and specificity. *J Clin Endocrinol Metab*, *89*, 5328–5336.
138. Wang, Y., Ozawa, A., Zaman, S., et al. (2011). The tumor suppressor protein menin inhibits AKT activation by regulating its cellular localization. *Cancer Res*, *71*, 371.
139. Kaji, H., Canaff, L., Lebrun J.-J., et al. (2001). Inactivation of menin, a Smad3-interacting protein, blocks transforming growth factor beta signaling. *Proc Natl Acad Sci U S A*, *98*, 3837–3842.
140. Heppner, C., Bilimoria, K. Y., Agarwal, S. K., et al. (2010). The tumor suppressor protein menin interacts with NF-kappa B proteins and inhibits NF-kappa B-mediated transactivation. *Oncogene*, *20*, 4917–4925.
141. Francis, J., Lin, W., Pozenblatt-Rosen, O., & Meyerson, M. (2011). The menin tumor suppressor protein is phosphorylated in response to DNA damage. *PLoS One*, *6*, e16119.
142. Turner, J. J. O., Leotela, P. D., Pannett, A. A. J., et al. (2002). Frequent occurrence of an intron 4 mutation in multiple endocrine neoplasia type 1. *J Clin Endocrinol Metab*, *87*, 2688–2693.
143. Arnold, A., & Marx, S. J. (2008). Familial hyperparathyroidism (including MEN, FHH, and HPT-JT). In C. J. Rosen (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (7th ed.) (pp. 361–366). Washington, DC: American Society of Bone and Mineral Metabolism.
144. Ozawa, A., Agarwal, S. K., Mateo, C. M., et al. (2007). The parathyroid/pituitary variant of multiple endocrine neoplasia type 1 usually has causes other than $p27^{Kip\ 1}$ mutations. *J Clin Endocrinol Metab*, *92*, 1948–1951.
145. Choi, S.-K., Yoon, S.-R., Calabrese, P., & Arnheim, N. (2012). Positive selection for new disease mutations in the human germline: evidence from heritable cancer syndrome Multiple Endocrine Neoplasia Type 2B. *PLoS Genet*, *8*, e1002420.
146. Lodish, M. B., & Stratakis, C. A. (2010). Rare and unusual endocrine cancer syndromes with mutated genes. *Semin Oncol*, *37*, 680–690.
147. Lumbroso, S., Paris, F., & Sultan, C. (2004). Activating Gsα mutations: analysis of 113 patients with signs of McCune-Albright syndrome: a European collaborative study. *J Clin Endocrinol Metab*, *89*, 2107–2113.
148. Chan, W., Sheehan, S., Miller, L. C., & Sadeghi-Najad, A. (2006). Hypercalcemia in a newly arrived international adoptee. *J Pediatr Endocrinol Metab*, *19*, 1249–1150.
149. Araki, T., Holick, M. F., Alfonso, B. D., et al. (2011). Vitamin D intoxication with severe hypercalcemia due to manufacturing and labeling errors of two dietary supplements in the United States. *J Clin Endocrinol Metab*, *96*, 3603–3608.
150. Kawaguchi, M., Mitsuhashi, Y., & Kondo, S. (2003). Iatrogenic hypercalcemia due to vitamin D3 ointment (1,24(OH)2D3) combined with thiazide diuretics in a case of psoriasis. *J Dermatol*, *30*, 801–804.
151. Adams, J. S., & Hewison, M. (2006). Hypercalcemia caused by granuloma-forming disorders. In M. J. Favus (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (6th ed.) (pp. 200 -202). Washington, DC: American Society for Bone and Mineral Research.
152. Jacobs, T. P., & Bilezikian, J. P. (2005). Clinical review: rare causes of hypercalcemia. *J Clin Endocrinol Metab*, *90*, 6316–6322.
153. Horwitz, M. J., Hodak, S. P., & Stewart, A. F. (2008). Non-parathyroid hypercalcemia. In C. J. Rosen (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (7th ed.) (pp. 307–312). Washington, DC: American Society of Bone and Mineral Metabolism.
154. El Saleeby, C. M., Grottkau, B. E., Friedmann, A. M., et al. (2011). Case 4–2011: a 4-year-old boy with back pain and hypercalcemia. *N Engl J Med*, *364*, 552–562.
155. Wysolmerski, J. J. (2006). Miscellaneous causes of hypercalcemia. In M. J. Favus (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism*, (6th ed.) (pp. 203-208). Washington, DC: American Society for Bone and Mineral Research.
156. Prince, R. L. (2006). Secondary and tertiary hyperparathyroidism. In M. J. Favus (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (6th ed.) (pp. 190–195). Washington, DC: American Society for Bone and Mineral Research.
157. Hruska, K. A., & Mathew, S. (2008). Chronic kidney disease bone mineral disorder (CKD-BMD). In C. J. Rosen (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (7th ed.) (pp. 343–349). Washington, DC: American Society of Bone and Mineral Metabolism.
158. Thakker, R. V. (2008). Genetic basis of renal stones. In C. J. Rosen (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism*, (7th ed.) (pp. 474–478). Washington, DC: American Society of Bone and Mineral Metabolism.
159. Shane, E., & Berenson, J. R. (2012). Treatment of hypercalcemia. *UpToDate*. Retrieved from http://www.uptodate.com/contents/treatment-of-hypercalcemia
160. D'Agostino, J. D., Diana, M., Vix, M., et al. (2012). Three-dimensional virtual neck exploration before parathyroidectomy. *N Engl J Med*, *367*, 1072–1073.
161. Ix, J. H., Quarles, L. D., & Chertow, G. M. (2006). Guidelines for disorders of mineral metabolism and secondary hyperparathyroidism should not be modified. *Nature Clin Pract Nephrol*, *2*, 337–339.
162. San-Cristobal, P., Dimke, H., Hoenderope, J. G. J., & Bindels, R. J. M. (2010). Novel molecular pathways in renal Mg^{2+} transport: a guided tour along the nephron. *Curr Opin Nephrol Hypertens*, *19*, 456–462.
163. Koo, W. W. K. (2007). Neonatal calcium, magnesium, and phosphorus disorders. In F. Lifshitz (Ed.), *Pediatric endocrinology* (5th ed, Vol. 2) (pp. 497–529). New York: Informa Healthcare.
164. Rude, R. K., & Gruber, H. E. (2004). Magnesium deficiency and osteoporosis: animal and human observations. *J Nutr Biochem*, *15*, 710–716.
165. Ferre, S., Hoenderop, J. G. J., & Bindels, R. J. M. (2011). Insight into renal Mg^{2+} transporters. *Curr Opin Nephrol Hypertens*, *20*, 169–176.
166. Walder, R. Y., Landau, D., Meyer, P., et al. (2002). Mutation of TRPM6 causes familial hypomagnesemia with secondary hypocalcemia. *Nature Genet*, *31*, 171–174.
167. Chubanov, V., Waldegger, S., Mederos y Schnitzler, M., et al. (2004). Disruption of TRPM6/TRPM7 complex formation by a mutation in the TRPM6 gene causes hypomagnesemia with secondary hypocalcemia. *Proc Nat Acad Sci*, *101*, 2894–2899.
168. Weber, S., Schneider, L., Peters, M., et al. (2001). Novel paracellin-1 mutations in 25 families with familial hypomagnesaemia with hypercalciuria and nephrocalcinosis. *J Am Soc Nephrol*, *12*, 1872–1881.
169. Kausalya, P. J., Amasheh, S., Gunzel, D., et al. (2006). Disease-associated mutations affect intracellular traffic and paracellular Mg^{2+} transport function of claudin-16. *J Clin Invest*, *116*, 878–891.
170. Groenestege, W. M. T., Thebault, S., van der Wijst, J., et al. (2007). Impaired basolateral sorting of pro-EGF causes isolated recessive renal hypomagnesemia. *J Clin Invest*, *117*, 2260–2267.
171. Konrad, M., Schaller, A., Seelow, D., et al. (2006). Mutations in the tight-junction gene claudin 19 (CLDN19) are associated with renal magnesium wasting, renal failure, and severe ocular involvement. *Am J Hum Genet*, *79*, 949–957.
172. Stuiver, M., Lainez, S., Will, C., et al. (2011). CNNM2, encoding a basolateral protein required for renal Mg^{2+} handling, is mutated in dominant hypomagnesemia. *Am J Hum Genet*, *88*, 333–343.
173. Glaudemans, B., van der Wijst, J., Scota, R. H., et al. (2009). A missense mutation in the Kv1. 1 voltage-gated potassium channel-encoding gene KCNA1 is linked to human autosomal dominant hypomagnesemia. *J Clin Invest*, *119*, 936–942.

174. Adalat, S., Woolf, A. S., Johnstone, K. A., et al. (2009). HNF1B mutations associate with hypomagnesemia and renal magnesium wasting. *J Am Soc Nephrol*, *20L*, 1123–1131.
175. Kaplan, W., Haymond, M. W., McKay, S., & Karaviti, L. P. (2006). Osteopenic effects of $MgSO_4$ in multiple pregnancies. *J Pediatr Endocrinol Metab*, *19*, 1225–1230.
176. Rauch, F., & Schoenau, E. (2002). Skeletal development in premature infants: a review of bone physiology beyond nutritional aspects. *Arch Dis Child Fetal Neonatal Ed*, *86*, F82–F85.
177. Abrams, S. & the Committee on Nutrition (AAP). (2013). Calcium and vitamin D requirements of enterally fed preterm infants. *Pediatrics*, *131*, e1676–e1683.
177a. Abrams, S. A. (2012). Calcium and phosphorus requirements in newborn infants. *UpToDate.* Retrieved from .
178. Ahmad, I., Nemet, D., Eliakim, A., et al. (2010). Body composition and its components in preterm and term newborns: a cross-sectional, multimodal investigation. *Am J Hum Biol*, *22*, 69–75.
179. Lam, H. S., So, K. V. V., & Ng, P. C. (2007). Osteopenia in neonates: a review. *HK J Paediatr*, *12*, 118–124.
180. Miller, M. E. (2003). The bone disease of preterm birth: a biomechanical perspective. *Pediatr Res*, *53*, 10–15.
181. Funke, S., Morava, E., Czako, M., et al. (2006). Influence of genetic polymorphisms on bone disease of preterm infants. *Pediatr Res*, *60*, 607–612.
182. Atabek, M. E., Pirgon, O., Yorulmaz, A., & Kurtoglu, S. (2006). The role of cord blood IGF-I levels in preterm osteopenia. *J Pediatr Endocrinol Metab*, *19*, 253–257.
183. Cakir, M., Mungan, I., Karahan, C., et al. (2006). Necrotizing enterocolitis increases the bone resorption in premature infants. *Early Hum Devel*, *82*, 405–409.
184. Aly, H., Moustafa, M. F., Amer, H. A., et al. (2005). Gestational age, sex, and maternal parity correlate with bone turnover in premature infants. *Pediatr Res*, *57*, 708–711.
185. Aladangady, N., Coen, P. G., White, M. P., et al. (2004). Urinary excretion of calcium and phosphate n preterm infants. *Pediatr Nephrol*, *19*, 1225–1231.
186. Shiff, Y., Eliakim, A., Shainkin-Kestenbaum, R., et al. (2001). Measurement of bone turnover markers in premature infants. *J Pediatr Endocrinol Metab*, *14*, 389–395.
187. Eliakim, A., & Nemet, D. (2005). Osteopenia of prematurity: the role of exercise in prevention and treatment. *Pediatr Endocrinol Rev*, *2*, 675–682.
188. Hammami, H., Koo, W. W. K., & Hockman, E. M. (2003). Body composition of neonates from fan beam duel energy X-ray absorptiometry measurements. *J Parenteral Enteral Nutr*, *27*, 423–426.
189. Rack, B., Lochmuller, E.-M., Janni, W., et al. (2012). Ultrasound for assessment of bone quality in preterm and term infants. *J Perinatol*, *32*, 218–226.
190. Chen, H.-L., Lee, C.-L., Tseng, H.-I., et al. (20120). Assisted exercise improves bone strength in very low birthweight infants by bone quantitative ultrasound. *J Paediatr Child Health*, *46*, 653–659.
191. Weiler, H. A., Yuen, C. K., & Seshia, M. M. (2002). Growth and bone mineralization of young adults weighing less than 1500 grams at birth. *Early Hum Devel*, *67*, 101–1012.
192. Yeste, D., Almar, J., Clemente, M., et al. (2004). Areal bone mineral density of the lumbar spine in 80 premature newborns. A prospective and longitudinal study. *J Pediatr Endocrinol Metab*, *17*, 959–966.
193. Marini, J. C. (2008). Osteogenesis imperfecta. In C. J. Rosen (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (7th ed.) (pp. 446-450). Washington, DC: American Society of Bone and Mineral Metabolism.
194. Bodian, D. L., Chan, T.-F., Poon, A., et al. (2009). Mutation and polymorphism spectrum in osteogenesis imperfecta type II: implications for genotype-phenotype relationships. *Hum Molec Genet*, *18*, 463–471.
195. Van Dijk, F. S., Huizer, M., Kariminejad, A., et al. (2010). Complete COL1A1 deletions in osteogenesis imperfecta. *Genet Med*, *12*, 736–741.
196. Rauch, F., & Glorieux, F. H. (2004). Osteogenesis imperfecta. *Lancet*, *363*, 1377–1385.
197. Rauch, F., & Glorieux, F. H. (2005). Bisphosphonate treatment in osteogenesis imperfecta: which drug, for whom, for how long? *Ann Med*, *37*, 295–302.
198. Marini, J. C., Cabral, W. A., & Barnes, A. M. (2010). Null mutations in LEPRE1 and CRTAP cause severe recessive osteogenesis imperfecta. *Cell Tissue Res*, *339*, 59–70.
199. Rohrbach, M., & Giunta, C. (2012). Recessive osteogenesis imperfecta: clinical, radiological, and molecular findings. *Am J Med Genet* (Part C), *160c*, 175–189.
200. Font-Llitjos, M., Rodriguez-Santiago, B., Espino, M., et al. (2009). Novel SLC7A7 large rearrangements in lysinuric protein intolerance patients involving the same AluY repeat. *Europ J Human Genet*, *17*, 71–79.
201. Sperandeo, M. P., Andria, G., & Sebastio, G. (2008). Lysinuric protein intolerance: update and extended mutation analysis of the SLC7A7 gene. *Hum Mutat*, *29*, 14–21.
202. Lips, P., van Schoor, N. M., & Bravenboer, N. (2008). Vitamin D–related disorders. In C. J. Rosen (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (7th ed.) (pp. 329–335). Washington, DC: American Society of Bone and Mineral Metabolism.
203. Rauch, F. (2012). Overview of rickets in children. *UpToDate*. Retrieved from http://www.uptodate.com/contents/overview-of-rickets-in-children
204. Girgis, C. M., Clifton-Bligh, R. J., Hamrick, M. W., et al. (2013). The roles of vitamin D in skeletal muscle: form, function, and metabolism. *Endocr Rev*, *34*, 33–83.
205. Pettifor, J. M. (2005). Rickets and vitamin D deficiency in children and adolescents. *Endocrinol Metab Clin North Am*, *34*, 537–553.
206. Aggarwal, V., Seth, A., Aneja, S., et al. (2012). Role of calcium deficiency in development of nutritional rickets in Indian children: a case control study. *J Clin Endocrinol Metab*, *97*, 3461–3466.
207. Aloia, J. F. (2011). The 2011 report on dietary reference intake for vitamin D: where do we go from here? *J Clin Endocrinol Metab*, *96*, 2987–2996.
208. Misra, M. (2012). Vitamin D insufficiency and deficiency in children and adolescents. *UpToDate*. Retrieved from http://www.uptodate.com/contents/vitamin-d-insufficiency-and-deficiency-in-children-and-adolescents
209. Holick, M. F., Binkley, M. C., Bischoff-Ferrari, H. A., et al. (2012). Guidelines for preventing and treating VitD deficiency and insufficiency revisited. *J Clin Endocrinol Metab*, *97*, 1153–1158.
210. Schnadower, D., Agarwal, C., Oberfield, S. E., et al. (2006). Hypocalcemic seizures and secondary bilateral femoral fractures in an adolescent with primary vitamin D deficiency. *Pediatrics*, *118*, 2226–2230.
211. Rauch, F. (2012). Etiology and treatment of calciopenic rickets. *UpToDate*. Retrieved from http://www.uptodate.com/contents/etiology-and-treatment-of-calcipenic-rickets-in-children
212. Ross, A. C., Manson, J. E., Abrams, S. A., et al. (2011). The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. *J Clin Endocrinol Metab*, *96*, 53–58.
213. Rovner, A. J., & O'Brien, K. O. (2008). Hypovitaminosis D among healthy children in the United States: a review of current literature. *Arch Pediatr Adolesc Med*, *162*, 513–519.
214. Gordon, C. M., DePeter, K. C., Feldman, H. A., et al. (2004). Prevalence of vitamin D deficiency among healthy adolescents. *Arch Pediatr Adolesc Med*, *158*, 531–537.
215. Holick, M. F., Binkley, N. C., Bischoff-Ferrari, H. A., et al. (2011). Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab*, *96*, 1911–1930.
216. Das, G., Crocombe, S., McGrath, M., et al. (2006). Hypovitaminosis D among healthy adolescent girls attending an inner school. *Arch Dis Child*, *91*, 569–572.
217. Tolppanson, A.-M., Fraser, A., Fraser, W. D., & Lawlor, D. A. (2012). Risk factors for variation in 25-hydroxyvitamin D_3 and D_2 concentrations and vitamin D deficiency in children. *J Clin Endocrinol Metab*, *97*, 1202–1210.
218. Vidailhet, M., Mallet, E., Bocquet, A., et al. (2012). Vitamin D: still a topical matter in children and adolescents. A position paper by the Committee on Nutrition of the French Society of Paediatrics. *Arch Pediatr*, *19*, 316–328.
219. DeLucia, M. C., Mitnick, M. E., & Carpenter, T. O. (2003). Nutritional rickets with normal circulating 25-hydroxyvitamin D: a call for reexamining the role of dietary calcium intake in North American infants. *J Clin Endocrinol Metab*, *88*, 3539–3545.

220. Sversky, A. L., Kumar, J., Abramowitz, M. K., et al. (2011). Association of glucocorticoid use and low 25-hydroxyvitamin D levels: results from the National Health and Nutrition Examination Survey (NHANES): 2001–2006. *J Clin Endocrinol Metab*, *96*, 3838–3845.
221. Baroncelli, G. I., Bertelloni, S., Ceccarelli, C., et al. (2000). Bone turnover in children with vitamin D deficiency rickets before and during treatment. *Acta Paediatr*, *89*, 513–518.
222. Tangpricha, V. (2007). Vitamin D deficiency in the southern United States. *South Med J*, *100*, 384–385.
223. Alemzadeh, R., Kichler, J., Barar, G., & Calhoun, M. (2008). Hypovitaminosis D in obese children and adolescents: relationship with adiposity, insulin sensitivity, ethnicity, and season. *Metabolism*, *57*, 183–191.
224. Reinehr, T., de Sousa, G., Alexy, U., et al. (2007). Vitamin D status and parathyroid hormone in obese children before and after weight loss. *Eur J Endocrinol*, *157*, 225–232.
225. Censani, M., et al. (June 2012). Vitamin D deficiency in morbidly obese adolescents [Abstract]. *Annual Meeting of the Endocrine Society*, Houston, TX.
226. Thacher, T. D., Fischer, P. R., Pettifor, J. M., et al. (1999). A comparison of calcium, vitamin D, or both for nutritional rickets in Nigerian children. *N Engl J Med*, *341*, 563–568.
227. Pettifor, J. M. (2006). Nutritional and drug-induced rickets and osteomalacia. In M. J. Favus (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (6th ed.) (pp. 330–338). Washington, DC: American Society for Bone and Mineral Research.
228. Agus, Z. S. (2012). Causes of hypophosphatemia. *UpToDate*. Retrieved from http://www.uptodate.com/contents/causes-of-hypophosphatemia
229. Malloy, P. J., & Feldman, D. (2010). Genetic disorders and defects in vitamin D actions. *Endocrinol Metab Clin North Am*, *39*, 333–346.
230. Cheng, J. B., Levine, M. A., Bell, N. H., et al. (2004). Genetic evidence that human CYPR1 enzyme is a key vitamin D 25-hydroxylase. *Proc Natl Acad Sci*, *101*, 7711–7715.
231. Tosson, H., & Rose, S. R. (2012). Absence of mutation in coding regions of CYP2R1 gene in apparent autosomal dominant vitamin D 25-hydroxylase deficiency. *J Clin Endocrinol Metab*, *97*, E796–E801.
232. Demay, M. B. (2006). Rickets caused by impaired vitamin D activation and hormone resistance: pseudovitamin D deficiency rickets and hereditary vitamin D–resistant rickets. In M. J. Favus (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (6th ed.) (pp. 338–341). Washington, DC: American Society for Bone and Mineral Research.
233. Edouard, T., Alos, N., Chabot, G., et al. (2011). Short and long-term outcome of patients with pseudo-vitamin D deficiency rickets treated with calcitriol. *J Clin Endocrinol Metab*, *96*, 82–89.
234. Skorija, K., Cox, M., Sisk, J. M., et al. (2005). Ligand-independent actions of the vitamin D receptor maintain hair follicle homeostasis. *Mol Endocrinol*, *19*, 855–862.
235. Nicolaidou, P., Tsitsika, A., Papadimitriou, A., et al. (2007). Hereditary vitamin D–resistant rickets in Greek children: genotype, phenotype, and long-term response to treatment. *J Pediatr Endocrinol Metab*, *20*, 245–430.
236. Tiosano, D., Hadad, S., Chen, Z., et al. (2011). Calcium absorption, kinetics, bone density, and bone structure in patients with hereditary vitamin D–resistant rickets. *J Clin Endocrinol Metab*, *96*, 3701–3709.
237. Chen, H., Hewison, M., Hu, B., & Adams, J. S. (2003). Heterogeneous nuclear ribonucleoprotein (hnRNP) binding to hormone response elements: a cause of vitamin D resistance. *Proc Natl Acad Sci U S A*, *100*, 6109–6114.
238. Chen, H., Hewison, M., & Adams, J. S. (2006). Functional characterization of heterogeneous nuclear ribonucleoprotein C1/C2 in vitamin D resistance: a novel response element-binding protein. *J Biol Chem*, *281*, 39114–39120.
239. Ruppe, M. D., & Jan de Beur, S. M. (2008). Disorders of phosphate homeostasis. In C. J. Rosen (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (7th ed.) (pp. 317–325). Washington, DC: American Society of Bone and Mineral Metabolism.
240. Alon, U. S. (2006). Hypophosphatemic vitamin D–resistant rickets. In M. J. Favus (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism*, (6th ed.) (pp. 342–345). Washington, DC: American Society for Bone and Mineral Research.
241. Scheinman, S. J., Agus, Z. S., & Drezner, M. K. (2012). Hereditary hypophosphatemic rickets and tumor-induced osteomalacia. *UpToDate*. Retrieved from http://www.uptodate.com/contents/hereditary-hypophosphatemic-rickets-and-tumor-induced-osteomalacia
242. Gattineni, J., & Baum, M. (2012). Genetic disorders of phosphate regulation. *Pediatr Nephrol*, *27*, 1477–1487.
243. Sabbagh, Y., Carpenter, T. O., & Demay, M. (2005). Hypophosphatemia leads to rickets by impairing caspase-mediated apoptosis of hypertrophic chondrocytes. *Proc Natl Acad Sci U S A*, *102*, 9637–9642.
244. Ecarot, B., & Desbarats, M. (1999). 1,25-$(OH)_2D_3$ down-regulates expression of PHEX, a marker of the mature osteoblast. *Endocrinology*, *140*, 1192–1199.
245. Gaucher, C., Walrant-Debray, O., Nguyen, T. M., et al. (2009). PHEX analysis in 118 pedigrees reveals new genetic clues in hypophosphatemic rickets. *Hum Genet*, *125*, 401–411.
246. Cho, H. Y., Lee, B. H., Kang, J. H., et al. (2005). A clinical and molecular genetic study of hypophosphatemic rickets in children. *Pediatr Res*, *58*, 329–333.
247. Martin, A., David, V., & Quarles, L. D. (2012). Regulation and function of the FGF23/klotho endocrine pathway. *Physiol Rev*, *92*, 131–155.
248. White, K. E., Larsson, T. E., & Econs, M. J. (2006). The roles of specific genes implicated as circulating factors in normal and disordered phosphate homeostasis: frizzled related protein-4, matric extracellular phosphoglycoprotein, and fibroblast growth factor 23. *Endocr Rev*, *27*, 221–241.
249. Benet-Pages, A., Lorenz-Depiereux, B., Zischka, H., et al. (2004). FGF23 is processed by proprotein convertases but not by PHEX. *Bone*, *35*, 455–462.
250. Rowe, P. S. N. (2012). Regulation of bone-renal mineral and energy metabolism: the PHEX, FGF23, DMP1, MEPE ASARM pathway. *Crit Rev Eukaryotic Gene Express*, *22*, 61–86.
251. Addison, W. N., Nakano, Y., Loisel, T., et al. (2008). MEPE-ASARM peptides control extracellular matrix mineralization by binding to hydroxyapatite: an inhibition regulated by PHEX cleavage of ASARM. *J Bone Miner Res*, *23*, 1638–1649.
252. Addison, W. N., Masica, D. L., Gray, J. J., & McKee, M. D. (2010). Phosphorylation dependent inhibition of mineralization by osteopontin ASARM peptides in regulated by PHEX cleavage. *J Bone Miner Res*, *25*, 695–705.
253. Martin, A., David, V., Laurence, J. S., et al. (2008). Degradation of MEPE, DMP1, and release of SIBLING ASARM-peptides (minhibins): ASARM-peptide(s) are directly responsible for defective mineralization in HYP. *Endocrinology*, *149*, 1757–1772.
254. Burnett, S. A. M., Gunawardene, S. C., Bringhurst, F. R., et al. (2006). Regulation of C-terminal and intact FGF-23 by dietary phosphate in men and women. *J Bone Miner Res*, *21*, 1187–1196.
255. Hori, M., Shimizu, Y., & Fukumoto, S. (2011). Minireview: fibroblast growth factor 23 in phosphate homeostasis and bone metabolism. *Endocrinology*, *152*, 4–10.
256. Goji, K., Ozaki, K., Sadewa, A. H., et al. (2006). Somatic and germline mosaicism for a mutation of the PHEX gene can lead to genetic transmission of X-linked hypophosphatemic rickets that mimics an autosomal dominant trait. *J Clin Endocrinol Metab*, *91*, 365–370.
257. Novais, E., & Stevens, P. M. (2006). Hypophosphatemic rickets: the role of hemiepiphysiodesis. *J Pediatr Orthop*, *26*, 238–244.
258. Grundberg, C. M., Simpson, C. A., & Insogna, K. L. (2011). Calcitonin administration in X-linked hypophosphatemia. *N Engl J Med*, *354*, 1678–1680.
259. Makitie, O., Doria, A., Kooh, S. W., et al. (2003). Early treatment improves growth and biochemical and radiographic outcome in X-linked hypophosphatemic rickets. *J Clin Endocrinol Metab*, *88*, 3591–3597.
260. Zivicnjak, M., Schnabel, D., Billing, H., et al. (2011). Age-related stature and linear body segments in children with X-linked hypophosphatemic rickets. *Pediatr Nephrol*, *26*, 223–231.
261. Haffner, D., Nissel, R., Wuhl, E., & Mehls, O. (2004). Effects of growth hormone treatment on body proportions and final height

among small children with X-linked hypophosphatemic rickets. *Pediatrics*, *113*, e593–e596.
262. Baroncelli, G. I., Bertelloni, S., Dati, E., & Cavallo, L. (2007). Linear growth during growth hormone treatment in X-linked hypophosphatemic rickets: report of two patients. *J Pediatr Endocrinol Metab*, *20*, 351–356.
263. Zivicnjak, M., Schnabel, D., Staude, H., et al. (2011). Three-year growth hormone treatment in short children with X-linked hypophosphatemic rickets. Effects on linear growth and body disproportion. *J Clin Endocrinol Metab*, *96*, E2097–E2105.
264. Ward, L. M. (2005). Renal phosphate-wasting disorders in childhood. *Pediatr Endocrinol Rev*, *2*, 342–350.
265. Brownstein, C. A., Adler, F., Nelson-Williams, C., et al. (2008). A translocation causing increased alpha-klotho level results in hypophosphatemic rickets and hyperparathyroidism. *Proc Natl Acad Sci U S A*, *105*, 3455–3460.
266. Segawa, H., Aranami, F., Kaneko, I., et al. (2009). The roles of Na/Pi-II transporters in phosphate metabolism. *Bone*, 45(*Suppl.* 1), S2–S7.
267. Magen, D., Berger, L., Coady, M. J., et al. (2010). A loss-of-function mutation in NaPi-IIa and renal Fanconi's syndrome. *N Engl J Med*, *362*, 1102–1109.
268. Courbebaisse, M., Leroy, C., Bakouh, N., et al. (2012). A new human NHERF1 mutation decreases renal phosphate transporter NPT2a expression by a PTH-independent mechanism. *Plos ONE*, *7*(4), e34764. doi:10. 1371/journal. pone. 0034764
269. Karim, Z., Gerard, G. B., Bakouh, N., et al. (2008). NHERF1 mutations and responsiveness of renal parathyroid hormone. *N Engl J Med*, *359*, 1128–1135.
270. Levi, M., & Breusegem, S. (2008). Renal phosphate-transporter regulatory proteins and nephrolithiasis. *N Engl J Med*, *359*, 1171–1173.
271. Zeger, M. D., Adkins, D., Fordham, L. A., et al. (2007). Hypophosphatemic rickets in opsismodysplasia. *J Pediatr Endocrinol Metab*, *20*, 79–86.
272. Below, J. E., Earl, D. L., Shively, K. M., et al. (2013). Whole-genome analysis reveals that mutations in inositol polyphosphate phosphatase-like 1 cause opsismodysplasia. *Am J Hum Genet*, *92*, 137–143.
273. Huber, C., Fageih, E. A., Bartholdi, D., et al. (2013). Exome sequencing identifies INPPL1 mutations as a cause of opsismodysplasia. *Am J Hum Genet*, *92*, 144–149.
274. Carpenter, T. O., Ellis, B. K., Insogna, K. L., et al. (2005). Fibroblast growth factor 7: an inhibitor of phosphate transport derived from oncogenic osteomalacia-causing tumors . *J Clin Endocrinol Metab*, *90*, 1012–1020.
275. Imel, E. A., Peacock, M., Pitukcheewanont, P., et al. (2006). Sensitivity of fibroblast growth factor 23 measurements in tumor-induced osteomalacia. *J Clin Endocrinol Metab*, *91*, 2055–2061.
276. Ward, L. M., Rauch, F., White, K. E., et al. (2004). Resolution of severe, adolescent-onset hypophosphatemic rickets following removal of an FGF-23-producing tumour of the distal ulna. *Bone*, *34*, 905–911.
277. Shulman, D. I., Hahn, G., Benator, R., et al. (2004). Tumor-induced rickets: usefulness of MR gradient echo recall imaging for tumor localization. *J Pediatr*, *144*, 381–385.
278. Feng, J. Q., Ward, L. M., Liu, S., et al. (2006). Loss of DMP1 causes rickets and osteomalacia and identifies a role for osteocytes in mineral metabolism. *Nature Genet*, *38*, 1310–1315.
279. Lorenz-Depiereux, B., Bastepe, M., Benet-Pages, A., et al. (2006). DMP1 mutations in autosomal recessive hypophosphatemia implicate a bone matrix protein in the regulation of phosphate homeostasis. *Nature Genet*, *38*, 1248–1250.
280. Levy-Litan, V., Hershkovitz, E., Avizov, L., et al. (2010). Autosomal-recessive hypophosphatemic rickets is associated with inactivation mutation in the ENPP1 gene. *Am J Hum Genet*, *86*, 273–278.
281. Lorenz-Depiereux, B., Schnabel, D., Tiosano, D., et al. (2010). Loss of function ENPP1 mutations cause both generalized arterial calcification of infancy and autosomal-recessive hypophosphatemic rickets. *Am J Hum Genet*, *86*, 267–272.
282. Rutsch, F., Rui, N., Vaingankar, S., et al. (2003). Mutations in ENPP1 are associated with 'idiopathic' infantile arterial calcification. *Nature Genet*, *34*, 379–381.
283. Tebben, P. J., Thomas, L. F., & Kumar, R. (2006). Fanconi syndrome and renal tubular acidosis. In M. J. Favus (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (6th ed.) (pp. 354–358). Washington, DC: American Society for Bone and Mineral Research, 2006.
284. Sprecher, E. (2010). Familial tumoral calcinosis: from characterization of a rare phenotype to the pathogenesis of ectopic calcification. *J Invest Dermatol*, *130*, 652–660.
285. Stevenson, D. A., Carey, J. C., & Coburn, S. P. (2008). Autosomal recessive hypophosphatasia manifesting in utero with long bone deformity but showing spontaneous postnatal improvement. *J Clin Endocrinol Metab*, *93*, 3443–3448.
286. Wenkert, D., McAlister, W. H., Coburn, S. P., et al. (2011). Hypophosphatasia: nonlethal disease despite skeletal presentation in utero (17 new cases and literature review). *J Bone Miner Res*, *26*, 2389–2398.
287. Lia-Baldini, A. S., Muller, F., Taillandier, A., et al. (2001). A molecular approach to dominance in hypophosphatasia. *Hum Genet*, *109*, 99–108.
288. Zankl, A., Mornet, E., & Wong, S. (2008). Specific ultraosonographic features of perinatal lethal hypophosphatasia. *Am J Med Genet A*, *146A*, 1200–1204.
289. Cahill, R. A., Wenkert, D., Perlman, S. A., et al. (2007). Infantile hypophosphatasia: transplantation therapy trial using bone fragments and cultured osteoblasts. *J Clin Endocrinol Metab*, *92*, 923–2930.
290. Greenberg, C. R., Krishnani, P., Landy, H., et al. (March 2009). Hypophosphatasia: enzyme replacement with ENB0040, a bone-targeted human recombinant tissue nonspecific alkaline phosphatase (TNSALP) fusion protein. *Presented at the 16th Annual Clinical Genetics Meeting of the American College of Medical Genetics*, Tampa, FL.
291. Whyte, M. P., Mumm, S., & Deal, C. (2007). Adult hypophosphatasia treated with teriparatide. *J Clin Endocrinol Metab*, *92*, 1203–1208.
292. Fawaz, R., & Israel, R. (2012). Transient hyperphosphatasemia of infancy and early childhood. *UpToDate*. Retrieved from http://www.uptodate.com/contents/transient-hyperphosphatasemia-of-infancy-and-early-childhood
293. Huh, S. Y., Feldman, H. A., Cox, J. E., & Gordon, M. (2009). Prevalence of transient hyperphosphatasemia among healthy infants and toddlers. *Pediatrics*, *124*, 703–709.
293a. Shroff, R. (2011). Dysregulated mineral metabolism in children with chronic kidney disease. *Curr Opin Nephrol Hyperten*, *20*, 233–240.
294. Chauhan, V., Keleporis, E., Chauhan, N., & Vaid, M. (2012). Current concepts and management strategies in chronic kidney disease-mineral and bone disorder. *South Med J*, *105*, 479–485.
295. Rauch, F. (2006). Watching bones at work: what we can see from bone biopsies. *Pediatr Nephrol*, *21*, 457–462.
296. Bueno de Oliveira, R., & Affonso-Moyses, R. M. (2010). FGF-23:state of the art. *J Bras Nefrol*, *32*, 316–323.
297. Shalhoub, V., Shatzen, E. M., Ward, S. C., et al. (2012). FGF23 neutralization improves chronic kidney disease-associated hyperparathyroidism yet increases mortality. *J Clin Invest*, *122*, 2543–2553.
298. Koch Nogueira, P. C., David, L., & Cochat, P. (2000). Evolution of secondary hyperparathyroidism after renal transplantation. *Pediatr Nephrol*, *14*, 342–346.
299. Reusz, G. S., Szabo, A. J., Peter, F., et al. (2000). Bone metabolism and mineral density following renal transplantation. *Arch Dis Child*, *83*, 146–151.
300. Henriksen, K., Bollerslev, J., Everts, V., & Karsdal, M. A. (2011). Osteoclast activity and subtypes as a function of physiology and pathology: implications for future treatment of osteoporosis. *Endocr Rev*, *32*, 31–63.
301. Ralston, .S (2008). Juvenile Paget's disease, familial expansile osteolysis and other genetic osteolytic disorders. *Best Pract Res Clin Rheumatol*, *22*, 101–111.
302. Middleton-Hardie, C., Zhu, Q., Cundy, H., et al. (2060). Deletion of aspartate 182 in OPG causes juvenile Paget's disease by impairing both protein secretion and binding to RANKL. *J Bone Miner Res*, *21*, 438–445.
303. Cundy, T., Davidson, J., Rutland, M. D., et al. (2005). Recombinant osteoprotegerin for juvenile Paget's disease. *N Engl J Med*, *353*, 918–923.

304. Polyzos, S. A., Anastasilakis, A. D., Litsas, J., et al. (2010). Profound hypocalcemia following effective response to zoledronic acid treatment in a patient with juvenile Paget's disease. *J Bone Miner Metab*, *28*, 706–712.
305. Crockett, J. C., Mellis, D. J., Scott, D. I., & Helfrich, M. H. (2011). New knowledge on critical osteoclast formation and activation pathways from study of rare genetic diseases of osteoclasts: focus on the RANK/RANKL axis. *Osteoporosis Intern*, *22*, 1–22.
306. Crockett, J. C., Mellis, D. J., Shennan, K. I., et al. (2011). Signal peptide mutations in RANK prevent downstream activation of NF-Kappa B. *J Bone Miner Res*, *26*, 1926–1938.
307. Guerrini, M. M., Sobacchi, C., Cassani, B., et al. (2008). Human osteoclast-poor osteopetrosis with hypogammaglobulinemia. *Am J Hum Genet*, *83*, 64–76.
308. Boyce, A. M., & Gafni, R. I. (2011). Approach to the child with fractures. *J Clin Endocrinol Metab*, *96*, 1943–1952.
309. Chevalley, T., Bonjour, J. P., van Rietbergen, B., et al. (2011). Fractures during childhood and adolescence in healthy boys: relation with bone mass and strength. *J Clin Endocrinol Metab*, *96*, 3134–3142.
310. Bachrach, L. K. (2005). Osteoporosis and measurement of bone mass in children and adolescents. *Endocrinol Metab NA*, *34*, 521–535.
311. Harvey, N., Dennison, E., & Cooper, C. (2008). Epidemiology of osteoporotic fractures. In C. J. Rosen (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (7th ed.) (pp. 198–203).Washington, DC: American Society of Bone and Mineral Metabolism.
312. Ferrari, S., Bianchi, M. L., Eisman, J. A., et al. (2012). Osteoporosis in young adults: pathophysiology, diagnosis, and management. *Osteoporos Int*, *23*, 2735–2748.
313. Cohen, A., & Shane, E. (2008). Premenopausal osteoporosis. In C. J. Rosen (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (7th ed.) (pp. 289–293). Washington, DC: American Society of Bone and Mineral Metabolism.
314. Baim, S., Binkley, N., Bilezikian, J. P., et al. (2008). Official positions of the International Society for Clinical Densitometry and executive summary of the 2007 ISCD Position Development Conference. *J Clin Densitom*, *11*, 75–91.
315. Bachrach, L. K., & Sills, I. N. (2011). Clinical report: bone densitometry in children and adolescents. *Pediatrics*, *127*, 189–194.
316. Rauch, F., Plotkin, H., DiMeglio, L., et al. (2008). Fracture prediction and the definition of osteoporosis in children and adolescents: the ISCD 2007 pediatric official positions. *J Clin Densit: Assess Clin Densit*, *11*, 22–28.
317. Kalkwarf, H. J., Zemel, B. S., Gilsanz, V., et al. (2007). The bone mineral density in childhood study: bone mineral content and density according to age, sex, and race. *J Clin Endocrinol Metab*, *92*, 2087–2099.
318. Zemel, B. S., Kalkwarf, H. J., Gilsanz, V., et al. (2011). Revised reference curves for bone mineral content and areal bone mineral density according to age and sex for black and non-black children: results of the Bone Mineral Density in Childhood Study. *J Clin Endocrinol Metab*, *96*, 3160–3169.
319. Hazell, T. J., Vanstone, C. A., Rodd, C. J., et al. (2013). Bone mineral density measured by a portable x-ray device agrees with dual-energy x-ray absorptiometry at forearm in preschool aged children. *J Clin Densitom*, *16*, 302–307.
320. Ward, K. A., Ashby, R. L., Roberts, S. A., et al. (2007). UK reference data for the Hologic QDR Discovery dual-energy x ray absorptiometry scanner in healthy children and young adults aged 6–17 years. *Arch Dis Child*, *92*, 53–59.
321. Leonard, M. B., Shults, J., Elliott, D. M., et al. (2004). Interpretation of whole body dual energy X-ray absorptiometry measures in children: comparison with peripheral quantitative computed tomography. *Bone*, *34*, 1044–1052.
322. Hsu, Y.-H., & Kiel, D. P. (2012). Genome-wide associations studies of skeletal phenotypes: what we have learned and where we are headed. J Clin Endocrinol Metab, 97, E1958–E1977.
323. Golden, N. H. (2000). Osteoporosis prevention: a pediatric challenge. *Arch Pediatr Adolesc Med*, *154*, 542–543.
324. Wyshak, G. (2000). Teenaged girls, carbonated beverage consumption, and bone fractures. *Arch Pediatr Adolesc Med*, *154*, 610–613.
325. Wosje, K. S., Khoury, P. R., Clayton, R. P., et al. (2009). Adiposity and TV viewing are related to less bone accrual in young children. *J Pediatr*, *154*, 79–84.
326. Janicka, A., Wren, T. A. L., Sanchez, M. M., et al. (2007). Fat mass is not beneficial to bone in adolescents and young adults. *J Clin Endocrinol Metab*, *92*, 143–147.
327. Kessler, J., Koebnick, C., Smith, N., & Adams, A. (2013). Childhood obesity is associated with increased risk of most lower extremity fractures. *Clin Orthop Relat Res*, *471*, 1199–1207.
328. Pitukcheewanont, P., Punyasavatsut, N., & Feuilee, M. (2012). Physical activity and bone health in children and adolescents. *Pediatr Endocrinol Rev*, 7, 275–282.
329. Specker, B., & Binkley, T. (2030). Randomized trial of physical activity and calcium supplementation on bone mineral content in 3- to 5-year old children. *J Bone Miner Res*, *18*, 885–892.
330. Winzenberg, T. M., Shaw, K., Fryer, J., & Jones, G. (2006). Calcium supplementation for improving bone mineral density in children. *Cochrane Database of Systematic Reviews*, *2*, CD005119.
331. Khadilkar, A., Kadam, N., Chiplonkar, S., et al. (2012). School-based calcium-vitamin D with micronutrient supplementation enhances bone mass in underprivileged Indian premenarchal girls. *Bone*, *51*, 1–7.
332. Carpenter, T. O., DeLucia, M. C., Zhang, J. H., et al. (2006). A randomized controlled study of the effects of dietary magnesium oxide supplementation on bone mineral content in healthy girls. *J Clin Endocrinol Metab*, *91*, 4866–4872.
333. Loro, L. M., Sayre, J., Roe, T. F., et al. (2000). Early identification of children predisposed to low peak bone mass and osteoporosis later in life. *J Clin Endocrinol Metab*, *85*, 3908–3918.
334. Stone, M., Briody, J., Kohn, M. R., et al. (2006). Bone changes in adolescent girls with anorexia nervosa. *J Adolesc Hlth*, *39*, 835–841.
335. Misra, M., Soyka, L. A., Miller, K. K., et al. (2003). Serum osteoprotegerin in adolescent girls with anorexia nervosa. *J Clin Endocrinol Metab*, *88*, 3816–3822.
336. DiVasta, A. D., & Gordon, C. M. (2006). Bone health in adolescents. *Adolesc Med*, *17*, 639–652.
337. Ackerman, K. E., Nazem, T., Chapko, D., et al. (2011). Bone microarchitecture is impaired in adolescent amenorrheic athletes compared with eumenorrheic athletes and nonathletic controls. *J Clin Endocrinol Metab*, *96*, 3123–3133.
338. Fernandez-Garcia, D., Rodriguez, M., Garcia Aleman, J., et al. (2009). Thin healthy women have a similar low bone mass to women with anorexia nervosa. *Br J Nutr*, *102*, 709–714.
339. Zacharin, M. (2040). Current advances in bone health of disabled children. *Curr Opin Pediatr*, *16*, 545–551.
340. Henderson, R. C., Lark, R. K., Grka, M. J., et al. (2002). Bone density and metabolism in children and adolescents with moderate to severe cerebral palsy. *Pediatrics*, *110*, e5.
341. Henderson, J. K., Kairalla, J. A., Barrington, J. W., et al. (2005). Longitudinal changes in bone density in children and adolescents with moderate to severe cerebral palsy. *J Pediatr*, *146*, 769–775.
342. Henderson, R. C., Lark, R. K., Kecskemethy, H. H., et al. (2002). Bisphosphonates to treat osteopenia in children with quadriplegic cerebral palsy: a randomized, placebo-controlled clinical trial. *J Pediatr*, *141*, 644–651.
343. Ali, O., Shim, M., Fowler, E., et al. (2007). Growth hormone therapy improves bone mineral density in children with cerebral palsy: a preliminary pilot study. *J Clin Endocrinol Metab*, *92*, 932–937.
344. Paksu, M., Vurucu, S., Karaoglu, A., et al. (2012). Osteopenia in children with cerebral palsy can be treated with oral alendronate. *Child Nerv Syst*, *28*, 283–286.
345. Caulton, J. M., Ward, K. A., Alsop, C. W., et al. (2004). A randomised controlled trial of standing programme on bone mineral density in non-ambulant children with cerebral palsy. *Arch Dis Child*, 89, 131–135.
346. Drake, M. T., & Khosla, S. (2008). Role of sex steroids in the pathogenesis of osteoporosis. In C. J. Rosen (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (7th ed.) (pp. 208–213).Washington, DC: American Society of Bone and Mineral Metabolism.
347. Bakalov, V. K., Foodim, J., & Bondy, C. A. (2006). Bone density and fractures in Turner syndrome. *Internat Cong Series*, *1298*, 160–167.

348. Sas, T. C., De Muinck Keizer-Schrama, S. M., Stijnen, T., et al. (2000). A longitudinal study on bone mineral density until adulthood in girls with Turner's syndrome participating in a growth hormone injection frequency-response trial. *Clin Endocrinol*, *52*, 531–536.
349. Antoniazzi, F., Bertoldo, F., Lauriola, S., et al. (1999). Prevention of bone demineralization by calcium supplementation in precocious puberty during gonadotropin releasing hormone agonist treatment. *J Clin Endocrinol Metab*, *84*, 1992–1996.
350. Van Staa, T. P. (2006). The pathogenesis, epidemiology and management of glucocorticoid-induced osteoporosis. *Calcif Tissue Int*, *79*, 129–137.
351. Dore, R. K. (2010). How to prevent glucocorticoid-induced osteoporosis. *Cleveland Clin J Med*, *77*, 529–536.
352. Sambrook, P. N. (2006). Glucocorticoid-induced osteoporosis. In M. J. Favus (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (6th ed.) (pp. 296–302). Washington, DC: American Society for Bone and Mineral Research.
353. Allen, D. B. (2006). Effects of inhaled steroids on growth, bone metabolism, and adrenal function. *Adv Pediatr*, *53*, 101–110.
354. Kelly, H. W., Van Natta, M. L., Covar, R. A., et al. (2008). Effect of long-term corticosteroid use on bone mineral density in children: a prospective longitudinal assessment in the Childhood Asthma Management Program (CAMP) study. *Pediatrics*, *122*, e53–e61.
355. Holm, I. A. (2009). Do short courses of oral corticosteroids and use of inhaled corticosteroids affect bone health in children? *Nature Clin Pract: Endocrinol Metab*, *5*, 132–133.
356. Wong, C. A., Walsh, L. J., Smith, C. J. P., et al. (2000). Inhaled corticosteroid use and bone mineral density in patients with asthma. *Lancet*, *355*, 1399–1403.
357. King, J. A., Wisniewski, A. B., Bankowski, B. J., et al. (2006). Long-term corticosteroid replacement and bone mineral density in adult women with classical congenital adrenal hyperplasia. *J Clin Endocrinol Metab*, *91*, 865–869.
358. Noguera, A., Ros, J. B., Pavia, C., et al. (2030). Bisphosphonates, a new treatment for glucocorticoid-induced osteoporosis in children. *J Pediatr Endocrinol Metab*, *16*, 529–536.
359. Giustina, A., Mazziotti, G., & Canalis, E. (2080). Growth hormone, insulin-like growth factors, and the skeleton. *Endocr Rev*, *29*, 535–559.
360. Tahimic, C. G. T., Wang, Y., & Bikle, D. D. (2013). Anabolic effects of IGF-1 signaling on the skeleton. *Front Endocrinol* (Lausanne), *4*, 6.
361. Maheshwari, H. G., Bouillon, R., Nijs, J., et al. (2003). The impact of congenital, severe, untreated growth hormone (GH) deficiency on bone size and density in young adults: insights from genetic GH-releasing hormone receptor deficiency. *J Clin Endocrinol Metab*, *88*, 2614–2618.
362. Hogler W, & Shaw, N. (2010). Childhood growth hormone deficiency, bone density, structures and fractures: scrutinizing the evidence. *Clin Endocrinol*, *72*, 281–289.
363. Robson, H., Siebler, T., Shalet, S. M., & Williams, G. R. (2003). Interactions between GH, IGF-I, glucocorticoids, and thyroid hormones during skeletal growth. *Pediatr Res*, *52*, 137–147.
364. Heap, J., Murray, M. A., Miller, M. C., et al. (2004). Alterations in bone characteristics associated with glycemic control in type 1 diabetes mellitus adolescents. *J Pediatr*, *144*, 56–62.
365. Moyer Mileur, L. J., Dixon, S. B., Quick, J. L., et al. (2004). Bone mineral acquisition in adolescents with type 1 diabetes. *J Pediatr*, *145*, 662–669.
366. Bechtold, S., Dirlenbach, I., Raile, K., et al. (2006). Early manifestation of type 1 diabetes in children is a risk factor for changed bone geometry: data using peripheral quantitative computed tomography. *Pediatrics*, *118*, 627–634.
367. Chaiban, J., Muwakkit, S., Arabi, A., et al. (2009). Modeling pathways for low bone mass in children with malignancies. *J Clin Densit: Assess Skeletal Health*, *12*, 441–449.
368. Ebeling P (2008). Transplantation osteoporosis. In C. J. Rosen (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (7th ed.) (pp. 279–285). Washington, DC: American Society of Bone and Mineral Metabolism.
369. Rovner, A., Zemel, B. S., Leonard, M. B., et al. (2005). Mild to moderate cystic fibrosis is not associated with increased fracture risk in children and adolescents. *J Pediatr*, *147*, 327–331.
370. Burnham, J. M., Shults, J., Semeao, E., et al. (2004). Whole body BMC in pediatric Crohn disease: independent effects of altered growth, maturation, and body composition. *J Bone Miner Res*, *19*, 1961–1968.
371. Larussa, T., Suraci, E., Nazionale, I., et al. (2012). Bone mineralization in celiac disease. *Gastroenterol Res Pract*. doi: 10.1155/2012/198025
372. Mora, S., Zamproni, I., Beccio, S., et al. (2004). Longitudinal changes of bone mineral density and metabolism in antiretroviral-treated human immunodeficiency virus-infected children. *J Clin Endocrinol Metab*, *89*, 24–28.
373. Arpradi, S., Horlick, M., & Shane, E. (2004). Editorial: metabolic bone disease in human immunodeficiency virus-infected children. *J Clin Endocrinol Metab*, *89*, 21–23.
374. Rouster-Stevens, K. A., & Klein-Gitelman, M. S. (2005). Bone health in pediatric rheumatic disease. *Curr Opin Pediatr*, *17*, 703–708.
375. Rauch, F., & Bishop, N. (2008). Juvenile osteoporosis. In C. J. Rosen (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (7th ed.) (pp. 264–267). Washington, DC: American Society of Bone and Mineral Metabolism.
376. Hartikka, H., Makitie, O., Mannikko, M., et al. (2005). Heterozygous mutations in the LDL receptor-related protein 5 (LRP5) gene are associated with primary osteoporosis in children. *J Bone Miner Res*, *20*, 783–789.
377. Glass, D. A., II, & Karsenty, G. (2007). Minireview: in vivo analysis of Wnt signaling in bone. *Endocrinology*, *148*, 2630–2634.
378. Baron, R., & Rawadi, G. (2007). Minireview: targeting the Wnt/β-catenin pathway to regulate bone formation in the adult skeleton. *Endocrinology*, *148*, 2635–2643.
379. Ke, H. Z., Richards, W. G., Li, X., & Ominsky, M. S. (2012). Sclerostin and Dikkopf-1 as therapeutic targets in bone diseases. *Endocr Rev*, *33*, 747–783.
380. Ai, M., Heeger, S., Barteks, C. F., et al. (2005). Clinical and molecular findings in osteoporosis-pseudoglioma syndrome. *Am J Hum Genet*, *77*, 741–753.
381. Balesman, W., & Van Hul, W. (2012). Minireview: the genetics of low density Lipoprotein Receptor-Related Protein 5 in bone: a story of extremes. *Endocrinology*, *148*, 2622–2629.
382. Tuysuz, B., Bursali, A., Alp, Z., et al. (2012). Osteoporosis-pseudoglioma syndrome: three novel mutations in the LRP5 gene and response to bisphosphonate treatment. *Horm Res Paediatr*, *77*, 115–120.
383. Zemel, B. S., Leonard, M. B., Kelly, A., et al. (2010). Height adjustment in assessing dual energy x-ray absorptiometric measurements of bone mass and density in children. *J Clin Endocrinol Metab*, *95*, 1265–1273.
384. Topor, L. S., Melvin, P., Giancaterino, C., & Gordon, C. M. (2013). Factors associated with low bone density in patients referred for assessment of bone health. *Intern J Pediatr Endocrinol*, *2013*, 4.
385. Rauchenzauner, M., Schmid, A., Heinz-Erian, P., et al. (2007). Sex- and age-specific reference curves for serum markers of bone turnover in healthy children 2 months to 18 years. *J Clin Endocrinol Metab*, *92*, 443–449.
386. Nishizawa, Y., Ohia, H., Miora, M., et al. (2013). Guidelines for the use of bone metabolic markers in the diagnosis and treatment of osteoporosis (2012 edition). *J Bone Miner Metab*, *31*, 1–15.
387. Kleerekoper, M. (2008). Overview of osteoporosis treatment. In C. J. Rosen (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (7th ed.) (pp. 220–221). Washington, DC: American Society of Bone and Mineral Metabolism.
388. Guo, B., Peng, S., Liang, C., et al. (2012). Recent developments in bone anabolic therapy for osteoporosis. *Expert Rev Endocrinol Metab*, *7*, 677–685.
389. Lindsay, R. (2008). Estrogens and SERMS. In C. J. Rosen (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (7th ed.) (pp. 234–236). Washington, DC: American Society of Bone and Mineral Metabolism.
390. Zaidi, M. (2007). Skeletal remodeling in health and disease. *Nature Med*, *13*, 791–801.
391. McClung, M. R., Lewiecki, E. M., Cohen, S. B., et al. (2006). Denosumab in postmenopausal women with low bone mineral density. *N Engl J Med*, *354*, 821–831.

392. Adami S (2008). Calcitonin. In C. J. Rosen (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (7th ed.) (pp. 250–251). Washington, DC: American Society of Bone and Mineral Metabolism.
393. Rogers, M. J., Crockett, J. C., Coxon, F. P., & Monkkonen, J. (2011). Biochemical and molecular mechanisms of action of bisphosphonates. *Bone*, *49*, 34–41.
394. Whyte, M. P., Wenkert, D., Clements, K. L., et al. (2003). Bisphosphonate-induced osteopetrosis. *N Engl J Med*, *349*, 457–463.
395. Odvina, C. Y., Zerwekh, J. E., Sudhaker, R., et al. (2005). Severely suppressed bone turnover: a potential complication of alendronate therapy. *J Clin Endocrinol Metab*, *90*, 1294–1301.
396. Chan, B., & Zacharin, M. (2006). Maternal and infant outcome after pamidronate treatment of polyostotic fibrous dysplasia and osteogenesis imperfecta before conception: a report of four cases. *J Clin Endocrinol Metab*, *91*, 2107–2020.
397. Bellido, T., Ali, A. A., Gubrij, I., et al. (2005). Chronic elevation of parathyroid hormone in mice reduces expression of sclerostin by osteocytes: a novel mechanism for hormonal control of osteoblastogenesis. *Endocrinology*, *146*, 4577–4583.
398. Horwitz, M. J., Tedesco, M. B., Grundberg, C., et al. (2003). Short-term, high-dose parathyroid hormone-related protein as a skeletal anabolic agent for treatment of postmenopausal osteoporosis. *J Clin Endocrinol Metab*, *88*, 569–572.
399. Prockop, D. J. (2012). New targets for osteoporosis. *N Engl J Med*, *367*, 2353–2354.
400. Van Dijk, F. S., Pals, G., Vam Rijn, R. R., et al. (2010). Classification of osteogenesis imperfecta revisited. *Eur J Med Genet*, *53*, 1–5.
401. Starr, S. B., Roberts, T. T., & Fischer, P. R. (2010). Osteogenesis imperfecta: primary care. *Pediatr Rev*, *31*, e54–e64.
402. Cabral, W. A., Makareeva, E., Colige, A., et al. (2005). Mutations near amino end of alpha-1(I) collagen cause combined osteogenesis imperfecta/Ehlers-Danlos syndrome by interfering with N-propeptide processing. *J Biol Chem*, *280*, 19259–19269.
403. Makareeva, E., Cabral, W. A., Marini, J. C., & Leikin, S. (2006). Molecular mechanism of alpha-1(I)-osteogenesis imperfecta/Ehlers-Danlos syndrome: unfolding of an N-anchor domain at the N-terminal end of the type I collagen triple helix. *J Biol Chem*, *281*, 6463–6470.
404. Lindahl, K., Barnes, A. M., Fratzi-Zelman, N., et al. (2011). COL1 C-propeptide cleavage site mutations cause high bone mass osteogenesis imperfecta. *Hum Mutat*, *32*, 598–609.
405. Morello, R., Bertin, T. K., Chen, Y., et al. (2006). CRTAP is required for prolyl 3-hydroxylation and mutations cause recessive osteogenesis imperfecta. *Cell*, *127*, 291–304.
406. Barnes, A. M., Chang, W., Morello, R., et al. (2006). Deficiency of cartilage-associated protein in lethal osteogenesis imperfecta. *N Engl J Med*, *355*, 2757–2764.
407. Amor, I. M., Rauch, F., Gruenwald, K., Weis, M., et al. (2011). Severe osteogenesis imperfecta caused by a small in-frame deletion in CRTAP (2011). *Am J Med Genet* (Part A), *155A*, 2865–2870.
408. Cabral, W. A., Chang, W., Barnes, A. M., et al. (2007). Prolyl 3-hydroxylase 1 deficiency causes a recessive metabolic bone disorder resembling lethal/severe osteogenesis imperfecta. *Nature Genet*, *39*, 359–365.
409. Willaert, A., Malfait, F., Symoens, S., et al. (2009). Recessive osteogenesis imperfecta cause by LEPRE1 mutations: clinical documentation and identification of the splice form responsible for prolyl 3-hydroxylation. *J Med Genet*, *46*, 233–241.
410. Cabral, W. A., Barnes, A. M., Adevemo, A., et al. (2012). A founder mutation in LEPRE1 carried by 1.5% of West Africans and 0.4% of African Americans causes lethal recessive osteogenesis imperfecta. *Genet Med*, *14*, 543–551.
411. Chang, W., Barnes, A. M., Cabral, W. A., et al. (2010). Prolyl 3-hydroxylase 1 and CRTAP are mutually stabilizing in the endoplasmic reticulum collagen prolyl 3-hydroxylation complex. *Hum Molec Genet*, *19*, 223–234.
412. Van Dijk, F. S., Nesbitt, I. M., Zwikstra, E. H., et al. (2009). PPIB mutations cause severe osteogenesis imperfecta. *Am J Hum Genet*, *85*, 521–527.
413. Barnes, A. M., Carter, E. M., Cabral, W. A., et al. (2010). Lack of cyclophilin B in osteogenesis imperfecta with normal collagen folding. *N Engl J Med*, *362*, 521–528.
414. Pyott, S. M., Schwarze, U., Christiansen, H. E., et al. (2011). Mutations in PPIB (cyclophilin B) delay type I procollagen chain association and result in perinatal lethal to moderate osteogenesis imperfecta phenotypes. *Hum Mol Genet*, *20*, 1595–1609.
415. Cho, T. J., Lee, K. E., Lee, S. K., et al. (2012). A single recurrent mutation in the 5′-UTR of IFITM5 causes osteogenesis imperfecta type V. *Am J Hum Genet*, *91*, 343–348.
416. Semler, O., Garbes, L., Keupp, K., et al. (2012). A mutation in the 5′-UTR of IFITM5 creates an in-frame start codon and causes autosomal-dominant osteogenesis imperfecta type V with hyperplastic callus. *Am J Hum Genet*, *91*, 349–357.
417. Rauch, F., Moffatt, P., Cheung, M., et al. (2013). Osteogenesis imperfecta type V: marked phenotypic variability despite the presence of the IFITM5 c. -14C-T mutation in all patients. *J Med Genet*, *13*, 21–24.
418. Hanagata, H., Li, X., Morita, H., et al. (2011). Characterization of the osteoblast-specific transmembrane protein IFITM5 and analysis of IFITM5-deficient mice. *J Bone Miner Metab*, *29*, 279–290.
419. Becker, J., Semler, O., Gilissen, C., et al. (2011). Exome sequencing identifies truncating mutations in human SERPINF1 in autosomal-recessive osteogenesis imperfecta. *Am J Hum Genet*, *88*, 362–371.
420. Homan, E. P., Rauch, F., Grafe, I., et al. (2011). Mutations in SERPINF1 cause osteogenesis imperfecta VI. *J Bone Miner Res*, *26*, 2798–2803.
421. Venturi, G., Gandini, A., Monti, E., et al. (2012). Lack of expression of SERPINF1, the gene coding for pigment epithelium-derived factor, causes progressively deforming osteogenesis imperfecta with normal type I collagen. *J Bone Miner Res*, *27*, 723–728.
422. Rauch, F., Husseini, A., Roughley, P., et al. (2012). Lack of circulating pigment epithelium-derived factor is a marker of osteogenesis imperfecta type VI. *J Clin Endocrinol Metab*, *97*, E1550–E1556.
423. Christiansen, H. E., Schwarze, U., Pyott, S. M., et al. (2010). Homozygosity for a missense mutation in SERPINH1, which encodes the collagen chaperone protein HSP47, results in severe recessive osteogenesis imperfecta. *Am J Hum Genet*, *86*, 389–398.
424. Masago, Y., Hosoya, A., Kawasaki, K., et al. (2012). The molecular chaperone Hsp47 is essential for cartilage and endochondral bone formation. *J Cell Sci*, *125*, 1118–1128.
425. Widmer, C., Gebauer, J. M., Brunstein, E., et al. (2012). Molecular basis for the action of collagen-specific Hsp47/SERPINH1 and its structure-specific client recognition. *Proc Natl Acad Sci U S A*, *109*, 13243–13247.
426. Alanay, Y., Avaygan, H., Camacho, N., et al. (2010). Mutations in the gene encoding the RER protein FKBP65 cause autosomal-recessive osteogenesis imperfecta. *Am J Hum Genet*, *86*, 551–559.
427. Kelley, B. P., Malfait, F., Bonafe, L., et al. (2011). Mutations in FKBP10 cause recessive osteogenesis imperfecta and Bruck syndrome. *J Bone Miner Res*, *26*, 666–672.
428. Venturi, G., Monti, E., Dalle Carbonare, L., et al. (2012). A novel splicing mutation in FKB10 causing osteogenesis imperfecta with a possible mineralization defect. *Bone*, *50*, 343–349.
429. Barnes, A. M., Cabral, W. A., Weis, M., et al. (2012). Absence of FKBP10 in recessive type XI osteogenesis imperfecta leads to diminished collagen cross-linking and reduced collagen deposition in extracellular matrix. *Hum Mutat*, *33*, 1589–1598.
430. Shaheen, R., Al-Owain, M., Faqeih, E., et al. (2011). Mutations in FKBP10 cause both Bruck syndrome and isolated osteogenesis imperfecta in humans. *Am J Med Genet*, *155A*, 1448–1452.
431. Lapunzina, P., Aglan, M., Temtamy, S., et al. (2010). Identification of a frameshift mutation in Osterix in a patient with recessive osteogenesis imperfecta. *Am J Hum Genet*, *87*, 110–114.
432. Zhou, X., Zhang, Z., Feng, J. Q., et al. (2010). Multiple functions of Osterix are required for bone growth and homeostasis in postnatal mice. *Proc Natl Acad Sci U S A*, *107*, 12919–12924.
433. Asharani, P. V., Keupp, K., Semler, O., et al. (2012). Attenuation of BMP1 function compromises osteogenesis leading to bone fragility in humans and zebrafish. *Am J Med Genet*, *90*, 661–674.
434. Martinez-Glen, V., Valencia, M., Caparros-Martin, J. A., et al. (2012). Identification of a mutation causing deficient BMP1/mTLD proteolytic activity in autosomal recessive osteogenesis imperfecta. *Hum Mutat*, *33*, 343–350.

435. Shaheen, R., Alazami, A. M., Alshammart, M. J., et al. (2012). Study of autosomal recessive osteogenesis imperfecta in Arabia reveals a novel locus defined by TMEM38B mutation. *J Med Genet*, *49*, 630–635.
436. Volodarsky, M., Markus, Cohen, I., et al. (2013). A deletion mutation in TMEM38B associated with autosomal recessive osteogenesis imperfecta. *Hum Mutat*, *34*, 582–586.
437. Puig-Hervas, M. T., Temtamy, S., Aglan, M., et al. (2012). Mutations in PLOD2 cause autosomal-recessive connective tissue disorders within the Bruck syndrome-osteogenesis imperfecta phenotypic spectrum. *Hum Mutat*, *33*, 1444–1449.
438. Beary, J. F., III, & Chines, A. A. (2012). Osteogenesis imperfecta: clinical features and diagnosis. Retrieved from http://www.com/contents/osteogenesis-imperfecta-clinical-features-and-diagnosis
439. Shapiro, J. R., & Sponsellor, P. D. (2010). Osteogenesis imperfecta: questions and answers. *Curr Opin Pediatr*, *21*, 709–716.
440. van Dijk, F. S., Byers, P. H., Dalgleish, R., et al. (2012). EMQN best practice guidelines I the laboratory diagnosis of osteogenesis imperfecta. *Eur J Hum Genet*, *20*, 11–19.
441. Beary, J. F., III, & Chines, A. A. (2012). Osteogenesis imperfecta: management and prognosis. *UpToDate*. Retrieved from http://www.uptodate.com/contents/osteogenesis-imperfecta-management-and-prognosis
442. Bachrach, L. K., & Ward, L. M. (2009). Clinical review: bisphosphonate use in childhood osteoporosis. *J Clin Endocrinol Metab*, *94*, 400–409.
443. Land, C., Rauch, F., Munns, C. F., et al. (2006). Vertebral morphometry in children and adolescents with osteogenesis imperfecta: effect of intravenous pamidronate treatment. *Bone*, *39*, 901–906.
444. Rauch, F., Travers, R., & Glorieux, F. H. (2006). Pamidronate in children with osteogenesis imperfecta: histomorphometric effects of long-term therapy. *J Clin Endocrinol Metab*, *91*, 511–516.
445. Rauch, F., Munns, C., Land, C., & Glorieux, F. H. (2006). Pamidronate in children and adolescents with osteogenesis imperfecta: effect of treatment discontinuation. *J Clin Endocrinol Metab*, *91*, 1268–1274.
446. Ljunggren, O., Lindahl, K., Rubin, C. J., & Kindmark, A. (2011). Allele-specific gene silencing in osteogenesis imperfecta. *Endocr Devel*, *21*, 85–90.
447. Collins, M. T., Riminucci, M., & Bianco, P. (2008). Fibrous dysplasia. In C. J. Rosen (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (7th ed.) (pp. 423–427). Washington, DC: American Society of Bone and Mineral Metabolism.
448. Riddle, N. D., & Bui, M. M. (2013). Fibrous dysplasia. *Arch Pathol Lab Med*, *137*, 134–138.
449. Weinstein, L. S. (2006). $G_s\alpha$ mutations in fibrous dysplasia and McCune-Albright syndrome. *J Bone Miner Res*, *21*(Suppl. 2), P–20–P124.
450. Boyce, A. M., Chung, W. H., Yao, J., et al. (2012). Denosumab treatment for fibrous dysplasia. *J Bone Miner Res*, *27*, 1462–1470.
451. Tolar, J., Teitelbaum, S. L., & Orchard, P. J. (2004). Osteopetrosis. *N Engl J Med*, *351*, 2839–2849.
452. Pangrazio, A., Cassani, B., Guerrini, M. M., et al. (2012). RANK-dependent autosomal recessive osteopetrosis: characterization of five new cases with novel mutations. *J Bone Miner Res*, *27*, 342–351.
453. Waguespack, S. G., Hui, S. L., DiMeglio, L. A., & Econs, M. J. (2007). Autosomal dominant osteopetrosis: clinical severity and natural history of 94 subjects with a chloride channel 7 gene mutation . J Clin Endocrinol Metab, *92*, 771–778.
454. Douni, E., Rinotas, V., Makrinou, E., et al. (2012). A RANKL G278R mutation causing osteopetrosis identifies a functional amino acid essential for trimer assembly in RANKL and TNF. *Hum Molec Genet*, *21*, 784–798.
455. Sobacchi, C., Frattini, A., Guerrini, N. M., et al. (2007). Osteoclast-poor human osteopetrosis due to mutations in the gene encoding RANKL. *Nat Genet*, *39*, 960–962.
456. Roberts, C. M., Angus, J. E., Leach, I. H., et al. (2010). A novel NEMO mutation causing osteopetrosis, hypohidrotic ectodermal dysplasia and immunodeficiency (OL-HED-ID). *Eur J Pediatr*, *169*, 1403–1407.
457. Almarzooqi, S., Reed, S., Fung, B., et al. (2011). Infantile osteopetrosis and juvenile xanthogranuloma presenting together in a newborn: a case report. *Pediatr Devel Pathol*, *14*, 307–312.
458. Pangrazio, A., Caldana, M. E., Sobacchi, C., et al. (2009). Characterization of a novel Alu-Alu recombination-mediated genomic deletion in the TCIRG1 gene in five osteopetrotic subjects. *J Bone Miner Res*, *24*, 162–167.
459. Gheorge, G., Galambos, C., Jain, S., et al. (2012). A novel TCRG1 mutation leads to severe osteopetrosis with altered content of monocytes/macrophages in several organs. *Pediatr Develop Pathology*, *15*, 156–159.
460. Aker, M., Rouvinski, A., Hashavia, S., et al. (2012). An SNX10 mutation causes malignant osteopetrosis of infancy. *J Med Genet*, *49*, 221–226.
461. Weinert, S., Jabs, S., Supanchart, C., et al. (2010). Lysosomal pathology and osteopetrosis upon loss of H+-driven lysosomal Cl- accumulation. *Science*, *328*, 1401–1403.
462. Schulz, A., & Kornak, U. (2010). CLCN7-related osteopetrosis. GeneReviews Bookshelf ID: NBK1127. PMID: 20301306.
463. Kantaputra, P. H., Thawanaphong, S., Issarangporn, W., et al. (2012). Long-term survival in infantile malignant autosomal recessive osteopetrosis secondary to homozygous p. Arg526Gln mutation in CLCN7. *Am J Med Genet* (Part A), *158A*, 909–916.
464. Lange, P. F., Wartosch, L., Jentsch, T. J., & Fuhrmann, J. C. (2006). ClC-7 requires Ostm1 as a β-subunit to support bone resorption and lysosomal function. *Nature*, *440*, 220–223.
465. Chalhoub, N., Benachenhou, N., Rajapurohitam, V., et al. (2003). Grey-lethal mutation indices severe malignant autosomal recessive osteopetrosis in mouse and human. *Nature Med*, *9*, 399–406.
466. Ramirez, A., Faupel, J., Goebel, I., et al. (2004). Identification of a novel mutation in the coding region of the grey-lethal gene OSTM1 in human malignant infantile osteopetrosis. *Hum Mutat*, *23*, 471–476.
467. Tabata, K., Matsunaga, K., Sakane, A., et al. (2010). Rubicon and PLEKHM1 negatively regulate the endocytic/autophagic pathway via a novel Rab7-binding domain. *Mol Biol Cell*, *21*, 4162–4172.
468. Van Wesenbeeck, L., Odgren, P. R., Coxon, F. P., et al. (2007). Involvement of PLEKHM1 in osteoclastic vesicular transport and osteopetrosis in incisor absent rats and humans. *J Clin Invest*, *117*, 919–930.
469. Pangrazio, A., Budin, E., Piters, E., et al. (2011). Identification of the first deletion in the LRP5 gene in a patient with autosomal dominant osteopetrosis type I. *Bone*, *49*, 568–571.
470. Demirel, F., Esen, I., Tunc, B., & Tavil, B. (2010). Scarcity despite wealth: osteopetrorickets. *J Pediatr Endocrinol Metab*, *23*, 931–934.
471. Gao, Y., Grassi, F., Ryan, M. R., et al. (2007). IFN-γ stimulates osteoclast formation and bone loss in vivo via antigen-driven T cell activation. *J Clin Invest*, *117*, 122–132.
472. Stark, Z., & Savarirayan, R. (2009). Osteopetrosis. *Orphanet J Rare Dis*, *4*, 5.
473. Steward, C. G. (2010). Hematopoietic stem cell transplantation in osteopetrosis. *Pediatr Clin, NA*, *57*, 171–180.
474. Shroff R Beringer, O., Rao, K., et al. (2012). Denosumab for post-transplant hypercalcemia in osteopetrosis. *N Engl J Med*, *367*, 1766–1767.
475. Susani, L., Pangrazio, A., Sobachhi, A., et al. (2004). TCIRG1-dependent recessive osteopetrosis: mutation analysis, functional identification of the splicing defects, and in vitro rescue by U1 snRNA. *Hum Mutat*, *24*, 225–235.
476. Askmyr, M., Flores, C., Fasth, A., & Richter, J. (2009). Prospects for gene therapy of osteopetrosis. *Curr Gene Ther*, *9*, 150–259.
477. Ramaiah, K. K. K., George, G. B., Padiyath, S., et al. (2011). Pyknodysostosis: report of a rare case with review of literature. *Imaging Sci Dent*, *41*, 177–181.
478. Boyden, L. M., Mao, J., Belsky, J., et al. (2002). High bone density due to a mutation in LDL-receptor-related protein 5. *N Engl J Med*, *346*, 1513–1521.
479. Van Wesenbeeck, L., Cleiren, E., Gram, J., et al. (2003). Six novel missense mutations in the LDL receptor-related protein 5 (LRP5) gene in different conditions with increased bone density. *Am J Hum Genet*, *72*, 763–771.
480. Van Lierop, A. H., Hamdy, N. A. T., van Bezooijen, R. L., et al. (2012). The role of sclerostin in the pathophysiology of sclerosing bone dysplasias. *Clinic Rev Bone Miner Metab*, *10*, 108–116.

481. Gardner, J. C., van Bezooijen, R. L., Mervis, B., et al. (2005). Bone mineral density in sclerosteosis: affected individuals and gene carriers. *J Clin Endocrinol Metab*, *90*, 6392–6395.
482. Loots, G. B., Kneissel, M., Keller, H., et al. (2005). Genomic deletion of a long-range bone enhancer misregulates sclerostin in Van Buchem disease. *Genome Res*, *15*, 928–935.
483. Leupin, O., Piters, E., Halleux, C., et al. (2011). Bone overgrowth-associated mutations in the LRP4 gene impair sclerostin facilitator function. *J Biol Chem*, *286*, 19489–19500.
484. Janssens, K., Vanhoenacker, F., Bonduelle, M., et al. (2007). Camurati-Engelmann disease: review of the clinical, radiological, and molecular data of 24 families and implications for diagnosis and treatment. *J Med Genet*, *43*, 1–11.
485. Ihde, L. L., Forrester, D. M., Gottsegen, C. J., et al. (2011). Sclerosing bone dysplasias: review and differentiation from other causes of osteosclerosis. *RadioGraphics*, *31*, 1865–1882.
486. Janssens, K., ten Dyke, P., Janssens, S., & Van Hul, W. (2005). Transforming growth factor-beta 1 to the bone. *Endocr Rev*, *26*, 743–774.
487. Shore, E. M., Xu, M., Feldman, G. J., et al. (2006). A recurrent mutation in the BMP type I receptor ACVR1 causes inherited and sporadic fibrodysplasia ossificans progressiva. *Nature Genet*, *38*, 525–527.
488. Hammond, P., Suttie, M., Hennekam, R. C., et al. (2012). The face signature of fibrodysplasia ossificans progressiva. *Am J Med Genet* (Part A), *158A*, 1368–1380.
489. Bocciardi, R., Bordo, D., Di Duca, M., et al. (2009). Mutational analysis of the ACVR1 gene in Italian patients affected with fibrodysplasia ossificans progressiva: confirmation and advancements. *Eur J Hum Genet*, *17*, 311–318.
490. Kaplan, F. S., Fiori, J., Serrano de la Pena, L., et al. (2006). Dysregulation of the BMP-4 signaling pathway in fibrodysplasia ossificans progressiva. *Ann NY Acad Sci*, *1068*, 54–65.
491. Barnett, C. P., Dugar, M., & Haan, E. A. (2011). Late-onset variant fibrodysplasia ossificans progressiva leading to misdiagnosis of ankylosing spondylitis. *Am J Med Genet* (Part A), *155A*, 1492–1495.
492. Kaplan, F. S., Glaser, D. L., Shore, E. M., et al. (2007). Hematopoietic stem-cell contribution to ectopic skeletogenesis. *J Bone Joint Surg*, *89*, 347–357.
493. Chan, I., Hamada, T., Hardman, C., et al. (2004). Progressive osseous heteroplasia resulting from a new mutation in the GNAS1 gene. *Clin Exper Dermatol*, *29*, 77–80.
494. Lebrun, M., Richard, N., Abeguile, G., et al. (2010). Progressive osseous heteroplasia: a model for the imprinting effects of GNAS inactivating mutations. *J Clin Endocrinol Metab*, *95*, 3028–3038.
495. Whyte, M. P. (2006). Extracellular (ectopic) calcification and ossification. In M. J. Favus (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (6th ed.) (pp. 436–437). Washington, DC: American Society for Bone and Mineral Research.
496. Shaw, N. (2008). Tumoral calcinosis: dermatomyositis. In C. J. Rosen (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (7th ed.) (pp. 433–435). Washington, DC: American Society of Bone and Mineral Metabolism.
497. Farrow, E. G., Imel, E. A., & White, K. E. (2011). Miscellaneous non-inflammatory musculoskeletal conditions. Hyperphosphatemic familial tumoral calcinosis (FGF23, GALNT3 and Klotho). *Best Pract Res Clin Rheumatol*, *25*, 735–747.
498. Slavin, R. E., Wen, J., & Barmada A (2012). Tumoral calcinosis—a pathogenetic overview: a histological and ultrastructural study with report of two new cases, one in infancy. *Int J Surg Pathol*, *20*, 462–473.
499. Ichikawa, S., Baujat, G., Sevahi, A., et al. (2010). Clinical variability of familial tumoral calcinosis caused by novel GALNT3 mutations. *Am J Med Genet*, *152A*, 896–903.
500. Frishberg, Y., Ito, N., Rinat, C., et al. (2007). Hyperostosis-hyperphosphatemia syndrome: a congenital disorder of O-glycosylation associated with augmented processing of fibroblast growth factor 23. *J Bone Miner Res*, *22*, 235–242.
501. Garringer, H. J., Fisher, C., Larsson, T. E., et al. (2006). The role of mutant UDP-N-acetyl-alpha-D-galactosamine-polypeptide N-acetylgalactosaminyltransferase 3 in regulating serum intact fibroblast growth factor 23 and matrix extracellular phosphoglycoprotein in heritable tumoral calcinosis. *J Clin Endocrinol Metab*, *91*, 4037–4041.
502. Ichikawa, S., Imel, E. A., Kreiter, M. L., et al. (2007). A homozygous missense mutation in human KLOTHO causes severe tumoral calcinosis. *J Clin Invest*, *117*, 2684–2691.
503. Chefetz, I., Ben Amital, D., Browning, S., et al. (2008). Normophosphatemic familial tumoral calcinosis is caused by deleterious mutations in SMD9, encoding a TNF-alpha responsive protein. *J Invest Dermatol*, *128*, 1423–1429.
504. Hershkovitz, D., Gross, Y., Nahum, S., et al. (2011). Functional characterization of SAMD9, a protein deficient in normophosphatemic familial tumoral calcinosis. *J Invest Dermatol*, *131*, 662–669.
505. Alanay, Y., & Rimoin, D. L. (2008). Chondrodysplasias. In C. J. Rosen (Ed.), *Primer on the metabolic bone diseases and disorders of mineral metabolism* (7th ed.) (pp. 428–429). Washington, DC: American Society of Bone and Mineral Metabolism.
506. Warman, M. L., Cormier-Daire, V., Hall, C., et al. (2011). Nosology and classification of genetic skeletal disorders: 2010 revision. *Am J Med Genet* (Part A), *155A*, 943–968.
507. Horton, W. A. (2006). Molecular pathogenesis of achondroplasia. *Growth Genet Horm*, *22*, 49–54.
508. Hari Kumar KVS, Shaikh, A., Sharma, R., & Prusty, P. (2011). SADDAN syndrome. *J Pediatr Endocrinol Metab*, *24*, 851–852.
509. Natacci, F., Baffico, M., Cavallari, U., et al. (2008). Germline mosaicism in achondroplasia detected in sperm DNA of the father of three affected sibs. *Am J Hum Genet*, *146A*, 784–786.
510. Pannier, S., Martinovic, J., Heuertz, S., et al. (2009). Thanatophoric dysplasia caused by double missense FGFR3 mutations. *Am J Hum Genet A*, *149A*, 1296–1301.
511. Heuertz, S., Le Merrer, M., Zabel, B., et al. (2006). Novel FGFR3 mutations creating cysteine residues in the extracellular domain of the receptor cause achondroplasia or severe forms of hypochondroplasia. *Eur J Hum Genet*, *14*, 1240–1247.
512. Toydemir, R. M., Brassington, A. E., Bayrack-Toydemir, P., et al. (2006). A novel mutation in FGFR3 causes camptodactyly, tall stature, and hearing loss (CATSHL) syndrome. *Am J Hum Genet*, *79*, 935–941.
513. He, L., Horton, W., & Hristova, K. (2010). Physical basis behind achondroplasia, the most common form of human dwarfism. *J Biol Chem*, *285*, 30103–30114.
514. Su, N., Sun, Q., Lu, X., et al. (2010). Gain-of-function mutation in FGFR3 in mice leads to decreased bone mass by affecting both osteoblastogenesis and osteoclastogenesis. *Hum Molec Genet*, *19*, 1199–1210.
515. Saito, H., Sekizawa, A., Morimoto, T., et al. (2000). Prenatal DNA diagnosis of a single gene disorder from maternal plasma. *Lancet*, *356*, 1170–1171.
516. Pitteloud, N., Acierno, J. S., Jr., Meysing, A., et al. (2006). Mutations in fibroblast growth factor receptor 1 cause both Kallmann syndrome and idiopathic hypogonadotropic hypogonadism. *Proc Natl Acad Sci U S A*, *103*, 6281–6286.
517. Nino, M., Matos-Miranda, C., Maeda, M., et al. (2008). Clinical and molecular analysis of arylsulfatase E in patients with brachytelephalangic chondrodysplasia punctata. *Am J Med Genet A*, *146A*, 997–1008.
518. Marchini, A., Hacker, B., Marttila, T., et al. (2007). BNP is a transcriptional target of the short stature homeobox gene SHOX. *Hum Molec Genet*, *16*, 3081–3087.
519. Benito-Sanz, S., Thomas, N. S., Huber, C., et al. (2005). A novel class of pseudoautosomal region 1 deletions downstream of SHOX is associated with Leri-Weill dyschondrosteosis. *Am J Hum Genet*, *77*, 534–544.
520. Thiel, C. T., Mortier, G., Kaitila, I., et al. (2007). Type and level of RMP functional impairment predicts phenotype in the cartilage hair hypoplasia-anauxetic dysplasia spectrum. *Am J Hum Genet*, *81*, 519–529.
521. Thiel, C. T., & Rauch, A. (2011). The molecular basis of the cartilage-hair hypoplasia-anauxetic dysplasia spectrum. *Best Pract Res Clin Endocrinol Metab*, *25*, 131–142.
522. Forbes, F. D., & Herman, G. E. (2011). Malformation syndromes caused by disorders of cholesterol synthesis. *J Lipid Res*, *52*, 6–34.
523. DeBarber, A. E., Eroglu, Y., Merkens, L. S., et al. (2011). Smith-Lemli-Opitz syndrome. *Expert Rev Molec Med*, *13*, e24.

524. Koo, G., Conley, S. K., Wassif, C. A., & Porter, F. D. (2010). Discordant phenotype and sterol biochemistry in Smith-Lemli-Opitz syndrome. *Am J Med Genet A*, *152A*, 2094–2098.
525. Schaaf, C. P., Koster, J., Katsonis, P., et al. (2011). Desmosterolosis-phenotypic and molecular characterization of a third case and review of the literature. *Am J Med Genet A*, *155A*, 1597–1604.
526. Zolotushko, J., Flusser, H., Markus, B., et al. (2011). The desmosterolosis phenotype: spasticity, microcephaly and micrognathia with agenesis of the corpus callosum and loss of white matter. *Eur J Hum Genet*, *19*, 942–946.
527. Wassif, C. A., Brownson, K. E., Sterner, A. L., et al. (2007). HEM dysplasia and ichthyosis are likely laminopathies and not due to 3 beta-hydroxysterol delta 14-reductase deficiency. *Hum Mol Genet*, *16*, 1176–1187.
528. Bilezikian, J. P., Khan, A., Potts, J. T., Jr., et al. (2011). Hypoparathyroidism in the adult: epidemiology, diagnosis, pathophysiology, target-organ involvement, treatment, and challenges for future research. *J Bone Mineral Res*, *26*, 2317–2337, Figure 2.
529. Steenkamp, D., & Lee, S. L. (2011). Hypercalcemic crisis in a young woman. *Endocrine Today*, October, Figure 7.
530. Imel, E. A., & Econs, M. J. (2012). Approach to the hypophosphatemic patient. *J Clin Endocrinol Metab*, *97*, 696–706.
531. Farrow, E. G., & White, K. E. (2010). Recent advances in renal phosphate handling. *Nature Rev/Nephrol*, *6*, 207–217.
532. Bishop, N. (2010). Characterising and treating osteogenesis imperfecta. *Early Human Devel*, *86*, 743–746.
533. Rauch, F., Lalic, L., Roughley, P., & Glorieux, F. H. (2010). Relationship between genotype and skeletal phenotype in children and adolescents with osteogenesis imperfecta. *J Bone Miner Res*, *25*, 1367–1374.
534. Nesbit, M. A., Hannan, F. M., Howles, S. A., et al. (2013). Mutations affecting G-protein subunit α_{11} in hypercalcemia and hypocalcemia. *N Engl J Med*, *368*, 2476–2486.
535. Mannstadt, M., Harris, M., Bravenboer, B., et al. (2013). Germline mutations affecting $G\alpha_{11}$ in hypoparathyroidism. *N Engl J Med*, *368*, 2532–2534.
536. Nesbit, M. A., Hannan, F. M., Howles, S. A., et al. (2013). Mutations in AP2S1 cause familial hypocalciuric hypercalcemia type 3. *Nature Genet*, *45*, 93–97.
537. Isojima T, Doi K, Mitsui J, et al. (2013) A recurrent de novo FAM111A mutation causes Kenny-Caffey syndrome type 2. *J Bone Miner Res*. doi: 10.1002/jbmr.2091.
538. Unger, S., Gorna, M.V.V., Le Bechec, A., et al. (2013). FAM111A mutations result in hypoparathyroidism and impaired skeletal development. *Am J Hum Genet*, *92*, 990–995.
539. Laine, C.M., Joeng, K.S., Campeau, P.M., et al. (2013). WNT1 mutations in early-onset osteoporosis and osteogenesis imperfecta. *New Eng J Med*, *368*, 1809–1816.
540. Pyott, S.M., Tran, T.T., Leistritz, D.F., et al. (2013). WNT1 mutations in families affected by moderately severe and progressive recessive osteogenesis imperfecta. *Am J Hum Genet*, *92*, 590–597.
541. Keupp, K., Beleggia, F., Kayserili, H., et al. (2013). Mutations in WNT1 cause different forms of bone fragility. *Am J Hum Genet*, *92*, 565–574.

QUESTIONS

1. Pathophysiologically, what causes hypocalcemia?
   a. Abnormalities in the packaging of parathyroid hormone (PTH) within the Golgi apparatus of the parathyroid chief cell
   b. A subnormal rate of transit of parathyroid hormone (PTH) through the endoplasmic reticulum to the plasma membrane
   c. Inhibition of *PTH* transcription
   d. Increased sensitivity of the calcitonin receptor to its endogenous ligand
   e. Impaired release of PTH from the parathyroid chief cell

Answer: e

2. Pseudohypoparathyroidism type IA is the result of monoallelic variations in which of the following?
   a. Paternal transcript of *PTH1R* (PTH/PTHrP receptor)
   b. Maternal genes encoding both adenylyl cyclase (*ADC1*) and protein kinase B (*AKT1*)
   c. Product of paternal *MAP2K1* (encoding mitogen-activated protein kinase kinase 1)
   d. Maternal Gαs transcript of *GNAS* (guanine nucleotide-binding protein, alpha-stimulating activity polypeptide)
   e. Maternal transcript of *TBCE* (encoding tubulin specific chaperone E)

Answer: d

3. In the majority of patients, neonatal severe hyperparathyroidism is the result of which genetic variation?
   a. Biallelic insertion of the amino acid glycine at codon 73 of paternal *CASR* (encoding the calcium sensing receptor)
   b. Monoallelic paternal activating mutation in *CASR*
   c. Biallelic loss-of-function mutations in *CASR*
   d. Monoallelic deletion of the entire maternal *CASR* gene
   e. Mutations in the 5′ regulatory region of *CASR*

Answer: c

4. What results from a loss of renal 24-hydroxylase activity?
   a. Hypophosphatemia
   b. Hypercalcemia
   c. Osteopenia
   d. Rickets
   e. Osteosclerosis

Answer: b

5. Which of the following describes the Williams-Beuren syndrome of infantile hypercalcemia?
   a. It is characterized by mitral valve prolapse, extremely fine facial features, and overgrowth.
   b. It is related to loss-of function of *BAZ1B* (encoding the Williams syndrome transcription factor).
   c. It is the result of increased functional activity of WINAC (WSTF including nucleoside assembly complex).
   d. It is dependent on increased activity of hepatic 25-hydroxylase.
   e. It is transmitted as a sex-linked recessive trait.

Answer: b

6. Which of the following is true about phosphopenic rickets due to loss-of-function mutations in *PHEX* (encoding phosphate-regulating endopeptidase homologue, X linked)?
   a. It is manifested radiographically by extremely fragile long bone with decreased cortical width.
   b. It is pathogenetically related to a functional abnormality of the vitamin D nuclear receptor.
   c. It is associated with decreased production of fibroblast growth factor-23 (FGF23).
   d. It adversely affects membrane bone formation primarily.
   e. It is due to failure to prevent the release and degradation of phosphorylated acidic serine aspartate-rich MEPE-associated motif (ASARM).

Answer: e

7. What causes the metabolic bone disease that develops in children with chronic renal insufficiency?
   a. Decreased secretion of FGF23
   b. Excessive osteoblast synthesis of collagen type I
   c. Impaired renal excretion of phosphate
   d. Increased renal tubular calcium reabsorption in response to α-klotho
   e. Subnormal activity of the parathyroid chief cells

Answer: c

8. Which of the following is true about bisphosphonates?
   a. In excess, they paradoxically decrease bone density.
   b. They are administered by deep intramuscular injection.
   c. They act by incorporating into bone marrow precursor cells, thus prolonging their life span.
   d. They augment bone mineralization by increasing osteoblastogenesis.
   e. They are retained in bone for many years.

Answer: e

CHAPTER 19

Diabetes Mellitus

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CHAPTER OUTLINE

INTRODUCTION

Diabetes mellitus (DM) is best defined as a syndrome characterized by inappropriate fasting or postprandial hyperglycemia, caused by absolute or relative insulin deficiency and its metabolic consequences, which include disturbed metabolism of protein and fat. This syndrome results from a combination of deficiency of insulin secretion and its action. Diabetes mellitus occurs when the normal constant of the product of insulin secretion times insulin sensitivity, a parabolic function termed the "disposition index" (Figure 19-1), is inadequate to prevent hyperglycemia and its clinical consequences of polyuria, polydipsia, and weight loss. At high degrees

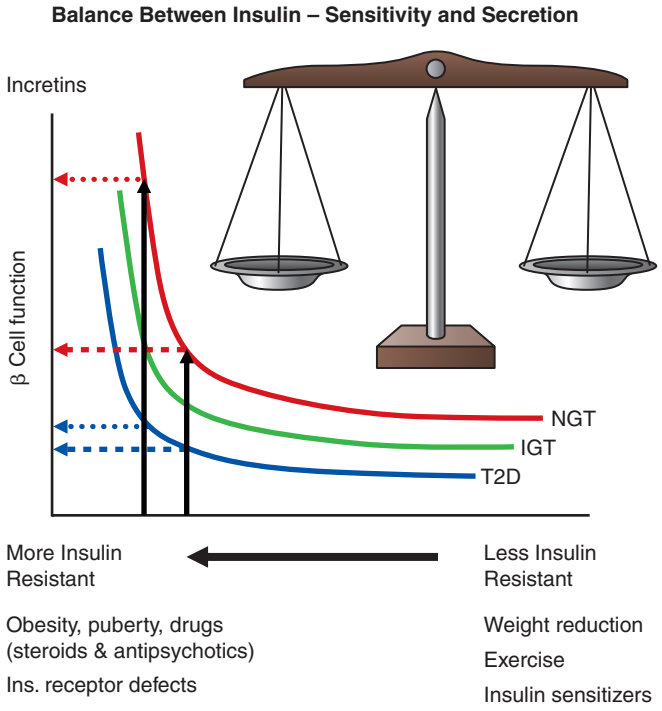


FIGURE 19-1 ■ **This figure shows the hyperbolic relationship of insulin resistance and beta cell function. On the y-axis is beta cell function as reflected in the first-phase insulin response during intravenous (IV) glucose infusion; on the x-axis is insulin sensitivity and its mirror image resistance. In a subject with normal glucose tolerance (NGT) and beta-cell reserve, an increase in insulin resistance results in increased insulin release and normal glucose tolerance. In an individual for whom the capacity to increase insulin release is compromised, increasing insulin resistance with partial or no beta-cell compensation results in progression from normal glucose tolerance, to impaired glucose tolerance (IGT), and finally to diabetes (T2D). Differences between these categories are small at high insulin sensitivity, which may be maintained by weight reduction, exercise, and certain drugs. At a critical degree of insulin resistance, due to obesity or other listed factors, only a further small increment in resistance requires a large increase in insulin output. Those that can increase insulin secretion to this extent retain normal glucose tolerance; those who cannot achieve this degree of insulin secretion (e.g., due to a mild defect in genes regulating insulin synthesis, insulin secretion, insulin action, or an ongoing immune destruction of beta cells) now unmask varying degrees of carbohydrate intolerance. The product of insulin sensitivity (the reciprocal of insulin resistance) and acute insulin response (a measurement beta-cell function) has been called the "disposition index." This index remains constant in an individual with normal beta cell compensation in response to changes in insulin resistance. IGT, impaired glucose tolerance; NGT, normal glucose tolerance; T2D, type 2 diabetes.** (From Ize-Ludlow, D., & Sperling, M. A. (2005). The classification of diabetes mellitus: a conceptual framework. *Pediatr Clin North Am, 52*, 1533–1552.)

of insulin sensitivity, small declines in the ability to secrete insulin cause only mild, clinically imperceptible defects in glucose metabolism. However, irrespective of insulin sensitivity, a minimum amount of insulin is necessary for normal metabolism. Thus, near absolute deficiency of insulin must result in severe metabolic disturbance as occurs in type 1 diabetes mellitus (T1DM). By contrast, with decreasing sensitivity to its action, higher amounts of insulin secretion are required for a normal disposition index. At a critical point in the disposition index curve (see Figure 19-1), a further small decrement in insulin sensitivity requires a large increase in insulin secretion; those who can mount these higher rates of insulin secretion retain normal glucose metabolism, whereas those who cannot increase their insulin secretion because of genetic or acquired defects now manifest clinical diabetes as occurs in type 2 diabetes (T2DM).

By simultaneously considering insulin secretion and insulin action in any given individual, it becomes possible to account for the natural history of diabetes in that person (e.g., remission in a patient with T1 diabetes or ketoacidosis in a person with T2DM). Thus, diabetes mellitus may be the result of absolute insulin deficiency, or of absolute insulin resistance, or a combination of milder defects in both insulin secretion and insulin action.[1] Collectively, the syndromes of diabetes mellitus are the most common endocrine/metabolic disorders of childhood and adolescence. The application of molecular biologic tools continues to provide remarkable insights into the etiology, pathophysiology, and genetics of the various forms of diabetes mellitus that result from deficient secretion of insulin or its action at the cellular level.

Morbidity and mortality stem from the metabolic derangements and from the long-term complications that affect small and large vessels, resulting in retinopathy, nephropathy, neuropathy, ischemic heart disease, and arterial obstruction with gangrene of extremities.[2] The acute clinical manifestations can be fully understood in the context of current knowledge of the secretion and action of insulin.[3] Genetic and other etiologic considerations implicate autoimmune mechanisms in the evolution of the most common form of childhood diabetes, known as type 1a diabetes.[4,5] Genetic defects in insulin secretion are increasingly recognized and understood as defining the causes of monogenic forms of diabetes such as maturity-onset diabetes of youth (MODY) and neonatal DM and contributing to the spectrum of T2DM.[6]

There is strong evidence that the long-term complications are related to the degree and duration of metabolic disturbances.[2] These considerations form the basis of standard and innovative therapeutic approaches to this disease that include newer pharmacologic formulations of insulin, delivery by traditional and more physiologic means, and evolving methods to continuously monitor blood glucose to maintain it within desired limits by linking these features to algorithm-driven insulin delivery pumps for an "artificial pancreas."

CLASSIFICATION

Diabetes mellitus is not a single entity but a heterogeneous group of disorders in which there are distinct genetic patterns as well as other etiologic and pathophysiologic mechanisms that lead to impairment of glucose tolerance.[1,7] Box 19-1 outlines an etiologic classification of diabetes mellitus in children, based on the "Report of the Expert Committee on the Classification and Diagnosis of Diabetes Mellitus," published by the American Diabetes Association in January of 2013.[7]

BOX 19-1 Etiologic Classification of Diabetes Mellitus

I. Type 1 Diabetes (Beta-Cell Destruction Ultimately Leading to Complete Insulin Deficiency)
- A. Immune mediated
- B. Idiopathic

II. Type 2 Diabetes (Variable Combinations of Insulin Resistance and Insulin Deficiency)
- A. Typical
- B. Atypical

III. Genetic Defects of Beta-Cell Function
- A. MODY syndromes
  1. MODY 1 chromosome 20, HNF-4α
  2. MODY 2 chromosome 7, glucokinase
  3. MODY 3 chromosome 12, HNF-1α, TCF-1
  4. MODY 4 chromosome 13, IPF-1
  5. MODY 5 chromosome 17, HNF-1β, TCF-2
  6. MODY 6 chromosome 2q32, neuro-D1/beta-2
- B. Mitochondrial DNA mutations (includes one form of Wolfram syndrome, Pearson syndrome, Kearns-Sayre, diabetes mellitus, deafness)
- C. Wolfram syndrome—DIDMOAD (diabetes insipidus, diabetes mellitus, optic atrophy, deafness): WFS1-Wolframin—chromosome 4p
  1. Wolfram locus 2—chromosome 4q22-24
  2. Wolfram mitochondrial
- D. Thiamine responsive megaloblastic anemia and diabetes

IV. Drug or Chemical Induced
- A. Antirejection—cyclosporine, sirolimus
- B. Glucocorticoids (with impaired insulin secretion; e.g., cystic fibrosis)
- C. L-Asparaginase
- D. β-Adrenergic blockers
- E. Vacor (rodenticide)
- F. Phenytoin (dilantin)
- G. Alfa-interferon
- H. Diazoxide
- I. Nicotinic acid
- J. Others

V. Diseases of Exocrine Pancreas
- A. Cystic fibrosis–related diabetes
- B. Trauma—pancreatectomy
- C. Pancreatitis—ionizing radiation
- D. Others

VI. Infections
- A. Congenital rubella
- B. Cytomegalovirus
- C. Hemolytic-uremic syndrome

VII. Variants of Type 2 Diabetes
- A. Genetic defects of insulin action
  1. Rabson-Mendenhall syndrome
  2. Leprechaunism
  3. Lipoatrophic diabetes syndromes
  4. Type A insulin resistance—acanthosis
- B. Acquired defects of insulin action
  1. Endocrine tumors—rare in childhood
- C. Pheochromocytoma
- D. Cushing
- E. Others
  1. Anti-insulin receptor antibodies

VIII. Genetic Syndromes with Diabetes and Insulin Resistance/Insulin Deficiency
- A. Prader-Willi syndrome, chromosome 15
- B. Down syndrome, chromosome 21
- C. Turner syndrome
- D. Klinefelter syndrome
- E. Others
  1. Bardet-Biedel
  2. Alstrom
  3. Werner

IX. Gestational Diabetes

X. Neonatal Diabetes
- A. Transient—chromosome 6q24, KCNJ11, ABCC8, INS, HNF1β, others
- B. Permanent—agenesis of pancreas—glucokinase deficiency, homozygous, KCNJ11, ABCC8, others (see Table 9-1)

Our classification is modified to reflect more accurately the major categories in childhood, including the emergence of type 2 diabetes mellitus, cystic fibrosis-related diabetes, and drug-induced diabetes—largely from the antirejection agents cyclosporine, sirolimus, and tacrolimus (formerly FK-506). Table 19-1 presents a summary of the classification originally proposed in 1979 but incorporates the newer criteria for blood glucose values used to diagnose diabetes, impaired glucose tolerance, and gestational diabetes.

Among the insulin-dependent forms, severe lack of insulin secretion results most commonly from presumed autoimmune destruction of islets in genetically predisposed hosts. This form is synonymous with type 1a diabetes, formerly called juvenile-onset diabetes.[4,5,8,9] Severe insulin-dependent diabetes mellitus, clinically indistinguishable from the autoimmune form, may, however, not have any evidence of autoimmunity and can result from mitochondrial or other gene defects that interfere with normal insulin secretion or rarely from pancreatic agenesis.[10-13]

The more severe forms of the MODY syndromes, subsequently detailed, also may require insulin.[12,13] Clinically similar forms of diabetes may occur secondary to cystic fibrosis[14,15] from toxic drugs such as the immunosuppressive agents cyclosporine, sirolimus and tacrolimus,[16,17] the rodenticide Vacor,[18] or streptozotocin as used for certain pancreatic islet cell tumors[19]; with the hemolytic uremic syndrome[20]; or after pancreatectomy, such as for persistent hyperinsulinemic hypoglycemia in infancy.[21] Childhood insulin-dependent diabetes is generally type 1a diabetes mellitus.

TABLE 19-1 **Summary of Classification of Diabetes Mellitus in Children and Adolescents**

Category	Criteria
Diabetes Mellitus	
Type 1	Typical symptoms: glucosuria, ketonuria; random plasma glucose > 200 mg/dL
Type 2	Fasting plasma glucose > 126 mg/dL with 2-hour intervening value > 200 mg/dL on OGTT more than once and in the absence of precipitating factors
Other types	Type 1 or 2 criteria with genetic syndrome, drug therapy; pancreatic disease or other known causes or associations
Impaired fasting glucose	Glucose > 110 mg/dL but < 126 mg/dL
Impaired glucose tolerance	Fasting plasma glucose < 126 mg/dL with 2-hour > 140 mg/dL but < 200 mg/dL on OGTT
Gestational diabetes	Two or more abnormal fasting plasma glucose levels > 105 mg/dL, 1-hour > 180 mg/dL, 2-hour > 155 mg/dL, 3-hour > 140 mg/dL on OGTT
Statistical Risk Classes	
Previous abnormality of glucose tolerance	Normal OGTT with previously abnormal OGTT, spontaneous hyperglycemia, or gestational diabetes
Potential abnormality of glucose tolerance	Genetic propensity (e.g., identical twin with diabetes mellitus); islet cell antibodies

Type 1 Diabetes Mellitus

This condition is characterized by severe insulinopenia and dependence on exogenous insulin to prevent ketosis and to preserve life. Thus, it was termed insulin-dependent diabetes mellitus (IDDM). The natural history of this disease indicates that there are preketotic non–insulin-dependent phases before and after the initial diagnosis. Although the onset is predominantly in childhood, the disease may occur at any age.[1] Therefore, such names as "juvenile diabetes," "ketosis-prone diabetes," and "brittle diabetes" were abandoned in favor of the term *type 1 diabetes*.

Type 1a diabetes is generally distinct by virtue of its association with certain histocompatibility locus antigens (HLAs) and other genetic markers the majority of which determine the response to self (or exogenous) antigens; by the presence of circulating antibodies to cytoplasmic and cell-surface components of islet cells; of antibodies to insulin in the absence of previous exposure to exogenous injection of insulin, of antibodies to glutamic acid decarboxylase (GAD, the enzyme that converts glutamic acid to γ-aminobutyric acid found abundantly in the innervation of pancreatic islets), of antibodies to IA-2 (an islet cell-associated phosphatase and antibodies to the zinc transporter molecule ZnT8); by lymphocytic infiltration of islets early in the disease; and by coexistence with other autoimmune diseases.[4,5] Occasionally, markers of autoimmunity are not found and yet there is profound insulinopenia and dependence on insulin without evidence of a mitochondrial or other genetic defect. In these cases, type 1 diabetes is considered idiopathic (type 1b). With the exceptions noted, diabetes in children is usually insulin dependent and fits the type 1a category.[1]

Type 2 Diabetes

Persons with this subclass of diabetes (formerly known as "adult-onset diabetes," "maturity-onset diabetes" [MOD], or "stable diabetes") may not be permanently insulin dependent and only occasionally develop ketosis. Some may, however, need insulin to correct symptomatic hyperglycemia—and ketosis may develop in some during severe infections or other stress. Therefore, this was previously called non–insulin-dependent diabetes mellitus (NIDDM).[1] This form of diabetes is becoming an increasing problem in overweight adolescents, especially those from vulnerable groups such as Africans, Mexicans, Native Indians, and other susceptible ethnic groups.[22,23]

Type 2 diabetes (T2DM) is not a single entity.[1] T2DM may be a primary disorder, with inadequate insulin secretion caused by mutations in one of several genes encoding enzymes or transcription factors important to islet cell development and insulin secretion. Several of these defects are now part of the spectrum of the syndromes commonly associated with maturity onset diabetes of youth (MODY), which has a dominant mode of inheritance.[12,13,24] However, some patients with MODY defects, which we term *monogenic diabetes of youth*, may require insulin from

the outset or as they grow older and become insulin resistant, exceeding their ability to compensate by increasing insulin secretion (see Figure 19-1). A defect in the gene regulating glucose transport into the pancreatic beta cell, the GLUT2 transporter, may be responsible for another form of type 2 diabetes.[24]

Defects in glycogen synthase have also been implicated.[25,26,] A primary defect in insulin receptors—often associated with acanthosis nigricans,[27] postreceptor defects (including Rad [Ras associated with diabetes]),[28] and milder mitochondrial gene defects[10]—also may result in type 2 diabetes. Secondary causes of type 2 diabetes mellitus include excessive counterregulatory hormones, especially pharmacologic doses of glucocorticoids, antibodies to the insulin receptor, and obesity with impaired insulin secretion.[29-40]

In type 2 diabetes mellitus, the serum concentration of insulin may be increased, normal, or moderately depressed depending on whether the defect is one of insulin action or secretion.[27-40] The onset of type 2 diabetes mellitus occurs in children generally around the time of puberty or shortly thereafter, but it is recognized, that it may occur at any age and is becoming increasingly frequent in childhood and adolescence.[22,23] In some instances, there appears to be adequate secretion of insulin but resistance to its actions, and in some individuals it may represent slowly evolving type 1 diabetes mellitus.[38] As an initial approach, weight reduction is indicated in children who are obese. In type 2 diabetes, there is no association with specific HLA antigens, autoimmunity, or various islet cell antibodies (ICAs).[40] However, several genetic abnormalities regulating insulin secretion are increasingly implicated in T2DM.[40]

TYPE 1 DIABETES MELLITUS

Epidemiology

The prevalence of diabetes mellitus is highly correlated with increasing age. Available data indicate a range of 1 case per 1430 children at 5 years of age to 1 case in 360 children at 16 years.[41-45] Data on incidence in relation to racial or ethnic backgrounds indicate a range of more than 50 new cases annually per 100,000 population in Finland and Sardinia to about 1 per 100,000 in China and parts of South America[42-46] (Figure 19-2). In all examined areas there appears to be an increasing incidence of T1DM of about 2% to 3% per year. However, the incidence of T1DM in Finland was reported to have peaked and slightly decined.[41]

In the United States, the occurrence of type 1 in blacks had previously been reported to be only between one third and two thirds of that in whites.[46] More recent data suggest that the incidence of diabetes mellitus in African Americans is increasing.[46,47] It is not clear, however, whether this increase in incidence among African Americans is exclusively type 1 or includes cases of type 2 presenting in ketoacidosis and thus misclassified.[46] The annual incidence in the United States is 20 to 25 cases per 100,000 of the childhood population[44] (see www.cdc.gov/diabetes/projects/diabetes/children.htm and Figure 19-2).

Males and females appear to be almost equally affected. There is no apparent correlation with socioeconomic status. Peaks of presentation occur in two age groups: at 5 to 7 years of age and at the time of puberty. The first peak corresponds to the time of increased exposure to infectious agents coincident with the beginning of school. The latter corresponds to the pubertal growth spurt induced by increased pubertal growth hormone secretion that antagonizes insulin action. The incidence of type 1 diabetes is increasing worldwide, most prominently in certain populations (e.g., Finland) and in certain age groups (especially those younger than age 5 years).[44,45] As mentioned, there appears to be a plateau in incidence reported from Finland.[41] In younger patients, onset appears to be more abrupt and the extent of immune markers is less apparent than in older children.[48] Type 1b diabetes with abrupt onset, less evidence of autoimmunity, and indicators of viral infection (including evidence of pancreatitis) has been described in Japan.[49,50]

Seasonal and long-term cyclical variations have been noted in the incidence of type 1 diabetes. Newly recognized cases appear to occur with greater frequency in the autumn and winter in the northern and southern hemispheres.[51] Seasonal variations are most apparent in the adolescent years.[51] There is no consistent pattern linking long-term cyclicity with the incidence of viral infections; however, there is a definite increased incidence of diabetes in children with congenital rubella.[52,53] These changing patterns in incidence and associations with viral infections suggest a potential role for viruses or other microbial agents or their products as direct or indirect triggering mechanisms for inducing T1DM in a susceptible host.[52-57]

Etiology, Pathogenesis, and Genetics

The cause of the initial clinical findings in this predominant form of diabetes in childhood is the sharply diminished secretion of insulin.[58] Although basal insulin concentrations in plasma may be normal in newly diagnosed patients, insulin production in response to a variety of potent secretagogues is blunted and usually disappears over a period of months to years. In certain individuals considered at high risk for the development of type 1 diabetes, such as the nonaffected identical twin of a diabetic, a progressive decline in insulin-secreting capacity has been noted for months to years before the clinical appearance of symptomatic diabetes that usually manifests when insulin-secreting reserve is 20% or less than normal for that individual (Figure 19-3).[4,5,58]

The mechanisms that lead to failure of the function of pancreatic beta cells point to autoimmune destruction of pancreatic islets in predisposed individuals. Type 1 diabetes has long been known to have an increased prevalence among persons with such disorders as Addison disease and Hashimoto thyroiditis, in whom autoimmune mechanisms are known to be pathogenic.[58] These conditions, as well as type 1 diabetes mellitus, are known to be associated with an increased frequency of genes involved in the regulation of immunity including the autoimmune regulator gene AIRE, PTPN22, CTLA4, and INS gene itself as well as certain histocompatibility loci antigens

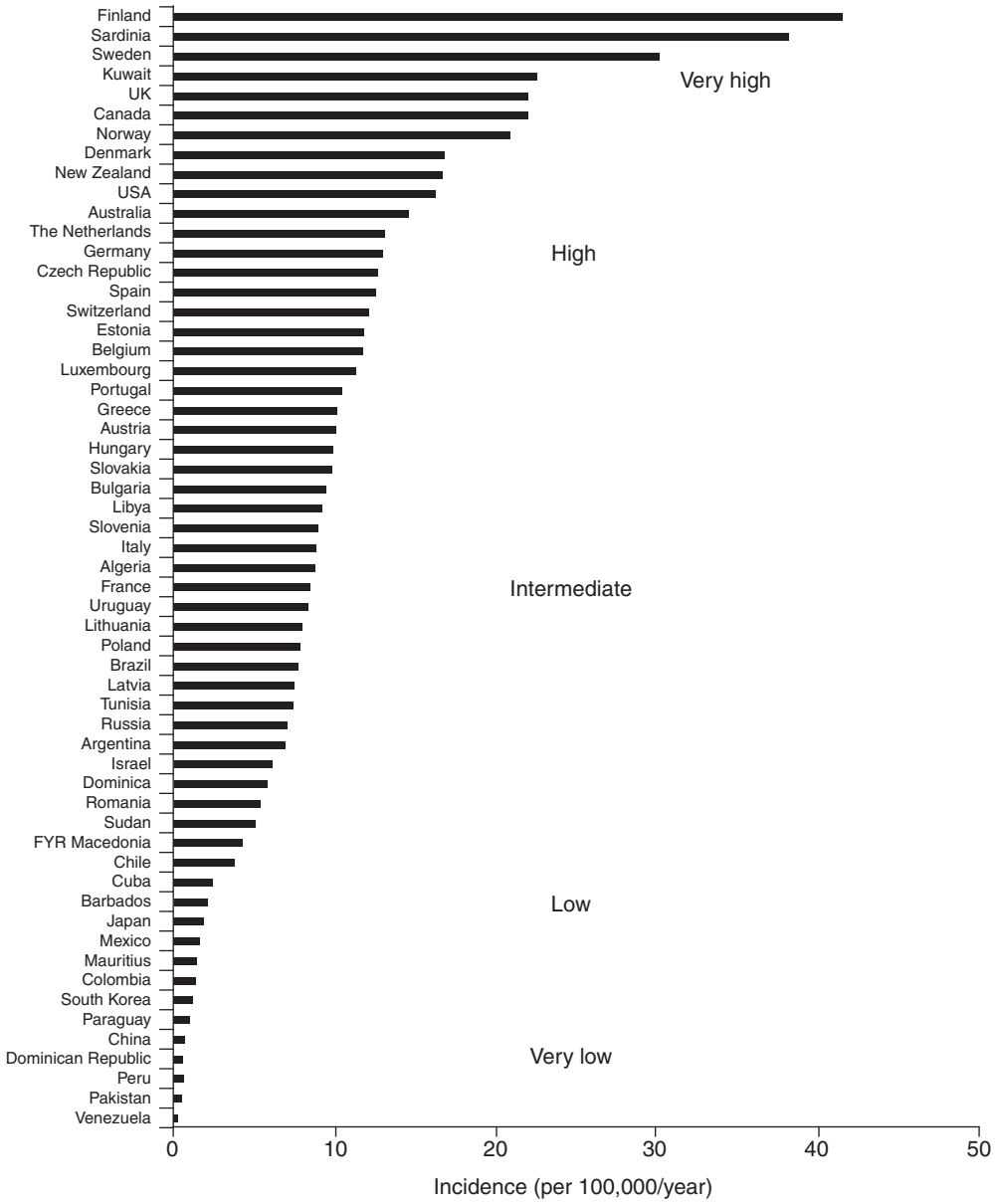


FIGURE 19-2 ■ Age-standardized incidence (per 100,000/year) of type 1 diabetes in children younger than 14 years of age in 100 populations. Data for boys and girls have been pooled. Countries are arranged in descending order according to the incidence. (Puerto Rico and the Virgin Islands are presented separately from other populations in the United States.) (From Diamond Project Group (2006). Incidence of trends of childhood type1 diabetes worldwide 1990-1999. *Diabet Med, 23,* 857–866.)

(HLAs)—in particular, DR3 and DR4.[58,59] Located on chromosome 6, the HLA system is the major histocompatibility complex—consisting of a cluster of genes that code transplantation antigens and play a central role in immune responses.[58-70]

Increased susceptibility to a number of diseases has been related to one or more of the identified HLA antigens. The inheritance of HLA DR3 or DR4 confers a twofold to threefold increased risk for developing type 1 diabetes. When both DR3 and DR4 are inherited, the relative risk for developing diabetes is increased sevenfold to tenfold. Application of newer molecular genetic techniques has revealed further heterogeneity in the HLA D region among individuals with and without diabetes despite possessing the DR3 or DR4 markers, suggesting the participation of other susceptibility loci within these markers.[58-70]

Extensive genome-wide scans of markers associated with type 1 diabetes mellitus have uncovered more than a 40 loci considered to confer susceptibility (Table 19-2). Some of these loci are confirmed and replicated by at least three different data sets. Others are suggestive but as yet not definitively linked. The strongest markers are those on chromosomes 6 and 11 (IDDM1 and IDDM2), respectively, linked to the HLA DQ β chain and the insulin gene itself.

In IDDM1, the homozygous absence of aspartic acid at position 57 of the HLA DQ *b* chain (non-Asp/non-Asp) confers an approximately 100-fold relative risk for developing type 1 diabetes. Those who are heterozygous

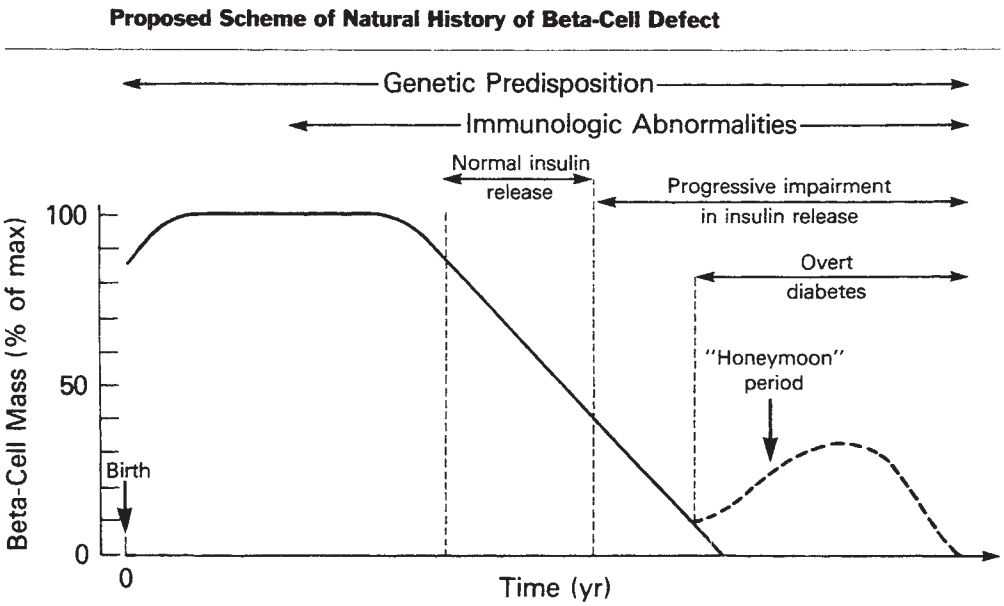


FIGURE 19-3 ■ Proposed scheme of natural history of the evolution of insulin-dependent diabetes mellitus with progressive beta-cell failure. (From Sperling, M. A. (Ed.) (1988). *Physician's guide to insulin-dependent (type 1) diabetes mellitus: diagnosis and treatment.* Alexandria, VA: American Diabetes Association.)

TABLE 19-2 **Genome-Wide Association Study and Meta-Analysis Find More Than 40 Loci Affect Risk of Type 1 Diabetes**

Loci	Approx. Relative Risk	Effect on
HLA	6.5	Immunity
INS	2.3	Insulin production and metabolism
PTPN22	2.0	Immunity
ILR2A	1.5	Immunity
SH2BE	1.3	Immunity
ERBB3	1.3	Insulin production and metabolism
PTPN2	1.25	Immunity
CLEC16A	1.20	Unknown function
CTLA4	1.20	Immunity
IL18RAP	1.20	Immunity
PTPN2	1.20	Immunity
OCR5	1.20	Immunity
IFIHI	1.20	Immunity
CTSH	1.20	Unknown
CD226	1.10	Immunity
IL2RA	1.10	Immunity
PRKCQ	1.10	Immunity
IL2	1.10	Immunity
BACH2	1.10	Immunity
UBASH3A	1.10	Immunity
RGS1	1.10	Immunity
IL7RA	1.10	Immunity
CITNF6	1.10	Unknown
TNFAIP3	1.10	Beta-cell apoptosis protection
TAGAP	1.10	Immunity

Adapted from Barrett, J. C., Clayton, D. G., Concannon, P., et al. for the Type 1 Diabetes Genetics Consortium (2009). Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes. Nat Genet, 41, *703–707.*

with a single aspartic acid at position 57 (non-Asp/Asp) are less likely to develop diabetes and are no more susceptible than individuals who contain aspartic acid on both DQ *b* chains (i.e., homozygous Asp/Asp; Table 19-3). Some studies suggest that type 1 diabetes mellitus is proportional to the gene frequency of non-Asp alleles in that population.[69] In addition, arginine at position 52 of the DQ *a* chain confers marked susceptibility to type 1.[63] Position 57 of the DQ *b* and position 52 of the DQ *a* chains are at critical locations of the HLA molecule that permit or prevent antigen presentation to T-cell receptors and activate the autoimmune cascade (Figure 19-4).[60-63]

IDDM2 is a polymorphic marker near the transcription start site of the insulin gene, giving rise to variable numbers of tandem repeats (VNTR) at the promoter end of the insulin gene on chromosome 11. Each tandem repeat element consists of an approximately 14-bp DNA segment with a consensus nucleotide sequence. The number of repeats ranges from about 25 to about 200, and the three classes of alleles are based on overall size. Class I insulin VNTR consists of 26 to 63 repeats and confers susceptibility, whereas class III consists of 140 to 200 or more repeats and is protective of diabetes. Together, the gene markers on chromosomes 6 and 11 (i.e., IDDM1 and IDDM2) account for 50% to 60% of the heritability of type 1 diabetes. However, combinations of certain DQ alleles in association with certain DR alleles may confer susceptibility or protection to the development of type 1 diabetes (see Table 19-3).

In addition, other as yet undefined genetic factors play a role because the same high-risk genotypes are about sixfold more likely to develop diabetes in an individual with a positive family history than in one without a family history without type 1 diabetes (Table 19-4). Investigation of four genome-wide linkage scans in close to 1500 families with more than one affected member having

TABLE 19-3 **HLA DR and DQ Phenotype Frequencies in Patients with Type 1 Diabetes Mellitus and Healthy Control Subjects**

Phenotype	Diabetic (%)	Nondiabetic	Odds Ratio (%)
DR (Serology)			
DR3/DR4	33	6	8.3
DR3/DR3	7	1	9.8
DR3/DRX	7	14	0.05
DR4/DR4	26	0	—
DR4/DRX	22	16	1.5
DRX/DRX	4	63	0.02
DQ (Molecular Probes)			
Non-Asp/non-Asp	96	19	107.2
Non-Asp/Asp	4	46	0.04
Asp/Asp	0	34	0

Based on Morel, P. A., Dorman, J. S., Todd, J. A., et al. (1988). Aspartic acid at position 57 of the HLA-DQ beta chain protects against type 1 diabetes: a family study. Proc Natl Acad Sci USA, 85, *8111.*

T1DM identified several susceptibility loci. Of these, about 40% can still be attributed to allelic variation of HLA loci, and the influence of the VNTR in the insulin gene was confirmed.[59,64]

In addition, the cytotoxic T-lymphocyte antigen 4 (CTLA4) gene on chromosome 2 and the protein tyrosine phosphatase nonreceptor 22 (PTPN22) gene on chromosome 1p13 were found to contribute significantly to predisposition to T1DM. However, the genome scan identified other potential loci conferring susceptibility on chromosomes 2q31-q33, 10p14-q11, and 16q220q24 and a locus on the long arm of chromosome 6 (6q21) distinct from the HLA region on 6p21. The precise genes in these regions that may predispose to T1DM have not been identified as yet, although some have been excluded[59] and newer candidate genes—such as CBLB interacting with CTLA4,[66] the decay-accelerating factor gene (DAF, a complement inhibitor),[67] and the interleukin 2A receptor IL2RA—are under scrutiny.[63]

These considerations provide a rational framework for the long-recognized association of type 1 diabetes with genetic factors on the bases of the increased incidence in some families, of the concordance rates in monozygotic twins, and of ethnic and racial differences in prevalence.[59,63,70] From multiple family pedigrees and HLA typing data, it has been estimated that if a sibling shares both HLA D haplotypes with an index case the risk for that individual is 12% to 20%; for a sibling sharing only one haplotype, the risk for IDDM is 5% to 7%; and with no haplotypes in common, the risk is only 1% to 2%.[70] HLA typing is not recommended for routine practice, but for purposes of genetic counseling it can be safely assumed that in whites the overall recurrence risks to siblings is approximately 6% if the proband is younger than 10 years of age and 3% if older at the time of diagnosis. The risk to offspring is 2% to 5%, with the higher risk in the offspring of a diabetic father. [63,70]

Factors other than pure inheritance must also be involved in evoking clinical diabetes. For example, DR3 or DR4 is found in approximately 50% of the general population and non-Asp/non-Asp is found in approximately 20% of white nondiabetics in the United States. However, the risk for type 1 diabetes in these subjects is only one tenth that in an HLA-identical sibling of an index case with type 1 diabetes possessing these markers.[70] Even siblings sharing only one haplotype have a sixfold to tenfold greater risk of developing type 1 compared with the normal population (see Table 19-4).

Importantly, approximately 10% to 15% of patients with type 1 do not have HLA DR3 or DR4 (see Table 19-3).[58] Most compelling is the fact that the concordance rate among identical twins of whom one has insulin-dependent diabetes is only about 50%, suggesting the participation of environmental triggering factors or other genetic factor such as the postnatal selection of certain autoreactive T-cell clones that bear receptors recognizing "self." This postnatal process occurs within the thymus and implies that identical twins are not identical with respect to the T-cell receptor repertoire they possess.

Triggering factors might include viral infections.[54-57] In animals, a number of viruses can cause a diabetic syndrome, the appearance and severity of which depend on the genetic strain and immune competence of the species of animal tested. In humans, epidemics of mumps, rubella, and coxsackievirus infections have been associated with subsequent increases in the incidence of type1 diabetes. The acute onset of diabetes mellitus, presumably induced by coxsackievirus B4, has been described.[57] The viruses may act by directly destroying beta cells, by persisting in pancreatic beta cells as slow viral infections, or by triggering a widespread immune response to several endocrine tissues.[57]

A superantigen response may be involved in triggering T cells, bypassing the classic presentation by antigen-presenting cells (APCs) of the processed antigen in the context of restricted HLA molecules to T-cell receptors.[71] Some viruses and certain endotoxins or exotoxins are capable of inducing a superantigen response. In addition, the virus may induce initial beta cell damage—which

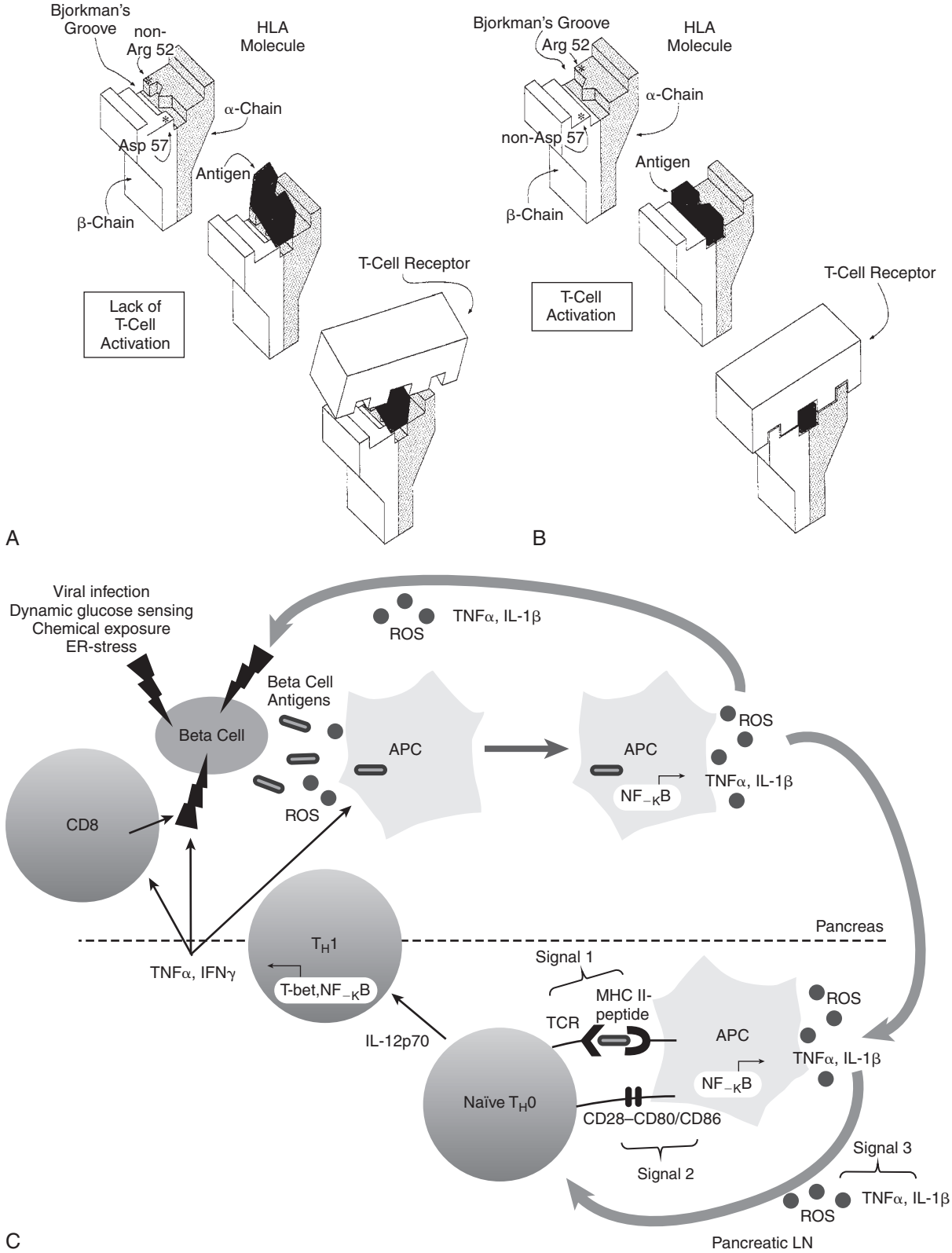


FIGURE 19-4 ■ Representation of the interaction between antigen presentation in the context of specific HLA-DQ subtypes and the T-cell receptor. **A,** Presence of aspartic acid at position 57 of the DQ *b* chain and an amino acid other than arginine at position 52 of the *a* chain prevents antigen lodging in the Bjorkman groove. Therefore, antigen presentation to the T-cell receptor is impaired—and in the absence of this "fit," T-cell activation is prevented. **B,** Lack of aspartic acid at position 57 of the DQ *b* chain and arginine at position 52 of the DQ *a* chain permits antigen to fit and be recognized by the T-cell receptor that is now activated. **C,** Role of redox in the immunopathology of type 1 diabetes. An initial genetic or environmental insult to the beta cell triggers the release of beta-cell antigens as well as the production of reactive oxygen species (ROS). Beta-cell antigens are phagocytosed, and ROS are able to stimulate redox-dependent transcription factors such as NF-κB, which leads to antigen presenting cell (APC) activation and cytokine secretion. ROS and proinflammatory cytokines secreted by APCs act as the third signal within the T-cell-APC immunologic synapse, which occurs in the pancreatic lymph node. ROS play a critical role in the progression of naïve TH0 cells to cytokine-secreting TH1 cells. Release of IFNγ by TH1 cells then works directly on the beta cells as well as activating more APCs and CD8 cells, all of which can impart deleterious effects on the islets. (**A** and **B** from Trucco M (1995). To be or not to be Asp 57, that is the question. Diabetes Care 15:705; Faas S, Trucco M (1995). The genes influencing the susceptibility to IDDM in humans. J Endocrinol Invest 17:477; **C** from Delmastro, M. M., & Piganelli, J. D. (2011). Oxidative stress and redox modulation potential in type 1 diabetes. *Clin Dev Immunol*, 2011. doi: 10.1155/2011/593863.)

TABLE 19-4 **Genetic Risk Estimates for HLA Class II in Type 1 Diabetes Mellitus**

High-Risk Genotypes	Risk in an Individual with This Genotype
DQB1p0302 (DQ3.2)	1 in 60
DQ3.2/DQ2 (DR3)	1 in 25
DQB1p03021 family history of IDDM	1 in 10
DQ3.2/DQ2 (DR3)1 family history of IDDM	1 in 4
Complete sharing of both HLA haplotypes	1 in 2*

*Individual is a sibling of patient with T1DM.

Adapted from Nepom, G. T. (1995). Class II antigens and disease susceptibility. Ann Rev Med, 46, *17; Aly, T. A., Ide, A., Jahromi, M. M., et al. (2006). Extreme genetic risk for type 1A diabetes.* Proc Natl Acad Sci U S A, 103, *14, 10474–10479.*

results in the presentation of previously masked or altered antigenic determinants. It is also possible that the virus shares some antigenic determinants with those present on or in beta cells, including GAD, such that antibodies formed in response to the virus may interact with these shared determinants of beta cells, resulting in their destruction, an example of molecular mimicry.[72-79]

Nitrosamines and early exposure to cow's milk have been suggested as factors that may trigger diabetes in those genetically at risk, thus explaining the reported lower incidence of diabetes among exclusively breastfed infants. This is the basis for one ongoing primary prevention study: the Trial to Reduce Insulin-dependent Diabetes in Those Genetically at Risk (TRIGR).[72-79] Antecedent stress and exposure to certain chemical toxins have been implicated in the development of type 1 diabetes. Although the rodenticide Vacor has been a cause of diabetes in individuals deliberately or inadvertently poisoned by this agent, some of these patients had islet cell antibodies (ICAs), suggesting that such antibodies are secondary to islet damage or that evolving type 1 disease preceded the drug ingestion. Nitrosamines in cured meat have also been implicated in type 1 diabetes, as have other environmental toxins.[72,80]

Evidence supports an autoimmune basis for the development of type 1 diabetes. but why the beta cell is the specific target remains a mystery.[43,81] Is the pancreatic beta cell the sole target of immune destruction (homicide) or a contributor to its own demise (suicide)? [43,81] Histologic examination of pancreas from patients with type 1 who die of incidental causes has revealed lymphocytic infiltration around the islets of Langerhans. Later the islets become progressively hyalinized and scarred, a process suggesting an ongoing inflammatory response that is possibly autoimmune.[82] However, these changes are often patchy in distribution, so that areas that appear to contain normal beta cells are interspersed with areas of beta-cell destruction, similar to the patchy distribution of depigmentation found in vitiligo.[58,83] Eighty percent to 90% of newly diagnosed patients with type 1 diabetes have ICA directed at cell surface or cytoplasmic determinants in their islet cells. The prevalence of these antibodies decreases with the duration of established disease. In contrast, after pancreatic transplantation ICA may reappear in patients whose sera had become negative for ICA before transplantation. Taken together, these findings suggest that ICA disappears as the antigens intrinsic to pancreatic islets are destroyed and reappear when fresh antigen (transplanted islets) is presented.

Studies in identical twins and in family pedigrees demonstrate that the existence of ICA may precede by months to years the appearance of symptomatic type 1 diabetes.[4] In vitro, ICA may impair insulin secretion in response to secretagogues and can be shown to be cytotoxic to islet cells—especially in the presence of complement or T cells from patients with type 1 diabetes. About 80% of patients may have antibodies to GAD, and 30% to 40% of newly diagnosed patients have spontaneous anti-insulin antibodies at initial diagnosis. These antibodies may be detected months to years before clinical diabetes becomes apparent.[4,60,75,81] A more recently described antibody, the zinc transporter, ZnT8, appears to be a major marker for progressive impairment of beta-cell function.[4] There is also evidence of abnormal T-cell function with an alteration in the ratio of suppressor (regulatory) to killer T cells at the onset of the disease.[4,5,8] Thus, the ability of Treg to modify the activity of T effector cells in causing beta-cell destruction is an area of investigation. These findings suggest that type 1 diabetes (akin to other autoimmune diseases such as Hashimoto thyroiditis) is a disease of "autoaggression" in which autoantibodies in cooperation with complement, T cells, cytokines, FAS, and FAS's ligand, and other factors, induce apoptosis or destruction of the insulin-producing islet cells.[5-8] Thus, the inheritance of certain genes (such as those associated with the HLA system on chromosome 6 or other immunoregulatory or immunomodulatory genes) appears to confer a predisposition for autoimmune disease—including diabetes—when triggered by an appropriate stimulus such as a virus.[4,5,9] Evidence of superantigen-triggered T-cell receptor activation was discussed earlier.[71]

Although it is understood that some insulin-dependent diabetic patients have none of the frequently associated HLA antigens, the evidence for an immune basis of islet cell destruction is sufficiently compelling to have fostered several studies of different immunosuppressive agents in the treatment of newly diagnosed diabetics (Table 19-5). None of these immunosuppressive or immunomodulatory agents has had long-term positive outcome, and some (e.g., cyclosporine) have proven toxic to beta cells.

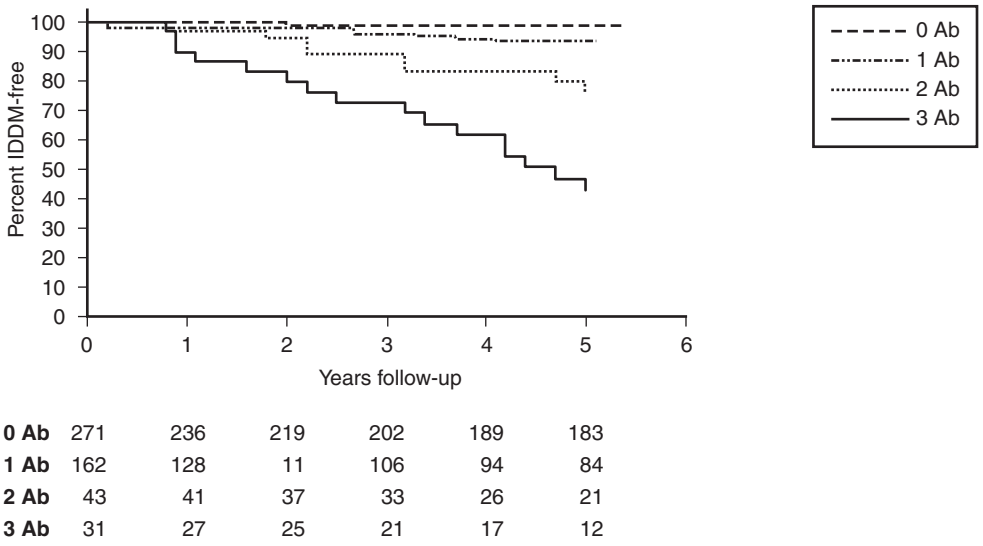
TABLE 19-5 **Partial List of Past and Ongoing Trials in Type 1 Diabetes***

Intervention	Outcome	Study Status
Prevention Trials		
Cow's milk protein avoidance (TRIGR)	Pilot, promising	Fully powered trial enrolled, results pending
Gluten avoidance	Pilot, inconclusive	
Omega-3 fatty acid supplementation (NIP)	Pilot, did not reach primary outcome	
Vitamin D supplementation	Pilot, promising	
Parenteral insulin (DPT-1)	No effect	
Oral insulin (DPT-1)	Did not reach primary outcome; promising in subset of subjects	Enrolling confirmatory study
Intranasal insulin	Finnish study, no effect	Study enrolling in Australia (INIT II)
Nicotinamide (ENDIT)	No effect	
Anti-CD3 mAb		Currently enrolling
CTLA-4 Ig		Currently enrolling
Preservation Trials		
Anti-CD3 mAb	Series of phase 2 and 3 studies, promising	
CTLA-4 Ig	Phase 2, promising	
LFA-3 Ig	Phase 2, promising, under-enrolled and just missed primary endpoint	
ATG, cyclophosphamide, G-CSF	Three phase 1 studies, promising efficacy but safety concerns	
ATG	Phase 2, did not meet primary endpoint	
G-CSF	Small phase 2	Fully enrolled with results pending
ATG + G-CSF	Small phase 2	Fully enrolled with results pending
IL-2 + rapamycin	Phase 1, transient decrease in C-peptide	Study discontinued
IL-2, low dose		Fully enrolled with results pending
Polyclonal regulatory T cells	Two phase 1 studies, promising	North American study currently enrolling
Umbilical cord blood	Phase 1, no effect	
Anti-CD20 mAb	Phase 2, promising	
Anti-IL - 1 mAb	Phase 2, no effect	
Anti-IL - 1 receptor	Phase 2, no effect	
Alpha - 1 antitrypsin	Pilot, promising	Phase 2, fully enrolled with results pending
GAD-alum vaccine	Phase 2, 3 studies, no effect	
Diapep277 vaccine	Phase 2, 3 studies, mixed results	
Sitagliptin + lansoprazole	Phase 2	Fully enrolled with results pending
Intense metabolic control	Phase 2, no effect	

ENDIT, European Nicotinamide Diabetes Intervention Trial; DPT-1, Diabetes Prevention Trial; GAD, glutamic acid decarboxylase; INIT II, Intranasal Insulin Trial II; mAb, monoclonal antibody; NIP, Nutritional Intervention to Prevent Type 1 Diabetes; TRIGR, Trial to Reduce Insulin-Dependent Diabetes Mellitus in the Genetically at Risk.

From Thomas, H. R., & Gitelman, S. E. (2013). Altering the course of type 1 diabetes: an update on prevention and new-onset clinical trials. Pediatr Diabetes, 14, *311–321.*

Although newer approaches are being attempted, all must be considered as experimental and not be viewed as established or recommended therapy.[82-89] The Diabetes Prevention Trial for T1DM (DPT1) was a multicenter randomized but nonblinded study using daily subcutaneous insulin and an annual admission for intravenous insulin infusion in first-degree relatives with proven risk factors for developing T1DM. Whereas prediction was highly accurate in identifying those most likely to develop T1DM within 5 years of entering the study, insulin injections had no protective effect to prevent the appearance of T1DM.[82-89] Figures 19-3 and 19-4 summarize current concepts of the cause of type1 diabetes as an autoimmune disease, the tendency for which is inherited and in which autoimmune destruction of beta cells is triggered by an as yet unidentified agent (possibly a virus). The slope of decline in insulin varies, and there may be periods of partial recovery such that the course of decline in insulin secretion is bumpy rather than smooth. The point at which clinical features appear corresponds to approximately 80% destruction of insulin secretory reserve. This process may take months to years in adolescent and



0 Ab	271	236	219	202	189	183
1 Ab	162	128	11	106	94	84
2 Ab	43	41	37	33	26	21
3 Ab	31	27	25	21	17	12

FIGURE 19-5 ■ Survival analysis showing conversion to insulin-dependent diabetes mellitus (IDDM) in a subset of first-degree relatives according to the presence of 0, 1, 2, or 3 autoantibodies to islet antigens (ICA, GAD65, and IA-2 autoantibodies). (From Rosenbloom, A. L., Schatz, D. A., Krischer, J. P., et al. (2000). Therapeutic controversy: prevention and treatment of diabetes in children. *J Clin Endocrinol Metab, 85*, 494. Copyright © The Endocrine Society.)

older patients, and weeks in the very young patient in whom acute destruction by non-autoimmune mechanisms may play a significant role. Higher titers of spontaneous anti-insulin antibodies and ICA are characteristic of more active islet cell destruction, typically in the younger patient, and may prove useful in predicting evolving diabetes.[82-89]

Prediction and Prevention

Although no presently available single marker or test can accurately predict type 1 diabetes mellitus, evidence suggests that a combination of immune and genetic markers for type1 diabetes may provide predictability.[82-89] Some authorities suggest that type1 diabetes is a predictable disease, but other authorities have raised objections because predictability is not as robust in their studies. Definitive preventive therapy is not available, thereby raising ethical dilemmas, and the majority of new cases occur sporadically in the absence of a positive family history in a first-degree relative.

Most predictive studies have been performed in first-degree relatives of patients with new-onset type 1 diabetes.[74] Nevertheless, there is increasing evidence that the presence of high titers of islet cell, GAD, IA2, ZnT8, and insulin autoantibodies combined with a consistently diminished first-phase response of insulin to a pulse of intravenous glucose (corresponding to the fifth percentile or less for age in insulin response) can be used to reliably predict the onset of type 1 disease.[82-89]

Figure 19-5 demonstrates that in a set of first-degree relatives, conversion to type1 diabetes was highly dependent on the number of antibodies detected in their sera. Of those with three antibodies, about half developed clinical diabetes within 5 years of follow-up. First-phase insulin response and genetic (HLA) markers may be used to augment the predictability. For example, Table 19-6 demonstrates that the relative risk of developing clinical diabetes within 4 years of detecting ICA is almost 230 among those who possess all four heterodimers in HLA DQ β that predispose to diabetes (i.e., Asp57 2/2 and Arg52 1/1).

As technologic improvements continue, it is likely that population-wide screening for antibody markers (alone or combined with specific genetic markers) will be available to identify those at risk for developing type 1 diabetes.

TABLE 19-6 **Influence of Diabetic Heterodimers (ASP57neg Arg52pos) and Islet Cell Antibody (ICA) Status on Relative Risk for Developing Diabetes after 4 Years**

	ICA Negative				ICA Positive			
Diabetic heterodimers (dH)	0	1	2	4	0	1	2	4
Developed IDDM after 4 years	12	16	37	12	12	18	29	15
Relative risk	1.0	2.9	8.6	25.4	9.0	26.5	78.0	229.3

Adapted from Friday, R. P., Trucco, M., & Pietropaolo, M. (1999). Genetics of type 1 diabetes mellitus. Diabetes Nutr Metab, 12, *3.*

Such population-wide screening would be ethically justified if prevention could be proven effective. Presently, the data are sufficiently persuasive to have fostered national trials in Europe and the United States to predict and possibly prevent the clinical onset of type 1 diabetes through immune intervention strategies (see Table 19-5).

The European Nicotinamide Diabetes Intervention Trial (ENDIT) was a multicenter trial that screened approximately 22,000 first-degree relatives of patients with type 1 diabetes to identify 500 considered to be at high risk for developing this disease.[86-89] These at-risk individuals were treated with nicotinamide or a placebo in a double-blind fashion. The purported advantage of nicotinamide was that it was not known to be toxic or harmful in humans at the recommended doses; its major disadvantage was that the proposed protective effects in delaying diabetes were based on a small sample cohort. The results of ENDIT were disappointing, with positive protection not apparent.

The U.S. Diabetes Prevention Trial for T1DM (DPT1) was based on promising pilot data that suggested preservation of insulin secretion and prevention of progression to diabetes mellitus in at-risk individuals treated with insulin.[85] Daily subcutaneous insulin, coupled with intensive intravenous insulin every 9 months, prevented diabetes for at least 3 years in five subjects considered to be at risk because of genetic markers, islet cell and insulin autoantibodies, and diminished first-phase insulin response. Among seven similar at-risk subjects who chose not to be treated, six developed insulin-dependent diabetes within 3 years. DPT-1 was concluded in 2001, and there was no difference in the rates of developing diabetes among the placebo and insulin-treated groups. However, the ENDIT and DPT1 studies proved that large-scale multicenter studies could be successfully undertaken and that the prediction of progression to clinical diabetes was remarkably accurate. Thus, in those at highest risk (such as first-degree relatives of patients with T1DM) prediction is feasible and the discovery of a successful means of arresting or reversing progression to clinical diabetes is the subject of intense research.

Another study (TRIGR) involves 3000 families in whom half will avoid cow's milk for the first 9 months of life to test the hypothesis that the ingestion of breast milk and avoidance of cow's milk formula (with its bovine serum albumin [BSA]) may protect participants from the appearance of diabetes.[78] Several other studies are examining the utility of antibodies to the IL2 receptor, CD3 antibodies, immune suppressors such as mycophenolate mofetil, and immune modulators in preventing diabetes, including oral insulin. These studies are conducted by worldwide consortia of participating institutions (see www2.diabetestrialnet.org).

In animal models, oral insulin or oral GAD has been successfully used to prevent diabetes.[90] It is postulated that ingestion of T lymphocyte–dependent antigens can establish immunologic tolerance. Such oral strategies have been proposed and oral insulin is being tested in humans.[90] The subjects of primary prevention trials and secondary intervention trials to preserve residual insulin secretion at initial diagnosis are of major interest to investigators and clinicians alike. Progress is likely, but at present all of these strategies must be viewed as experimental and not currently in the domain of daily clinical practice.

Insulin Biosynthesis

Insulin is synthesized on the ribosomes of pancreatic islet beta cells and released into the circulation as a molecule composed of two separate straight polypeptide chains linked by disulfide bridges between and within these chains.[91-98] The two chains are not synthesized separately but are derived from a larger precursor, proinsulin, a single coiled chain in which the NH_2 terminus of the A chain is linked to the COOH terminus of the B chain by a connecting peptide, known as C-peptide (Figure 19-6). An even larger precursor (preproinsulin, containing an additional peptide chain on the NH_2 terminus of the A chain) is first synthesized, but this additional piece (important to the initiation of synthesis) is rapidly excised. Further processing of proinsulin within the beta cell cleaves the C-peptide, consisting of 31 amino acids, from the insulin molecule at the sites indicated in the figure.

Defects in these cleavage sites are inherited in an autosomal-dominant manner and result in insulin molecules with less-than-normal biologic activity that can give rise to two types of familial hyperproinsulinemia. One defect yields B-C proinsulin, cleaved at site 1 but not at site 2 (see Figure 19-6). This intermediate has 50% of the biologic activity of insulin, which is sufficient to prevent any abnormality in carbohydrate metabolism. The defect at site 1 yields A-C proinsulin, cleaved at site 2 but not at site 1, which has inadequate biologic activity to prevent carbohydrate intolerance. A structural mutation in the proinsulin molecule, between the C-peptide and insulin, has been confirmed.[90-96] In addition, a defect also occurs in the enzymatic conversion of a normal proinsulin molecule to insulin—yielding hyperproinsulinemia and mild carbohydrate intolerance.[91]

The proconvertases responsible for correct conversion of proinsulin to insulin are also involved in the processing of other hormones. Thus, impaired prohormone processing may lead to obesity and secondary hypocortisolism owing to defective processing of proopiomelanocortin (POMC) and to hypogonadotropic hypogonadism.[97-98] Native proinsulin has less than 5%, whereas C-peptide has none, of the biologic activity of insulin. During synthesis, the role of C-peptide appears to be the provision of the spatial arrangement necessary in the formation of the disulfide bonds. Other defects have been described in insulin biosynthesis involving substitution of amino acids in the B chain that lead to impaired glucose tolerance in the presence of hyperinsulinemia.[91-99]

The insulin gene has been cloned and localized to chromosome 11, and genetic defects in insulin synthesis may be associated with diabetes—especially the syndromes MODY1, -3, -5, and -6 with candidate genes for MODY7.[12,13] By some estimates, MODY syndromes may constitute 2% to 5% of all lean persons developing clinical diabetes between the ages of 10 and 30 years. The association of VNTRs in the insulin gene with genetic predisposition for diabetes was described earlier.

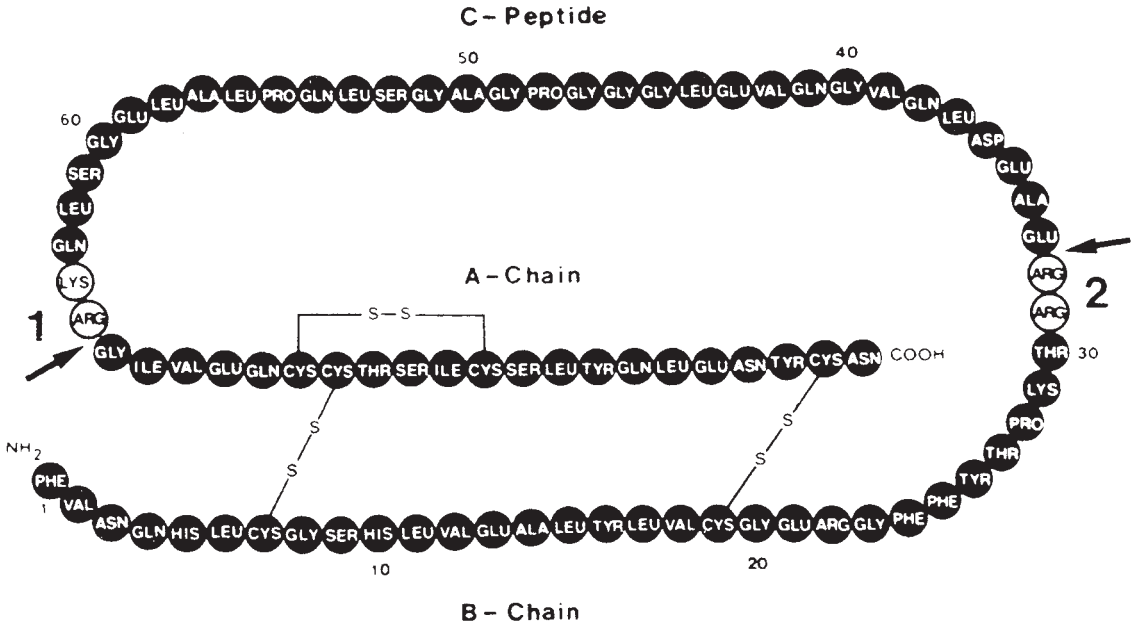


FIGURE 19-6 ■ Structure of proinsulin. Arrows 1 and 2 indicate the two sites of normal cleavage that yield insulin and C-peptide when the amino acid residues indicated in the open circles are removed. These cleavage points are known mutation sites, are inherited in an autosomal-dominant manner, and can yield two types of familial hyperproinsulinemia. During insulin secretion, equimolar amounts of insulin and C-peptide are released.

Under normal circumstances, only small quantities of proinsulin are released into the circulation—amounting to less than 15% of total insulin as measured by radioimmunoassay (RIA). Even smaller quantities of proinsulin intermediates are also released. However, during insulin secretion induced by all stimuli, one molecule of C-peptide is released with each molecule of insulin. Thus, the plasma of normal individuals contains small amounts of proinsulin, proinsulin intermediates, and almost equimolar amounts of insulin and C-peptide. The plasma metabolic half-life of C-peptide is, however, longer than that of insulin. Therefore, the molar ratio of C-peptide to insulin in peripheral plasma is always greater than 1 and the peak of C-peptide secretion or the nadir after suppression of release appears to occur later than that of insulin. Although standard RIA of insulin will also measure proinsulin, C-peptide will not be measured because it is immunologically distinct.

Separation of proinsulin from insulin can be achieved by chromatography to separate the larger proinsulin before assay. This is done with the use of an enzyme that degrades insulin but not proinsulin, or with the use of a C-peptide assay that will also measure proinsulin but not insulin. Because C-peptide is immunologically distinct, RIAs for this substance can be used to assess beta cell secretory reserve even in the presence of insulin antibodies formed in response to injections of bovine-porcine or human insulin.

Endogenous insulin secretion is accompanied by C-peptide release, whereas exogenous insulin administration suppresses endogenous insulin (and therefore C-peptide) secretion in all circumstances except insulinoma. Results of standard RIA with double-antibody precipitation are high in both circumstances. These attributes are important in distinguishing abuse of individuals by injection of exogenous insulin (high insulin, low C-peptide) from insulinomas or dysregulated insulin secretion (high insulin, high C-peptide) in cases of hypoglycemia. Measurements of C-peptide kinetics or of urinary excretion of C-peptide can be used as an index of endogenous insulin secretion.[29]

Insulin Secretion

Insulin secretion is governed by the interaction of nutrients, hormones, and the autonomic nervous system. Glucose, as well as certain other sugars metabolized by islets, stimulates insulin release. Basal and peak insulin levels are closely related to the glucose concentration, and prolonged fasting will further reduce glucose and insulin levels—which, however, remain in the measurable range at 2 to 5 mU/mL. There is evidence that a product or products of glucose metabolism may be involved in maintaining insulin secretion and that sugars not metabolized by islet cells do not promote insulin release.[100-102]

The initial steps of glucose-stimulated insulin release are depicted in Figure 19-7 and discussed in detail in Chapter 6 in connection with mutations in the sulfonylurea receptor (SUR)-Kir6 (inward-rectifying potassium channel) complex of the adenosine triphosphate-regulated potassium channel K_{ATP}, along with the subsequent steps that may cause activation of glucose or amino acid-stimulated insulin secretion.[103] This schema involves glucose transport into the beta cell through the GLUT2 glucose transporter and phosphorylation of glucose by means of glucokinase. Defects in the former are associated with type 2 diabetes, whereas heterozygous

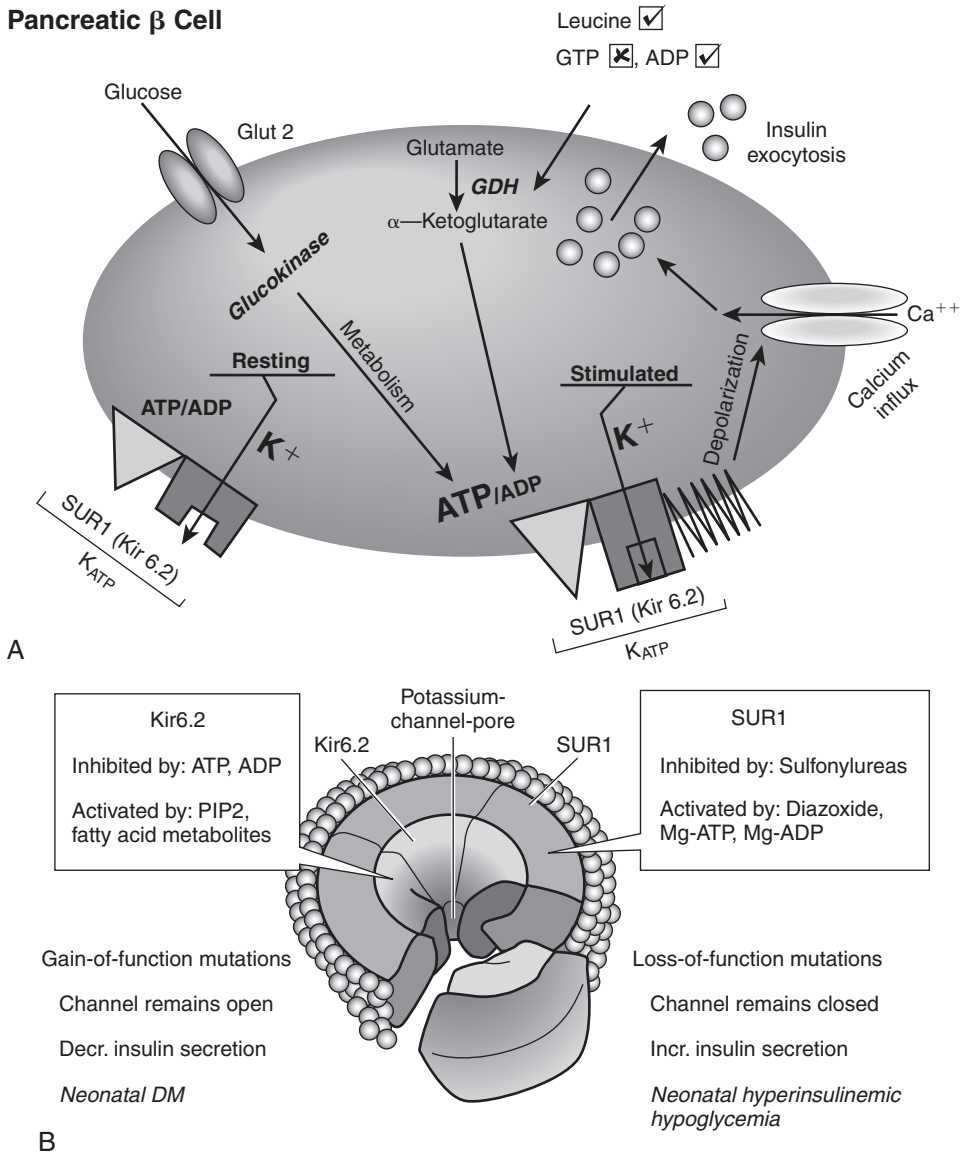


FIGURE 19-7 ■ **A,** Model of insulin secretion by pancreatic beta cell. Glucose transported into the beta cell by the insulin-independent glucose transporter (Glut 2) undergoes phosphorylation by glucokinase and is metabolized. This results in an increase in the ATP/ADP ratio with subsequent closure of the KATP channel and initiation of a cascade of events that is characterized by decreased flux of potassium across the membrane, membrane depolarization, calcium influx, and release of insulin from storage granules. Leucine stimulates insulin secretion by allosterically activating glutamate dehydrogenase (GDH) and by increasing the oxidation of glutamate; this increases the ATP/ADP ratio and closure of the KATP channel. The check mark sign (√) indicates stimulation of insulin secretion; the cross sign (x) indicates inhibition of insulin secretion. Diazoxide inhibits insulin secretion by interacting with the sulfonylurea receptor; somatostatin and calcium channel blockers interfere with calcium signaling. **B,** Regulation of insulin secretion. The Kir6.2–SUR1 complex and its regulation and genetic variability. The panel shows the detailed subunit structure of the KATP channel, which is composed of four small subunits, Kir6.2, that surround a central pore and four larger regulatory subunits constituting SUR1. In the normal resting state, the potassium channel is open, modulated by the ratio of ATP to ADP. PIP2 denotes phosphatidylinositol-4,5-bisphosphate. Kir6.2 denotes the inward rectifying potassium channel 6.2; SUR1 denotes the sulfonylurea receptor 1. ADP is adenosine diphosphate; ATP is adenosine triphosphate. As noted, in gain-of-function mutations, the channel remains open leading to decreased insulin secretion so that neonatal diabetes may result. With loss-of-function mutations, the channel remains closed, leading to persistent insulin secretion and hence is a cause of neonatal hyperinsulinemic hypoglycemia. (Redrawn from Sperling, M. A. (2006). ATP-sensitive potassium channels—neonatal diabetes mellitus and beyond. *New Engl J Med, 355,* 507–510.)

mutations in the latter are associated with MODY. Homozygous mutations in glucokinase result in permanent neonatal diabetes mellitus (as described in detail in Chapter 9). Glucokinase defects are generally associated with normal insulin release at higher glucose concentrations, and therefore with a milder type of diabetes.[12,13] After intravenous glucose infusion in normal persons, insulin secretion is biphasic—with an initial spike followed by a sustained plateau. It is proposed that the initial spike represents preformed insulin, whereas the sustained plateau represents newly synthesized insulin.

Cyclic adenosine monophosphate (AMP) is involved in stimulating insulin release. Therefore, agents that inhibit phosphodiesterase and reduce cyclic AMP destruction

(such as theophylline) augment insulin release. Translocation of calcium ions into the cytoplasm from the exterior, as well as from the intracellular organelles (see Figure 19-7) plays a key role in the contractile forces that propel insulin to the cell surface.[103] There, the membrane of the insulin vesicle fuses with the cell membrane—allowing extrusion of insulin granules into the surrounding vascular space, a process known as emiocytosis. Other ions, including potassium and magnesium, are involved in the insulin secretion.[103-106] The sulfonylurea receptor is closely linked to potassium channels in the beta cell.[103-106] Amino acids also stimulate insulin release, although the potency of individual amino acids varies.[107] A group of amino acids is more potent than any single one, and the insulin-secretory response is potentiated in the presence of glucose.[107] Free fatty acids and ketone bodies may also stimulate insulin release.[107] Insulin responses to oral glucose administration are always greater than responses to intravenous administration of glucose that result in the same blood glucose profile a finding that led to the concept that gut factors (incretins) modulate and increment insulin secretion.[108] Although a variety of gut hormones participate in promoting insulin release,[108] gastrointestinal polypeptide (GIP) pancreatic glucagon and the glucagon-like peptides (GLP) play a major role in stimulating insulin release.[108] These properties have found application as agents, collectively named incretins, in augmenting insulin secretion in persons with T2DM and in some persons with T1DM. Somatotropin release-inhibiting factor (somatostatin), produced in the delta cells of islets, inhibits insulin and glucagon release and reduces splanchnic blood flow. These properties have found application to reduce insulin secretion in neonates with hyperinsulinemic hypoglycemia of infancy (see Chapter 6). Together, these factors may finely regulate nutrient intake and its disposition and form an enteroinsular axis for metabolic homeostasis.[108] In addition to these gut hormones, several other hormones modulate insulin secretion. Growth hormone is involved in insulin synthesis and storage. Persons with congenital growth hormone deficiency have subnormal basal and stimulated insulin responses, whereas in acromegaly basal and stimulated insulin levels are increased. Human chorionic somatomammotropin (also known as human placental lactogen), structurally related to growth hormone, likewise affects insulin release. The stimulatory effect of each hormone on insulin secretion is antagonized by the anti-insulin effect at the peripheral level, however. Similarly, glucocorticoids and estrogens evoke greater insulin secretion while inducing peripheral insulin resistance—in part by decreasing insulin receptors on target cells.

Insulin secretion is constantly modulated by the autonomic nervous system.[102,109] The parasympathetic arm, through the vagus, directly stimulates insulin release. Modulation of insulin secretion by the sympathetic arm depends on whether α- *or* β-adrenergic receptors are activated. Activation of β_2 receptors by agents such as isoproterenol stimulates insulin secretion by a process that involves cyclic AMP generation. Blockade of β-adrenergic receptors by propranolol blunts basal and stimulated insulin release. Conversely, activation of α-adrenergic receptors blunts insulin secretion, and blockade of these receptors by agents such as phentolamine augments basal and glucose-stimulated insulin release. Epinephrine and norepinephrine stimulate predominantly α-adrenergic receptors in islets, resulting in impaired insulin secretion—as observed during stress or in patients with pheochromocytoma.[102]

In summary, in normal humans insulin secretion is constantly modulated by the quantity, quality, and frequency of nutrient intake; by the hormonal milieu; and by autonomic impulses. The ingestion of nutrients, principally carbohydrate and protein, produces intestinal hormonal signals that prime and initiate insulin release. The entry of glucose into the beta cell, the phosphorylation of glucose, and the generation of adenosine triphosphate (ATP) by this or other nutrients result in insulin release. This sequence involves cyclic AMP, β-adrenergic receptors, and ions—principally calcium and potassium. Glucose metabolism within the beta cell provides energy for further synthesis and release of insulin.[103]

Insulin Action

Insulin action on target cells in tissues such as liver, adipocytes, and muscle begins by binding to specific insulin receptors located on the cell membrane. Binding to these receptors is saturable, occurs with a high energy of association (affinity), and is pH and temperature dependent.[110-114] The insulin receptor is a heterodimeric glycoprotein consisting of two α and two β subunits linked by disulfide bonds (Figure 19-8). The α subunit, with a molecular mass of approximately 125,000 kd, acts as the binding site—whereas the β subunit, with a molecular mass of approximately 90,000 kd, possesses tyrosine kinase activity for endogenous and exogenous substrates (see Figures 19-8 and 19-9).

This ability to phosphorylate proteins may underlie some of the manifold actions of insulin. Among the classes of proteins phosphorylated are insulin receptor substrates 1 through 3 (considered an important insulin-signaling effector molecule) and pp 185, another substrate of the insulin receptor (see Figure 19-9). Other insulin mediators may be involved in insulin action. This action may also be mediated in part by hydrolysis of glycan phosphoinositides in the cell plasma membrane. The insulin receptor gene has been cloned and localized to chromosome 19, whereas the structurally related insulin-like growth factor-1 (IGF-1) receptor has been localized to chromosome 15.[110-114]

Under normal conditions, only a small proportion of the total available cell receptors need be occupied to achieve maximal biologic response. Thus, ordinarily there are spare receptors. Insulin receptors display two phenomena: down-regulation, in which high ambient insulin concentrations reduce the number of available receptors, and negative cooperativity, in which the occupancy of a receptor reduces the affinity of adjoining receptor sites. Scatchard analysis of insulin-binding data in in vitro systems reveals curvilinear plots compatible with negative cooperativity or with two classes of receptors: high-affinity/low-capacity and low-affinity/high-capacity. Total receptor number and the affinities of both classes of receptor sites can be calculated with use of these Scatchard plots.

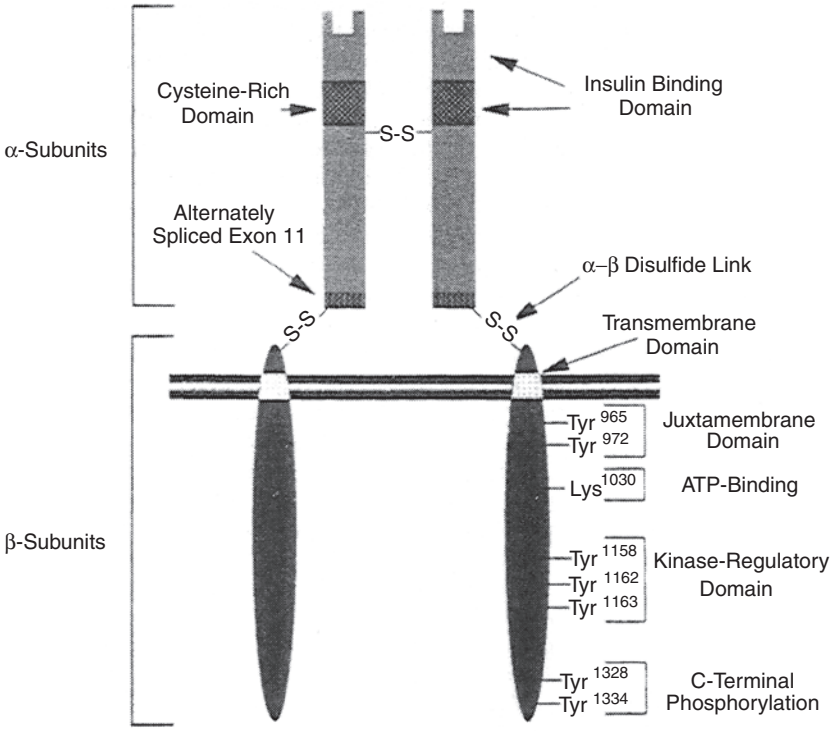


FIGURE 19-8 ■ Structure of the insulin receptor. ATP, adenosine triphosphate. (From Cheatham, B., Kahn, C. R (1995). Insulin action and the insulin signaling network. *Endocr Rev*, *16*, 117.).

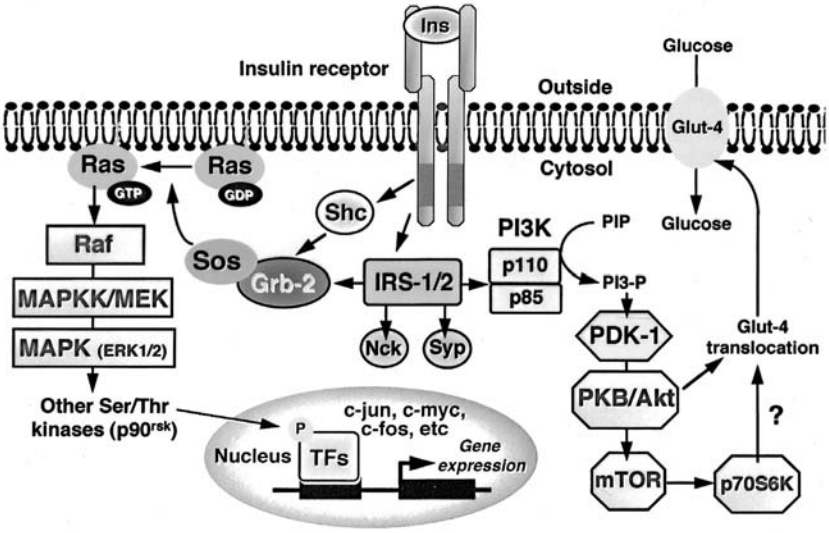


FIGURE 19-9 ■ Schema of insulin receptor signal transduction pathways. ERK, extracellular regulated kinase; Glut-4, glucose transporter 4; Grb-2, growth factor receptor binding protein 2; Ins, insulin; IRS, insulin receptor substrate; MAPK, mitogen-activated protein kinase; MAPKK, mitogen-activated protein kinase kinase; MEK, map/Erk kinase; mTOR, target of rapamycin; P13K, phosphoinositol-3-kinase; p70S6K, p70S6 kinase (small ribosomal subunit of protein-6-kinase); PDK-1, phospholipid-dependent kinase 1; PKB, protein kinase B; Ras, rat sarcoma; Shc, Sh2 cytosolic adaptor; SOs, son of sevenless; Syp, now called SHP-2 (for SH-2-containing phosphatase). (Courtesy of L. Mandarino, PhD, University of Texas Health Science Center, San Antonio.)

After binding to the cell surface, the receptor-insulin complex is internalized within the cell and processed by lysosomal enzymes, with release of free insulin and potential recycling of the receptor back to the cell membrane. Binding of insulin to the cell surface receptor, perhaps with the participation of internalization that permits insulin action at the level of the nucleus, leads to the complex biochemical processes characteristic of insulin action in a given tissue. The ultimate mechanism or mechanisms by which insulin exerts its effects beyond receptor binding and phosphorylation remain unknown.

With postreceptor events assumed to be normal, however, the biologic response to insulin in a tissue is a function of the number of receptor/insulin complexes formed—which in turn is directly related to the circulating insulin concentration and to the receptor concentration. Thus, a

reduction in receptor number could be compensated for by an increase in insulin concentration as long as the critical number of receptors necessary to produce maximal biologic response remains. Conversely, reduced insulin concentration could be compensated for by an increase in receptor number, provided the minimum amount of insulin necessary to produce a maximal biologic response is present.

Insulin receptors and their signaling proteins (see Figures 19-8 and 19-9) are widely distributed in various tissues. Using targeted deletions of individual components or various combinations of components of the insulin receptor pathway has provided remarkable insight into the contribution of liver, muscle, fat, the beta cell, and brain to overall glucose homeostasis.[110-114] Key concepts that have emerged are that the insulin receptor signal cascade on beta cells is critically important in maintaining normal insulin secretion. Thus, mutations causing insulin resistance at the beta cell eventually lead to relative hypoinsulinemia that can interact with the insulin resistance in peripheral tissues to produce the hallmark of type 2 diabetes (i.e., peripheral insulin resistance plus relative insulinopenia).

In addition, studies with targeted deletion of the insulin receptor in the brain—the so-called NIRKO mouse (neuron-specific insulin receptor knockout)—demonstrate that these animals developed obesity, increased body fat, insulin resistance with modest hyperinsulinemia, and elevated levels of triglycerides. Reproductive function in both males and females is impaired as a result of abnormal regulation of luteinizing hormone secretion, and serum leptin levels are elevated.[114] Thus, insulin signaling in the brain joins the emerging list of factors important in regulating energy homeostasis and reproduction.[114]

Primary defects in insulin receptor number or affinity may produce the same profound derangements in intermediary metabolism as deficient insulin secretion, and similar disturbances may result despite normal insulin concentration and normal receptor characteristics if postreceptor steps are defective.[115] Insulin signaling for the regulation of metabolism has been the subject of considerable research and is extensively reviewed.[113,115] Examples of each type of defect in the individual components of this integrated system that comprises insulin biosynthesis, secretion, and action exist and can account for the metabolic abnormalities that characterize diabetes mellitus. An approach based on the principles of insulin biosynthesis secretion and action also permits a rational classification of diabetes mellitus.

Pathophysiology

Normal insulin secretion in response to feeding is exquisitely modulated by the interplay of neural, hormonal, and substrate-related mechanisms to permit controlled disposition of ingested foodstuff as energy for immediate or future use. Mobilization of energy during the fasted state depends on low plasma levels of insulin. Thus, in normal metabolism there are regular swings between the postprandial high-insulin anabolic state and the fasted low-insulin catabolic state that affect three major tissues: liver, muscle, and adipose tissue (Table 19-7).

Insulin is the key anabolic hormone that promotes the synthesis and storage of carbohydrates, lipids, and proteins while simultaneously restraining their degradation. The uptake of glucose, fatty acids, and amino acids is stimulated—as is the activity or expression of enzymes that promote glycogen, fat, and protein synthesis. Conversely, the activity or expression of enzymes that break down these metabolites is restrained. All of these anabolic actions of insulin are reversed during the low-insulin state of starvation. Type 1 diabetes mellitus, as it evolves, becomes a permanent low-insulin catabolic (starvation) state in which feeding cannot reverse but rather exaggerates these catabolic processes.

It is important to emphasize that liver is more sensitive than muscle or fat to a given concentration of insulin. That is, endogenous glucose production from the liver

TABLE 19-7 **Influence of Feeding (High Insulin) or Fasting (Low Insulin) on Some Metabolic Processes in Liver, Muscle, and Adipose Tissue***

	High Plasma Insulin (Postprandial State)	Low Plasma Insulin (Fasted State)
Liver	Glucose uptake	Glucose production
	Glycogen synthesis	Glycogenolysis
	Absence of gluconeogenesis	Gluconeogenesis
	Lipogenesis	Absence of lipogenesis
	Absence of ketogenesis	Ketogenesis
Muscle	Glucose uptake	Absence of glucose uptake
	Glucose oxidation	Fatty acid and ketone oxidation
	Glycogen synthesis	Glycogenolysis
	Protein synthesis	Proteolysis and amino acid release
Adipose tissue	Glucose uptake	Absence of glucose uptake
	Lipid synthesis	Lipolysis and fatty acid release
	Triglyceride uptake	Absence of triglyceride uptake

*Insulin is considered the major factor governing these metabolic processes. Diabetes mellitus may be viewed as a permanent low-insulin state that if untreated results in exaggerated fasting.

by means of glycogenolysis and gluconeogenesis can be restrained at insulin concentrations that do not fully augment glucose utilization by peripheral tissues. Consequently, with progressive failure of insulin secretion the initial manifestation is postprandial hyperglycemia. Fasting hyperglycemia is a late manifestation that reflects severe insulin deficiency and indicates excessive endogenous glucose production. [116]

Although insulin deficiency is the primary defect, several secondary changes that involve the stress hormones (i.e., epinephrine, cortisol, growth hormone, and glucagon) accelerate and exaggerate the rate and magnitude of metabolic decompensation.

Increased plasma concentrations of these counterregulatory hormones magnify metabolic derangements by further impairing insulin secretion (e.g., epinephrine), by antagonizing its action (e.g., epinephrine, cortisol, and growth hormone), and by promoting glycogenolysis, gluconeogenesis, lipolysis, and ketogenesis (e.g., glucagon, epinephrine, growth hormone, and cortisol) while decreasing glucose utilization and glucose clearance (e.g., epinephrine, growth hormone, and cortisol).[117] With progressive insulin deficiency, especially with concurrently elevated stress hormones, excessive glucose production and impairment of its utilization result in hyperglycemia with glucosuria when the renal threshold of approximately 180 mg/dL is exceeded.

The resultant osmotic diuresis produces polyuria, urinary losses of electrolytes, dehydration, and compensatory polydipsia. These evolving manifestations, especially dehydration, represent physiologic stress—resulting in hypersecretion of epinephrine, glucagon, cortisol, and growth hormone that amplifies and perpetuates metabolic derangements and accelerates metabolic decompensation. The acute stress of trauma or infection may likewise accelerate metabolic decompensation to ketoacidosis in evolving or established diabetes. [117]

Hyperosmolality, commonly encountered as a result of progressive hyperglycemia, contributes to the symptomatology—especially to cerebral obtundation in diabetic ketoacidosis. Serum osmolality can be estimated with the following formula.

$$\text{Serum osmolality (mOsm/kg)} = (\text{serum Na [mEq/L]} + \text{K [mEq/L]}) \times 2 + \text{Glucose mmol/L (Note that 1 mmol/L of glucose is equivalent to 18 mg/dL.)}$$

Consideration of serum osmolality has important implications in the treatment of diabetic ketoacidosis. The combination of insulin deficiency and elevated plasma values of the counterregulatory hormones is also responsible for accelerated lipolysis and impaired lipid synthesis, with resulting increased plasma concentrations of total lipids, cholesterol, triglycerides, and free fatty acids. The hormonal interplay of insulin deficiency and glucagon excess shunts the free fatty acids into ketone body formation. The rate of formation of these ketone bodies, principally β-hydroxybutyrate and acetoacetate, exceeds the capacity for peripheral utilization and for their renal excretion. Accumulation of these ketoacids results in metabolic acidosis and in compensatory rapid deep breathing in an attempt to excrete excess carbon dioxide (Kussmaul respiration).

Acetone, formed by nonenzymatic conversion of acetoacetate, is responsible for the characteristic fruity odor of the breath. Ketones are excreted in the urine in association with cations and thus further increase losses of water and electrolytes (Figure 19-10 and Tables 19-8 and 19-9). With progressive dehydration, acidosis, hyperosmolality, and diminished cerebral oxygen utilization, consciousness becomes impaired—with the patient ultimately becoming comatose. Thus, insulin deficiency produces a profound catabolic state—an exaggerated starvation in which all of the initial clinical features can be explained on the basis of known alterations in intermediary metabolism mediated by insulin deficiency in combination with counterregulatory hormone excess. Because the counterregulatory hormonal changes are usually secondary, the severity and duration of the symptoms reflect the extent of primary insulinopenia. [116,117]

Clinical Manifestations of Diabetes Mellitus

The classic presentation of diabetes in children is a history of polyuria, polydipsia, polyphagia, and weight loss. Polyuria may be heralded by the recurrence of bedwetting in a previously toilet trained child and polydipsia by a child constantly requesting fluids to drink. Unexplained weight loss should raise suspicion of the existence of diabetes that should be confirmed or excluded by measurement of blood glucose concentration first in the postprandial and later in the fasting state. The urine should also be checked for the presence of glucosuria. The duration of these symptoms varies but is often less than 1 month. Most children who are diagnosed with T1DM have been seen by a physician within a week or so of diagnosis. However, diabetes was not considered, and a glucose measurement in blood or urine was not performed.[118,119]

An insidious onset with lethargy, weakness, and weight loss is also quite common. The loss of weight despite increased dietary intake is readily explicable by the following example. The average healthy 10-year-old child has a daily intake of 2000 or more calories, of which approximately 50% are derived from carbohydrates. With the development of diabetes, daily losses of water and glucose may be as much as 5 L and 250 g, respectively. This represents 1000 calories lost in the urine, or 50% of average daily caloric intake. Therefore, despite the child's compensatory increased intake of food and water, the calories cannot be utilized, excessive caloric losses continue, and increasing catabolism and weight loss ensue.

Pyogenic skin infections and candidal vaginitis in girls or candidal balanitis in uncircumcised boys are occasionally present at the time of diagnosis of diabetes. They are rarely the sole clinical manifestations of diabetes in children, and a careful history will invariably reveal the coexistence of polyuria, polydipsia, and perhaps weight loss. Ketoacidosis is responsible for the initial presentation of many (about 25% to 40%) diabetic children. Ketoacidosis is likely to be present more often in children younger than 5 years of age because the diagnosis may not be suspected

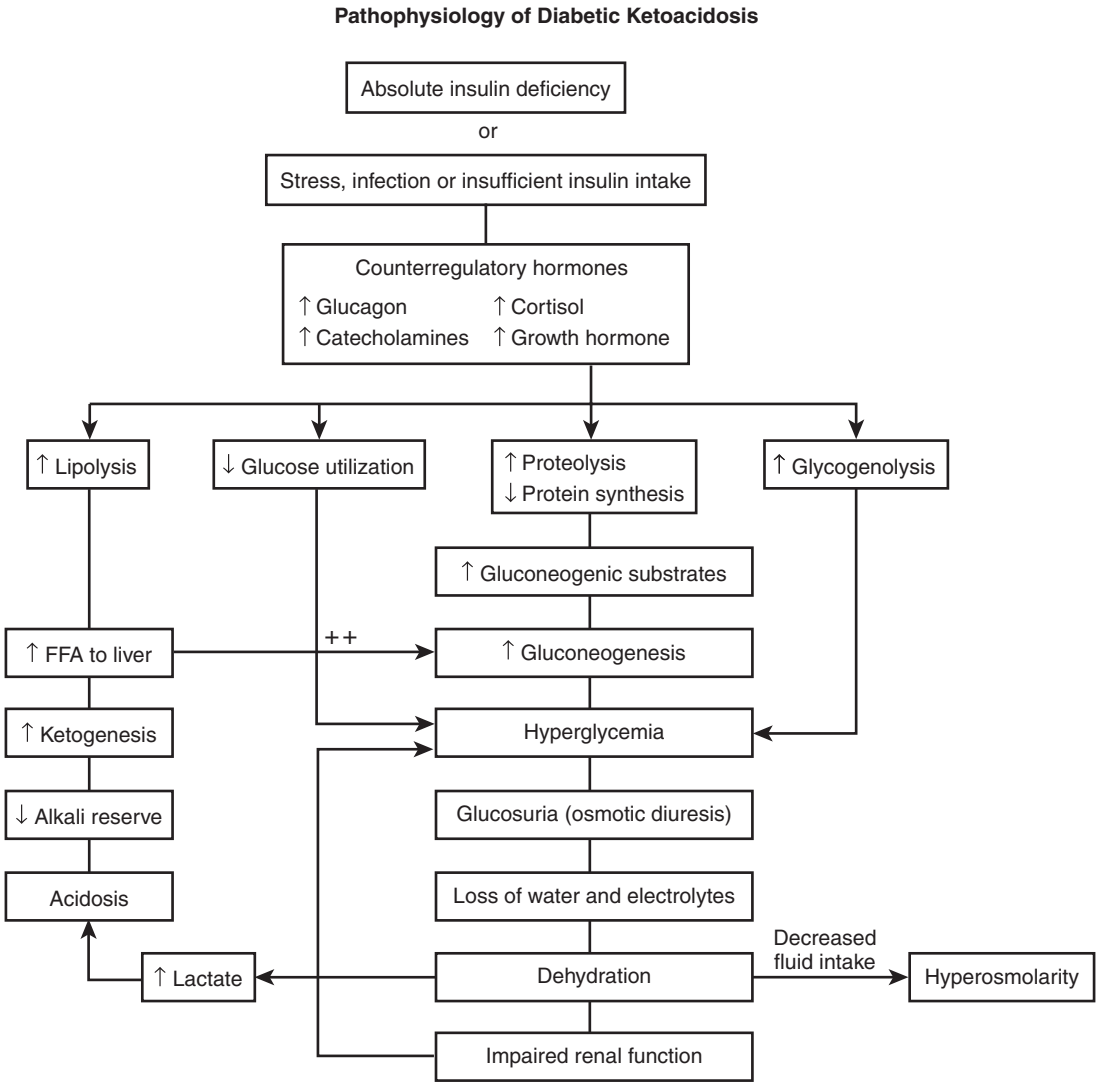


FIGURE 19-10 ■ The pathophysiology of diabetic ketoacidosis is illustrated as a function of absolute insulin deficiency or insufficient insulin in the presence of major stress such as an infection, which leads to increases in the four major counterregulatory hormones. Together, these changes increase glucose production via glycogenolysis and gluconeogenesis, which together result in hyperglycemia, osmotic diuresis, and dehydration. Simultaneously increased lipolysis leads to ketone body production and acidosis in combination with increased lactic acid from dehydration. See text for greater detail.

TABLE 19-8 **Fluid and Electrolyte Maintenance Requirements and Estimated Losses in Diabetic Ketoacidosis**

	Approximate Daily Maintenance Requirements*	Approximate Accumulated Losses†
Water	1500 mL/m^2	100 mL/kg (range, 60-100 mL/kg)
Sodium	45 mEq/m^2	6 mEq/kg (range, 5-13 mEq/kg)
Potassium	35 mEq/m^2	5 mEq/kg (range, 4-6 mEq/kg)
Chloride	30 mEq/m^2	4 mEq/kg (range, 3-9 mEq/kg)
Phosphate	10 mEq/m^2	3 mEq/kg (range, 2-5 mEq/kg)

*Maintenance is expressed in surface area to permit uniformity because fluid requirements change as weight increases.
†Losses are expressed per unit of body weight because the losses remain relatively constant in relation to total body weight.

and a history of polyuria and polydipsia may be difficult to elicit.[118-126] The early manifestations may be relatively mild and consist of vomiting, polyuria, and dehydration.

In more prolonged and severe cases, Kussmaul respiration is present—and there is an odor of acetone on the breath. Kussmaul respiration may be confused with bronchiolitis or asthma and be treated with steroids or adrenergic agents that worsen diabetes. Abdominal pain or rigidity may be present and may mimic appendicitis or pancreatitis. Cerebral obtundation and (ultimately) coma ensue and are related to the degree of hyperosmolarity. Laboratory findings include glucosuria, ketonuria, hyperglycemia, ketonemia, and metabolic acidosis. Leukocytosis is common, and nonspecific serum amylase levels may be elevated. The serum lipase level is usually not elevated. In those with abdominal pain, it should not be assumed that these findings are evidence of a surgical emergency before

TABLE 19-9 **Fluid Electrolyte Therapy for Diabetic Ketoacidosis: Recommendations for Replacement of Fluid**

Replacement Fluids	Approximate Accumulated Losses with 10% Dehydration	Approximate Requirements for Maintenance (36 Hours)	Approximate Totals for Replacement and Maintenance (36 Hours)
Water (mL)	3000	2250	5500
Sodium (mEq)	180	65	250
Potassium (mEq)	150	50	200
Chloride (mEq)	120	45	165
Phosphate (mEq)	90	15	100

Replacement Schedule (Continuous Intravenous Infusion)

Approximate Duration	Fluid (Composition)	Sodium (mEq)	Potassium (mEq)	Chloride (mEq)	Phosphate (mEq)
Hour 1	500 mL of 0.9% NaCl (isotonic saline)	75	—	75	—
Hour 2p	500 mL of 0.45% NaCl (0.5 isotonic saline) plus 20 mEq of KCl	35	20	55	—
Hour 3-12 (200 mL/hr for 10 hours)	2000 mL of 0.45% NaCl with 30 mEq/L of potassium phosphate	150	60	150	40
Subtotal initial 12 hours	3000 mL	260	80	280	40
Next 24 hours 100 mL/hr	2400 mL of 5% glucose in 0.2% NaCl with 40 mEq/L of potassium phosphate	75	100	75	60
Total over 36 hours	5400 mL	335	180	355	100

Note: Maintenance requirements remain the same. Losses and for maintenance of a 30-kg (surface area, 1.0 m^2) child with assumed 10% dehydration (duration of treatment, 36 hours). All replacement values should be halved if dehydration is estimated to be 5%.
Additional guidelines:

- A diabetic flow sheet with laboratory data appropriately recorded must be maintained in the patient's chart.
- Insulin therapy by continuous low-dose intravenous method: priming dose—bolus injection of 0.1 U/kg of regular insulin IV followed immediately by continuous IV infusion of 0.1 U/kg/hr of regular insulin beginning with second hour.
- Directions for making insulin infusion: Add 50 units of regular insulin to 500 mL of isotonic saline. Flush 50 mL through the tubing to saturate insulin-binding sites. For 30-kg patient, infuse at rate of 30 mL/hr. When the blood glucose concentration approaches 300 mg/dL, continue the insulin infusion at a reduced rate or add glucose to the infusate until acidosis is resolved and then start insulin therapy by subcutaneous injections of 0.2 to 0.4 U/kg of insulin at intervals of 6 hours.
- Bicarbonate therapy: For pH ≥ 7.10, no therapy is necessary. For pH 7.00 to 7.10, 40 mEq/m^2 of bicarbonate over 2 hours; then reevaluate. For pH ≤ 7.00, 80 mEq/m^2 of bicarbonate over 2 hours; then reevaluate. For new diabetics younger than 2 years of age with diabetic ketoacidosis and 10% dehydration or any diabetic with pH ≤ 7.00, coma or blood glucose ≥ 1000 mg/dL should be managed in an intensive care unit or equivalent setting.

a period of appropriate fluid, electrolyte, and insulin therapy to correct dehydration and acidosis. The abdominal manifestations frequently disappear after several hours of such treatment.

Diagnosis

The diagnosis of diabetes mellitus must be considered in children who have the following manifestations: those who have a history suggestive of diabetes, especially symptoms of polyuria with polydipsia, and failure to gain weight or a weight loss despite a healthy appetite; those who have confirmed glucosuria; and those who have clinical manifestations of metabolic acidosis with or without stupor or coma. In all instances, the diagnosis of diabetes mellitus is dependent on the demonstration of hyperglycemia in association with glucosuria with or without ketonuria. When classic symptoms of polyuria and polydipsia are associated with hyperglycemia and glucosuria that meet the criteria for diagnosing diabetes mellitus as defined by the American Diabetes Association (ADA) or World Health Organization (WHO), a glucose tolerance test is contraindicated.

Renal glucosuria may be an isolated congenital disorder or a manifestation of the Fanconi syndrome and other renal tubular disorders owing to severe heavy metal intoxication, ingestion of certain drugs (e.g., outdated tetracycline), or inborn errors of metabolism (e.g., cystinosis). When vomiting, diarrhea, and inadequate intake of food are complicating factors in any of these conditions, starvation ketosis may ensue and simulate diabetic ketoacidosis. The absence of hyperglycemia eliminates the possibility of diabetes. It is also important to recognize that not all urinary sugar is glucose, and infrequently galactosemia, pentosuria, and the fructosuria will require consideration as diagnostic possibilities.

The discovery of glucosuria, with or without a mild degree of hyperglycemia, during a hospital admission for trauma or infection (or even during the associated emotional upheaval) may herald the existence of diabetes. In most of these instances, the glucosuria remits during recovery.[1] Because this circumstance may indicate a

limited capacity for insulin secretion, which is unmasked by elevated plasma concentrations of stress hormones, these patients should be rechecked at a later date for the possibility of hyperglycemia, clinical features of diabetes mellitus, and family history of diabetes.

A family history of diabetes mellitus in two preceding generations should suggest the possibility of a MODY syndrome; absence of the common antibodies to beta cells such as IA2, GAD65, or ICA, strengthens the possibility of a diagnosis of MODY.[12,13] Under these circumstances, a glucose tolerance test may be useful to establish a diagnosis. Glucose tolerance testing should be performed several weeks after recovery from the acute illness, with a glucose loading dose adjusted for weight. Evidence indicates that the test is most likely to be abnormal in those with HLA DR3 and DR4, in whom ICA or insulin autoantibodies are detected, or who have MODY.[12,13] Transient hyperglycemia is common in patients with asthma treated with epinephrine and steroids. Further testing in such patients is not indicated. Screening procedures, such as postprandial determinations of blood glucose or oral glucose tolerance tests, have yielded low detection rates in children—even among those considered at risk, such as siblings of diabetic children. Accordingly, such screening procedures are not recommended in children.

Diabetic Ketoacidosis

Diabetic ketoacidosis (DKA) is a medical emergency and represents a life-threatening decompensation of metabolism that requires prompt recognition and appropriate treatment, with careful monitoring of clinical and biochemical indices. DKA must be differentiated from acidosis and coma from other causes. These include hypoglycemia, uremia, gastroenteritis with metabolic acidosis, lactic acidosis, salicylate intoxication, encephalitis, and other intracranial lesions. Diabetic ketoacidosis exists when there is hyperglycemia (glucose 300 mg/dL), ketonemia (β-OH butyrate $>$ 4 mEq/L) via formal laboratory or bedside ketone meter measurement or ketones strongly positive at greater than 1:2 dilution of serum, acidosis (pH 7.30 or less and bicarbonate 15 mEq/L or less), glucosuria, and ketonuria in addition to the clinical features of tachypnea (Kussmaul respiration) and cerebral obtundation. The severity of DKA is defined by the degree of acidosis; pH 7.2 to 7.3 or HCO3 10 to 15 mEq/L is defined as mild; pH 7.1 to 7.2 or HCO3 5 to 10 mEq/L is moderate; pH $<$ 7.1 or HCO3 $<$ 5 is defined as severe.

Precipitating factors, even for the initial presentation, include stress (e.g., trauma), infections, vomiting, and major psychological disturbances. Recurrent episodes of ketoacidosis in established diabetics often represent deliberate errors in recommended insulin dosage, unusual stress responses that indicate psychological disturbances, and at times pleas to be removed from a home environment perceived to be stressful or intolerable. Diabetic ketoacidosis should also be distinguished from nonketotic hyperosmolar coma. which occurs in children with T2DM and is increasingly recognized.[118-139]

Nonketotic hyperosmolar coma is a syndrome characterized by severe hyperglycemia (blood glucose concentration of more than 600 mg/dL), absence of or only very slight ketosis, nonketotic acidosis, severe dehydration, depressed sensorium or frank coma, and various neurologic signs that may include grand mal seizures, hyperthermia, hemiparesis, and positive Babinski signs. Respiration is usually shallow, but coexistent metabolic (lactic) acidosis may be manifested by Kussmaul breathing. Serum osmolarity is commonly 350 mOsm/kg or higher. In children, this condition is relatively infrequent and when reported there has been a high incidence of preexisting neurologic damage. The profound hyperglycemia may develop over a period of days, and initially the obligatory osmotic polyuria and dehydration may be partially compensated for by increased fluid intake. With progression, thirst becomes impaired—possibly because of alteration of the hypothalamic thirst center by hyperosmolarity and possibly in some instances because of a preexisting defect in the hypothalamic osmo-regulating mechanism.[118-140] Nonketotic hyperglycemia with coma has been a feature of presentation in youth with T2DM and is often associated with morbid obesity. Some of these obese patients may present with ketoacidosis as the initial clinical presentation.

The low production of ketones is attributed mainly to the hyperosmolarity, which in vitro blunts the lipolytic effect of epinephrine, and to the concomitant antilipolytic effect of residual insulin. Blunting of lipolysis by the therapeutic use of β-adrenergic blockers may also contribute to the syndrome. The key difference between diabetic ketoacidosis and hyperglycemic hyperosmolar nonketotic coma appears to be the degree of insulinopenia, which is nearly absolute in ketoacidosis but with sufficient residual activity in hyperglycemic hyperosmolar nonketotic coma to limit lipolysis in adipose tissue but inadequate to permit normal peripheral glucose utilization at a time of increased glucose production induced by the stress hormones. As indicated, prerenal azotemia and decreased thirst contribute to this syndrome in adults and in children. Depression of consciousness is closely correlated with the degree of hyperosmolarity in this condition, as well as in diabetic ketoacidosis. Hemoconcentration may also predispose to cerebral arterial and venous thromboses.[126-127]

Treatment of nonketotic hyperosmolar coma is directed at repletion of the vascular volume deficit and correction of the hyperosmolar state (see "Diabetic Ketoacidosis" for management). Patients who are hypotensive should be started on isotonic saline (0.9% NaCl) until the condition is stable. Then, treatment should proceed as for DKA with the following caveats: extending the period of fluid repair to 36 to 48 hours; Infuse insulin at half the amount used in DKA (i.e., 0.05 U/kg/hr).[125,126] When the blood glucose concentration approaches 300 mg/dL, the hydrating fluid should be changed to 5% dextrose in 0.2 N saline. Approximately 20 mEq/L of potassium chloride, or more if indicated, should be added to each of these fluids to prevent hypokalemia. Serum potassium and plasma glucose concentrations should be monitored at 2-hour intervals for the first 12 hours and at 4-hour intervals for the next 24 hours to permit appropriate adjustments of administered potassium and insulin.[126-127]

Insulin can be given by continuous intravenous infusion, beginning with the second hour of fluid therapy. Because blood glucose may decrease dramatically with fluid therapy alone, the intravenous loading dose

should be 0.05 U/kg of fast-acting insulin followed by 0.05 U/kg/hr of the same insulin—rather than 0.1 U/kg/hr as advocated for diabetic ketoacidosis. Most now advocate that the initial insulin loading dose be omitted. During the recovery period, therapy with insulin and diet and monitoring of the patient are as described for patients recovering from diabetic ketoacidosis (see Table 19-9).

Treatment of Diabetes Mellitus

The management of type 1 diabetes may be divided into three phases, depending on the initial presentation: ketoacidosis, the postacidotic transition period for establishment of metabolic control, and the continuing phase of guidance of the diabetic child and his or her family for daily living with diabetes integrating insulin regimens, nutritional intake, exercise, and glucose monitoring to achieve as near normal glucose control as is feasible. Each of these phases has separate goals, although in practice they merge into a continuum. For purposes of management, the transition period corresponds to presentations with polyuria, polydipsia, and weight loss but without biochemical decompensation to ketoacidosis.

Treatment of Diabetic Ketoacidosis

The pathophysiology and treatment of diabetic ketoacidosis were extensively reviewed and published as a consensus conference. The views and recommendations are endorsed by the Lawson Wilkins Pediatric Endocrine Society, the European Society for Pediatric Endocrinology, the International Society of Pediatric and Adolescent Diabetes (ISPAD), and others. Similar guidelines for management have now also been recommended by the American Diabetes Association. These recommendations have been incorporated into the guidelines published and regularly updated by ISPAD.[119]

The immediate aims of therapy are expansion of intravascular volume; correction of deficits in fluid, electrolyte, and acid-base status; initiation of insulin therapy to correct intermediary metabolism; and the exclusion of a treatable precipitating event such as infection or trauma. Treatment should be instituted as soon as the clinical diagnosis is confirmed by the presence of hyperglycemia and ketonemia. Determinations of blood pH and electrolytes should also be obtained. An electrocardiogram is useful to provide a rapid reference for the existence of hyperkalemia.

If sepsis is suspected as a possible precipitating factor, a blood culture should be obtained and the urine should be examined for the presence of bacteria and leukocytes. A flow sheet is essential to record chronologically the rate and composition of fluid input and urine output, the amount of insulin administered, and the acid-base and electrolyte values of the blood. Catheterization of the bladder is not routinely recommended in children. Bag collection or condom drainage permits an assessment of urinary output, but catheterization may be indicated in comatose patients.[118-139]

Fluid and Electrolyte Therapy

The expansion of reduced intravascular volume and correction of depleted fluid and electrolyte stores are important in the treatment of diabetic ketoacidosis (see Table 19-9 and Box 19-2). It must be stressed, however,

BOX 19-2 Steps in Management of Diabetic Ketoacidosis

1. Confirm diagnosis.
Obtain:
   - Blood glucose
   - Serum electrolytes
   - Acid-base status: pH, HCO_3^2

Consider:
   - Urine microscopy/culture
   - Chest radiograph
   - Blood culture
   - Throat culture
   - Electrocardiogram

Intensive care–like setting:
   - pH ≤ 7.00
   - Age 2 years
   - Unconscious
   - Blood glucose ≥ 1000 mg/dL

2. Begin intravenous fluids.
   - 20 mL/kg of 0.9% (normal), saline (NaCl) over 1 hour
3. Reassess the patient: What precipitated this episode?
   - Delayed diagnosis
   - Infection
   - Noncompliance
   - Trauma
4. Follow protocol guidelines in Table 10-11. Begin insulin 0.1 U/kg/hr with hour 2 of IV therapy as outlined in Table 10-11.
5. Measure glucose every 2 hours; electrolytes/acid-base every 2 to 4 hours for the first 24 hours.
6. Continue treatment with insulin even if glucose approaches 300 mg/dL (17 mM) as long as acidosis persists. Consider adding 5% to 10% glucose in IV line or occasionally reducing insulin to 0.05 U/kg/hr.
7. If acidosis is not resolving (or improving) despite fluids and insulin of 0.1 U/kg/hr, consider:
   - Severe sepsis causing lactic acidosis and/or insulin degradation
   - Error in insulin dose
8. In children younger than 10 years (especially, 5 years), anticipate possible clinical cerebral edema after 4 to 6 hours of treatment. The following herald evolving edema:
   - Headache
   - Change in consciousness level/response
   - Unequal dilated pupils
   - Delirium
   - Incontinence
   - Vomiting
   - Bradycardia
9. If cerebral edema is clinically apparent:
   - Reduce IV rate.
   - Give mannitol 1 g/kg IV (10-20 g/m^2).
   - Repeat in 2 to 4 hours if indicated.

that exogenous insulin is essential to arrest further metabolic decompensation and restore intermediary metabolism.[141-145] Dehydration is commonly approximately 10%. Initial fluid therapy can be based on this estimate, with subsequent adjustments to be related to clinical and laboratory data. The initial hydrating fluid should be isotonic saline (0.9%). Because of the hyperglycemia, hyperosmolarity is universal in diabetic ketoacidosis. Thus, even 0.9% saline is hypotonic relative to the patient's serum osmolality. Some investigators maintain infusion with fluids that are isotonic such as 0.9% saline and report low morbidity and mortality with such regimens.[118-123]

A gradual decline in osmolality is desirable because too rapid a decline has been implicated in the development of cerebral edema, one of the major complications of therapy in children. For the same reason, the rate of fluid replacement is adjusted to provide the total calculated deficit plus maintenance requirement over 48 hours after the initial rehydration with normal isotonic saline. In addition, glucose (5% solution in 0.2 to 0.5 N saline) is administered when blood glucose concentration approaches 300 mg/dL to limit the decline of serum osmolality in an attempt to reduce the risk of developing cerebral edema (see Box 19-2). Insulin should be continued until ketoacidosis is resolved and the pH is equal to or greater than 7.3 or until the bicarbonate concentration in plasma is equal to or greater than 18 mEq/L, because blood glucose corrects more rapidly than ketoacidosis. As long as acidosis persists, insulin infusion should be maintained at the same rate (and, if necessary, with 5% to 10% glucose added to the infusate) to maintain blood glucose at approximately 300 mg/dL.

Cerebral Edema

Cerebral edema is an unusual but potentially devastating complication of diabetic ketoacidosis and its management in childhood. There is considerable debate and several hypotheses have been proposed, but there is presently no consensus as to the precise cause of this syndrome. It remains unproved whether cerebral edema during treatment of diabetic ketoacidosis can be predicted on the basis of clinical and biochemical indices and whether physicians contribute to it by their mode of management through the choice and composition of the hydrating fluid, its rate of administration, and the controlled rate of decline of blood glucose.

The incidence of cerebral edema is reported to occur in 0.5% to 1.5% of all episodes of diabetic ketoacidosis. It is more common in younger children, especially at initial presentation.[118,137] The duration and severity of symptoms and signs before initiating treatment are the major identifiable factors.[121] If cerebral edema develops during treatment for diabetic ketoacidosis, mortality and morbidity are high. Although mortality has declined from previously reported rates of 40% to 90% to a more recently reported rate of 20%, cerebral edema remains the cause of about half of all deaths in children with diabetes mellitus. Morbidity from cerebral edema also remains high. About one fourth of the survivors may have permanent neurologic sequelae.[119] Prompt recognition and intervention with mannitol or other hyperosmolar agents, respiratory support by means of endotracheal intubation, and hyperventilation may be lifesaving. Awareness, early recognition, and treatment are responsible for the reported decline in mortality cited previously.[140] Subclinical cerebral edema may be more common than hitherto appreciated.[123] Randomized prospective trials of management of diabetic ketoacidosis are not ethically feasible, but in the most rigorous and extensive retrospective analysis involving some 6000 episodes of diabetic ketoacidosis, the following risk factors were identified: young age, duration and severity of symptoms before starting treatment, low PCO_2, high serum urea nitrogen, lack of an increase in serum sodium during therapy, and treatment with bicarbonate.[118] All of the biochemical risk factors in this study may reflect the initial severity of biochemical derangement. Thus, avoidance of diabetic ketoacidosis (especially in young children) by early recognition of the signs and symptoms of diabetes mellitus is the best way to prevent cerebral edema.

The duration and severity of symptoms before therapy may predispose to cerebral ischemia, to which younger children would be more prone because of the higher metabolic rate and hence higher oxygen requirement of a child's brain relative to an adult's. In addition, there may be a longer time to accumulate so-called idiogenic osmoles in the brain. Many children may have evidence of raised intracranial pressure on imaging studies (e.g., computed tomography), but only a minority develop clinical cerebral edema. [134]

Moreover, early studies in dogs and in adult men demonstrated that intracranial pressure rose in all during fluid replacement therapy for diabetic ketoacidosis.[135] Thus, given the hyperbolic pressure/volume relationship of the intracranial space and the likely shift of water into the intracellular compartment during therapy, it is considered prudent to advocate the use of normal saline (or equivalent volume expanders) during initial therapy to limit the rate of infusion to no more than twice-daily maintenance requirement and not to exceed 4 L/m^2/day, to carefully monitor the decline in blood glucose not to exceed about 100 mg/dL/hr, and to carefully monitor the clinical status of the patient. The managing team must be prepared to intervene at the bedside with mannitol or other volume expanders.

Finally, it should be noted that abnormalities in the clotting-fibrinolytic pathway (e.g., protein S and C deficiency, factor V Leiden) are not uncommon in the general population and may contribute to cerebral thrombosis/ischemia in the setting of dehydration, hyperviscosity, and increased platelet adhesiveness that exists in diabetic ketoacidosis.[118,137] There is also evidence that the osmotic mechanisms described previously, the so-called cytotoxic mechanism, may not be the only operative theory. Vasogenic mechanisms with disruption of vascular permeability in the cerebral blood-brain barrier may also be operative. The latter comes from studies via perfusion and diffusion magnetic resonance imaging (MRI) that show a decrease in the apparent diffusion coefficient after recovery (i.e., more pericellular and not intracellular water during DKA treatment).[122] Moreover, in that study one

in five episodes of cerebral edema was evident at initial presentation before any fluid or insulin therapy could have contributed to water accumulation in the brain. The two proposed mechanisms are not mutually exclusive and may allow more treatment options in time.

Cerebral edema may be present at initial presentation or early in the course of treatment, but it usually becomes apparent several hours after therapy has begun and when there is improvement in blood glucose level, acid-base status, and clinical state of hydration. Both early and late appearances of cerebral edema have been reported.[124] A previously alert patient may become drowsy, complain of headache, have abnormal neurologic findings (including papilledema), progress to coma, and have respiratory arrest with herniation of the brainstem. Recovery may occur with prompt recognition and treatment with intravenous mannitol. Survival and neurologic outcome are markedly improved with prompt recognition and intervention as outlined (Table 19-10). Although clinically apparent cerebral edema is often fatal, subclinical cerebral edema is present in many patients during therapy for ketoacidosis.[123] A series of bedside clinical observations may facilitate the recognition and prediction of those developing cerebral edema during therapy for DKA. The series of major and minor criteria proposed by Muir and colleagues remains uncorroborated.[133]

Electrolytes

Potassium should be administered early. Total body potassium may be considerably depleted during acidosis, even when the serum potassium concentration is normal or elevated. Although potassium moves from intracellular to extracellular sites during acidosis, the reverse occurs during correction of acidosis—particularly when exogenous insulin and glucose are available in the circulation. This shift of potassium back to the intracellular compartment may result in life-threatening hypokalemia. Therefore, after the initial fluid replacement of approximately 20 mL/kg of isotonic saline (0.9%) has been provided, potassium should be added to subsequent infusates if urinary output is adequate. Serum potassium concentration should then be monitored, initially at 1 to 2 hours intervals and periodically thereafter. Complete replacement of body potassium stores may not occur until after the patient has resumed oral feeding.

An electrocardiogram provides a rapid assessment of serum potassium concentration. T waves are peaked in hyperkalemia and are low and associated with U waves in hypokalemia. Because the total potassium deficit cannot be replaced within the initial 24 hours of treatment, potassium supplementation should continue as long as fluids are administered intravenously (see Table 19-9).

It is almost inevitable that the patient will receive an excess of chloride, which may aggravate acidosis. The extent of acidosis, however, can be reduced by substitution of phosphate—which is also significantly depleted in diabetic ketoacidosis. Moreover, phosphate in conjunction with glycolysis is essential to the formation of 2,3-diphosphoglycerate (2,3-DPG)—which governs the oxygen dissociation curve. During deficiency of 2,3-DPG, the oxygen dissociation curve is shifted to the left (i.e., more oxygen is retained by hemoglobin and less is available to tissues, a situation that predisposes to lactic acidosis).

Acidosis per se tends to shift the oxygen dissociation curve toward the right (Bohr effect) and thus partially "compensates" for 2,3-DPG deficiency. As acidosis resulting from the accumulation of ketones is corrected by the provision of insulin, with or without administration of bicarbonate, the effects of 2,3-DPG deficiency may no longer be compensated for and the release of oxygen to tissues may again be impaired. Exogenous phosphate, by contributing to the formation of 2,3-DPG, permits the oxygen dissociation curve to shift to the right and thus facilitates release of oxygen to tissues and aids in the correction of acidosis. Furthermore, resistance to insulin action is associated with hypophosphatemia. Therefore, the use of phosphate is recommended (the administration of potassium phosphate is outlined in Box 19-2). Because the excessive use of phosphate may result in hypocalcemia, serum calcium should be measured periodically.[136] Symptomatic hypocalcemia should be corrected with calcium gluconate, and some of the potassium chloride should be temporarily substituted for potassium phosphate.

TABLE 19-10 **Pharmacodynamic Properties of Common Insulin Formulations**

Category	Onset (Hour)	Peak (Hour)	Duration (Hour)
Rapid-acting insulin lispro, aspart, and glulisine	0.25-0.5	0.5-1	3-5
Short-acting regular	0.5-1	2-4	4-8
Intermediate-acting NPH	2-4	4-10	12-18
Long-acting insulin glargine and detemir	2-4	N/A	18 > 24
Premixed			
Mix 25 (75% NPH/25% lispro)	0.25-0.5	1-2	12-18
NovoLog mix (70% NPH/30% aspart) from living with diabetes	0.25-0.5	1-2	12-18

NPH, Neutral protamine Hagedorn.

Alkali Therapy

With provision of fluids, electrolytes, glucose, and insulin, metabolic acidosis is usually corrected through the interruption of ketogenesis, the metabolism of ketones to bicarbonate, and the generation of bicarbonate by the distal renal tubule. Concern regarding the therapeutic administration of bicarbonate centers on four issues: alkalosis, by shifting the oxygen dissociation curve to the left, may diminish the release of oxygen to tissues and therefore predispose to lactic acidosis; alkalosis accelerates the entry of potassium into cells and therefore may produce hypokalemia; the provision of bicarbonate according to the calculated base deficit overcorrects and may result in alkalosis; and perhaps most important, bicarbonate may lead to worsening of cerebral acidosis during the time when the plasma pH is being restored to normal because HCO_3^- combines with H^+ and dissociates to CO_2 and H_2O to form H_2CO_3.

Although bicarbonate passes the blood-brain barrier slowly, CO_2 diffuses freely, thereby potentially exacerbating cerebral acidosis and possibly cerebral depression. On the other hand, severe acidosis (with blood pH of about 7.1) diminishes respiratory minute volume, may produce hypotension by peripheral vasodilation, impairs myocardial function, and may be a factor in insulin resistance. For these reasons, administration of bicarbonate is recommended only at pH of about 7.1 or less (see Table 19-9). At pH of 7 to 7.1, we recommend that 40 mEq of HCO_3^-/m^2 (at pH of less than 7, 80 mEq of HCO_3^-/m^2) should be infused over a period of 2 hours. Acid-base status should then be reevaluated before further alkali therapy. Bicarbonate should not be given by bolus infusion because it may precipitate cardiac arrhythmias. [118,119,137]

Insulin Therapy

The continuous low-dose intravenous infusion method, in which a priming dose of 0.1 U/kg of regular insulin is followed by a constant infusion of 0.1 U/kg/hr, is outlined in Table 19-9. This method is effective, simple, and physiologically sound—and has gained acceptance as the recommended method for administering insulin during diabetic ketoacidosis. It provides a constant steady concentration of insulin in plasma that approximates the peak attained in normal individuals during an oral glucose tolerance test. Some practitioners recommend insulin to be given at 0.05 U/kg/hr and claim equal efficacy in correcting acidosis without the rapid decline in glucose concentration. Controlled trials comparing the 0.1 U/kg/hr with the smaller 0.05 U/kg/hr have not been performed. Also, the priming dose of insulin is not recommended as essential.[118]

Presumably, the same steady concentration is attained at the cellular level and permits a steady metabolic response without the fluctuations that must occur with intermittent injections of insulin. Concern that the insulin may adhere to glass and tubing has proved to be unfounded, and effective delivery of insulin can be provided without the addition of albumin or gelatin to the infusate. Although insulin infusion can be provided by gravity drip without the use of a special pump, such a pump is helpful and used in most intensive care unit (ICU) settings. A separate infusion set for insulin connected to the infusion line used for fluid and electrolyte therapy is recommended so that adjustments in the dosage of each can be made independently. After the amount of insulin has been calculated, for the initial 6 to 8 hours this quantity is diluted in a 50-mL syringe containing 0.9% saline infused via a separate pump but connected to the fluid infusate (see Table 19-9 for specific instructions).

When the blood glucose concentration approaches 300 mg/dL, the ongoing potassium requirement is added to 5% glucose in 0.2 to 0.5 N saline (see Table 19-9) and the rate of insulin infusion may be reduced from 0.1 to 0.05 U/kg/hr, provided acidosis is correcting. The rate of insulin infusion should, however, be periodically adjusted based on recovery of acidosis and the blood glucose response of each individual. [118,119,137]

In treating diabetic ketoacidosis, it is regularly observed that blood glucose concentration corrects more quickly than pH or plasma bicarbonate.[137,138] Insulin must be provided by infusion or subcutaneous injection as long as acidosis persists, even if glucose approaches 300 mg/dL. It may be necessary to add glucose to the infusate while continuing insulin infusion at a rate of 0.05 to 0.1 U/kg/hr until acidosis is corrected. If acidosis persists despite these measures, a cause such as gram-negative sepsis should be considered.

The essential steps in managing diabetic ketoacidosis are summarized in Table 19-9 and Box 19-2. When acidosis is corrected, the continuous infusion may be discontinued and insulin immediately given by subcutaneous injection at a dosage of 0.2 to 0.4 U/kg every 6 to 8 hours while the glucose infusion is maintained until the child can fully tolerate food. Subcutaneous injections of regular insulin at dosages of 0.2 to 0.4 U/kg every 6 to 8 hours before meals should be continued for a full 24-hour day after the child is eating. The blood glucose level should be monitored before and 2 hours after each meal, with the insulin dose adjusted to maintain blood glucose concentration in the range of 80 to 180 mg/dL. The total dose of regular or fast-acting insulin used in this representative day serves as a guide for subsequent insulin treatment with a combination of intermediate of long-acting as well as short-acting insulin, as described later in the chapter.

Ketonemia and ketonuria may persist despite clinical improvement. The nitroprusside reaction routinely used to measure ketones reacts with acetoacetate and weakly with acetone but not with β-hydroxybutyrate. The usual ratio of β-hydroxybutyrate to acetoacetate is approximately 3:1 but is commonly 8:1 or more in diabetic ketoacidosis. With correction of acidosis, β-hydroxybutyrate dissociates to acetoacetate—which is identified by the nitroprusside reaction. Therefore, persistence of ketonuria for 1 or more days may not reliably reflect the clinical improvement and should not be interpreted as an index of poor therapeutic response. Bedside meters for measuring β-OH butyrate in blood have been introduced in some units.[139] A campaign of advertising the symptoms and signs of diabetes in childhood to schools and primary care providers

was reported to markedly reduce the rates of DKA at diagnosis over a period of 8 years.[140]

TREATMENT OF TYPE 1 DIABETES MELLITUS (T1DM)

General Principles

Optimal management of the child with T1 and T2DM requires an integrated approach, taking into account the overall level of functioning of the child and family, the nutritional and lifestyle patterns specific to that child, and attention to the overall developmental stages of childhood and adolescence. There is no one appropriate insulin regimen or meal plan. The overriding principle should be that the diabetes care plan should fit wherever possible into the surrounding home and school environments, and that the primary childhood tasks of education, socialization, growth, and maturity continue unhindered by the extra responsibilities diabetes care entails.

This daunting task of assisting families in managing diabetes is best accomplished by a multidisciplinary team, consisting of physicians, nurse educators and practitioners, dietitians, and mental health professionals, all trained and experienced in the nuances of diabetes care in children. Children with diabetes should be seen by the team at frequent intervals (usually every 3 months in established patients) for assessment of glycemic control, growth, and development; evaluation for related disorders and complications; education; troubleshooting; problem solving; and screening for adjustment problems that may affect diabetes or the overall health of the child.

Goals of Therapy

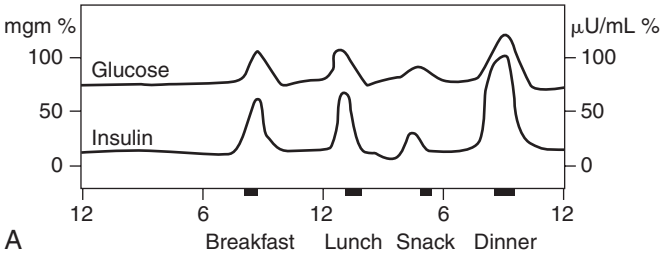
The Diabetes Control and Complications Trial (DCCT) established that intensive glycemic management leading to near normal glucose and A1c levels significantly reduced the risk of developing long-term complications and the benefits of this reduced risk outweighed the threefold increase in the risk of severe hypoglycemia. [141] Slowing the development of early retinopathy was observed in the DCCT subgroup of intensively treated adolescents, as well as in adults in the study.[142] In the Epidemiology of Diabetes Interventions and Complications (EDIC) Study, the observational follow-up to the DCCT, a continued increase in risk of complications was seen in the former conventional treatment group years after the end of the randomized control trial despite improved metabolic control in the former conventional treatment group, indicating that near normal glucose and A1c levels should be achieved and maintained in patients with T1D as early in the course of the disease as possible.[143] As a result of this evidence, current recommendations mandate that youth with T1DM should aim to achieve plasma glucose and HbA1c levels as close to normal as possible, as early in the course of the disease as possible, and with as few severe hypoglycemic events as possible.

With respect to specific targets, the American Diabetes Association treatment guidelines indicate that HbA1c targets should be adjusted based on the age of the child: namely $< 8.5\%$ in children < 8 years of age, $< 8\%$ in 7- to 12-year-olds, and $< 7.5\%$ in teenagers.[144] Higher HbA1c levels were suggested for very young children because of the potential risk of recurrent hypoglycemia on the developing brain. There are many problems with this approach, including the emerging evidence that chronic hyperglycemia is also detrimental to the developing brain. In contrast, the International Society for Pediatric and Adolescent Diabetes (ISPAD) guidelines recommend a target HbA1c of $< 7.5\%$ for all pediatric patients with strong emphasis placed on individualizing glucose targets to promote normoglycemia while preventing severe or frequent hypoglycemia.[145]

Types of Insulin

Currently available insulins are classified based on their duration of action as rapid, short, intermediate, and long acting, and each is available in a concentration of 100 U/mL (U-100). A higher concentration (U-500) of human regular insulin is available for the patient who has severe insulin resistance. Appropriate dilutions can be prepared for younger patients requiring low doses. The development of recombinant DNA technology to synthesize human insulin and human insulin analogs has changed the face of insulin treatment.

The aim of insulin replacement therapy is to simulate the normal pattern of insulin secretion as closely as possible (Figure 19-11 and 19-12). This aim can best be achieved



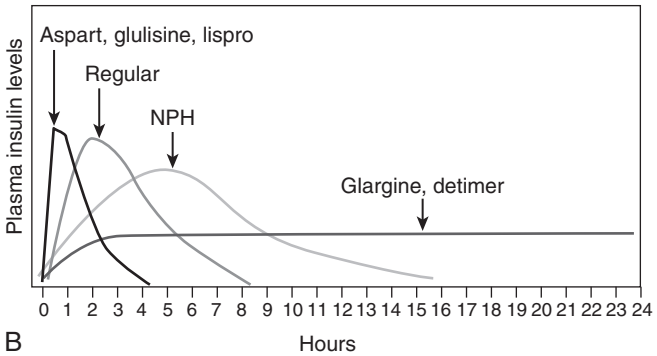


FIGURE 19-11 ■ **A** and **B,** Representation of the normal relationships among food intake, blood glucose, and serum insulin concentration. Note that glucose concentration is maintained between 80 and 140 mg/dL. Note also the precise release of insulin that has passed through the portal circulation synchronous with and proportional to the food-induced glycemic excursions. Compare and contrast these patterns with the time pattern of insulin action after subcutaneous injection of Aspart/glulisine/lispro, Regular, NPH, and glargine/detemir insulins.

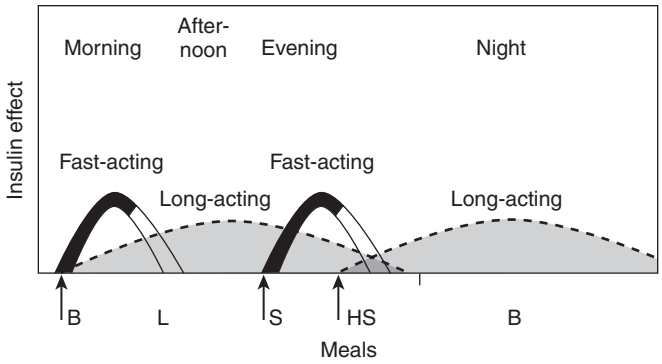


FIGURE 19-12 ■ Three-dose insulin regimen. The morning dose is composed of a combination short- and intermediate-acting insulin of one half to two thirds of the total daily dose. The short-acting dose before supper covers the anticipated glycemic elevation with dinner. The long-acting insulin is not given until bedtime so that the peak effect is delayed. (Redrawn from Schade, D. S., et al. (1983). *Intensive insulin therapy.* Belle Mead, NJ: Excerptal Medica.)

through the use basal-bolus therapy using multiple daily injections (MDI) or continuous subcutaneous insulin infusion (CSII) pump therapy. Until recently, options for insulin formulations were limited. Today, there are more than 10 varieties of biosynthetic human and analog insulins, including human regular insulin, human NPH insulin, long-acting insulin analogs, rapid-acting insulin analogs, and several kinds of premixed insulins (see Table 19-10).

Human Regular Insulin

Human regular insulin was a mainstay of insulin management of youth with T1D until the early 2000s when the advent of rapid-acting insulin analogs virtually eliminated its use in children and adolescents, except for intravenous administration. The delayed absorption and prolonged duration of action of the large premeal bolus doses of regular insulin that are required by adolescents with T1D to overcome the insulin resistance of puberty contributed to problems with hyper- and hypoglycemia in this age group.[142] Regular insulin remains the insulin of choice for intravenous infusion in the treatment of diabetic ketoacidosis. A special U-500 (500 units/mL) formulation of regular insulin as is available for use in patients with severe insulin resistance who require very large daily doses of insulin.

Rapid-Acting Analogs

Lispro (Eli Lilly), aspart (Novo Nordisk), and glulisine (Sanofi) insulins are produced by amino acid substitutions in the C-terminal region of the B-chain that reduce the affinity of insulin molecules to self-aggregate into hexamers. These modifications allow more rapid absorption of the analog into the bloodstream after subcutaneous injection. In comparison to regular insulin, the faster absorption of rapid-acting analogs results higher and sharper peaks and shorter duration of action, pharmacokinetic and pharmacodynamic effects that reduce the risk of late postprandial hypoglycemia and temper early postmeal hyperglycemia.[146,147] Puberty does not appear alter the pharmacokinetics of premeal boluses of rapid analogs, but the insulin resistance of puberty reduces the ability of these insulins to stimulate glucose metabolism.[148] Rapid-acting insulin may be safely mixed with intermediate-acting insulins, but mixing with long-acting insulin is not recommended, as such mixing markedly attenuates the peak action of the rapid-acting analog.[149,150] All three rapid-acting analogs may be used in an insulin pump, and studies have shown them to be safe and effective.[151,152] Lispro and aspart insulin may be diluted to concentrations less than U-100 such as U-50 or U-25 using diluents obtained from the manufacturer. Diluted insulin is used in very young children and others who are sensitive to insulin and could benefit from the more accurate dosing. Rapid-acting insulin analogs can be given intravenously, but they have not been shown to be superior to regular insulin.[153]

Intermediate-Acting Insulin

Neutral protamine Hagedorn (NPH) is now the only available intermediate-acting insulin. Although the delay in peak action of NPH given before breakfast provides a means to cover lunchtime glucose excursions, NPH is less than satisfactory for overnight basal insulin replacement.[154] As with regular insulin, the advent of long-acting insulin analogs has largely replaced NPH in basal-bolus MDI treatment in pediatrics. However, the combination of NPH in the morning before breakfast and long-acting insulin before dinner has been shown to be an effective initial treatment of newly diagnosed children and adolescents with T1DM.[155]

Long-Acting Insulin Analogs

Glargine (Lantus manufactured by Sanofi) and detemir (Levemir manufactured by Novo Nordisk) were the first two commercially available long-acting insulin analogs that were developed to meet the body's basal insulin requirements for regulating hepatic glucose production. Insulin glargine is an analog of human insulin with C-terminal elongation of the beta chain by two arginine residues and replacement of asparagine in position A21 by glycine. This analog is soluble in the acidic solution in which it is packaged but relatively insoluble in the physiologic pH of the extracellular fluid. Consequently, microprecipitates of glargine insulin are formed following subcutaneous injection, which markedly delays its absorption into the systemic circulation. The action of insulin detemir is prolonged by the addition of a fatty acid side chain that promotes reversible binding with albumin in the interstitial fluid and in the circulation.

Pharmacokinetic and pharmacodynamic studies have demonstrated that these long-acting insulin analogs have flat and prolonged time-action profiles. However, compared to glargine, detemir has been shown to have a more consistent pharmacokinetic profile in children and a more consistent pharmacodynamic profile in adults with T1DM.[156,157] Anecdotal evidence suggests that basal insulin replacement with insulin detemir is more likely

to require two daily injections more frequently than treatment with insulin glargine.

Insulin degludec is a new, ultra-long-acting insulin that is a product of coupling of Des-B30 threonine insulin to fatty acid side chains. After injection, degludec self-associates, forming long chains of insulin that dissociate very slowly. Plasma insulin levels rise reach peak concentrations 10 to 12 hours after an injection and the half-life is between 17 to 21 hours, roughly double the duration of action of insulin glargine. There is little information regarding the use of this insulin in pediatrics at this time.

Premixed Insulin

Various premixed insulin formulations, combining either rapid-acting insulin analogs or regular insulin with NPH or intermediate-acting insulin analogs, are currently available. The percentage of rapid acting/regular to intermediate acting varies with the formulation. The practical advantage of premixed insulins to cover both short- and intermediate-term insulin needs in a single shot is also their greatest limitation. There is less flexibility when using premixed insulin for adjusting the individual insulin components, and this can be particularly problematic in young children or those with variable food intake. Use of premixed insulin has been generally limited to patients and families who have difficulties managing more complicated treatment regimens.

Syringes versus Pens

Insulin syringes come in a variety of sizes that are particularly useful in young children. In addition to the traditional 100-unit (1-mL) syringe marked in 2-unit increments, 50-unit (0.5-mL) and 30-unit (0.3-mL) syringes are available that are marked in single-unit increments, and 30-unit syringes with ½-unit markings can also be ordered. These syringes are available with needle lengths from 8 to 13 mm. Insulin pens are also available, both in as disposable pens and a reusable pens with disposable cartridges. Currently, the disposable pens only allow for 1-unit doses, which may be problematic for very young children who might need ½-unit dosing. On the other hand, there are reusable cartridge pens that allow for ½-unit dosing.

Initiating Insulin Therapy

Most newly diagnosed patients who present with symptoms of diabetes or are in diabetic ketoacidosis (DKA) require upward of 1 U/kg/day at the start of insulin treatment. For asymptomatic patients who are "incidentally" diagnosed with diabetes during a routine sports physical or a well-child exam, starting doses may only need to be ~0.5 U/kg/day. The goal of initial insulin therapy in children with T1D should be to achieve near normoglycemia over the first few weeks of treatment in order to give the child the opportunity to enter the "honeymoon," or partial remission phase. The honeymoon period results from a combination of improved function of residual beta cells and reversal of the insulin resistance that accompanies uncontrolled diabetes. Prompt achievement of optimal control rather than the methods used to achieve good control appears to be the most important factor in obtaining these outcomes.[158] The ability to achieve target A1c levels with little or no severe hypoglycemia is greatly enhanced in T1D patients with residual beta-cell function.[159]

Some centers admit all newly diagnosed patients to the hospital for initial diabetes education and insulin dose titration, but others have effective outpatient treatment protocols. During the 2 to 3 weeks following discharge, insulin doses are titrated toward target premeal glucose values of 70 to 130 mg/dL during daily telephone contact; patients are seen in follow-up clinic visits, approximately 2, 6, 13, 26, 39, and 52 weeks following diagnosis.

INSULIN REGIMENS

Plasma insulin levels in nondiabetic individuals are characterized by relatively low basal levels on which are superimposed meal-stimulated spikes in insulin concentrations. Current intensive treatment regimens attempt to simulate this pattern of plasma insulin fluctuations by employing a basal-bolus approach to insulin replacement. Because exogenous insulins are injected or infused subcutaneously rather than directly into the portal vein, their rates of absorption may be variable. Also, the dose that is injected is determined empirically, so it lacks the precision of endogenously secreted insulin. Thus, no insulin replacement regimen will precisely duplicate the pattern of normal insulin secretion; there will be periods of increased plasma insulin concentrations that may produce hypoglycemia and periods of low insulin levels that lead hyperglycemia. Therefore, the goal of current insulin regimens is to minimize the frequency and severity of excursions into the hyper- and hypoglycemic range.

The DCCT and EDIC studies established basal-bolus therapy using either MDI or CSII as the gold standard of treatment of T1D. However, insulin only works if the youngster receives it, and other factors must be addressed when determining the best insulin regimen for an individual patient. These factors include the availability of an adult parent/guardian to supervise insulin administration, the ability to count carbohydrates and monitor blood glucose (BG) levels, and the willingness to wear a pump or take four or more injections of insulin daily.

Multiple Daily Injection (MDI) Regimens

MDI regimens attempt to replicate normal insulin secretion through the use of a long-acting insulin analog to replace basal insulin needs along with bolus injections of rapid-acting insulin analog to cover food intake and to correct elevations in blood glucose levels. Either once or twice daily glargine or detemir may be used for basal insulin coverage. Typically, basal insulin accounts for approximately 40% to 50% of the insulin total daily dose (TDD). However, children younger than 5 years of age often require basal insulin doses that are 30% to 40% of TDD, and there is considerable variability between patients. Once-daily glargine or detemir may be given

either in the morning or in the evening. Although this has not been well studied in pediatric patients, patients treated with detemir may be more likely to need twice-daily injections than those treated with glargine. Some pediatric practitioners mix long- and rapid-acting insulin in order to reduce the number of injections, but studies indicate that such mixing markedly blunts and delays the absorption and action of the rapid-acting insulin component of the mixture.[149,150]

Any one of the rapid-acting insulin analogs may be used to cover bolus insulin needs. Ideally, the rapid-acting insulin bolus is given 10 to 15 minutes before eating, but this is a difficult goal to achieve in many youth with T1D. For the most precise dosing of bolus insulin, it is necessary to use an insulin-to-carbohydrate (ICR) ratio and insulin sensitivity or correction factor, as well as the rate and direction of change in sensor glucose levels in patients using continuous glucose monitoring devices. The ICR is defined as the grams of carbohydrate that 1 unit of insulin will cover. This ratio may be determined using the 500 rule; namely, dividing 500 by the total daily insulin dose (TDD) will give a starting point for the carbohydrate ratio. For example, in a teenager with a TDD of 50 units, 1 unit of insulin will cover 10 grams of carbohydrate (500/50 = 10). It should also be noted that the ICR frequently differs by time of day, as more insulin is often needed per gram of carbohydrate before breakfast and less may be given to cover bedtime snacks. Accuracy in determining the carbohydrate content of meals is of utmost importance but often a problem in adolescents. Teens tend to underestimate the number of carbohydrates in their meal, and refresher meetings with a dietitian can reinforce the importance of maintaining mastery of this skill.

In addition to meal coverage, a correction dose of rapid-acting insulin should be given at meal times in order to "fix" an elevated blood glucose levels. Correction doses may also be given at other points in the day for the same reason. Traditional "sliding scale" insulin doses based on blood glucose levels have given way to a more sophisticated dosing algorithms based on an insulin sensitivity factor (ISF); namely, how much will blood glucose be lowered by 1 unit of insulin. The ISF may be determined using the 1800 rule: divide 1800 by the TDD. The correction dose can then be calculated according to the following equation:

$$\frac{\text{(Actual BG} - \text{Target BG)/ISF}}{\text{Number of units of insulin}} =$$

No matter how they are initially calculated, ICRs and ISFs are subsequently adjusted based on glucose monitoring results.

Particular advantages of basal-bolus MDI regimens are that they try to mirror the physiologic model of insulin secretion, increase flexibility in the timing and size of meals, and provide more opportunities to "course correct" throughout the day in response to abnormal glucose excursions. A particular disadvantage of these regimens is that they require a large number of daily injections. Indeed, because of the flat time-action profile of the long-acting basal insulin analogs, basal-bolus MDI treatment puts a premium on compliance with premeal bolus dosing. Without bolus doses of rapid-acting insulin, long-acting basal insulin alone cannot prevent marked postprandial hyperglycemia. However, strict adherence to premeal bolus dosing is difficult for teenagers with T1D, even when it is made as easy as possible to accomplish with an insulin pump. These are some of the reasons why mean A1c levels in pediatric patients, especially adolescents, with T1DM, remain well above the target levels recommended by the ADA and ISPAD (Box 19-3).[160]

Insulin Pump Therapy

The first successful studies of effectiveness of CSII were carried out in children in the 1980s,[161] but it was not until the early 2000s that the use of CSII in pediatrics took hold.[162] Since then, CSII has been shown to be more effective in lowering A1c compared to injection therapy in randomized and nonrandomized pediatric studies and to be associated with improved patient satisfaction and reduced frequency of hypoglycemia.[163] Some of the practical benefits of CSII compared to MDI are listed in Box 19-4. From the pediatric practitioner perspective, the memory function that records all pump-related activities is especially important. A specific risk of pump therapy is that prolonged accidental or purposeful interruption of insulin delivery over several hours can lead to the development of ketones and DKA because the patients are only receiving rapid-acting insulin.[164] This risk can be reduced or eliminated by regular blood glucose testing and standard patient protocols for managing hyperglycemia.

Indications for Pumps in Pediatrics

In 2006, an international consensus conference of leading pediatric diabetes specialists was convened to develop

BOX 19-3 Levels of Treatment: Biochemical and Clinical Characteristics

Minimal

- HbA1c 11%-13%, GHb 13%-15%
- Many SMBG values of 300 mg/dL
- Almost constantly positive urine glucose tests
- Intermittent spontaneous ketonuria

Average

- HbA1c 8%-9%, GHb 10%-11%
- Premeal SMBG 160-200 mg/dL
- Intermittent positive urine glucose
- Rare ketonuria

Intensive

- HbA1c 6%-7%, GHb 7%-9%
- Premeal SMBG 70-120 mg/dL; postmeal SMBG, 180 mg/dL
- Essentially no positive urine glucose or ketones

GHb, glycosylated hemoglobin; HbA1c, glycohemoglobin; SMBG, self-monitored blood glucose.

BOX 19-4 Practical Benefits of CSII

- Basal infusion rates adjustable up to every 30 minutes
- Programmable temporary basal rates
- Preprogrammable alternate basal rate profiles
- One site insertion every 2 to 3 days (versus many injections each day)
- Dose calculator
- Pump history functions and ability to upload data to data management systems
- Customizable square wave and dual wave boluses
- Ability to program temporary basal rates for sick days and during and after exercise
- Ability to deliver very small (0.025 to 0.05 units) doses of insulin

treatment guidelines for the use of CSII in children and adolescents.[165] These experts agreed that CSII was indicated for pediatric patients who had the following conditions:

- Elevated A1c levels on injection therapy
- Frequent severe hypoglycemia
- Widely fluctuating glucose levels (regardless of A1c)
- A treatment regimen that compromises lifestyle
- The presence of microvascular complications or risk factors for macrovascular complications

CSII was also thought to be beneficial in athletes, very young children, adolescents with eating disorders, patients with a pronounced dawn effect, ketosis-prone patients, pregnant teens (ideally preconception), and children with pronounced needle phobia.[165]

Ideal candidates for CSII include motivated families who are committed to monitoring the BG at least four times per day and have a working understanding of basic diabetes management, especially carbohydrate counting and using ICR and ISF to calculate insulin doses. HbA1c level also plays a role in determining readiness for CSII, although it is less important than the factors listed earlier. We generally prefer to have A1c levels below 8.5% before transition to pump treatment. The age of the patient and duration of diabetes should not be factors in determining when patients will transition from injections to CSII. In fact, it is increasingly clear that infants, toddlers, and preschoolers are probably the ideal pediatric patients for CSII, because it lowers A1c, reduces the frequency of severe hypoglycemic episodes, and improves the quality of life of parents.[166,167] Moreover, starting newly diagnosed patients immediately on pump therapy is an increasingly common practice.

Pump Features

Insulin pump therapy uses only a rapid-acting insulin analog to deliver 24/7 insulin coverage. A reservoir for the pump is filled with several days' worth of insulin. In conventional pumps, the reservoir attaches to a variable length of tubing, which in turn attaches to a small catheter or steel needle that is inserted into the subcutaneous tissue. Most common sites for insertion include the buttocks, abdomen, upper leg/hip, and some children use their arms. Insertion of the infusion set is done by either the child or caregiver and should be done every 2 to 3 days or whenever persistently high BG values indicate a potential site failure. The Omni Pod is a "patch pump" in which both the mechanics to drive the pump and the insulin are contained in a disposable "pod." This pod is attached to the surface of the skin and includes an integrated catheter, which allows for subcutaneous delivery of the insulin. Insulin doses programmed by the patient/caregiver throughout the day are delivered by way of a hand held device, which wirelessly communicates with the patch.

Most currently available insulin pumps are "smart" pumps into which both ICRs and ISFs are programmed to create a bolus calculator. Some pumps have the ability to receive and integrate blood glucose values into their bolus calculator through a wireless link with a specific blood glucose meter. Bolus doses can be administered over a few minutes or as square-wave and dual-wave boluses over a longer period of time. Ideally, bolus doses should be delivered 10 to 15 minutes before the meal in order to minimize postmeal excursions.[168] In young children and picky eaters, a small priming bolus of insulin can be given before the meal, followed by additional bolus doses depending on how many carbohydrates are actually consumed.

Basal insulin needs are covered by the rapid-acting insulin, which is delivered through a preprogrammed "basal pattern." This pattern can be made up of multiple different rates, which allow for a waxing/waning pattern of basal insulin delivery. In addition, most pumps allow for multiple 24-hour basal patterns to be preprogrammed and stored in the memory. For example, some of our adolescents have a basal pattern for school days and one for weekends to account for their tendency wake up much later on weekends and holidays. Finally, temporary changes can be made to the basal insulin pattern, which can be an effective tool for dealing with exercise or sick day management.

NPH-Based Treatment Regimens

The "split-mixed" regimen consisting of two daily doses of NPH and regular insulin that were mixed together in the same syringe was a standard approach to insulin replacement in pediatrics for decades. The regular insulin component was replaced by rapid-acting insulin analogs when lispro insulin was introduced. Patients started on this approach generally receive two thirds of the total daily dose before breakfast and one third before dinner. Each injection starts with approximately two thirds of NPH and one third of rapid-acting analog. Individual components of the regimen are subsequently adjusted based on blood glucose testing results.

This conventional treatment regimen is almost always inadequate for patients with T1DM who have no residual endogenous insulin secretion due the limitations of both the pre-breakfast dose of NPH to cover lunch and the pre-dinner dose of NPH to provide basal insulin replacement during the overnight period. The conventional twice a day regimen can still play a role in newly diagnosed patients—who frequently go through a "honeymoon" or partial remission period of diabetes.

During the honeymoon, insulin requirements rapidly decrease and the doses of rapid-acting insulin may even be discontinued due to low pre-lunch and bedtime glucose levels. A major reason the two daily injections regimen is effective during the honeymoon period is that stimulation of endogenous insulin secretion curbs postmeal glucose excursions and it provides much of the overnight basal insulin requirements, leading to normal fasting blood glucose values. Conversely, increased and more labile pre-breakfast glucose levels often herald the loss of the relatively small amount of residual endogenous insulin secretion required for overnight glucose control. An alternative to the twice-a-day NPH is to retain the pre-breakfast mixture of NPH and rapid-acting insulin, cover dinner with rapid-acting analog, and use glargine or detemir (given before supper or bedtime) for overnight basal replacement. This modified twice-a-day conventional insulin regimen has been shown to be effective in achieving target A1c levels during the first year of treatment of T1D.[155]

Blood Glucose Monitoring

The safety and success of any insulin regimen depend on frequent monitoring of blood glucose levels. Intensive diabetes control would have been impossible without the development of accurate and easy-to-use home glucose meters and HbA1c levels in adults and children with T1DM are inversely correlated with the daily frequency of blood glucose monitoring. Many brands of glucose meters are commercially available, most of which are accurate to within about 5% to 10% of laboratory measurements.[169,170] Current models utilize glucose-oxidase-based electrochemical methods. The meters are fast and require very small volumes of blood (0.1 mL). The smaller blood volume requirement has allowed alternate site testing (e.g., forearm), which may minimize discomfort and improve adherence to self-monitoring regimens.

A key component of intensive insulin management is regular adjustment of insulin doses based on changes in glucose control and on daily activity. Frequent blood glucose monitoring, with a minimum of four tests per day, premeals, and at bedtime, should be the goal. In addition to the traditional four blood glucose tests, many patients benefit from strategically adding further tests, such as 2 hours after meals, overnight and before/after exercise in order to develop a more robust picture of daily glucose trends. Testing should also be done whenever the symptoms of hypoglycemia occur.

The special challenge of managing T1D during childhood and adolescence is that insulin requirements are always changing. Thus, simply measuring blood glucose and giving immediate correction doses are insufficient for long-term glycemic control in pediatrics. Instead, parents and patients need to be instructed on how to recognize trends that indicate that patients have outgrown their insulin dose(s) and how to make their own adjustments in the regimen.[171] Pattern recognition requires maintaining and reviewing an electronic of written log of blood glucose levels over time. Unfortunately, evidence indicates that too few families are downloading and reviewing glucose meter data and making insulin self-adjustments.[172]

Continuous Glucose Monitoring

Even when families regularly test blood glucose levels, they only see the "tip of the iceberg" when it comes to daily fluctuations in glucose levels.[173] Thus, the introduction of real-time continuous glucose monitoring (CGM) systems has the potential to revolutionize insulin management. Currently available CGM devices are inserted subcutaneously and measure interstitial fluid glucose concentrations using glucose-oxidase–based electrochemical methods. Sensor glucose are reported in real time ~ every 5 minutes with rate of change values and 1- to 24-hour retrospective profiles. This wealth of information allows for adjustments in insulin doses based on a retrospective analysis as well as immediate, in the moment adjustments. Web-based data managing systems are available to assist patients and clinicians in evaluating sensor data alone or in combination with insulin pump data.[174]

JDRF CGM Randomized Control Trials demonstrated that adults who used CGM the on a nearly daily basis significantly lowered HbA1c levels without increasing the risk of hypoglycemia and those who have already achieved A1c targets can better maintain this level of control with the use of CGM.[175] This held true for youth who were willing to wear the sensor almost every day as well. Unfortunately, far fewer children and adolescents were able to achieve the goal of almost daily wear. Indeed, in current pediatric practice in the United States, < 5% of pediatric patients are currently using CGM.[172] Thus, improvements in the CGM devices, including integration in sensor-augmented pump systems, are needed for these devices to fulfill their promise in improving clinical outcomes in children and adolescents with T1D.

Medical Nutrition Therapy

Proper nutritional management is critical to the short- and long-term health of children with diabetes. Generally, terms such as *diet* should be avoided in favor of *meal plan* or *healthy food choices*—both for the negative connotation associated with the former and for the simple fact that nutritional requirements for normal growth and development are the same in diabetic and nondiabetic children. Moreover, reasonably accurate estimations of the carbohydrate content of meals are important to optimal glycemic control with current basal-bolus treatment regimes. Indeed, the popularity of basal-bolus therapies and the use of carbohydrate counting to adjust the dose of rapid-acting insulin taken with each meal have fundamentally changed the treatment paradigm.

The traditional approach of adjusting the patient's lifestyle around fixed insulin doses and fixed amounts of carbohydrate intake with each meal has been replaced by a more flexible approach that attempts to adjust the insulin regimen to the patient's lifestyle. The day-to-day variations in appetite in children and adolescents make the latter approach more likely to be successful. It is

important to note that in some patients and some families, the traditional approach of trying to consume consistent carbohydrate servings per meal/snack may be more successful because it fits their personalities and lifestyle better.

The currently favored model of nutritional therapy is carbohydrate counting, based on the conceptual model of matching carbohydrate "doses" to insulin doses.[176,177] As the total carbohydrate content of foods (rather than the type of carbohydrate) has the greatest impact on blood glucose, the amount of carbohydrates ingested per meal or snack needs to be estimated as accurately as possible. Although the protein and fat contents of meals do affect the pattern of postmeal glucose excursions,[178,179] they are not usually counted in order to simplify the procedure. Food labeling requirements have simplified the process, as most foods are clearly labeled with the number of carbohydrate grams per serving and the serving size. Foods less easily quantified may be weighed or estimated, and eating out may be a particular problem. Although the prohibition of "sweets" has been done away with, we still recommend that patients drink diet rather than regular sodas.

In the flexible approach to nutrition counseling, there is no set intake. Rather, the child and parents decide the meal content. The carbohydrates are counted and an insulin dose is calculated based on a ratio of the number of insulin units per grams of carbohydrate determined by the empirical trial-and-error method. Actual insulin-carbohydrate ratios vary from child to child and in the same child according to the time of day. Breakfast often requires relatively more insulin than lunch or dinner. Carbohydrate counting can also be used in the traditional approach to dietary treatment to provide consistent carbohydrate servings per meal/snack. Indeed, a simple approach that stresses consistency in the timing and size of meals may be effective at the time of diagnosis of diabetes—when parents and patients are too overwhelmed to be able to learn more advanced nutritional concepts.[180]

The American Diabetes Association's recommendations regarding general nutritional principles in diabetes also take into consideration the long-term goal of preventing the macrovascular and microvascular complications of diabetes.[180] Consequently, heart-healthy diets low in cholesterol and saturated fats are encouraged. Long-term goals of nutritional management of diabetes include maintenance of nutrient intake balance of about 50% carbohydrate, 20% protein, and 30% fat (of which no more than 10% should be saturated). Most important, growth and weight gain should be monitored—and regular follow-up with a dietitian trained in diabetes management should be encouraged to individualize a meal plan for each child based on his or her needs and food preferences. We face an epidemic of childhood obesity in developed countries, and one of the adverse consequences of intensive insulin treatment is that 30% to 35% of pediatric patients with T1D in the United States are overweight or obese.[172] Thus, any tendency for body mass index (BMI) Z scores to increase excessively needs to be dealt with promptly.

Exercise

Establishment and maintenance of an active lifestyle should be a goal for all children, but especially children with diabetes, in order to enhance cardiovascular health. Exercise and increased physical fitness are associated with improved insulin sensitivity and glucose utilization, the clinical correlates of which are lower insulin requirements, lower blood pressures, and better lipid profiles. Improvements in physical fitness are also frequently associated with greater self-esteem and increased motivation to participate in diabetes care.

Despite its benefits, acute bouts of exercise in children with T1DM actually make regulation of blood glucose levels more difficult. Hypoglycemia is a common complication during exercise,[181] and excessive snacking to prevent hypoglycemia can result in hyperglycemia and negate some of the metabolic and cardiovascular benefits of exercise. These difficulties are compounded by the irregular pattern of physical activity that characterizes most youth who are not participating in organized sports or regimented training programs and by conventional methods of diabetes management that feature fixed basal insulin replacement doses. The Diabetes Research in Children Network demonstrated that the risk of nocturnal hypoglycemia nearly doubled in youth with T1D following antecedent afternoon exercise versus sedentary days.[182]

The effects of exercise must be carefully considered in the context of the entire diabetes care plan. Children participating in school sports or other programs should be counseled to monitor blood glucose before, during, and after exercise in order to optimize glycemic management. In addition, patients and parents should be aware that late hypoglycemic effects of afternoon exercise are often observed 7 to 11 hours after the bout of exercise in the afternoon,[183] a phenomenon that appears to be due to an increase in nonoxidative glucose disposal during sleep, which may serve to support repletion of muscle glycogen stores.[183]

In pump patients, simply suspending the basal infusion rate can markedly reduce the risk of hypoglycemia during exercise[181]—and similar benefits may accrue from reducing the overnight basal rates after very active days. Studies that have examined methods of managing glycemia during exercise illustrate that there is an almost infinite number of combinations of conditions that need to be considered. Because of this complexity, trial and error remains the principal method of managing glucose levels during and after exercise in children and adolescents with T1DM.[184]

Residual β-Cell Function (The Honeymoon Period)

After the initiation of insulin treatment in newly diagnosed patients, secretory function of residual beta cells improves and the insulin resistance and glucotoxicity of decompensated diabetes are reversed—heralding the onset of the partial remission (honeymoon period) of T1DM.[185] Most patients require a progressive reduction in their daily dose of insulin from ≥ 1 to ≤ 0.5 U/kg.

A minority of children (fewer than 5% of patients) can even maintain normoglycemia for a time without any administered insulin. Although opinion varies, we do not discontinue insulin treatment unless a daily dose of 0.1 U/kg still causes hypoglycemia.

The duration of the honeymoon phase in children with T1DM is variable but the lowest HbA1c levels and total daily insulin doses (TDD) are usually observed between 3 and 6 months postdiagnosis and both often rise between 9 and 15 months.[186] With current MDI and CSII treatment methods, the large majority of youth with T1D retain substantial residual insulin secretion, as measured by C-peptide responses to mixed meal tolerance tests, during the first year of their disease, and this can be extended for several years thereafter with maintenance of optimal glycemic control.[187] In comparison to patients who are C-peptide negative, patients with residual beta-cell function have lower HbA1c levels and total daily insulin doses—as well as a reduced risk of hypoglycemia.[188] These clinical and metabolic benefits serve as a strong rationale for current research involving immune interventions directed at preserving beta-cell function in newly diagnosed patients.[189] On the other hand, earlier and more aggressive application of sensor-augmented pump therapy around the time of diagnosis does not appear to confer any advantages over standard MDI and CSII with respect to HbA1c levels or C-peptide responsiveness in youth with T1DM.[158]

Hypoglycemia

The dark side of intensive treatment regimens that are effective in lowering HbA1c levels is that they also increase the risk of hypoglycemia. In the DCCT, intensive therapy was associated with an approximate threefold increased risk for severe hypoglycemia versus conventional treatment and, irrespective of treatment group, the rate of severe hypoglycemia was about 50% higher for adolescents than for adults.[143] Hypoglycemia has become the most significant barrier to the pursuit and maintenance of tight glycemic control among people with T1DM, and effectively managing of the risk for hypoglycemia is especially important in the treatment of children and adolescents with this disease.

The American Diabetes Association defines biochemical hypoglycemia (with or without symptoms) as any plasma glucose level < 70 mg/dL.[190] In nondiabetic adults, this is the plasma glucose level at which counterregulatory hormone responses engage and at which awareness of symptoms normally occurs. It should be noted, however, that such responses may be triggered at higher glucose levels in healthy 8- to 16-year-olds and in children and adolescents with T1DM who have poor glycemic control.[191]

The two main mechanisms that cause symptoms and signs of hypoglycemia are an outpouring of catecholamines (which results in pallor, sweating, apprehension, trembling, and tachycardia) and the effects of cerebral glucopenia, which include hunger, drowsiness, mental confusion, seizures, and coma. Mood and personality changes may be more subtle cerebral glucopenic effects that provide an early clue that plasma glucose has fallen to a dangerous level. Symptomatic episodes in which patients are able to treat themselves without the assistance of others are considered minor or mild hypoglycemia, whereas episodes in which there is sufficient cognitive impairment that treatment requires the assistance of another person are considered major or severe hypoglycemic events. Hypoglycemic events that cause seizures or coma are usually classified as a subset of severe hypoglycemia. In severe hypoglycemia, ingestion of carbohydrates may be precluded due to loss of consciousness, seizures, or coma—and treatment may require administration of a glucagon injection or IV glucose infusion.

In nondiabetic children, the initial response to falling plasma glucose levels is a prompt suppression of insulin secretion. If plasma glucose continues to fall and threshold values for release of anti-insulin counterregulatory hormones are reached, there are abrupt increases in circulating concentrations of glucagon and epinephrine. Plasma growth hormone and cortisol levels also increase, but these hormones are less important in acutely counteracting the effects of insulin. Defective counterregulation occurs in patients with T1DM because exogenously supplied insulin levels do not decrease in response to low blood glucose levels and the ability to secrete glucagon in response to hypoglycemia is lost early in the course of the disease.[185,192] Consequently, patients with T1DM depend on sympathetic nervous system responses—especially increases in plasma epinephrine levels—to prevent hypoglycemia.

The episodes of mild hypoglycemia that frequently accompany intensive treatment blunt catecholamine responses and symptom awareness to subsequent hypoglycemic challenges. This phenomenon has been called hypoglycemia-associated autonomic failure (HAAF).[193,194] Studies have shown that such defects in counterregulatory hormone responses are common in both young children and adolescents with T1DM who are well controlled.[195] Catecholamine responses to hypoglycemia are also impaired during sleep, which is an important reason why most of the severe hypoglycemia events occur during the night.[196] As noted earlier, bouts of exercise in the afternoon sharply increase the risk of hypoglycemia on the following night.[184]

The use of basal-bolus therapy with insulin analogs and insulin pumps has reduced the frequency of severe hypoglycemia somewhat but has by no means eliminated this problem. HbA1c levels can be reduced in patients who use CGM with or without integration in sensor-augmented pump system without increasing the risk of hypoglycemia, but severe hypoglycemia remains a significant problem.[175] In the T1D Exchange Clinic Registry that includes >10,000 children with T1D, one in 20 patients reported having at least one seizure or coma event related to hypoglycemia in the prior 12 months.[172] It is also noteworthy that the risk of severe hypoglycemia in patients with HbA1c levels $> 9.5\%$ in this cohort was similar to that in patients with HaA1c levels $< 8\%$. These data indicate that poor-control, per se, is not an effective strategy for reducing the risk of hypoglycemia.[172]

It is important that the patient and family recognize early symptoms and signs of hypoglycemia and known

precipitating factors. Mild episodes should be immediately treated with 10 to 15 g of carbohydrate (e.g., glucose tablets, juice, or glucose gel). Parents and school nurses also need to be instructed on how to perform injections of glucagon (0.5 to 1.0 mg) when the patient has lost consciousness and is unable to swallow exogenous carbohydrate. If exercise has been the precipitating factor, the patient should be instructed about preventive measures.

Sick Day Management

Children with intercurrent illnesses, such as infections or vomiting, should be closely monitored for elevations in blood glucose levels and ketonuria. On sick days, blood glucose levels should be checked every 2 hours—and the urine should be checked for ketones with every void. Supplemental doses of rapid-acting insulin (0.1 to 0.3 U/kg) should be given every 2 to 4 hours for elevations in glucose or ketones. Even in the absence of marked hyperglycemia, the presence of ketones indicates insulin deficiency and therefore the need for supplemental insulin.

Adequate fluid intake is essential to prevent dehydration and hasten the excretion of ketones. Fluids such as flat soda, clear soups, popsicles, and gelatin water are recommended to provide some electrolyte and carbohydrate replacement. In the child tolerating oral rehydration, a fluid "dose" of 1 ounce per year of age per hour serves as a rough guideline, the sugar content of which depends on the serum glucose. For blood glucose values $>$ 200 mg/dL, sugar-free fluids should be given. For those levels between 140 and 200 mg/dL, a mixture of sugar-free and sugar-containing fluids should be given. For blood sugar $<$ 140 mg/dL, only sugar-containing fluids should be given.

If emesis precludes normal oral intake, the intermediate or long-acting insulin should be discontinued and small frequent doses of short- or rapid-acting insulin should be given. In pump-treated patients, higher or lower temporary basal rates may also be used, depending on changes in glucose and ketone levels. Once the ketones have cleared and the child is tolerating an oral diet, the family may resume the normal routine. If vomiting is persistent and ketones remain moderate or large after several supplemental insulin doses, arrangements should be made for potential hydration and evaluation in a hospital emergency department.

Special attention should be paid to persistent hyperglycemia in patients using pump therapy, as use of this modality of insulin administration is a risk factor for diabetic ketoacidosis (DKA). Because only rapid-acting insulin is administered, an interruption of insulin delivery will result in increases in blood and urine ketone levels in 4 to 6 hours.[164] These interruptions are most commonly caused by problems with the infusion set such as kinking or occlusion of the subcutaneous catheter. Thus, assessment of hyperglycemia and any symptoms of nausea and vomiting must include an evaluation of the integrity of the infusion set and infusion site.

Associated Autoimmune Diseases

Patients with T1DM are at increased risk for other autoimmune diseases. These are detailed in Chapter 20 , in the discussion of multiple endocrine deficiency syndromes. Chronic lymphocytic thyroiditis is frequently associated with T1DM in children, and as many as one in five may have thyroid antibodies in their serum. Only a small proportion of these patients develop clinical hypothyroidism, however. The interval between the diagnosis of diabetes and that of thyroid disease averages about 5 years. Physicians should anticipate the possibility of hypothyroidism in patients with T1DM by periodic examination of the thyroid gland and measurement of serum thyroid-stimulating hormone (TSH) concentration.

When diabetes and thyroid disease coexist, the possibility of adrenal insufficiency should be considered. This may be heralded by decreased insulin requirements, increased pigmentation of the skin and buccal mucosa, salt craving, weakness, and postural hypotension. Rarely, frank Addisonian crisis is the first evidence of adrenal failure. This syndrome generally occurs in the second decade of life or later.

Celiac disease affects from 1.5% to 4.5% of children with T1DM, and most of them are not aware of any gastrointestinal symptoms. Early diagnosis requires screening of tissue transglutaminase antibodies, which have high degrees of reproducibility, specificity, and sensitivity if total circulating IgA concentrations are not abnormally low. Antibody-positive children should be referred to a pediatric gastroenterologist for confirmation by small bowel biopsy, as well as for counseling and disease management. When typical signs and symptoms of malabsorption are present or the patient has frequent hypoglycemia or unexplained behavioral mood swings, dietary intervention is indicated. When symptoms are minimal or absent, the decision to introduce gluten-free diets with their additional restrictive and inconvenient burdens on the patient and family are less clear-cut.[197] To guide their decision, the parents and older children need to be made aware of the risk of bowel malignancies in untreated celiac disease in later life and of other long-term complications of this disease.

Associated Psychosocial Problems

Diabetes in a child affects the lifestyle and interpersonal relationships of the entire family. Guidelines for psychosocial management and support of families with childhood diabetes have been published. Feelings of anxiety and guilt are common in parents. Similar feelings, coupled with denial and rejection, are equally common in children—particularly during the rebellious teenage years. These issues are not unique to T1DM but are observed in families with children who have other chronic disorders difficult to treat. These stresses are often exaggerated in single-parent low-income families and impair their ability to effectively carry out needed self-management tasks, resulting in poor metabolic control.[198] Language and cultural barriers are additional obstacles in immigrant families.[199]

Psychosocial difficulties and conflict between patients and parents may result in nonadherence to instructions regarding healthy food choices, insulin therapy, and frequency of blood glucose monitoring. In this era of basal-bolus insulin therapy, missing premeal bolus doses is the most common cause of elevated HbA1c levels, especially in adolescents. Consequently, one of the major advantages of CSII over MDI in pediatric patients is that the bolus history function of current insulin pumps provides a record of the number of daily bolus doses that were actually administered.[200] Deliberate overdosage with insulin resulting in hypoglycemia or omission of insulin to limit weight gain (also referred to as "diabolemia") may be pleas for psychological help. Occasionally, they may be manifestations of suicidal intent. Frequent admissions to the hospital for ketoacidosis or hypoglycemia should arouse suspicion of underlying emotional and family conflict. Feelings of being different or of being alone are also common.

The clinician managing a child or adolescent with diabetes should be aware of his or her pivotal role as counselor and advisor and should anticipate the common emotional problems of the patient. When emotional problems are clearly responsible for poor compliance with the medical regimen, referral for psychological help is indicated. In pediatric centers, psychologists form part of the management team for children with diabetes. Although clinical depression is one of the most common comorbidities in youth with T1DM, problems in this area may not be evident during routine clinic visits, especially in preteens who may present as somewhat withdrawn rather than rebellious.[201] As a result, treatment guidelines suggest that screening questionnaires for depressive symptoms should be administered to youth with T1DM on a regular basis.[202] However, it has been difficult to implement this recommendation in most clinical practices.

Outpatient Care

The importance of frequent follow-up by the diabetes health care team cannot be overemphasized. Children and adolescents with T1DM should be routinely cared for at a diabetes center that uses a multidisciplinary team knowledgeable about and experienced in the management of young patients. This team should ideally consist of pediatric diabetologists, diabetes nurse specialists, nutritionists, and social workers or psychologists.

The American Diabetes Association has published guidelines for care of the child and adolescent with diabetes.[144] Regular follow-up visits with the physician or diabetes nurse specialist/practitioner every 2 to 3 months are recommended for most patients. The main purpose of these visits is to ensure that the patient is achieving primary treatment goals. Outpatient visits provide an opportunity to review glucose monitoring (and CGM profiles), to adjust the treatment regimen, and to assess child and family adjustment. At each visit, glucose records should be reviewed and appropriate dosage or schedule changes should be instituted. The nutritionist and psychologist or social worker also gives follow-up advice and support.

A detailed interim history should include questions relating to general health, energy, fatigue, polyuria or nocturia, intercurrent illnesses, hypoglycemic episodes, and the presence of symptoms such as abdominal pain, bloating, or diarrhea. It is important to remember the comorbidities of other autoimmune disorders that occur with an increased frequency in children with T1DM: thyroiditis, adrenal insufficiency, and celiac disease. The child should be weighed and measured at each visit, and blood pressure documented. Physical examination of the child should focus on the general organ systems and on examination of the skin and insulin injection/pump insertion sites for signs of lipohypertrophy or pigmentary changes, palpation of the thyroid, and determination of the stage of sexual development stage.

Lipoatrophy was commonly observed in the past but is now a rare complication of insulin therapy.[203] A deceleration in growth, delay in sexual development, or finding of goiter may herald hypothyroidism. The astute clinician should also consider that frequent unexplained hypoglycemia or reduction in insulin requirements in the absence of exercise or activity might be subtle indicators of hypothyroidism or adrenal insufficiency. Similarly, although a history of having frequent foul-smelling greasy stools is a more obvious indicator of celiac disease, the majority of cases are asymptomatic.

Measurement of glycosylated hemoglobin (HbA1c) provides the gold standard by which to judge the adequacy of the insulin regimen and use of point-of-service methods that can be performed in the office in a few minutes offers the opportunity to make immediate changes in the insulin regimen while the patient is being seen. Results of point of care measurements compare favorably with laboratory methods.[204] Even more important, the results of this test delivered during face-to-face encounters with the clinician serve as the quarterly "report cards" for the child and the parents. Teenagers may not be able to identify with the concept of working hard on their diabetes to be healthier many years in the future, but most are able to understand good grades. Thus, the HbA1c level provides a tangible outcome with which they can identify. The goal of treatment is to achieve HbA1c levels as close to normal as possible. Based on DCCT results and ISPAD recommendations, our general goal of therapy is to try to keep all patients under 7.5%. HbA1c levels are determined at least every 3 months.

Routine screening for associated autoimmune diseases with T4, thyroid-stimulating hormone (TSH), and tissue transglutaminase IgA every 1 to 2 years is recommended. Monitoring of the diabetic child for potential complications and cardiovascular risk factors is another important function of the clinic visit. The normal urinary albumin to creatinine ratio is < 30 mg albumin to gm creatinine, microalbuminuria is 30 to 300 mg/gm, and macroalbuminuria is > 300 mg/gm. Elevated spot samples should be confirmed at least a second positive. Preferably this would be either a first morning void or a timed overnight collection rule out benign orthostatic proteinuria. If the confirmatory test also demonstrates microalbuminuria, treatment with angiotensin-converting enzyme inhibitor or angiotensin receptor blocker therapy or referral to

pediatric nephrologist is indicated. All diabetic children with hypertension, regardless of albumin excretion status, should be considered candidates for these medications, as well. Although there are no specific guidelines for diabetic children,[205] current recommendations for nondiabetic children are that blood pressures above the 90th percentile for age warrant lifestyle intervention and those about the 95th merit the use of pharmacologic agents.

Other screening studies for complications of diabetes include measurement of serum lipid concentrations. The current standards of care for adults with diabetes indicate that low-density lipoprotein (LDL) concentrations should be maintained below 100 mg/dL, whereas pharmacologic therapy for dyslipidemia in children with diabetes is not recommended unless dietary interventions fail to lower LDL concentrations below 130 mg/dL. The American Diabetes Association recommends that annual dilated retinal examinations be obtained in patients who are over 10 years or age and have had T1DM for 3 or more years. On the other hand, the yield from such examinations is very low in children and adolescents who have normal blood pressure, HbA1c levels that meet current targets, and who are without microalbuminuria.[206]

Management During Surgery

Management objectives during surgery are the prevention of hypoglycemia, excessive loss of fluids, and ketosis during anesthesia. The regimens described here are generally applicable, but vigilance and individual adjustments for each patient are necessary to achieve these goals. Evidence-based, controlled studies of perioperative care have not been carried out in children, but detailed expert reviews of management have been published in the anesthesia and pediatric diabetes literature.[207,208]

The most reliable and straightforward approach to achieving management objectives during major elective or emergency surgery is to use intravenous infusions of glucose and insulin during the perioperative period. For surgical emergencies that can be briefly delayed, such as acute appendicitis, rehydration and metabolic balance should be restored before the operation. Elective major operations should be performed first thing in the morning, and the glucose and insulin infusions should be started 2 hours or more prior to proceeding to the operating room. For elective surgeries, an infusion of 5% glucose in 0.45% or 0.9% saline solution is begun on the morning of surgery—and 1 unit of regular insulin is infused intravenously for each 4 to 6 g of administered glucose. One unit of regular insulin for every 2 to 4 g of exogenous glucose may be required in surgical emergencies due to elevated circulating concentrations of stress hormones or in insulin-resistant obese diabetic patients.

The rate at which intravenous fluids are administered should be sufficient to provide maintenance fluid requirements plus estimated losses during surgery and other fluid deficits. The blood glucose concentration should be monitored at periodic intervals before, during, and after surgery. Concentrations of 120 to 150 mg/dL should be the goal. This can be achieved by varying the rate of infusion of the glucose and electrolyte mixture or the rate of insulin administration. The intravenous insulin and glucose infusions can be continued until the patient is awake and capable of taking regular meals, at which time their usual injection or insulin pump regimen can be reinstituted. Use of continuous glucose monitoring devices during surgery in children and adolescents has not been adequately studied.

In patients who receive NPH insulin in the morning and are undergoing surgery of short duration, a standard and effective approach is as follows: on the morning of surgery, half of the usual morning dose of NPH insulin is administered subcutaneously, the usual dose of rapid-acting insulin is omitted unless needed to correct hyperglycemia, and a maintenance intravenous infusion of the electrolyte and glucose solution is initiated if needed. Similarly, in patients on insulin pump therapy who are undergoing short procedures, the continuous subcutaneous infusion of insulin (CSII) can be continued at the usual or slightly reduced overnight basal rate. Insulin pump-treated patients can also be maintained on CSII for major procedures as long as the integrity of the infusion and infusion site is ensured.

The nighttime dose of glargine or detemir insulin may provide sufficient basal insulin coverage for surgery in patients who receive these long-acting insulins before dinner or bedtime. A reduction in the glargine or detemir dose by 20% to 30% on the night prior to surgery should be considered in patients who have had a tendency to low pre-breakfast plasma glucose levels. With all three regimens, a correction dose of rapid-acting insulin can be given subcutaneously immediately after the procedure if needed for hyperglycemia—and repeated as needed to balance initial oral intake (e.g., carbohydrate-containing clear liquids). When the intravenous infusion is discontinued and the patient is ready to resume regular meals, the usual treatment regimen is reinstituted.

THE FUTURE IS NOW: CLOSED-LOOP INSULIN DELIVERY

A number of investigator groups and pump and sensor manufacturers are developing closed-loop systems that combine external insulin pumps with current CGM devices. These systems utilize controller algorithms that automatically regulate insulin infusion rates delivered by the pump based on sensor glucose readings every 1 to 5 minutes. These automated insulin infusion systems will have to be easily managed by the patient, protected against system problems that lead to an overdelivery of insulin, and able to respond to challenges of human physiology during normal daily activities, such as exercise and psychological stress. Although it has already been demonstrated that closed-loop systems can control glucose levels effectively in short-term, inpatient, clinical research center studies,[168] much work needs to be done to ensure the safety of these systems before they will be ready for outpatient use.

The Paradigm Veo (Medtronic) insulin pump represents the first small step toward automated control of insulin delivery. The low-glucose-suspend feature of this sensor-augmented pump system allows the basal rate of

the patient's pump to be suspended for up to 2 hours if the patient fails to respond to the sensor's low-glucose alarm. This system has been tested in adults and children with T1D and has been found to significantly decrease the extent and duration of nocturnal hypoglycemia in patients with T1DM.[209] Importantly, almost all of the study participants reported that they felt safer and less anxious at night.[209]

Because of their increasingly important role in managing T1DM in childhood, a detailed description and discussion of pump therapy follows this section; Box 19-4 and Tables 19-11 and 19-12 summarize several key elements of pump therapy.

Non-autoimmune Type 1 Diabetes

Not all forms of apparently classic type 1 diabetes mellitus have associated markers of autoimmunity.[210] In one report, among children presenting with newly diagnosed diabetes mellitus, fewer of those younger than 5 years of age at diagnosis had positive titers of islet cell and GAD antibodies—and fewer had a honeymoon phase within 6 months—compared with a group with onset at a mean age of 10 years.[48] Similarly, Japanese investigators have reported that some patients with idiopathic type 1 diabetes mellitus have a non-autoimmune fulminant disorder with abrupt onset characterized by absence of circulating antibodies, evidence of insulinitis in pancreatic biopsies, and high concentrations of pancreatic enzymes—suggesting an acute inflammatory process in the pancreas.[49,50]

MODY syndromes may also be initially considered to be T1DM.[12,13] However, a strong family history of vertically transmitted DM in two to three generations, absence of autoimmune markers, and relatively milder features of diabetes should alert the physician to the possibility of a monogenic form of diabetes such as MODY.

CSII-Based Treatment Regiments

The use of insulin pumps (continuous subcutaneous insulin infusion [CSII]) has increased dramatically in children and adolescents with type 1 diabetes (T1D) since the 1990s. The T1D Exchange study group has reported that 33% of children < 6 years of age, 47% of children aged 6 to 12 years, 50% of adolescents aged 13 to 17 years, and 52% of patients aged 18 to 25 years used an insulin pump in the cohort of 20,555 participants (11,641 younger than age 18 years) from the United States.[211] This is matched by the 36.6% of 30,708 children and adolescents with T1D from Germany and Austria,[212] and with many other countries in the European Union and Israel reporting similar insulin

TABLE 19-11 **Pump Options and Features**

Pump	Minimal Basal Rate Increments (U/hr)	Minimal Bolus Dose Increments (Units)	Maximum Bolus Dose (Units)	Insulin Reservoir Capacity (Units)	Additional Features
Accu-Chek Spirit	0.1	0.1	25	315	Reversible display Includes personal digital assistant (PDA) device to calculate boluses and w/ database of foods Comes with backup pump AA battery Accu-Chek 360°(not Windows 7/Mac)
Animas One Touch Ping	0.025	0.05	35	200	Meter-remote can wirelessly beam blood glucose and deliver insulin within 10 feet CalorieKing database on meter Waterproof up to 12 feet AA battery (meter remote AAA battery) ezManager Max software (not Windows 7)
Insulet Omnipod	0.05	0.05	30	200	1000 common foods in PDA Freestyle meter in PDA component Pod is waterproof up to 8 feet PDM requires AAA battery Abbott's CoPilot software (not Windows 7/Mac)
Medtronic Paradigm Revel 523/723	0.025	0.025 (to 0.975) then 0.05	25	180 (523) or 300 (723)	Available with real-time continuous glucose monitoring (CGM) AAA battery CareLink Personal software (not Mac)

TABLE 19-12 The Stepwise Initiation of Pump Therapy at the Yale Children's Diabetes Program

2-6 Weeks before Start
- Review pros/cons of pump therapy
- Determine which model is best
- Initiate insurance process to secure coverage of pump
- Refer patient to on-line training resources or give them manual to review

1-2 Weeks before Start
- Have patient (depending on age) and/or caregivers complete on-line or written training
- Invite school nurse, baby sitters, other caregivers to pump training
- Family is contacted by official trainer from pump company to arrange the 1st of 2 visits
- Family receives insulin pump and should take pump out of box and start practicing button pushing techniques
- 1st visit with official trainer to review ins/outs of pump model, practice button pushing, site insertion and review DM management principles for pump users (may be done at family home or nearby site for convenience)

Day of Start
- Typically scheduled for a Monday or Tuesday morning
- Usually done at clinic building
- Hold AM long-acting insulin
- Initiate pump with insulin and family performs first site change
- Families instructed to test BG: before meals, 2H PP, HS, 12A and 3A

Week Following Start
- Daily phone contact with nurse practitioner to titrate doses
- Follow-up visit and/or phone contact with official trainer for first site change

2-4 weeks after Start
- Clinic visit to review DM management principles for pumpers
- Identify any areas for reinforcement
- Return to regular clinic schedule for quarterly follow-up

pump use in children and adolescents. Several tertiary pediatric centers report the use of insulin pumps as the predominant treatment modality in their pediatric population.[213] CSII has several practical benefits over MDI, including programmable basal insulin rates with multiple basal rate profiles and the possibility of temporary basal rates, adjustable "dual-wave" boluses, same injection site over 2 to 3 days (versus multiple daily injections), dose calculators, and the possibility of data upload, all of which facilitate the day-to-day management of the disease.

The use of insulin pumps is associated with improved metabolic control, lower glycated hemoglobin, or less hypoglycemia in many,[214,215] but not all,[216,217] clinical trials. Large cohort studies including several thousand pediatric patients have described a significant correlation between better metabolic control and the use of insulin pumps.[211,212] Additionally, the quality of life assessed by PedsQL-T1DM questionnaire is significantly associated with the use of insulin pumps,[218] and preliminary data suggest better cognition, mood, and behavior.[219,220]

Selection criteria for insulin pump therapy[221] in the pediatric patient population broadened as experience and data on its use accumulated.[222] Commonly, poor glycemic control, frequent hypoglycemia, increased glucose variability regardless of HbA1c, compromised quality of life, and increased risk of chronic complications are listed. Self-blood glucose monitoring (SBGM) is still an important prerequisite, as is parental support. Young age, frequent SBGM, and lower HbA1c at pump initiation are associated with better long-term metabolic control.[223] Conversely, female gender, age older than 10 years in girls, and poor metabolic control at pump initiation are associated with higher risk for attrition from insulin pump therapy.[224] Interestingly, children with inadequate metabolic control on MDI may experience the highest decrease in their HbA1c when switched to an insulin pump.[225] In preschool children, initiating the insulin pump therapy at the disease onset may help patients to maintain lower HbA1c for up to 8 years.[226]

Insulin pump therapy initialization is a structured multistep process involving the whole (extended) patient's family and a diabetes team ideally including a pediatric endocrinologist, a pediatric diabetes nurse educator, a specialized dietitian, a child psychologist, and a social worker. Additionally, adequate training of daycare personnel, a nanny, or school personnel is of crucial importance.[227]

Basal Insulin Rates

A continuous sequence of small boluses approximately every 10 minutes of a short-acting insulin analog provides basal insulin required for suppressing liver gluconeogenesis and ketogenesis and maintaining basal normoglycemia. Age-specific daily patterns of basal rates differ considerably in dose and distribution.[228,229] As insulin resistance increases with age, so does the insulin dose. Although preschool children often need highest basal rates between 10 p.m. and 2 a.m., this pattern changes as puberty approaches when the highest insulin requirement usually starts after 4 a.m. and lasts till wakeup.

When switching a patient from MDI to CSII, the total basal rate dose usually is calculated based on the previous dose of the long-acting insulin analog and is usually reduced by 10% to 20% if HbA1c was $< 8\%$. Alternatively, the total daily insulin dose is reduced by 20% to 30% and then half is administered as the basal rate. Some clinicians calculate the basal rate dose arbitrarily as approximately 0.4 units of insulin per kilogram body weight, especially when the dose of the long-acting insulin analog or the total daily insulin dose when treated with injections was evidently not appropriate and achieved poor metabolic control.

Programming multiple basal rates in the pediatric population seems prudent, as this was associated with better metabolic control.[230] Several initial approaches are used[231] with likely similar success, as basal rates should be subsequently verified and personalized.

In infants and toddlers, a flat initial basal rate may be appropriate and tailored to individual need in the first week of insulin pump use. As modern insulin pumps can deliver as little as 0.025 units of insulin per hour, diluting insulin is rarely needed. Intermittent programming of 0 units of insulin per hour for shorter periods of up to 2 hours is sometimes used for neonates. A higher basal insulin rate will be needed around midnight with a lower rate applied in the afternoon. For older children and adolescents, the day may be divided into five parts: from midnight to 4 a.m., covering the period with least basal insulin requirement, from 4 to 7 a.m. covering the dawn phenomenon period, from 7 a.m. to 1 p.m. covering the morning, from 1 to 8 p.m. covering the afternoon, and from 8 p.m. to midnight covering the evening.[231] Sometimes, an additional time frame for the dusk phenomenon may be needed from 5 to 8 p.m.[232] Several basal rate patterns may be needed for different situations: a standard pattern for regular weekdays, a weekend or leisure pattern with lower basal insulin rates during the morning (e.g., less stress from school or work or more activity), and a "sick days" basal rate pattern with 30% more insulin throughout the 24 hours.[233]

Verifying Basal Insulin Rate Settings

The ideal basal rate will keep the blood glucose levels within the desire range during fasting. The need for basal insulin changes with time and depends on insulin sensitivity that varies with age, lifestyle, growth, and several other fluctuating influences. Basal insulin rates can be best verified when food intake is omitted. The nighttime basal insulin rates may be verified first, as the overnight glucose concentration profile is usually of greatest concern. On an uneventful day after an early dinner (at 5 to 6 p.m.) that was meticulously covered by an appropriate bolus, SBGM is performed every 2 to 3 hours from 10 p.m. till wakeup. If the BG changes for more than ±40 mg/dL (2 mM), the basal rate approximately 1 hour prior to the BG change is adjusted for ±10% to 20%. Similarly, a morning basal SBGM profile is performed after an uneventful night, skipping the breakfast and morning snack with SBGM every 2 to 3 hours until lunch. Finally, an afternoon basal SBGM profile is performed after an uneventful morning, skipping lunch, with SBGM every 2 to 3 hours until dinner. Basal insulin rates are adjusted as described for the night basal profile. If the BG level fells below or above the usual range between 70 to 180 mg/dL, the condition is treated, the basal profile testing is discontinued, the basal rate is adjusted accordingly, and the basal rate SBGM profile retested in a couple of days. In preschool children basal rates can be verified in shorter time intervals, as only one meal per day can be omitted. Some verify basal profiles without omitting meals but rather after eating standardized small meals covered by appropriate boluses. Continuous glucose monitoring (CGM) can be of obvious assistance and can reduce the frequency of SBGM. Frequent phone contacts with the diabetes team are prudent in the first weeks after insulin pump initialization to individually fine-tune basal insulin rates.[233]

Temporary Basal Rates and Basal Insulin Suspend

With little insulin in the body between boluses, reducing or stopping the basal insulin rate is an efficient way to prevent hypoglycemia during physical activity or sport.[234] Smart pumps can show the amount of remaining insulin in the body after a bolus (so-called active insulin), which can be of considerable help in preventing exercise-associated hypoglycemia, as "active insulin" can be covered with additional carbohydrates.

Bolus Insulin Dosages

Careful and more frequent dosing of insulin for covering food and correcting high BG levels is associated with better metabolic control when using insulin pumps.[235] Modern insulin pumps can assist calculations of bolus insulin reducing errors[236] and incorporating physiologic variability of insulin sensitivity during the course of the day.

Bolus Insulin for Food Coverage

Principles of calculating boluses for food are same as for MDI, with the additional possibility of more precise dosing down to 0.025 units of insulin in some insulin pumps. Solid practical knowledge of carbohydrate counting is paramount. The arbitrary formula of 500 divided by the total daily insulin dose for older children and adolescents (300 or 200 for preschool children or toddlers, respectively) can help in determining the initial insulin-to-carbohydrates ratio (ICR). The ICR can be higher (one unit of insulin covers fewer carbohydrates) in the morning, sometimes by 30% to 100% as compared to midday, and slightly higher again in the evening. Bolus calculators are incorporated into modern pumps and do all required calculations based on preset ICRs, the current BG, the planned amount of carbohydrate ingestion, and the glucose target range, typically between 70 and 120 mg/dL (3.9 and 6.5 mM).

Advanced food-bolus determination incorporates protein counting (15 grams of carbohydrates for each additional 100 g of protein after the first 100 g)[237] and the use of prolonged or "dual-wave" bolus administration, which is associated with better postprandial glucose control after ingestion of food with low glycemic index.[238]

Preferably, the bolus is administrated 15 to 20 minutes before the meal as the postprandial peak of BG precedes the peak of rapid-acting insulin analog action.[239,240] As eating habits in smaller children may be unpredictable, a "split-bolus" approach may be used for optimal postprandial control with only 10% to 20% of the calculated bolus administrated 15 to 20 minutes prior to the meal for covering "certainly" ingested food, and the rest administrated after the meal according to the amount actually consumed.

ICRs should be verified on a regular basis with hourly postprandial SBGM or CGM, aiming at BG < 180 mg/dL (10 mM).

Missed prandial boluses, particularly during the school hours, are the most common cause of suboptimal metabolic control.[241] Parental and school-personnel supervision and support is valuable.

Bolus Insulin for Correction of Hyperglycemia

Insulin sensitivity factor (ISF) can be estimated by dividing 1800 by the total daily dose in older children and adolescents (2000 or 2200 in preschool children and toddlers, respectively). Again, bolus calculators incorporated into smart insulin pumps calculate correction boluses based on the preset ISF, current BG level, preset BG target range, and the amount of "active insulin" from previously administered correction boluses. The amount of "active insulin" calculated by the insulin pump bolus calculator depends on the setting of the insulin action time, commonly set to 3 hours for boluses used in pediatric population. A shorter insulin action time setting allows for more aggressive correction bolusing.

Also ISFs are regularly verified with SBGM 2 to 4 hours post correction or with CGM. It is advisable to avoid correction boluses less than 2 hours apart as insulin accumulation (stacking) may occur and provoke hypoglycemia.[242]

Specific Considerations and Acute Complications of Insulin Pump Therapy

Regular physical activity is strongly advised but challenging in youngsters with T1D, particularly in relation to hypoglycemia during and after it.[243,244] The biggest advantage of CSII may be related to its possibility of reducing physical activity–related hypoglycemia.[245,246] Basal insulin rate can be stopped or diminished by setting a temporary basal rate immediately prior and during the physical activity, depending on its intensity and duration. The presence of "active insulin" from previous boluses can be verified and an appropriate carbohydrate snack added prior to the physical activity in addition to basal insulin rate modification, effectively preventing hypoglycemia. Finally, strenuous or prolonged exercise often results in delayed hypoglycemia, which can be prevented by a diminished temporary basal insulin rate programmed for several hours post physical activity. Hypoglycemia can therefore be prevented without an excessive use of additional carbohydrates and thus a negative energy balance can be maintained, which is of particular importance for body weight management.

Reduction of hypoglycemia with sensible use of insulin pump therapy was demonstrated in several studies[247,248] and has many important practical implications that must be discussed during the training of the family. The possibilities of insulin stacking after too frequent correction bolusing, inappropriate counting of carbohydrates, and insulin modifications related to an acute illness (e.g., vomiting), eating disorder, or to physical activity are the most important issues that should be taken into consideration.

Diabetic ketoacidosis (DKA) was traditionally considered as a risk associated with CSII; however, studies report less DKA with the use of modern insulin pump.[248,249] As only rapid-acting insulin analogs are used in insulin pumps, significant ketonemia may develop 4 to 6 hours after discontinuation of CSII.[250] Partial or complete disconnection of the infusion set and plugging or kinking of the subcutaneous catheter are the most common causes. A DKA rescue plan must be included in the structured education provided to patients and caregivers. Blood or urine ketones must be checked if two consecutive BG levels or the fasting BG levels are $>$ 275 mg/dL ($>$ 15 mM), and the possibility of infusion set problems considered. Infusion set must be replaced and an appropriate correction bolus administered, alternatively with an insulin pen injector, if the functioning of the infusion set is not certain. If ketonemia is $>$ 1.5 mM, an increased temporary basal rate of 150% to 200% may be needed until blood ketones are normalized. Additional fluid intake is advisable. If ketonemia is associated with an acute illness or remains unexplained, contact with the on-call diabetes team is warranted.

Maintaining normoglycemia during acute severe illness has been demonstrated in adults,[251] along with the danger of hypoglycemia. Despite the lacking published evidence for children, teams in PICU and NICU need appropriate training for maintaining stable glycemic control with CSII during acute severe illness.[252] Anesthesiologists often suggest a switch to intravenous insulin during prolonged surgery, which with frequent BG level determination provides a well-established and safe treatment modality. CSII should be restarted just prior to the termination of intravenous insulin administration. Discontinuing CSII during intermittent hospitalizations on various pediatric or surgery wards is usually associated with poor metabolic control and should be discouraged. A pediatric endocrinology team should be involved in the appropriate care of hospitalized children with diabetes.

Insulin Pump Therapy in Kindergarten and School

Most schools in developed societies accept chronically ill children and provide necessary care either enforced through law and regulations or voluntarily. A structured plan for school personnel and diabetes-related education similar to the one for caregivers facilitate cooperation among parents, the diabetes team, and the school. A cooperative spirit aimed at the best interests of the child with T1D usually yields optimum long-term results. The diabetes team serves as facilitator and coordinator in the collaboration between parents and the school personnel. Ideally, basic training for school personnel includes but is not limited to SBGM and appropriate recording, management of hypoglycemia including discontinuation of insulin delivery, rescue carbohydrates and administration of glucagon, management of hyperglycemia including correction boluses and ketones checking, carbohydrate counting and determining boluses, and management of physical activity.[227,253,254]

Despite significant improvements in metabolic control with the routine use of CSII in pediatric populations, most young people with type 1 diabetes still do not reach target metabolic control,[255] with hypoglycemia and DKA remaining as problems.[256] More emphasis on psychosocial support and successful parenting[257] are needed in view of challenging modern environments to which children and adolescents are often exposed.

TYPE 2 DIABETES MELLITUS

Typical

Type 2 diabetes mellitus, formerly known as NIDDM, is a heterogeneous disorder characterized by defective insulin secretion that progressively fails to compensate for insulin resistance.[258] The cause of insulin resistance is usually obesity,[258-263] although agents such as growth hormone and cortisol also antagonize insulin action and may unmask inadequately compensated insulin secretion. The high concentrations of placental growth hormones during mid to late gestation likewise may unmask inadequate insulin secretion, resulting in gestational diabetes—a harbinger of permanent diabetes later in life.

The mechanism of insulin resistance caused by obesity may in part relate to changes in fatty acid metabolism that interfere with normal glucose metabolism, and in part to factors synthesized within fat cells that antagonize insulin action.[264] Most notable are the hormones adiponectin, leptin, and resistin,[264] which are produced by fat cells and are a likely major link between obesity and diabetes. The sensing of insulin resistance induced by obesity results in the production by the liver and fat cells of a protein, betatrophin, which stimulates beta cell expansion and increased insulin secretion in mice by altering the expression of genes regulating the cell cycle. Genetic regulation of the ability to overcome insulin resistance by producing this and other proteins may be another mechanism that predisposes or protects from T2DM.[265,266] Thus, in addition to the genetic components responsible for impaired insulin secretion (which are being investigated, progressively identified, and presently cannot be changed),[267-271] the key modifiable factor responsible for the epidemic of type 2 diabetes in children is the epidemic of obesity increasingly recognized throughout the world.[271-275]

Type 2 diabetes mellitus is being increasingly recognized in children, particularly obese adolescents and especially but not exclusively in certain ethnic groups such as Native American Indians, African Americans, Mexican Americans, and Southeast Asians in the developing world.[271-275] Here, too, an epidemic of obesity is responsible for a rapid increase in the proportion of patients with type 2 diabetes—representing up to half of the newly presenting cases in one major medical center in the United States.[271,272] These patients often have a family history of type 2 diabetes, may have acanthosis nigricans, are more commonly girls, and often have poor metabolic control that predisposes them to the earlier appearance of microvascular and macrovascular complications.[272] They may present initially in diabetic ketoacidosis suggesting type 1 diabetes, but after recovery they may manifest a prolonged "honeymoon phase"—the so-called atypical diabetes mellitus (ADM), or type 2 diabetes, as documented by significant insulin or C-peptide levels not consistent with type 1 diabetes. They also lack markers of islet autoimmunity and the classic HLA associations. [272]

In those with a family history of type 2 diabetes mellitus, insulin resistance (as shown by impaired insulin-stimulated glucose disposal and higher insulin values during oral glucose tolerance or so-called hyperglycemic clamps) is demonstrable in the first decade of life before clinically demonstrable changes in glucose tolerance.[271-272] The sites of this presumably genetic impairment in insulin sensitivity has not yet been identified but clearly precedes clinical diabetes mellitus brought about by obesity-induced insulin resistance.[271,272] There may be other factors that induce insulin resistance.

There also are an emerging number of newly identified genetic factors associated with impaired insulin secretion and action. Genome-wide scans have consistently identified three major genetic linkages: the ATP-regulated potassium channel KATP, especially the Kir 6.2 subunit encoded by the KCNJ11 gene; the peroxisome proliferator-activated receptor γ (PPARG); and transcription factor 7 like 2 (TCF7L2.[274-276] The development of sophisticated arrays that permit genotyping of literally hundreds of thousands of polymorphisms has revealed additional loci of genetic markers in T2DM, including a polymorphism in the zinc transporter SLC30A8 (expressed only on pancreatic β cells) and genes potentially involved in pancreatic development (IDE, KIF11, HHEX) or function (EXT2-ALX4).[268-270,272-275] These sophisticated genetic screening approaches are likely to identify more of the complex genetic traits that underlie T2DM. From a practical point of view, type 2 diabetes mellitus in children and adolescents should be viewed as a major public health issue—and without effective lifestyle interventions (such as weight reduction combined with regular exercise), treatment options are limited and only modestly successful.[271,272] Exogenous insulin may be necessary to control blood glucose initially, but its appetite-stimulating effects challenge attempts at weight reduction. Sulfonylureas may be temporarily helpful, as may meglitinide—which acutely increase insulin secretion.

Metformin, which sensitizes tissue to insulin action and diminishes hepatic glucose production, is the most frequently used agent for treating type 2 diabetes in children. The U.S. Food and Drug Administration (FDA) has approved it for use for those over 10 years of age. The FDA has not approved for use in children thiazolidinediones (glitazones) that sensitize tissue to insulin, but nevertheless they have reportedly been used.[276,277] These agents are potentially hepatotoxic, and the original product was withdrawn for this reason. Newer glitazones (such as rosiglitazone) are under investigation in adolescents with T2DM, although rosiglitazone itself is under scrutiny for potential harmful effects on cardiac function.[276] Alpha-glucosidase inhibitors that slow carbohydrate absorption and lipase inhibitors to diminish fat absorption are available, but none has undergone clinical trials in children/adolescents for efficacy and compliance. This represents a major deficiency in our ability to treat type 2 diabetes mellitus in children.[271,272]

GENETIC DEFECTS OF BETA CELL FUNCTION

MODY Syndromes

Although MODY was originally conceptualized as a form of maturity-onset (i.e., type 2) diabetes, the MODY syndromes are best considered a group of disorders of

monogenic defect in beta cell function. Affected patients may have modest elevation of glucose, may remain asymptomatic for many years, and may become clinically apparent during intercurrent illness or pregnancy that unmasks the limited insulin secretion.[12,13] Clinical criteria used to establish the diagnosis include the following.

- Dominant inheritance with at least two (and preferably three) consecutive affected generations
- Onset before age 25 to 30 years
- Evidence of significant but impaired residual insulin secretion reflected in C-peptide levels whether or not the patient is being treated with insulin

At least six specific genetic defects have been identified (Table 19-13), although more are included in the Online Mendelian Inheritance in Man (OMIM) database. Moreover, milder defects in the K_{ATP} genes KCNJ11 and ABCC8 as well as defects in the insulin gene itself have been found in patients who fulfill the criteria for a diagnosis of MODY. Of these genetic defects that together account for no more than 2% to 5% of type 2 diabetes, about two thirds (65%) are MODY 3 (HNF1α), 10% are MODY 2 (glucokinase defect), and the remainder constitute the other defects. Mild stable hyperglycemia may be present from birth and not require treatment except during stress, such as infections in an infant or child or pregnancy in a young adult.

With glucokinase deficiency, microvascular complications of diabetes are rare. In the other common forms of MODY, such as MODY3 and MODY1, however, onset is usually in the early teens to 20s, glucose intolerance may become progressively worse and hence require treatment, and microvascular complications may develop later in life. Renal cysts or other reno pelvic anomalies may occur in MODY 5 (HNF1β). Notably, if the genetic defect in IPF1 (insulin promoter factor-1, MODY 4) is homozygous, pancreatic agenesis results and this is a cause for permanent neonatal diabetes associated with exocrine as well as endocrine insufficiency (see Chapter 9). Likewise, homozygous mutations in glucokinase have been associated with congenital diabetes. By contrast, gain-of-function mutations in glucokinase cause persistent hyperinsulinemic hypoglycemia of infancy (see Chapter 6 ***). Thus, the MODY syndromes are monogenic defects of islet cell formation (MODY 4) or of transcription factors (MODY 1, 3, 5, and 6)—or a defect in the functional glucose sensor glucokinase (MODY 2).[12,13,277]

Table 19-14 compares and contrasts the four most common types of diabetes found in adolescents: type 1, type 2, atypical diabetes mellitus, and MODY. The monogenic MODY syndromes have been extensively reviewed in terms of chemical, biochemical, and molecular analyses.[12,13,277] Other monogenic defects reportedly associated with a type 2 clinical picture include mutation in GLUT2 and the glycogen synthase genes.[278,279]

Other Forms of Monogenic Diabetes

Mitochondrial Diabetes

Mitochondrial genetic defects that cause diabetes are commonly but not invariably associated with neuromuscular disorders, including deafness, migraine, seizures, and mental retardation. For example, the MELAS (mitochondrial encephalopathy, lactic acidosis, and strokelike episodes) syndrome may initially present in childhood with short stature, go on to deafness in teen years, and develop diabetes and encephalopathy in midlife. Diabetes mellitus may be the only manifestation of a mitochondrial disorder encoded by a gene defect within the mitochondria

TABLE 19-13 **Protein/Gene Mutations Causing Maturity-Onset Diabetes of the Young**

MODY Type	Protein Mutated	Gene/Gene Location	Gene Function with Respect to the Beta Cell
MODY 1	Hepatocyte nuclear factor-4α	HNF4α; 20q12-q13.1	Binds to HNF1α and IPF1 promoter; regulates HNF1α and IPF1 gene transcription
MODY 2	Glucokinase	GCK; 7p15-p13	Catalyzes conversion of glucose to glucose 6-phosphate
MODY 3	Hepatocyte nuclear factor-1α	HNF1α; 12q24.2	Binds to A3/A4 box of insulin gene promoter; regulates insulin gene transcription
MODY 4	Insulin promoter factor-1	IPF1; 13q12.1	Binds to A5, A3/A4, A2, and A1 boxes of insulin gene promoter; regulates insulin gene transcription
MODY 5	Hepatocyte nuclear factor-1β	HNF1β, TCF2; 17cen-q21.3	Regulates HNF4α gene transcription
MODY 6	Neuro-D1/beta-2	Neuro-D1; 2q32; beta-2	Transcription factor for normal insulin secretion and normal development of pancreatic islets

TCF2, transcription factor-2, hepatic; also known as LF-B3, variant hepatic nuclear factor.

From Winter, W. E., Nakamura, M., & Hause, D. (2001). Monogenic diabetes mellitus in youth. Endocrinol Metab Clin North Am, 28, *765; Fajans, S. S., Bell, G. I., & Polonsky, K. S. (2001). Molecular mechanisms and clinical pathophysiology of maturity-onset diabetes of the young.* N Engl J Med, 345, *971.*

TABLE 19-14 **Comparison of the Common Forms of Youth-Onset Diabetes**

Characteristic	Type 1 Diabetes	Type 2 Diabetes	Classic MODY	Atypical Diabetes Mellitus
Age at onset	Peaks at 5 and 15 years of age	Teenage years, young adults	< 25 years of age	> 40 years of age
Predominant ethnic groups affected	White	Hispanic, African American, Native American	White	African American
Male-to-female ratio	1.1:1	1:1.5	1:1	1:3
Severity at onset	Acute, severe, insulin required	Subtle, insulin not required	Subtle, insulin not required	Acute, severe, insulin required
Islet autoimmunity	Present	Absent	Absent	Absent
HLA-DR3, -DR4	Very common	No increased frequency	No increased frequency	No increased frequency
Ketosis, DKA	Common	Uncommon	Rare	Common at onset
Long-term course	Insulin-dependent	Non-insulin-dependent	Non-insulin-dependent	Non-insulin-dependent
Prevalence of obesity	Uncommon	≥ 90%	Uncommon	40%
Proportion of cases of 100% youth-onset diabetes	Most common form of youth-onset diabetes	Rising in frequency; ± as common as type 1 diabetes in specific populations	≤ 5% of youth-onset diabetes in whites	≤ 10% of cases of youth-onset diabetes in African Americans
Percentage of probands with an affected first-degree relative	≤ 15%	Variable but common	100%	> 75%
Mode of inheritance	Nonmendelian, generally sporadic	Nonmendelian but strongly familial	Autosomal dominant	Autosomal dominant
Number of genes controlling inheritance	Polygenic	Polygenic	Monogenic	Monogenic
Pathogenesis	Autoimmune beta cell destruction: insulinopenia	Insulin resistance plus relative insulinopenia	Insulinopenia	Insulinopenia

DKA, diabetic ketoacidosis; MODY, maturity-onset diabetes of the young.

Adapted from Winter, W. E., Nakamura, M., & Hause, D. (1999). Monogenic diabetes mellitus in youth. Endocrinol Metab Clin North Am, 28, *765–785.*

(all of which are maternally inherited) or a nuclear DNA-encoded gene necessary to the oxidative phosphorylation sequence within mitochondria.

This defective energy pathway leads to progressive impairment of insulin secretion, and thus an initially mild hyperglycemia may progressively worsen. This is the case with the most common form of diabetes caused by a mitochondrial gene mutation at nucleotide pair (np) 3243 of the mitochondrial genome, often associated with deafness. Remarkably, this same genetic defect may be associated with the MELAS syndrome. Initially, patients with np 3243 mutations can be controlled by diet alone but later may require insulin. Diabetes mellitus presenting in infancy and severe from the outset and requiring insulin may be associated with mitochondrial DNA deletions, as seen in the Kearns-Sayre syndrome and Pearson syndrome.[280,281] Figure 19-7 identifies the critical role of energy production by the mitochondrial oxidative phosphorylation pathway for normal insulin secretion.

Defects in this pathway may be responsible for transient or permanent neonatal diabetes mellitus, especially those that involve activating mutations in the KATP channel subunits Kir 6.2(KCNJ11) and its regulatory subunit sulfonylurea receptor SUR1 (ABCC8). Inactivating mutations in the KATP subunits cause neonatal hyperinsulinemic hypoglycemia of variable severity, as described in detail in Chapter 6. Diabetes in the mitochondrial syndromes is generally well controlled by exogenous insulin. [281]

Wolfram Syndrome

Wolfram syndrome is characterized by diabetes insipidus, diabetes mellitus, optic atrophy, and deafness (DIDMOAD).[282,283] There is a selective loss of beta cells, which is responsible for the diabetes mellitus. Genetic linkage studies in consanguineous families with autosomal-recessive inheritance led to the positional cloning of a gene on the short arm of chromosome 4 (termed *WFS1*, and now identified as Wolframin).

Although the function of this gene is not entirely understood, it is expressed in many tissues (most abundantly in beta cells, compared with the exocrine pancreas). Mutations in Wolframin have been identified in many families with Wolfram syndrome. Affected individuals are usually compound heterozygotes. The Wolframin gene may have a role in beta cell and neural tissue survival, and there does not appear to be a correlation between the observed mutation and severity of disease. Defects in Wolframin have been implicated in the idiopathic common nonimmune form of type 1 diabetes mellitus.[282] A second locus of Wolfram syndrome has been mapped

to the long arm of chromosome 4 in several consanguineous Jordanian families.[282] In these patients, diabetes insipidus was not a feature—but upper gastrointestinal bleeding and ulceration were prominent.

Although a mitochondrial form of Wolfram syndrome has been proposed, a defect in mitochondrial DNA could not be confirmed in one large cohort.[283] It has been suggested that diabetes mellitus (before age 15) and progressive optic atrophy are highly predictive of Wolfram syndrome. The sequence of appearance of the stigmata is non-autoimmune type 1 diabetes in the first decade of life, central diabetes insipidus and sensorineural deafness in two thirds to three fourths of the patients in the second decade, renal tract anomalies in approximately half in the third decade, and neurologic complications such as cerebellar ataxia and myoclonus in half to two-thirds in the fourth decade. Other features include primary gonadal atrophy in the majority of males and a progressive neurodegenerative course with neurorespiratory death at a median age of 30 years. Depression has been reported as a frequent feature of relatives of patients with Wolfram syndrome. [282]

Thiamine-Responsive Diabetes Mellitus (Roger Syndrome)

This syndrome is characterized by megaloblastic anemia, diabetes mellitus, and sensorineural deafness—all of which may respond to vitamin B_1 (thiamine). Diabetes mellitus is mild to moderate, insulin secretion may improve with thiamine therapy, and there are no associated autoimmune markers. It may be caused by a defect in the thiamine transporter,[284,285] which has now been identified as being caused by mutations in the SLC19A gene. This gene encodes the membrane-bound thiamine transporter THTR-1.

Uptake of thiamine by the combined pathways of an active high-affinity carrier and a passive low-affinity carrier leads to accumulation of intracellular thiamine, which is then converted to its active form, thiamine pyrophosphate. This cofactor enables proper function of transketolase, which is important in the pentose phosphate shunt, the key to ribose synthesis and hence nucleic acid production and of pyruvate dehydrogenase, α ketoglutarate dehydrogenase, and branched chain acid dehydrogenase—all of which are key to oxidative decarboxylation. Mutations in the high-affinity transporter THTR-1 lead to cell death in those cells that have a high rate of nucleic acid turnover (such as bone marrow cells) and activity (such as pancreatic beta cells), thereby explaining the association of thiamine-responsive anemia with diabetes in those affected by this mutation.

Drug or Chemical Induced

A number of drugs and chemical agents may be toxic to the beta cell. Best known for the diabetogenic effects are the immunosuppressive drugs cyclosporine, sirolimus, and tacrolimus, which are toxic to beta cells—causing insulin-dependent diabetes in a significant proportion of patients treated with these agents for organ transplantation. Their toxicity to pancreatic beta cells is compounded by the use of immunosuppressive glucocorticoids, which antagonize insulin action to unmask diabetes.

Streptozotocin and the rodenticide Vacor are also beta cell toxic, causing diabetes. Box 19-1 lists other agents that may induce diabetes. Among these, L-asparaginase (used in chemotherapy for leukemia) and diazoxide (used to treat persistent hyperinsulinemic hypoglycemia in infancy) may cause diabetes. All of these agents, especially glucocorticoids, may combine to unmask clinical diabetes mellitus.[286-288]

Diseases of the Exocrine Pancreas

Cystic fibrosis (CF) is one of the most common inborn errors of metabolism, involving the chloride channel encoded on chromosome 7 and affecting approximately 1 in 2500 live-born white infants. The increasing survival of patients with CF and the increased use of glucocorticoids to suppress bronchopulmonary inflammation have brought to the fore an entity termed *cystic fibrosis–related diabetes* (CFRD), characterized by variable impairment of carbohydrate intolerance.

Some have mild diabetes mellitus clinically apparent only while receiving steroids, whereas others require insulin for a nonimmune form of insulinopenic diabetes mellitus. Up to 75% of adults with CF have CFRD. Islet amyloid is prominent, both insulin secretion and insulin sensitivity may be impaired, and diabetic ketoacidosis may occur—as may microvascular complications. When fasting hyperglycemia (7 mM = 126 mg/dL or higher) is documented, therapy with insulin is indicated. Insulin also facilitates optimal nutrition and growth and hence promotes a sense of well-being. The diagnostic and management criteria for CFRD have been extensively reviewed.[15,289]

Ionizing Radiation to the Abdomen

Ionizing radiation to the abdomen during childhood for a condition such as nephroblastoma has been associated with the development of diabetes mellitus some 20 years later in 5% to 10% of children so treated. [290]

Pancreatectomy

Extensive pancreatectomy performed for the management of severe hyperinsulinemic hypoglycemia of infancy is associated with diabetes in approximately 50% or more of long-term survivors.[21]

Virus Infections

Several viruses have been implicated in the cause of type 1 diabetes mellitus in children. Coxsackievirus B_4 has been shown in one case report as likely fulfilling Koch's postulates for a direct beta cell toxic effect in causing acute fulminant diabetes mellitus. In other cases of coxsackievirus infections (as well as with the established association among rubella, cytomegaloviruses, and enteroviruses), molecular mimicry between antigenic determinants in the virus and certain islet cell antigens has been implicated as the mechanism leading to an

autoimmune form of type 1 diabetes mellitus. Finally, a superantigen-triggered immune response has been suggested for some viral infections and may be the mechanism related to the acute onset of diabetes mellitus with the hemolytic-uremic syndrome.[20,57,291,292]

GENETIC DEFECTS IN INSULIN ACTION

Type A Insulin Resistance with Acanthosis Nigricans

This syndrome is characterized by severe insulin resistance, acanthosis nigricans in the absence of obesity, or lipoatrophy. Affected females also have hyperandrogenism, possibly as a secondary manifestation of the hyperinsulinemia with stimulation of androgen synthesis by ovarian theca cells. Glucose intolerance is variable and includes symptomatic diabetes. The hyperandrogenism presents as clinical and biochemical findings suggestive of polycystic ovary syndrome.

Some patients (predominantly black females with obesity, acanthosis nigricans, and accelerated growth suggestive of gigantism) may represent insulin resistance owing to obesity, with down-regulation of the insulin receptor. The gigantism may represent a "spillover" effect of insulin acting through the insulin-like growth factor-1 receptor rather than the insulin receptor.[293,294]

Type B Insulin Resistance

Type B insulin resistance is a rare syndrome associated with evidence of immune dysfunction (such as the defined autoimmune disease rheumatoid arthritis) or of nonspecific features of autoimmunity (such as elevated sedimentation rate or high levels of antinuclear antibodies). As with other autoimmune disorders, females are predominantly affected. An insulin-resistant diabetes develops together with acanthosis nigricans and features of PCOS (such as hirsutism).

The syndrome is due to serum autoantibodies against the insulin receptor whose function becomes impaired. However, the receptor may be activated by the presumed conformational changes induced by the antibody and cause severe hypoglycemia rather than diabetes. Treatment may require high doses of insulin to try to control the hyperglycemia, along with immunosuppressive drugs to suppress antibody production.

Leprechaunism (Donohue Syndrome)

Leprechaunism is a syndrome characterized by intrauterine growth retardation, fasting hypoglycemia, and postprandial hyperglycemia in association with profound resistance to insulin in a patient whose serum concentrations of insulin may be 100-fold that of comparable age-matched infants during an oral glucose tolerance test. Various defects of the insulin receptor have been described, thereby attesting to the important role of insulin and its receptor in fetal growth and possibly in morphogenesis. Even a probable complete absence of functional insulin receptors caused by homozygous inheritance of missense mutation in the insulin receptor, however, resulted in normal organogenesis and a live-born infant who had a severe form of leprechaunism. Most of these patients die during the first year of life. [295]

Rabson-Mendenhall Syndrome

The Rabson-Mendenhall syndrome is defined by clinical features that appear to be intermediate between those of acanthosis nigricans with insulin resistance type A and leprechaunism. Features include extreme insulin resistance, acanthosis nigricans, abnormalities of the teeth and nails, and pineal hyperplasia. It is not clear whether this syndrome is entirely distinct from leprechaunism. However, patients with Rabson-Mendenhall syndrome tend to live beyond the first year of life. Defects in the insulin receptor gene have been described in this syndrome.[296]

Lipoatrophic Diabetes

Lipoatrophic diabetes presents an interesting paradox. Whereas classic type 2 diabetes mellitus is generally associated with an excess of fat and its metabolic consequences (as described previously), a paucity of fat also causes severe insulin resistance and marked metabolic disturbances. Table 19-15 lists the genetic syndromes associated with lipoatrophic diabetes. A primary cause has been identified in the form called familial partial lipoatrophy (also known as Dunnigan syndrome). This is a gene defect localized to chromosome 1q21-22 and its product lamin A/C.

This autosomal disease usually manifests peripubertally as subcutaneous fat in the extremities and trunk but with progressively more fat in the face and neck as puberty progresses. Visceral and interfascicular fat also increases. Females seem to develop diabetes mellitus and dyslipidemia earlier and more severely than males. It is unclear why and how the lamin A/C mutations cause this lipoatrophy syndrome, especially as mutations in this gene also are associated with a progressive form of muscular dystrophy (Emery-Dreifuss syndrome), cardiomyopathy, and cardiac conduction defects.[297,298] The gene altered in Berardinelli-Seip congenital lipodystrophy has been localized to chromosome 11q13.274.[298] In addition, defects in genes encoding the enzyme AGPAT2, the endoprotease ZMPSTE24, the kinase AKT2, the nuclear receptor PPARγ, and the protein BSCL 2 have been found in patients with lipodystrophies. [297,298]

ACQUIRED DEFECTS IN INSULIN ACTION

These defects range from hormonal disorders such as pheochromocytoma and Cushing syndrome that antagonize insulin action to disease to drug-acquired forms of lipodystrophy. Anti-insulin receptor antibodies may be found in some collagen vascular disorders and can cause a type 2 diabetes mellitus syndrome characterized by acanthosis nigricans and severe insulin resistance. This is generally referred to as acanthosis nigricans with insulin resistance type B. The type A syndrome consists of a variety of insulin receptor mutations, some of which were

TABLE 19-15 **Genetic Syndromes of Lipoatrophy**

Syndrome	Lipoatrophy	Gene/Locus	Inheritance	OMIM
Primary Lipoatrophy Syndromes				
Congenital generalized lipoatrophy (Seip-Berardinelli)	Generalized See text for details.	9q34 Gmg3lg; 11q13p	AR	269700
Dunnigan syndrome	Familial partial See text for details.	1q21-22 Lamin A/C	AD	151660
Others	Numerous distributions	Unknown	AD/AR	N/A
Complex Syndromes Associated with Lipoatrophy				
Mandibuloacral dysplasia	Congenital, partial Involves extremities	Unknown	AR	248370
Werner syndrome	Congenital, partial Involves extremities	8p12 Werner's helicase	AR	277700
Cockayne syndrome	Congenital, partial Involves extremities	5 CSA	AR	216400
Carbohydrate-deficient glycoprotein syndrome	Transient, partial Buttocks	16p13.3 PMM1 and 2	AR	212065
SHORT syndrome[†]	Generalized, congenital	Unknown	AR	269880
AREDYLD syndrome[‡]	Generalized, congenital	Unknown	Unknown	207780

Evidence for genetic heterogeneity.
[†]Short stature, hyperextensibility, hernia, ocular depression, Rieger anomaly, and teething delay.
[‡]Acrorenal field defect, ectodermal dysplasia, and lipoatrophic diabetes; not clear if this is a variation of Seip-Berardinelli syndrome.
AD, autosomal dominant; AR, autosomal recessive; OMIM, Online Mendelian Inheritance in Man, database providing information about genetic syndromes; and PMM1, PMM2, phosphomannomutase 1 and 2.
From Arioglu, E., Rother, K. I., Reitman, M. L., et al. (2000). Lipoatrophy syndromes. Pediatr Diabetes 1:155; Magre J, Delepine M, Khallouf E, et al. (2001). Identification of the gene altered in Berardinelli-Seip congenital lipodystrophy on chromosome 11q13. Nat Genet, 28, *365.*

described earlier. The type B syndrome is rarely described in childhood.[299]

GENETIC SYNDROMES WITH DIABETES AND INSULIN RESISTANCE OR INSULIN DEFICIENCY

A number of genetic syndromes are associated with diabetes mellitus. In children, four relatively common genetic syndromes may be associated with diabetes. In trisomy 21 (Down syndrome) and Turner syndrome (a single normal X chromosome) there is an increased incidence of autoimmune disorders, especially of the thyroid. Type 1 diabetes mellitus also has a higher prevalence in patients with Down syndrome than in the general population.

In Turner syndrome, insulin secretory reserve may be limited such that treatment with growth hormone (now common) can result in impaired glucose tolerance or type 2 diabetes. In Klinefelter syndrome (XXY), insulin resistance is a major feature—but autoimmune associations have been described. In Prader-Willi syndrome, the reported high frequency of diabetes mellitus may not be caused simply by the insulin resistance as part of the obesity of this syndrome but possibly by a primary defect in insulin secretion.[300-303]

Alström syndrome consists of retinal dystrophy, sensorineural deafness, obesity and associated diabetes, cardiomyopathy, hypertriglyceridemia, liver disease, and urologic abnormalities. Severe insulin resistance may lead to acanthosis and diabetes. Mutations in the gene ALMS1 have been identified in these patients.[304] Bardet-Biedl syndrome also has atypical retinitis pigmentosa as a key feature, along with central obesity, polydactyly, mental retardation, hypogonadism, and renal dysfunction. Eleven loci have been linked to this syndrome, with abnormalities in cilia-like structures a central theme.[305] The association of these and other syndromes with diabetes (listed in Box 19-1) can be ascertained by searching the Online Mendelian Inheritance in Man database (OMIM, available at www.ncbi.nlm.nih.gov/omim).

Gestational Diabetes

Gestational diabetes is a disease of the second and third trimesters of pregnancy that is due to congenital or acquired defects in insulin secretion that result in the inability to compensate for the increased demands of insulin as a result of the counter-insulin effects of placental growth hormones.[306]

Neonatal Diabetes

Discoveries in the molecular basis for pancreas formation and regulation of insulin secretion have propelled the syndromes of neonatal diabetes from the backwater of rarity to the forefront of research. These syndromes probably occur more frequently than previously considered, and they may have an overall incidence of about

1:100,000 births.[307-323] Moreover, some of those with activating mutations in KATP channel subunits Kir 6.2 or SUR1 lend themselves to treatment with oral sulfonylurea hypoglycemic drugs such as glibenclamide—which results in endogenous insulin secretion with near-normal glycemic control and at least partial reversal of neuromuscular manifestations.[12,307-323] These entities are described in detail in Chapter 9.

Impaired Glucose Tolerance

The term *impaired glucose tolerance* is used to characterize individuals who have a plasma glucose concentration in excess of 140 mg/dL but less than 200 mg/dL at 2 hours after initiation of the standard oral glucose tolerance test but do not have symptoms of diabetes or fasting hyperglycemia. Such a constellation may represent the earliest phase of a gene defect in insulin secretion or action or be a step in evolving diabetes mellitus type 1. The hyperglycemia may be a chance discovery during an intercurrent illness, during therapy with corticosteroids, or as part of a screening of close relatives of patients with defined genetic syndromes.

In those who have impaired glucose tolerance but do not have fasting hyperglycemia, repeated oral glucose tolerance tests are not recommended. Investigations in such children indicate that the degree of impaired glucose tolerance tends to remain stable except in those who have markedly subnormal insulin response.[324] The arbitrarily designated response that identifies impaired glucose tolerance is defined as a fasting plasma glucose value of less than 110 mg/dL and a value at 2 hours of more than 140 mg/dL. The determination of serum insulin responses during the glucose tolerance test is not a prerequisite for reaching a diagnosis. Because the magnitude of the insulin response may have prognostic value, however, some investigators perform insulin determination tests.[237]

Pancreas and Islet Transplantation

In an attempt to cure insulin-dependent diabetes, transplantation of a segment of the pancreas or of isolated islets has been increasingly performed in humans.[325,326] These procedures are technically demanding and associated with the risks and complications of rejection and its treatment by immunosuppression. Therefore, segmental pancreas transplantation is generally performed in association with transplantation of a kidney for a patient with end-stage renal disease owing to diabetic nephropathy in whom the immunosuppressive regimen is indicated for the renal transplant.

Several thousand such transplants have been performed worldwide since the late 1980s. With experience and newer immunosuppressive agents, functional survival of the pancreatic graft may be achieved for as long as several years—during which patients may be in metabolic control with no or minimal exogenous insulin and reversal of some of the microvascular complications. Because children and adolescents with diabetes mellitus are not likely to have end-stage renal disease as a result of diabetes, however, pancreas transplantation as a primary treatment cannot be recommended (nor its risks justified) in children.

Attempts to transplant isolated islets have been equally challenging because of techniques to harvest sufficient islets and the issue of rejection.[327] Some of the newer antirejection drugs, notably cyclosporine and tacrolimus, are toxic to the islets of Langerhans—impairing insulin secretion and even causing diabetes.[327] The Edmonton protocol for islet transplantation avoids steroid and uses an anti-IL-2 receptor antibody and tacrolimus instead of other immunosuppressants.[326] Initial success had been promising, but long-term survival of the graft beyond 3 to 5 years is most unusual—and there have been serious side effects of the procedure as well as failure to restore hypoglycemia unawareness.[326] Hence, this islet transplantation protocol cannot be recommended in children.

Research continues to improve techniques for the yield, viability, and loss of immunogenicity of islets of Langerhans for transplantation. Transplantation has been investigated of islets coated or microencapsulated with a film of protective chemicals that permit diffusion of insulin and nutrients but prevent T-cell contact and therefore avoid rejection.[328] These novel approaches have been frustrated in the long term by overgrowth of fibroblasts that progressively impair glucose sensing by the islets and insulin diffusion from them. Should these technical problems be overcome, or methods to avoid rejection become established, transplantation of pancreas or islets as primary treatment for diabetes may be entertained after their risks are carefully compared with and weighed against potential benefits—especially in children. Islet regeneration by modulating the immune response is also under scrutiny.[329,330]

CONCLUDING REMARKS

Progress continues to be spectacular in understanding and treating diabetes mellitus. We have moved from a glimmer of understanding that diabetes is a syndrome of broad categories (insulin dependent and non-insulin dependent) to an understanding of disease susceptibility and disease causality at a molecular level. Type 1 diabetes is acknowledged to be an autoimmune disease with major susceptibility loci in the HLA complex. Positional cloning and genome wide association techniques have identified other gene markers, and we now recognize the importance of mitochondrial gene defects in some types of insulin-dependent diabetes that may not be autoimmune. The predisposition of the beta cell to autoimmune destruction (homicide) versus a predisposition to apoptosis (suicide) is the topic of intensive debate and investigation and raises questions about our accepted paradigms of this disease.[43,44,81] More spectacular has been the unraveling of the molecular basis of neonatal diabetes and its treatment, including the use of sulfonylureas. Not surprisingly, this knowledge is being rapidly applied to predict the likelihood of disease appearance in individuals whose susceptibility can be quantified by the presence of certain antibodies and by limitations in first-phase insulin response. Population surveys in individuals not known to be at risk for diabetes have just begun. We are in the early

stages of attempts to prevent the disease, reminiscent of the early trials examining the relationship between control and microvascular complications.

The monumental DCCT and European studies have irrefutably established the link, requiring new standards of care. Progress and understanding of insulin secretion and insulin action have been equally spectacular for the insights provided in defining non–insulin-dependent forms of diabetes at physiologic, biochemical, and molecular levels. Therapy with human insulin is now standard. Trials with improved insulin-delivery systems, including computerized artificial pancreas as well as pancreas and islet transplantation, are in progress. The FDA in the United States has approved one artificial pancreas system for use, and improvements in these devices are bound to change the course and treatment of T1DM. The beneficiaries of these advances are our patients, whose interests will continue to be best served by bidirectional scientific inquiry from bench to bedside.

REFERENCES

1. Ize-Ludow, D., & Sperling, M. A. (2005). The classification of diabetes Mellitus: a conceptual Framework. *Pediatr Clin N Am*, *52*, 1533–1552.
2. Pambianco, G., Cousacou, T., Ellis, D., et al. (2006). The 30 year natural history of type 1 diabetes complications: the Pittsburgh Epidemiology of Diabetes Complications Study experience. *Diabetes*, *55*, 1463–1469.
3. Saltiel, A. R., & Kahn, C. R. (2001). Insulin signaling and the regulation of glucose and lipid metabolism. *Nature*, *414*, 799–806.
4. Atkinson, M. A., Eisenbarth, G. S., & Michels, A. W. (2013). Type 1 diabetes. Lancet, S0140-6736, 60591–60597.
5. Eisenbarth, G. S. (2007). Update in type 1 diabetes. *J Clin Endocrinol Metab*, *92*, 2403–2407.
6. Hattersley, A., Bruining, J., Shield, J., et al. (2009). The diagnosis and management of monogenic diabetes in children and adolescents. *Pediatr Diabetes*, *10*(Suppl. 12), 33–42.
7. American Diabetes Association (2013). Diagnosis and classification of diabetes mellitus. *Diabetes Care*, *36*(Suppl. 1), S67–S74.
8. Wang, J., Miao, D., Babu, S., et al. (2007). Prevalence of autoantibody-negative diabetes is not rare at all ages and increases with older age and obesity. *J Clin Endocrinol Metab*, *92*, 88–92.
9. Trucco, M., & Giannoukakis, N. (2007). Immunoregulatory dendritic cells to prevent and reverse new-onset type 1 diabetes mellitus. *Expert Opin Biol Ther*, *7*, 951–963.
10. Stark, R., & Roden, M. (2007). ESCI Award 2006, mitochondrial function and endocrine disease. *Eur J Clin Invest*, *37*, 236–248.
11. Nunnari, J., & Suomalainen, A. (2012). Mitochondria: in sickness and in health. *Cell*, *148*, 1145–1159.
12. Murphy, R., Ellard, S., & Hattersley, A. T. (2008). Clinical implications of a molecular genetic classification of monogenic beta-cell diabetes. *Nat Clin Pract Endocrinol Metab*, *4*, 200–213.
13. Fajans, S. S., Bell, G. I., & Polonsky, J. S. (2001). Molecular mechanisms and clinical pathophysiology of maturity-onset diabetes of the young. *N Engl J Med*, *345*, 971–980.
14. Kelly, A., & Moran, A. (2013). Update on cystic fibrosis-related diabetes. *J Cyst Fibros*, *4*, 318–331.
15. Moran, A., Becker, D., Casella, S. J., et al. (2010). Epidemiology, pathophysiology, and prognostic implications of cystic fibrosis-related diabetes: a technical review. *Diabetes Care*, *33*, 2677–2683.
16. Goodyer, W. R., Gu, X., Liu, Y., et al. (2012). Neonatal β cell development in mice and humans is regulated by calcineurin/NFAT. *Dev Cell*, *23*, 21–34.
17. Barlow, A. D., Nicholson, M. L., & Herbert, T. P. (2013). Evidence for rapamycin toxicity in pancreatic β-cells and a review of the underlying molecular mechanisms. *Diabetes*, *62*, 2674–2682.
18. Esposti, M. D., Ngo, A., & Myers, M. A. (1996). Inhibition of mitochondrial complex 1 may account for IDDM induces by intoxication with the rodenticide Vacor. *Diabetes*, *45*, 1531–1534.
19. Strosberg, J., Hoffe, S., Gardner, N., et al. (2007). Effective treatment of locally advanced endocrine tumors of the pancreas with chemoradiotherapy. *Neuroendocrinology*, *85*, 216–220.
20. Nesmith, J. D., & Ellis, E. (2007). Childhood hemolytic uremic syndrome is associated with adolescent-onset mellitus. *Pediatr Nephrol*, *22*, 294–297.
21. de Lonlay-Debeney, P., Poggi-Travert, F., Fournet, J. C., et al. (1999). Clinical features of 52 neonates with hyperinsulinism. *N Engl J Med*, *340*, 1169–1175.
22. Bacha, F., Gungor, N., Lee, S., & Arslanian, S. (2013). Progressive deterioration of β-cell function in obese youth with type 2 diabetes. *Pediatr Diabetes*, *14*, 106–111.
23. Imperatore, G., Boyle, J. P., Thompson, T. J., et al. (2012) Projections of type 1 and type 2 diabetes burden in the U.S. population aged < 20 years through 2050: dynamic modeling of incidence, mortality, and population growth. *Diabetes Care*, *35*, 2515–2520.
24. Santer, R., & Calado, J. (2010). Familial renal glucosuria and SGLT2: from a mendelian trait to a therapeutic target. *Clin J Am Soc Nephrol*, *5*, 133–141.
25. Zouali, H., Velho, G., & Frouguel, P. (1993). Polymorphism of the glycogen synthase gene and non-insulin dependent diabetes mellitus. *N Engl J Med*, *328*, 1568.
26. Henriksen, E. J., & Dokken, B. B. (2006). Role of glycogen synthase kinase-3 in insulin resistance and type 2 diabetes. *Curr Drug Targets*, *7*, 1435–1441.
27. Taylor, S. I., Cama, A., Accili, D., et al. (1992). Mutations in the insulin receptor gene. *Endocr Rev*, *13*, 566–595.
28. Reynet, C., & Kahn, C. R. (1993). Rad: a member of the Ras family overexpressed in muscle of type 2 diabetic humans. *Science*, *262*,1441–1444.
29. Polonsky, K. S., Sturis, J. P., & Bell, G. I. (1996). Non-insulin-dependent diabetes mellitus: a genetically programmed failure of the beta cell to compensate for insulin resistance. *N Engl J Med*, *334*, 777–783.
30. Voight, B. F., Scott, L. J., Steinthorsdottir, V., et al. (2010). Twelve type 2 diabetes susceptibility loci identified through large-scale association analysis. *Nat Genet*, *42*, 579–589.
31. Kahn, B. B., & McGraw, T. E. (2010). Rosiglitazone, PPARγ, and type 2 diabetes. *N Engl J Med*, *363*, 2667–2669.
32. Saltiel, A. R. (2012). Insulin resistance in the defense against obesity. *Cell Metab*, *15*, 798–870.
33. Owen, K. R., & McCarthy, M. I. (2007). Genetics of type 2 diabetes. *Curr Opin Genet Dev*, *17*, 239–244.
34. Bergman, R. N., & Ader, M. (2000). Free fatty acids and pathogenesis of type 2 diabetes mellitus. *Trends Endocrinol Metab*, *11*, 351–356.
35. Duggirala, R., Blangero, J., Almasy, L., et al. (2001). A major locus for fasting insulin concentrations and insulin resistance on chromosome 6q with strong pleiotropic effects on obesity-related phenotypes in nondiabetic Mexican-Americans. *Am J Hum Genet*, *68*, 1149–1164.
36. Robertson, R. P., Harmon, J., Tran, P. O., & Poitout, V. (2004). Beta-cell glucose toxicity, lipotoxicity, and chronic oxidative stress in type 2 diabetes. *Diabetes*, *53*, S119–S124.
37. Sladek, R., Rocheleau, G., Rung, J., et al. (2007). A genome-wide association study identifies novel risk loci for type 2 diabetes. *Nature*, *445*, 881–885.
38. Schranz, D. B., Bekris, L., Landin-Olsson, M., et al. (2000). Newly diagnosed latent autoimmune diabetes in adults (LADA) is associated with low level glutamate decarboxylase (GAD65) and IA-2 autoantibodies. *Horm Metab Res*, *32*, 133–138.
39. Libman, I. M., & Becker, D. J. (2003). Coexistence of type 1 and type 2 diabetes mellitus: "double" diabetes? *Pediatr Diabetes*, *4*, 110–113.
40. Florez, J. C. (2008). Clinical review: the genetics of type 2 diabetes: a realistic appraisal in 2008. *J Clin Endocrinol Metab*, *93*, 4633–4642.
41. Harjutsalo, V., Sund, R., Knip, M., & Groop, P. H. (2013). Incidence of type 1 diabetes in Finland. *JAMA*, *310*, 427–428.
42. Forlenza, G. P., & Rewers, M. (2011). The epidemic of type 1 diabetes: what is it telling us? *Curr Opin Endocrinol Diabetes Obes*, *18*, 248–251.
43. Soleimanpour, S. A., & Stoffers, D. (2013). The pancreatic β cell and type 1 diabetes: innocent bystander or active participant? *Trends Endocrinol Metab*, *24*, 324–331.
44. Tuomilehto, J. (2013). The emerging global epidemic of type 1 diabetes. Curr Diab Rep. Advance online publication. doi: 10.1007/s11892-013-0433-5

45. Ziegler, A. G., Rewers, M., Simell, O., et al. (2013). Seroconversion to multiple islet autoantibodies and risk of progression to diabetes in children. *JAMA*, *309*, 2473–2479.
46. Libman, I. M., LaPorte, R. E., Becker, D. J., et al. (1998). Was there an epidemic of diabetes in nonwhite adolescents in Allegheny County, Pennsylvania? *Diabetes Care*, *21*, 1278–1281.
47. Pinhas-Hamiel, O., & Zeitler, P. (2007). Acute and chronic complications of type 2 diabetes mellitus in children and adolescents. *Lancet*, *369*, 1823–1831.
48. Hathout, E. H., Sharkey, J., Racine, M., et al. (2000). Diabetic autoimmunity in infants and pre-schoolers with type 1 diabetes. *Pediatr Diabetes*, *1*, 131–134.
49. Imagawa, A., Hanafusa, T., Miyagawa, J. I., et al. (2000). A novel subtype of type 1 diabetes mellitus characterized by a rapid onset and an absence of diabetes-related antibodies. *N Engl J Med*, *342*, 301–307.
50. Urakami, T., Inami, I., Morimoto, S., et al. (2002). Clinical characteristics of non-immune-mediated, idiopathic type 1 (Type 1B) diabetes mellitus in Japanese children and adolescents. *J Pediatr Endocrinol Metab*, *15*, 283–288.
51. Knip, M., Veijola, R., Virtanen, S. M., et al. (2005). Environmental triggers and determinants of type 1 diabetes. *Diabetes*, *54*, S125–S136.
52. Menser, M. A., Forrest, J. M., & Bransby, R. D. (1978). Rubella infection and diabetes mellitus. *Lancet*, *1*, 57–60.
53. Ou, D., Mitchell, L. A., Metzger, D. L., et al. (2000). Cross-reactive rubella virus and glutamic acid decarboxylase (65 and 67) protein determinants recognized by T cells of patients with type 1 diabetes mellitus. *Diabetologia*, *43*, 750–762.
54. Laitinen, O. H., Honkanen, H., Pakkanen, O., et al. (2013). Coxsackievirus B1 is associated with induction of β-cell autoimmunity that portends type 1 diabetes. *Diabetes*. Advance online publication. doi: 10.2337/db13-0619
55. Elding Larsson, H., Vehik, K., Gesualdo, P., et al. (2013). Children followed in the TEDDY study are diagnosed with type 1 diabetes at an early stage of disease. *Pediatr Diabetes*. Advance online publication. doi: 10.1111/pedi.12066
56. Hiemstra, H. S., Schloot, N. C., van Veelen, P. A., et al. (2001). Cytomegalovirus in autoimmunity: T cell cross reactivity to viral antigen and autoantigen glutamic acid decarboxylase. *Proc Natl Acad Sci USA*, *98*, 3988–3991.
57. Craig, M. E., Nair, S., Stein, H., & Rawlinson, W. D. (2013). Viruses and type 1 diabetes: a new look at an old story. *Pediatr Diabetes*, *14*, 149–158.
58. Eisenbarth, G. S. (2010). Banting lecture 2009: an unfinished journey: molecular pathogenesis to prevention of type 1A diabetes. *Diabetes*, *59*, 759–774.
59. Barrett, J. C., Clayton, D. G., Concannon, P., et al. (2009). Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes. *Nat Genet*, *41*, 703–707.
60. Pietropaolo, M., Towns, R., & Eisenbarth, G. S. (2012). Humoral autoimmunity in type 1 diabetes: prediction, significance, and detection of distinct disease subtypes. *Cold Spring Harb Perspect Med*, *2*, a012831.
61. Sosenko, J. M., Skyler, J. S., Palmer, J. P., et al. (2013). The prediction of type 1 diabetes by multiple autoantibody levels and their incorporation into an autoantibody risk score in relatives of type 1 diabetic patients. *Diabetes Care*, *36*, 2615–2620.
62. Daneman, D. (2006) Type 1 diabetes. *Lancet*, *367*, 847–858.
63. Bluestone, J. A., Herold, K., & Eisenbarth, G. (2010). Genetics, pathogenesis and clinical interventions in type 1 diabetes. *Nature*, *464*, 1293–1300.
64. Concannon, P., Erlich, H. A., & Julier, C. (2005). Type 1 diabetes: evidence for susceptibility loci from four genome-wide linkage scans in 1,435 multiplex families. *Diabetes*, *54*, 2995–3001.
65. Nejentsev, S., Smink, L. J., Smyth, D., et al. (2007). Sequencing and association analysis of the type 1 diabetes-linked region on chromosome 10p12-q11. *BMC Genet*, *8*, 24.
66. Payne, F., Cooper, J. D., Walker, N. M., et al. (2007). Interaction analysis of the CBLB and CTLA4 genes in type 1 diabetes. *J Leukoc Biol*, *81*, 581–583.
67. Taniguchi, H., Lowe, C. E., Cooper, J. D., et al. (2006). Discovery, linkage disequilibrium and association analyses of polymorphisms of the immune complement inhibitor, decay-accelerating factor gene (DAF/CD55) in type 1 diabetes. *BMC Genet*, *7*, 22.
68. Todd, J. A. (2006). Statistical false positive or true disease pathway? *Nat Genet*, *38*, 731–733.
69. Dorman, J., LaPorte, R., Stone, R., & Trucco, M. (1990). Worldwide differences in the incidence of type 1 diabetes are associated with amino acid variation at position 57 of the HLA-DQ beta chain. *Proc Natl Acad Sci USA*, *87*, 7370–7374.
70. Allen, C., Palta, M., & D'Alessio, D. J. (1991). Risk of diabetes in siblings and other relatives of IDDM subjects. *Diabetes*, *40*, 83–86.
71. Conrad, B., & Trucco, M. (1994). Superantigens as etiopathogenetic factors in the development of insulin-dependent diabetes mellitus. *Diabetes Metab Rev*, *10*, 309.
72. Symon, D. N., Hennessy, E. R., & Smail, P. J. (1984). Smoked foods in the diets of mothers of diabetic children. *Lancet*, *2*, 514.
73. Eisenbarth, G. S. (2012). Prevention of type 1A diabetes mellitus. *Endocr Pract*, *18*, 745–749.
74. Skyler, J. S., & Ricordi, C. (2011). Stopping type 1 diabetes: attempts to prevent or cure type 1 diabetes in man. *Diabetes*, *60*, 1–8.
75. Thomas, H. R., & Gitelman, S. E. (2013). Altering the course of type 1 diabetes: an update on prevention and new-onset clinical trials. *Pediatr Diabetes*, *14*, 311–321.
76. Atkinson, M. A., Bowman, M. A., Kao, K. J., et al. (1993). Lack of immune responsiveness to bovine serum albumin in insulin-dependent diabetes. *N Engl J Med*, *329*, 1853–1858.
77. Lamb, M. M., Simpson, M. D., Seifert, J., et al. (2013). The association between IgG4 antibodies to dietary factors, islet autoimmunity and type 1 diabetes: the Diabetes Autoimmunity Study in the Young. *PLoS One*, *8*, e57936.
78. Knip, M., Virtanen, S. M., Becker, D., et al. (2011). Early feeding and risk of type 1 diabetes: experiences from the trial to reduce insulin-dependent diabetes mellitus in the genetically at risk (TRIGR). *Am J Clin Nutr*, *94*, 1814S–1820S.
79. Frederiksen, B., Kroehl, M., Lamb, M. M., et al. (2013). Infant exposures and development of type 1 diabetes mellitus: the diabetes autoimmunity study in the young (DAISY). *JAMA Pediatr*, *167*, 808–815.
80. Hathout, E. H., Beeson, W. L., Ischander, M., et al. (2006). Air pollution and type 1 diabetes in children. *Peditr Diabetes*, *7*, 81–87.
81. Atkinson, M. A., Bluestone, J. A., Eisenbarth, G. S., et al. (2011). How does type 1 diabetes develop?: the notion of homicide or β-cell suicide revisited. *Diabetes*, *60*, 1370–1379.
82. Skyler, J. S. (2013). Primary and secondary prevention of type 1 diabetes. *Diabet Med*, *30*, 161–169.
83. Campbell-Thompson, M., Wasserfall, C., Kaddis, J., et al. (2012). Network for pancreatic organ donors with diabetes (nPOD): developing a tissue biobank for type 1 diabetes. *Diabetes Metab Res Rev*, *28*, 608–617.
84. Verge, C. F., Gianani, R., Kawasaki, E., et al. (1996). Prediction of type 1 diabetes in first-degree relatives using a combination of insulin, GAD, and ICA512bdc/IA-s autoantibodies. *Diabetes*, *45*, 926.
85. Diabetes Prevention Trial–Type 1 Diabetes Study Group (2002). Effects of insulin in relatives of patients with type 1 diabetes mellitus. *N Engl J Med*, *346*, 1685–1691.
86. Gale, E. A. (2002). Can we change the course of beta-cell destruction in type 1 diabetes? *N Engl J Med*, *346*, 1740–1742.
87. Gale, E. A., Bingley, P. J., Emmett, C. L., et al. (2004). European Nicotinamide Diabetes Intervention Trial (ENDIT): a randomized controlled trial of intervention before the onset of type 1 diabetes. *Lancet*, *363*, 925–931.
88. Wang, P. H. (2004). Commentary: growing pains in the pursuit of diabetes prevention. *Lancet*, *363*, 901.
89. Barker, J. M., & Eisenbarth, G. S. (2007). Primary prevention of type 1A diabetes: are we there yet? *Pediatr Diabetes*, *8*, 115–116.
90. Barker, J. M., McFann, K. K., & Orban, T. (2007). Effect of oral insulin on insulin autoantibody levels in the Diabetes Prevention Trial Type 1 oral insulin study. *Diabetologia*, *50*, 1603–1606.
91. Gruppuso, P. A., Gorden, P., Kahn, C. R., et al. (1984). Familial hyperproinsulinemia due to a proposed defect in conversion of proinsulin to insulin. *N Engl J Med*, *311*, 629–634.
92. Tager, H. S. (1984). Abnormal products of the human insulin gene. *Diabetes*, *33*, 693–699.
93. O'Rahilly, S., Gray, H., Humphreys, P. J., et al. (1995). Impaired processing of prohormones associated with abnormalities of glucose homeostasis and adrenal function. *N Engl J Med*, *333*, 1386–1390.
94. Gabbay, K. H. (1980). The insulinopathies. *N Eng J Med*, *302*, 165–167.

95. Steiner, D. F., Tager, H. S., Chan, S. J., et al. (1990). Lessons learned from molecular biology of insulin-gene mutations. *Diabetes Care*, *13*, 600–609.
96. Steiner, D. F. (2004). The proinsulin C-peptide: a multirole model. *Exp Diabesity Res*, *5*, 7–14.
97. Jackson, R. S., Creemers, J. W. M., Ohagi, S., et al. (1997). Obesity and impaired prohormone processing associated with mutations in the human prohormone convertase 1 gene. *Nat Genet*, *16*, 30–63.
98. Wardlaw, S. L. (2001). Obesity as a neuroendocrine disease: lessons to be learned from pro-opiomelanocortin and melanocortin receptor mutations in mice and men. *J Clin Endocrinol Metab*, *86*, 1442–1446.
99. Nanjo, K., Miyano, M., Kondo, M., et al. (1987). Insulin Wakayama: familial mutant insulin syndrome in Japan. Diabetologia, 30, 87–92.
100. Felig, P., Wahren, J., Sherwin, R., et al. (1976). Insulin, glucagon, and somatostatin in normal physiology and diabetes mellitus. *Diabetes*, *25*, 1091–1099.
101. Unger, R. H., & Orci, L. (1981). Glucagon and the A cell: physiology and pathophysiology. *N Engl J Med*, *304*, 1575–1580.
102. Woods, S. C., & Porte, D., Jr. (1974). Neural control of the endocrine pancreas. *Physiol Rev*, *54*, 596–619.
103. Ashcroft, F. M., & Rorsman, P. (2013). KATP channels and islet hormone secretion: new insights and controversies. *Nat Rev Endocrinol*. Advance online publication. doi: 10.1038/nrendo.2013.166
104. Aguilar-Bryan, L., Nichols, C. B., & Wechsler, S. W. (1995). Cloning of the beta cell high-affinity sulfonylurea receptor: a regulator of insulin secretion. *Science*, *268*, 423–426.
105. Philipson, L. H., & Steiner, D. F. (1995). Pas de deux or more: the sulfonylurea receptor and K^1 channels. *Science*, *268*, 372–373.
106. Thomas, P. M., Cote, G. J., Wohlik, N., et al. (1995). Mutations in the sulfonylurea receptor gene in familial persistent hyperinsulinemic hypoglycemia of infancy. Science, 268, 426–429.
107. Liang, Y., & Matschinsky, F. M. (1994). Mechanisms of action of nonglucose insulin secretagogues. *Annu Rev Nutr*, *14*, 59–81.
108. Campbell, J. E., & Drucker, D. J. (2013). Pharmacology, physiology, and mechanisms of incretin hormone action. *Cell Metab*, *17*, 819–837.
109. Porte, A., Baskin, D. G., & Schwartz, M. W. (2005). Insulin signaling in the central nervous system: a critical role in metabolic homeostasis and disease from C. elegans to humans. *Diabetes*, *54*, 1264–1276.
110. Cheatham, B., & Kahn, C. R. (1995). Insulin action and the insulin signaling network. *Endocr Rev*, *16*, 117–142.
111. Kido, Y., Nakae, J., & Accili, D. (2001). Clinical review 125: the insulin receptor and its cellular targets. *J Clin Endocrinol Metab*, *86*, 972–979.
112. Bevan, P. (2001). Insulin signaling. *J Cell Sci*, *114*, 1429–1430.
113. Taniguchi, C. M., Emanuelli, B., & Kahn, C. R. (2006). Critical nodes in signalling pathways: insights into insulin action. *Nat Rev Mol Cell Bio*, *7*, 85–96.
114. Bruning, J. C., Gautam, D., Burks, D. J., et al. (2000). Role of brain insulin receptor in control of body weight and reproduction. *Science*, *289*, 2122–2125.
115. Biddinger, S. B., & Kahn, C. R. (2006). From mice to men: insights into the insulin resistance syndromes. *Ann Rev Physiol*, *68*, 123–158.
116. Rizza, R. A., Mandarino, L. J., & Gerich, J. E. (1981). Dose-response characteristics for effects of insulin on production and utilization of glucose in man. *Am J Physiol*, *240*, E630–E639.
117. Schade, D. S., & Eaton, R. P. (1980). The temporal relationship between endogenously secreted stress hormones and metabolic decompensation in diabetic man. *J Clin Endocrinol Metab*, *50*, 131–136.
118. Wolfsdorf, J., Glaser, N., & Sperling, M. for the American Diabetes Association (2006). Diabetes ketoacidosis in infants, children and adolescents: a consensus statement from the American Diabetes Association. *Diabetes Care*, *29*, 1150–1159.
119. Wolfsdorf, J., Craig, M. E., Daneman, D., et al. (2009). Diabetic ketoacidosis in children and adolescents with diabetes. *Pediatr Diabetes*, *10*, 118–133.
120. Foster, D. W., & McGarry, J. D. (1983). The metabolic derangements and treatment of diabetic ketoacidosis. *N Engl J Med*, *309*, 159–169.
121. Glaser, N., Barnett, P., McCaslin, I., et al. (2001). Risk factors for cerebral edema in children with diabetic ketoacidosis. *N Engl J Med*, *344*, 264–269.
122. Glaser, N. (2006). New perspectives on the pathogenesis of cerebral edema complicating diabetic ketoacidosis in children. *Pediatr Endocrinol Rev*, *3*, 379–386.
123. Sperling, M. A. (2006). Cerebral edema in diabetes ketoacidosis: an underestimated complication? *Pediatr Diabetes*, *7*, 73–74.
124. White, P. C., & Dickson, B. A. (2013). Low mortality and mortality in children with diabetic ketoacidosis treated with isotonic fluids. *J Pediatr*, *163*, 761–766.
125. Decourcey, D. D., Steil, G. M., Wypij, D., & Agus, M. S. (2013). Increasing use of hypertonic saline over mannitol in the treatment of symptomatic cerebral edema in pediatric diabetic ketoacidosis: an 11-year retrospective analysis of mortality. *Pediatr Crit Care Med*. Advance online publication. doi: 10.1097/PCC.0b013e3182975cab
126. Zeitler, P., Hagg, A., Rosenbloom, A., et al. (2011). Hyperglycemic hyperosmolar syndrome in children: pathophysiological considerations and suggested guidelines for treatment. *J Pediatr*, *158*, 9–14.
127. Fourtner, S. H., Weinzimer, S. A., & Levitt Katz, L. E. (2005). Hyperglycemic hyperosmolar non-ketotic syndrome in children with type 2 diabetes. *Pediatr Diabetes*, *6*, 129–135.
128. Adrogue, H. J., Wilson, H., Boyd, A. E., et al. (1982). Plasma acid-base patterns in diabetic ketoacidosis. *N Engl J Med*, *307*, 1603–1610.
129. Arieff, A. I. (1989). Pathogenesis of lactic acidosis. *Diabetes Metab Rev*, *5*, 637–649.
130. Assadi, F. K., John, E. G., Fornell, M. T., et al. (1982). Falsely elevated serum creatinine concentration in ketoacidosis. *J Pediatr*, *107*, 562–564.
131. Halperin, M. L., Maccari, C., Kamel, K. S., et al. (2006). Strategies to diminish the danger of cerebral edema in a pediatric patient presenting with diabetes ketoacidosis. *Pediatr Diabetes*, *7*, 191–195.
132. Hoorn, E. J., Carlotti, A. P., Costa, L. A., et al. (2007). Preventing a drop in effective plasma osmolality to minimize the likelihood of cerebral edema during treatment of children with diabetes ketoacidosis. *J Pediatr*, *150*, 467–473.
133. Muir, A. B., Quisling, R. G., Yang, M. C., et al. (2004). Cerebral edema in childhood diabetes ketoacidosis: natural history, radiographic findings and early identification. *Diabetes Care*, *27*, 1541–1546.
134. Krane, E. J., Rockoff, M. A., Wallman, J. K., et al. (1985). Subclinical brain swelling in children during treatment of diabetic ketoacidosis. *N Engl J Med*, *312*, 1147.
135. Clements, R. S., Jr., Blumenthal, S. A., Morrison, A. D., & Winegrad, A. I. (1971). Increased cerebrospinal-fluid pressure during treatment of diabetic ketosis. *Lancet*, *2*, 671–675.
136. Winter, R. J., Harris, C. J., Phillips, L. S., et al. (1979). Diabetic ketoacidosis: induction of hypocalcemia and hypomagnesemia by phosphate therapy. *Am J Med*, *67*, 897–900.
137. Dunger, D. B., Sperling, M. A., Acerini, C. L., et al. (2004). ESPE/LWPES consensus statement on diabetic ketoacidosis in children and adolescents. *Arch Dis Child*, *89*, 188–194.
138. Schade, D. S., & Eaton, R. P. (1977). Dose response to insulin in man: differential effects on glucose and ketone body regulation. *J Clin Endocrinol Metab*, *44*, 1038–1053.
139. Yu, H. Y., Agus, M., & Kellogg, M. D. (2011). Clinical utility of Abbott Precision Xceed Pro ketone meter in diabetic patients. *Pediatr Diabetes*, *12*, 649–655.
140. Vanelli, M., Chiari, G., Lacava, S., & Iovane, B. (2007). Campaign for DKA prevention still effective eight years later. *Diabetes Care*, *30*, e12.
141. DCCT Research Group (1993). The effects of intensive diabetes treatment on the development and progression of long-term complication in insulin-dependent diabetes mellitus: the Diabetes Control and Complications Trial. *New Engl J Med*, *329*, 977–986.
142. DCCT Research Group (1994). The effect of intensive diabetes treatment on the development and progression of long-term complications in adolescents with insulin-dependent diabetes mellitus: the Diabetes Control and Complications Trial. *J Ped*, *1994*, 177–188.
143. DCCT Research Group, EDIC Research Group (2001). Beneficial effects of intensive therapy of diabetes during adolescence: outcomes after the conclusion of the diabetes control and complications trial (DCCT). *J Pediatr*, *139*, 804–812.

144. American Diabetes Association (2011). Standards of medical care in diabetes—2011. *Diabetes Care*, *34*(Suppl.), S11–S61.
145. Rewers, M., Pihoker, C., Donaghue, K., et al. (2009). Assessment and monitoring of glycemic control in children and adolescents with diabetes. *Pediatr Diabetes*, *10*(Suppl. 12), 71–81.
146. Miles, H. L., & Acerini, C. L. (2008). Insulin analog preparations and their use in children and adolescents with type 1 diabetes mellitus. *Pediatr Drugs*, *10*, 163–176.
147. Vajo, Z., Fawcett, J., & Duckworth, W. C. (2001). Recombinant DNA technology in the treatment of diabetes: insulin analogs. *Endocrine Rev*, *22*, 706–717.
148. Swan, K. L., Weinzimer, S. A., Steil, G., et al. (2008). Effect of puberty on the pharmacodynamic and pharmacokinetic properties of insulin pump therapy in youth with TIDM. *Diabetes Care*, *31*, 44–46.
149. Cengiz, E., Swan, K. L., Tamborlane, W. V., et al. (2012). The alteration of aspart insulin pharmacodynamics (PD) when mixed with detemir insulin. *Diabetes Care*, *35*, 690–692.
150. Cengiz, E., Tamborlane, W., Martin-Fredericksen, M., et al. (2010). Early pharmacokinetic and pharmacodynamic effects of mixing lispro with glargine insulin. *Diabetes Care*, *33*, 1009–1012.
151. Weinzimer, S. A., Ternand, C., Campbell, H., et al. (2008). A randomized trial comparing continuous subcutaneous insulin infusion of insulin aspart versus insulin lispro in children and adolescents with type 1 diabetes. *Diabetes Care*, *31*, 210–215.
152. Van Bon, A. C., Bode, B. W., Sert-Langeron, C., et al. (2011). Insulin glulisine compared to insulin aspart and insulin lispro administered by continuous subcutaneous insulin infusion in patients with type 1 diabetes: a randomized control trial. *Diabetes Technol Ther*, *13*, 607–614.
153. Bangstad, H. J., Danne, T., Deeb, L. C., et al. (2009). Insulin treatment in children and adolescents with diabetes. *Pediatr Diabetes*, *10*(Suppl. 12), 82–99.
154. Lepore, G., Dodesini, A. R., Nosari, I., & Trevisan, R. (2003). Both continuous subcutaneous insulin infusion and a multiple daily insulin injection regimen with glargine as basal insulin are equally better than traditional multiple daily insulin injection treatment. *Diabetes Care*, *26*, 1321–1322.
155. Cengiz, E., Sherr, J. L., Erkin-Cakmak, A., et al. (2011). A bridge to insulin pump therapy: bid regimen with NPH and detemir insulin during initial treatment of youth with type 1 diabetes (T1D). *Endoc Pract*, *6*, 1–17.
156. Danne, T., Datz, N., Endahl, L., et al. (2008). Insulin detemir is characterized by a more reproducible pharmacokinetic profile than insulin glargine in children and adolescents with type 1 diabetes: results from a randomized, double-blind controlled trial. *Pediatr Diabetes*, *9*, 554–560.
157. Heise, T., Nosek, L., Ronn, B. B., et al. (2004). Lower within-subject variability of insulin detemir in comparison to NPH insulin and insulin glargine in people with type 1 diabetes. *Diabetes*, *53*, 1614–1620.
158. Kordonouri, O., Pankowska, E., Rami, B., et al. (2010). Sensor-augmented pump therapy from the diagnosis of childhood type 1 diabetes: results of the Paediatric Onset Study (ONSET) after 12 months of treatment. *Diabetologia*, *53*, 2487–2495.
159. Sherr, J., Tamborlane, W. V., Tsalikian, E., et al. (2012). Hypoglycemia and lower glucose variability. *Diabetes Care*, *35*, 817–820.
160. Wood, J. R., Miller, K. M., Maahs, D. M., et al. (2013). Most youth with type 1 diabetes in the T1D Exchange Clinic Registry do not meet American Diabetes Association or International Society for Pediatric and Adolescent Diabetes clinical guidelines. *Diabetes Care*, *36*, 2035–2037.
161. Tamborlane, W. V., Sherwin, R. S., Genel, M., & Felig, P. (1979). Reduction to normal of plasma glucose in juvenile diabetics by subcutaneous administration of insulin with a portable infusion pump. *N Engl J Med*, *300*, 573–578.
162. Ahern, J. H., Boland, E. A., Doane, R., et al. (2002). Insulin pump therapy in pediatrics: a therapeutic alternative to safely lower therapy HbA1c levels across all age groups. *Pediatr Diabetes*, *3*, 10–15.
163. Sherr, J., & Tamborlane, W. V. (2008). Past, present, and future of insulin pump therapy: a better shot at diabetes control. *Mt Sinai J Med*, *75*, 352–361.
164. Attia, N., Jones, T. W., Holcombe, J., & Tamborlane, W. V. (1998). Comparison of human regular insulin and lispro insulin after interruption of CSII and in the treatment of acutely decompensated IDDM. *Diabetes Care*, *21*, 817–821.
165. Phillip, M., Battelino, T., Rodriguez, H., et al. (2007). Use of insulin pump therapy in the pediatric age-group consensus statement from the European Society for Paediatric Endocrinology, the Lawson Wilkins Pediatric Endocrine Society and the International Society for Pediatric and Adolescent Diabetes, endorsed by the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetes Care*, *30*, 1653–1662.
166. Weinzimer, S. A., Ahern, J. H., Doyle, E. A., et al. (2004). Persistence of benefits of continuous subcutaneous insulin infusion (CSII) in very young children with type 1 diabetes mellitus. *Pediatrics*, *114*, 1601–1605.
167. Sullivan-Bolyai, S., Knafl, K., Tamborlane, W., & Grey, M. (2004). Parents' reflections on the use of the insulin pump with their young children. *J Nursing Scholarship*, *6*, 316–323.
168. Weinzimer, S. A., Steil, G. M., Swan, K. L., et al. (2008). Fully automated closed-loop insulin delivery vs. semi-automated hybrid control in pediatric patients with type 1 diabetes using an artificial pancreas. *Diabetes Care*, *31*, 934–939.
169. Ziegler, R., Heidtmann, B., Hilgard, D., et al. (2011). Frequency of SMBG correlates with HbA1c and acute complications in children and adolescents with type 1 diabetes. *Pediatr Diabetes*, *12*, 11–17.
170. The Diabetes Research in Children Network Study Group (2005). Accuracy of newer generation home blood glucose meters in a Diabetes Research in Children Network (DirecNet) inpatient exercise study. *Diabetes Technol Ther*, 7, 675–680.
171. Niedel, S., Traynor, M., Acerini, C., et al. (2013). Framework for development of self-management expertise: health professional guidance of the development of parental expertise following diagnosis of childhood Type 1 Diabetes. *J Health Services Research Policy*. Advance online publication. doi: 10.1177/1355819613475602
172. Beck, R. W., Tamborlane, W. V., Bergenstal, R. R., et al. (2012). The T1D Exchange Clinic Registry: overview of the project and characteristics of initial 20,119 participants. JCEM 97, 4383–4389.
173. Boland, E. A., DeLucia, M., Brandt, C., et al. (2001). Limitations of conventional methods of self blood glucose monitoring: lessons learned from three days of continuous glucose monitoring in pediatric patients with type I diabetes. *Diabetes Care*, *24*,1858–1862.
174. Bergenstal, R. M., Tamborlane, W. V., Ahmann, A., et al. (2010). Effectiveness of sensor-augmented insulin pump therapy in type 1 diabetes. *N Engl J Med*, *363*, 311–320.
175. JDRF CGM Study Group (2008). Impact of continuous glucose monitoring in optimizing intensive treatment of type 1 diabetes in adults and children. *N Engl J Med*, *359*, 1464–1476.
176. Gregory, R. P., & Davis, D. L. (1994). Use of carbohydrate counting for meal planning in type 1 diabetes. *Diabetes Educator*, *20*, 406.
177. Aslander-van Vliet, E., Smart, C., & Waldron, S. (2007). Nutrition management of childhood and adolescent diabetes. *Pediatr Diabetes*, *8*, 323–339.
178. Ahern, J., Gatcomb, P. M., Held, N. A., et al. (1993). Exaggerated post-prandial hyperglycemia after a pizza meal in well-controlled diabetes. *Diabetes Care*, *14*, 578–580.
179. Pańkowska, E., & Błazik, M. (2010). Bolus calculator with nutrition database software, a new concept of prandial insulin programming for pump users. *J Diabetes Sci Technol*, *4*, 571–576.
180. American Diabetes Association Task Force for Writing Nutrition Principles and Recommendations for the Management of Diabetes and Related Complications (2002). American Diabetes Association position statement: evidence-based nutrition principles and recommendations for the treatment and prevention of diabetes and related complications. *J Amer Diet Assoc*, *102*, 109.
181. The Diabetes Research in Children Network (DirecNet) Study Group. (2006). The effects of aerobic exercise on glucose and counterregulatory hormone concentrations in children with type 1 diabetes. *Diabetes Care*, *29*, 20–25.
182. The Diabetes Research in Children Network (DirecNet) Study Group (2005). Impact of exercise on overnight glycemic control in children with type 1 diabetes. *J Pediatr*, *147*, 528–534.
183. McMahon, S. K., Ferreira, L. D., Davey, R. J., et al. (2007). Glucose requirements to maintain euglycemia following moderate intensity afternoon exercise in adolescents with type 1 diabetes are increased in a biphasic manner. *J Clin Endocrinol Metab*, *92*, 963–968.
184. Tamborlane, W. V. (2007). Triple jeopardy for hypoglycemia on nights following exercise in youth with type 1 diabetes. *J Clin Endocrinol Metab*, *92*, 815–816.

185. Sherr, J., Xing, D., Ruedy, K. J., et al. (2013). Lack of association between residual insulin production and glucagon response to hypoglycemia in youth with short duration of type 1 diabetes. *Diabetes Care*, *36*, 1470–1476.
186. Cengiz, E., Connor, C., Ruedy, K. J., et al. (2013). Pediatric diabetes consortium T1D New Onset (NeOn) study: clinical outcomes during the first year following diagnosis. *Pediatr Diabetes*. Advance online publication. doi: 10.1111/pedi.12068
187. The DCCT Research Group (1987). Effects of age, duration and treatment of IDDM on residual β-cell function: a study of the DCCT population. *J Clin Endocrinol Metab*, *65*, 30-36.
188. The DCCT Research Group (1998). The effect of intensive diabetes treatment in the DCCT on residual insulin secretion in IDDM. *Ann Int Med*, *28*, 517–523.
189. Herold, K. C., Hagopian, W., Auger, J. A., et al. (2002). Anti-CD3 monoclonal antibody in new-onset type 1 diabetes mellitus. *N Engl J Med*, *346*, 1692–1698.
190. American Diabetes Association Workgroup on Hypoglycemia (2005). Defining and reporting hypoglycemia in Diabetes. *Diabetes Care*, *28*, 1245–1249.
191. Jones, T. W., Boulware, S. D., Kraemer, D. T., et al. (1991). Independent effects of youth and poor diabetes control on responses to hypoglycemia in children. *Diabetes*, *40*, 358–363.
192. Ryan, C., Gurtunca, N., & Becker, D. (2005). Hypoglycemia: a complication of diabetes therapy in children. *Pediatr Clin N Am*, *52*, 1705–1733.
193. Cryer, P. E. (2005). Mechanisms of hypoglycemia-associated autonomic failure and its component syndromes in diabetes. *Diabetes*, *54*, 3592–3601.
194. Cryer, P. E. (2004). Diverse causes of hypoglycemia-associated autonomic failure in diabetes. *N Engl J Med*, *350*, 2272–2279.
195. The Diabetes Research in Children Network (DirecNet) Study Group (2009). Blunted counter-regulatory hormone responses to hypoglycemia in young children and adolescents with well-controlled type 1 diabetes. *Diabetes Care*, *32*, 1954–1959.
196. Jones, T. W., Porter, P., Sherwin, R. S., et al. (1998). Decreased epinephrine responses to hypoglycemia during sleep. *N Engl J Med*, *338*, 1657–1662.
197. Freemark, M., & Levitsky, L. L. (2003). Screening for celiac disease in children with type 1 diabetes: two views of the controversy. *Diabetes Care*, *26*, 1932–1939.
198. Springer, D., Dziura, J., Tamborlane, W. V., et al. (2006). Optimal control of type 1 diabetes in youth receiving intensive treatment. *J Pediatr*, *149*, 227–232.
199. Palau-Collazo, M. M., Rose, P., Sikes, K., et al. (2013). Effectiveness of a Spanish language clinic for Hispanic youth with type 1 diabetes. *Endocr Pract*, *19*, 800–804.
200. Doyle, E. A., Weinzimer, S. A., Steffen, A. L., et al. (2004). A randomized, prospective trial comparing continuous subcutaneous insulin infusion and with multiple daily injections using insulin glargine in youth with type 1 diabetes mellitus. *Diabetes Care*, *27*, 1554–1558.
201. Grey, M., Genel, M., & Tamborlane, W. V. (1980). Psychosocial adjustment of latency-aged diabetics: determinants and relationship to control. *Pediatrics*, *65*, 69–67.
202. Silverstein, J., Klingensmith, G., Copeland, K., et al. (2005). Standards of care of children and adolescents with type 1 diabetes: a statement of the American Diabetes Association. *Diabetes Care*, *28*, 186–212.
203. Griffin, M. E., Feder, A., Tamborlane, W. V. (2001). Lipoatrophy associated with lispro insulin in insulin pump therapy: an old complication, a new cause? *Diabetes Care*, *24*, 174.
204. Wood, J. R., Kaminski, B. M., Kollman, C., et al. (2012). The accuracy and precision of the Axis-Shield Afinion hemoglobin A1c measurement device. *J Diabetes Sci Technol*, *6*, 380–386.
205. Update on the 1987 task force report on high blood pressure in children and adolescents: a working group report for the national high blood pressure education program (1996). *Pediatrics*, *98*, 649–657.
206. Huo, B., Steffen, A. T., Swan, K., et al. (2007). Clinical outcomes and cost-effectiveness of retinopathy screening in youth with type 1 diabetes mellitus. *Diabetes Care*, *30*, 362–363.
207. Rhodes, E. T., Ferrari, L. R., & Wolsdorf, J. I. (2005). Perioperative management of pediatric surgical patients with diabetes mellitus. *Anesth Analg*, *101*, 986–999.
208. Betts, P., Brink, S. J., Swift, P. G. F., et al. (2007). Management of children with diabetes requiring surgery. *Pediatr Diabetes*, *8*, 242–247.
209. Choudhary, P., Shin, J., Wang, Y., et al. (2011) Insulin pump therapy with automated insulin suspension in response to hypoglycemia: reduction in nocturnal hypoglycemia in those at greatest risk. *Diabetes Care*, *34*, 2023–2025.
210. Iserman, B., Ritzel, R., Zorn, M., et al. (2007). Autoantibodies in diabetes mellitus: current utility and perspectives. *Exp Clin Endocrinol Diabetes*, *115*, 483–490.
211. Miller, K. M., Beck, R. W., Bergenstal, R. M., et al. (2013). Evidence of a strong association between frequency of self-monitoring of blood glucose and hemoglobin A1C levels in T1D exchange clinic registry participants. *Diabetes Care*. Advance online publication. doi: 10.2337/dc12-1770
212. Rosenbauer, J., Dost, A., Karges, B., et al. (2012). Improved metabolic control in children and adolescents with type 1 diabetes: a trend analysis using prospective multicenter data from Germany and Austria. *Diabetes Care*, *35*, 80–86.
213. Tamborlane, W. V., & Sikes, K. A. (2012). Insulin therapy in children and adolescents. *Endocrinol Metab Clin North Am*, *41*, 145–160.
214. Misso, M. L., Egberts, K. J., Page, M., et al. (2010). Continuous subcutaneous insulin infusion (CSII) versus multiple insulin injections for type 1 diabetes mellitus. *Cochrane Database Syst Rev*. doi: 10.1002/14651858.CD005103
215. Pańkowska, E., Błazik, M., Dziechciarz, P., et al. (2009). Continuous subcutaneous insulin infusion vs. multiple daily injections in children with type 1 diabetes: a systematic review and meta-analysis of randomized control trials. *Pediatr Diabetes*, *10*, 52–58.
216. Fox, L. A., Buckloh, L. M., Smith, S. D., et al. (2005). A randomized controlled trial of insulin pump therapy in young children with type 1 diabetes. *Diabetes Care*, *28*, 1277–1281.
217. Weintrob, N., Benzaquen, H., Galatzer, A., et al. (2003). Comparison of continuous subcutaneous insulin infusion and multiple daily injection regimens in children with type 1 diabetes: a randomized open crossover trial. *Pediatrics*, *112*, 559–564.
218. Lawrence, J. M., Yi-Frazier, J. P., Black, M. H., et al. (2012). Demographic and clinical correlates of diabetes-related quality of life among youth with type 1 diabetes. *J Pediatr*, *161*, 201–207.
219. Knight, S., Northam, E., Donath, S., et al. (2009). Improvements in cognition, mood and behaviour following commencement of continuous subcutaneous insulin infusion therapy in children with type 1 diabetes mellitus: a pilot study. *Diabetologia*, *52*, 193–198.
220. Knight, S. J., Northam, E. A., Cameron, F. J., & Ambler, G. R. (2011). Behaviour and metabolic control in children with Type 1 diabetes mellitus on insulin pump therapy: 2-year follow-up. *Diabet Med*, *28*, 1109–1112.
221. Pickup, J. C. (2012). Insulin pump therapy for type 1 diabetes mellitus. *New Engl J Med*, *366*, 1616–1624.
222. Pankowska, E., Blazik, M., Dziechiarz, P., et al. (2009). Continuous subcutaneous insulin infusion vs. multiple daily injections in children with type 1 diabetes: a systematic review and meta-analysis of randomized control trials. *Pediatr Diabetes*, *10*, 52–58.
223. Shalitin, S., Gil, M., Nimri, R., et al. (2010). Predictors of glycaemic control in patients with Type 1 diabetes commencing continuous subcutaneous insulin infusion therapy. *Diabet Med*, *27*, 339–347.
224. de Vries, L., Grushka, Y., Lebenthal, Y., et al. (2011). Factors associated with increased risk of insulin pump discontinuation in pediatric patients with type 1 diabetes. *Pediatr Diabetes*, *12*, 506–512.
225. Nimri, R., Weintrob, N., Benzaquen, H., et al. (2006). Insulin pump therapy in youth with type 1 diabetes: a retrospective paired study. *Pediatrics*, *117*, 2126–2131.
226. Sulmont, V., Souchon, P. F., Gouillard-Darnaud, C., et al. (2010). Metabolic control in children with diabetes mellitus who are younger than 6 years at diagnosis: continuous subcutaneous insulin infusion as a first line treatment? *J Pediatr*, *157*, 103–107.
227. Bratina, N., & Battelino, T. (2010). Insulin pumps and continuous glucose monitoring (CGM) in preschool and school-age children: how schools can integrate technology. *Pediatr Endocrinol Rev*, *7*(Suppl. 3), 417–421.
228. Bachran, R., Beyer, P., Klinkert, C., et al. (2012). Basal rates and circadian profiles in continuous subcutaneous insulin infusion (CSII) differ for preschool children, prepubertal children, adolescents and young adults. *Pediatr Diabetes*, *13*, 1–5.
229. Holterhus, P. M., Bokelmann, J., Riepe, F., et al. (2013). Predicting the optimal basal insulin infusion pattern in children and adolescents on insulin pumps. *Diabetes Care*, *36*, 1507–1511.

230. Nabhan, Z. M., Rardin, L., Meier, J., et al. (2006). Predictors of glycemic control on insulin pump therapy in children and adolescents with type I diabetes. *Diabetes Res Clin Pract*, *74*, 217–221.
231. Danne, T., von Schütz, W., Lange, K., et al. (2006). Current practice of insulin pump therapy in children and adolescents: the Hannover recipe. *Pediatr Diabetes*, 7(Suppl. 4), 25–31.
232. Battelino, T. (2006). Risk and benefits of continuous subcutaneous insulin infusion (CSII) treatment in school children and adolescents. *Pediatr Diabetes*, 7(Suppl. 4), 20–24.
233. Battelino, T. (2009). CSII and continuous glucose monitoring in children and adolescents. In J. Pickup (Ed.), ODL insulin pump therapy and continuous glucose monitoring. (pp. 53–68). Oxford: Oxford University Press.
234. Shalitin, S., & Phillip, M. (2008). Hypoglycemia in type 1 diabetes: a still unresolved problem in the era of insulin analogs and pump therapy. *Diabetes Care*, *31*(Suppl. 2), S121–S124.
235. Danne, T., Battelino, T., Jarosz-Chobot, P., et al. (2008). Establishing glycaemic control with continuous subcutaneous insulin infusion in children and adolescents with type 1 diabetes: experience of the PedPump Study in 17 countries. *Diabetologia*, *51*, 1594–1601.
236. Enander, R., Gundevall, C., Strömgren, A., et al. (2012). Carbohydrate counting with a bolus calculator improves post-prandial blood glucose levels in children and adolescents with type 1 diabetes using insulin pumps. *Pediatr Diabetes*, *13*, 545–551.
237. Kordonouri, O., Hartmann, R., Remus, K., et al. (2012). Benefit of supplementary fat plus protein counting as compared with conventional carbohydrate counting for insulin bolus calculation in children with pump therapy. *Pediatr Diabetes*, *13*, 540–544.
238. O'Connell, M. A., Gilbertson, H. R., Donath, S. M., & Cameron, F. J. (2008). Optimizing postprandial glycemia in pediatric patients with type 1 diabetes using insulin pump therapy: impact of glycemic index and prandial bolus type. *Diabetes Care*, *31*, 1491–1495.
239. Scaramuzza, A. E., Iafusco, D., Santoro, L., et al. (2010). Timing of bolus in children with type 1 diabetes using continuous subcutaneous insulin infusion (TiBoDi Study). *Diabetes Technol Ther*, *12*, 149–152.
240. Cobry, E., Messer, L., VanderWel, B., & Chase, H. P. (2010). Timing of meal insulin boluses to achieve optimal postprandial glycemic control. *Diabetes Technol Ther*, *12*, 173–177.
241. VanderWel, B., Messer, L., Horton, L., et al. (2010). Missed insulin boluses for snacks in youth with type 1 diabetes. *Diabetes Care*, *33*, 507–508.
242. Wilkinson, J., McFann, K., & Chase, H. P. (2010). Factors affecting improved glycaemic control in youth using insulin pumps. *Diabet Med*, *27*, 1174–1177.
243. Aman, J., Skinner, T. C., de Beaufort, C. E., et al. (2009). Associations between physical activity, sedentary behavior, and glycemic control in a large cohort of adolescents with type 1 diabetes: the Hvidoere Study Group on Childhood Diabetes. *Pediatr Diabetes*, *10*, 234–239.
244. Weinzimer, S. A., Beck, R. W., Chase, H. P., et al. (2005). Accuracy of newer-generation home blood glucose meters in a Diabetes Research in Children Network (DirecNet) in patient exercise study. *Diabetes Technol Ther*, 7, 675–680.
245. The Diabetes Research in Children Network (DirecNet) Study Group (2006). Prevention of hypoglycemia during exercise in children with type 1 diabetes by suspending basal insulin. *Diabetes Care*, *29*, 2200–2204.
246. Admon, G., Weinstein, Y., Falk, B., et al. (2005). Exercise with and without an insulin pump among children and adolescents with type 1 diabetes mellitus. *Pediatrics*, *116*, e348–e355.
247. Scrimgeour, L., Cobry, E., McFann, K., et al. (2007). Improved glycemic control after longterm insulin pump use in pediatric patients with type 1 diabetes. *Diabetes Technol Ther*, *9*, 421–428.
248. Jakisch, B. I., Wagner, V. M., Heidtmann, B., et al. (2008). Comparison of continuous subcutaneous insulin infusion (SII) and multiple daily injections (MDI) in paediatric type 1 diabetes: a multicentre matched-pair cohort analysis over 3 years. *Diabet Med*, *25*, 80–85.
249. Wolfsdorf, J., Glaser, N., Sperling, M. A., & American Diabetes Association (2006). Diabetic ketoacidosis in infants, children, and adolescents: a consensus statement from the American Diabetes Association. *Diabetes Care*, *29*, 1150–1159.
250. Guerci, B., Meyer, L., Sallé, A., et al. (1999). Comparison of metabolic deterioration between insulin analog and regular insulin after a 5-hour interruption of a continuous subcutaneous insulin infusion in type 1 diabetic patients. *J Clin Endocrinol Metab*, *84*, 2673–2678.
251. Finfer, S., Chittock, D. R., Su, S. Y., et al. (2009). Intensive versus conventional glucose control in critically ill patients. *N Engl J Med*, *360*, 1283–1297.
252. Van Herpe, T., Vanhonsebrouck, K., Mesotten, D., et al. (2012). Glycemic control in the pediatric intensive care unit of Leuven: two years of experience. *J Diabetes Sci Technol*, *6*, 15–21.
253. Maahs, D. M., Horton, L. A., & Chase, P. (2012). The use of insulin pumps in youth with type 1 diabetes. *Diabetes Technol Ther*, *12*(Suppl. 1), S59–S65.
254. Bratina, N., & Battelino, T. (2013). Establishing a good pump clinic: successes and challenges. *Diabetes Manag*, *3*, 131–143.
255. Wood, J. R., Miller, K. M., Maahs, D. M., et al. (2013). Most youth with type 1 diabetes in the T1D Exchange Clinic Registry do not meet American Diabetes Association or International Society for Pediatric and Adolescent Diabetes clinical guidelines. *Diabetes Care*, *36*, 2035–2037.
256. Cengiz, E., Xing, D., Wong, J. C., et al. (2013). Severe hypoglycemia and diabetic ketoacidosis among youth with type 1 diabetes in the T1D Exchange Clinic registry. *Pediatr Diabetes*, *14*, 447–454.
257. Shorer, M., David, R., Schoenberg-Taz, M., et al. (2011). Role of parenting style in achieving metabolic control in adolescents with type 1 diabetes. *Diabetes Care*, *34*, 1735–1737.
258. Libman, I. M., & Arslanian, S. A. (2007). Prevention and treatment of type 2 diabetes in youth. *Horm Res*, *67*, 22–24.
259. Gungor, N., Hannon, T., Libman, I., et al. (2005). Type 2 diabetes mellitus in youth: the complete picture to date. *Pediatr Clin North Am*, *52*, 1579–1609.
260. Weiss, R., & Caprio, S. (2006). Altered glucose metabolism in obese youth. *Pediatr Endocrinol Rev*, *3*, 233–238.
261. Savoye, M., Shaw, M., Dziura, J., et al. (2007). Effects of a weight management program on body composition and metabolic parameters in overweight children: randomized controlled trial. *JAMA*, *297*, 2697–2704.
262. Weiss, R., Dziura, J., Burgert, T. S., et al. (2004). Obesity and the metabolic syndrome in children and adolescents. *N Engl J Med*, *350*, 2362–2374.
263. Weiss, R., Caprio, S., Trombetta, M., et al. (2005). Beta-cell function across the spectrum of glucose tolerance in obese youth. *Diabetes*, *54*, 1735–1743.
264. Kralisch, S., Sommer, G., Deckert, C. M., et al. (2007). Adipokines in diabetes and cardiovascular diseases. *Minerva Endocrinol*, *32*, 161–171.
265. Yi, P., Park, J. S., & Melton, D. A. (2013) Betatrophin: a hormone that controls pancreatic β cell proliferation *Cell*, *153*, 1–12.
266. Spiegelman, B. M. (2013) Banting Lecture 2012: regulation of adipogenesis; towards new therapeutics for metabolic disease. *Diabetes*, *62*, 1774–1782.
267. Imamura, M., & Maeda, S. (2011). Genetics of type 2 diabetes: the GWAS era and future perspectives (Review). *Endocr J*, *58*, 723–739.
268. Min Xu, Yufang Bi, Bin Cui, et al. (2013). The new perspectives on genetic studies of type 2 diabetes and thyroid diseases. *Cur Genomics*, *14*, 33–48.
269. Zeggini, E., Weedon, M. N., Lindgren, C. M., et al. (2007). Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. *Science*, *316*, 1336–1341.
270. Scott, L. J., Mohlke, K. L., Bonnycastle, L. L., et al. (2007). A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. *Science*, *316*, 1341–1345.
271. Pinhas-Hamiel, O., Standiford, D., Hamiel, D., et al. (1999). A setting for development and treatment of adolescent type 2 diabetes mellitus. *Arch Pediatr Adolesc Med*, *153*, 1063–1067.
272. Rosenbloom, A. L. (2007). Distinguishing type 1 and type 2 diabetes at diagnosis: what is the problem? *Pediatr Diabetes*, *8*, 51–52.
273. Frayling, T. M. (2007). Genome-wide association studies provide new insights into type 2 diabetes aetiology. *Nat Rev Genet*, *8*, 657–662.
274. Hattersley, A. T. (2007). Prime suspect: the TCF7L2 gene and type 2 diabetes risk. *J Clin Invest*, *117*, 2077–2079.
275. Salonen, J. T., Uimari, P., Aalto, J. M., et al. (2007). Type 2 diabetes whole-genome association study in four populations: the DiaGen consortium. *Am J Hum Genet*, *81*, 338–345.
276. Solomon, D. H., & Winkelmayer, W. C. (2007). Cardiovascular risk and the thiazolidinediones: deja vu all over again? *JAMA*, *98*, 1216–1218.

277. Hattersley, A. T., & Pearson, E. R. (2006). Pharmacogenetics and beyond: the interaction of therapeutic response, beta-cell physiology, and genetics in diabetes. *Endocrinology*, *147*, 2657–2663.
278. Berry, G. T., Baynes, J. W., Wells-Knecht, K. J., et al. (2005). Elements of diabetec nephropathy in a patient with GLUT 2 deficiency. *Mol Genet Metab*, *4*, 473–477.
279. Macaulay, K., Dobie, B. W., Patel, S., et al. (2007). Glycogen synthase kinase 3alpha-specific regulation of murine hepatic glycogen metabolism. *Cell Metab*, *6*, 329–337.
280. Whittaker, R. G., Schaeffer, A. M., McFarland, R., et al. (2007). Diabetes and deafness: is it sufficient to screen for the mitochondrial 3243A>G mutation alone? *Diabetes Care*, *30*, 2238–2239.
281. Mancuso, M., Filosto, M., Choub, A., et al. (2007). Mitochondrial DNA-related disorders. *Biosci Rep*, *27*, 31–37.
282. Khanim, F., Kirk, J., Latif, F., et al. (2001). WFS1/wolframin mutations, Wolfram syndrome, and associated diseases. *Hum Mutat*, *17*, 357–367.
283. Barrett, T. G., Scott-Brown, M., Seller, A., et al. (2000). The mitochondrial genome in Wolfram syndrome. *J Med Genet*, *37*, 463–466.
284. Labay, V., Raz, T., Baron, D., et al. (1999). Mutations in SLC19A2 cause thiamine-responsive megaloblastic anaemia associated with diabetes mellitus and deafness. *Nat Genet*, *22*, 300–304.
285. Olsen, B. S., Hahnemann, J. M., Schwartz, M., et al. (2007). Thiamine-responsive megalobastic anaemia: a cause of syndromic diabetes in childhood. *Pediatr Diabetes*, *8*, 239–241.
286. Ferner, R. E. (2006). Drug-induced diabetes. *Baillieres Clin Endocrinol Metab*, *6*, 849–866.
287. Penfornis, A., & Kury-Pauliln, S. (2006). Immonosuppressive drug- induced diabetes. *Diabetes Metab*, *32*, 539–546.
288. Dufresne, R. L. (2007). Weighing in: emergent diabetes mellitus and second-generation antipsychotics. *Ann Pharmacother*, *41*, 1725–1727.
289. Costa, M., Potvin, S., Bethiaume, Y., et al. (2005). Diabetes: a major co-morbidity of cystic fibrosis. *Diabetes Metab*, *31*, 221–232.
290. Teinturier, C., Tournade, M. F., Caillat-Zucman, S., et al. (1995). Diabetes mellitus after abdominal radiation therapy. *Lancet*, *346*, 633–634.
291. Jun, H. S., & Yoon, J. W. (2003). A new look at viruses in type 1 diabetes. *Diabetes Metab Res Rev*, *19*, 8–31.
292. Andreoli, S., & Bergstein, J. (1987). Exocrine and endocrine pancreatic insufficiency and calcinosis after hemolytic uremic syndrome. *J Pediatr*, *110*, 816–817.
293. Low, L., Chernausek, S. D., & Sperling, M. A. (1989). Acromegaloid patients with type A insulin resistance: parallel defects in insulin and insulin-like growth factor-1 receptors and biological responses in cultured fibroblasts. *J Clin Endocrinol Metab*, *69*, 329–337.
294. Flier, J. S., Moller, Moses, A. C., et al. (1993). Insulin-mediated pseudoacromegaly: clinical and biochemical characterization of a syndrome of selective insulin resistance. *J Clin Endocrinal Metab*, *76*, 1533–1541.
295. Chernausek, S. D. (2006). Mendelian genetic causes of the short child born small for gestational age. *J Endocrinol Invest*, *29*, 16–20.
296. Musso, C., Cochran, E., Moran, S. A., et al. (2004). Clinical course of genetic diseases of the insulin receptor (type A and Rabson-Mendenhall syndromes): a 30-year prospective. *Medicine* (Baltimore), *83*, 209–222.
297. Hegele, R. A., Joy, T. R., Al-Attar, S. A., et al. (2007). Lipodystrophies: windows on adipose biology and metabolism. *J Lipid Res*, *48*, 1433–1444.
298. Agrwal, A. K., & Garg, A. (2006). Genetic basis of lipodystrophies and management of metabolic complications. *Annu Rev Med*, *57*, 297–311.
299. Flier, J. S., Kahn, C. R., & Roth, J. (1979). Receptors, antireceptor antibodies and mechanisms of insulin resistance. *N Engl J Med*, *300*, 413–419.
300. Crino, A., DiGiorgio, G., Manco, M., et al. (2007). Effects of growth hormone therapy on glucose metabolism and insulin sensitivity indices in prepubertal children with Prader-Willi syndrome. *Horm Res*, *68*, 83–90.
301. Gillespie, K. M., Dix, R. J., Williams, A. J., et al. (2006). Islet autoimmunity in children with Down's syndrome. *Diabetes*, *55*, 3185–3188.
302. Bakalov, V. K., Cooley, M. M., Quon, M. J., et al. (2004). Impaired insulin secretion in the Turner metabolic syndrome. *J Clin Endocrinol Metab*, *89*, 3516–3520.
303. Aoki, N. (1999). Klinefelter's syndrome, autoimmunity and associated endocrinopathies. *Intern Med*, *38*, 838–839.
304. Minton, J. A., Owen, K. R., Ricketts, C. J., et al. (2006). Syndromic obesity and diabetes: changes in body composition with age and mutation analysis if ALMS1 in 12 United Kingdom kindreds with Alstrom syndrome. *J Clin Endocrinol Metab*, *91*, 3110–3116.
305. Benzinou, M., Walley, A., Lobbens, S., et al. (2006). Bardet-Biedl syndrome gene variants are associated with both childhood and adult common obesity in French Caucasians. *Diabetes*, *55*, 2876–2882.
306. Buchanan, T. A., & Xiang, A. H. (2005). Gestational diabetes mellitus. *J Clin Invest*, *115*, 485-491.
307. Sperling, M. A. (2006). ATP-sensitive potassium channels-neonatal diabetes mellitus and beyond. *N Engl J Med*, *355*, 507–510.
308. Sperling, M. A. (2006). The genetic basis of neonatal diabetes mellitus. *Pediatr Endocrinol Rev*, *4*, 71–75.
309. Gloyn, A. L., Pearson, E. R., Antcliff, J. F., et al. (2004). Activating mutations in the gene encoding the ATP-sensitive potassium-channel subunit Kir6.2 and permanent neonatal diabetes. *N Eng J Med*, *350*, 1838–1849.
310. Pearson, E. R., Flechtner, I., Njolstad, P. R., et al. (2006). Switching from insulin to oral sulfonyurease in patients with diabetes due to Kir6.2 mutations. *N Engl J Med*, *355*, 467–477.
311. Babenko, A. P., Polak, M., Cave, H., et al. (2006). Activating mutations in the ABCC8 gene in neonatal diabetes mellitus. *N Engl J Med*, *355*, 456–466.
312. Hattersley, A. T., & Ashcroft, F. M. (2005). Activating mutations in Kir6.2 and neonatal diabetes: new clinical syndromes, new scientific insights and new therapy. *Diabetes*, *54*, 2503–2513.
313. Ellard, S., Flanagan, S. E., Girard, C. A., et al. (2007). Permanent neonatal diabetes caused by dominant, recessive or compound heterozygous SUR1 mutations with opposite functional effects. *Am J Hum Genet*, *81*, 375–382.
314. Nakhla, M., & Polychronakos, C. (2005). Monogenic and other unusual causes of diabetes mellitus. *Pediatr Clin North Am*, *52*, 1637–1650.
315. Olinder, A. L., Kernell, A., & Smide, B. (2006). Treatment with CSII in two infants with neonatal diabetes mellitus. *Pediatr Diabetes*, *7*, 284–288.
316. Hermann, R., Laine, A. P., Johansson, C., et al. (2000). Transient but not permanent neonatal diabetes mellitus is associated with paternal uniparental isodisomy of chromosome 6. *Pediatrics*, *105*, 49–52.
317. Varrault, A., Bilanges, B., Mackay, D. J., et al. (2001). Characterization of the methylation-sensitive promoter of the imprinted ZAC gene supports its role in transient neonatal diabetes mellitus. *J Biol Chem*, *276*, 18653–18656.
318. Hattersley, A. T. (2004). Unlocking the secrets of the pancreatic beta cell: man and mouse provide the key. *J Clin Invest*, *114*, 314–316.
319. Verwest, A. M., Poelman, M., Dinjens, W. N., et al. (2000). Absence of a PDX-1 mutation and normal gastroduodenal immunohistology in a child with pancreatic agenesis. *Virchows Arch*, *437*, 680–684.
320. Hani, E. H., Stoffers, D. A., Chevre, J. C., et al. (1999). Defective mutations in the insulin promoter factor-1 (IPF-1) gene in late-onset type 2 diabetes mellitus. *J Clin Invest*, *104*, R41–R48.
321. Njolstad, P. R., Sovik, O., Cuesta-Munoz, A., et al. (2001). Neonatal diabetes mellitus due to complete glucokinase deficiency. *N Engl J Med*, *344*, 1588–1592.
322. Thornton, C. M., Carson, D. J., & Stewart, F. J. (1997). Autopsy findings in the Wolcott-Rallison syndrome. *Pediatr Pathol Lab Med*, *17*, 487–496.
323. Zhang, W., Feng, D., Li, Y., et al. (2006). PERK EIF2AK3 control of pancreatic beta cell differentiation and proliferation is required for postnatal glucose homeostasis. *Cell Metab*, *4*, 491–497.
324. Zimmet, P., Alberti, K. G., Kaufman, F., et al. (2007). The metabolic syndrome in children and adolescents: an IDF consensus report. *Pediatr Diabetes*, *8*, 299–306.
325. Robertson, R. P., Davis, C., Larsen, J., et al. (2006). Pancreas and islet transplantation in type 1 diabetes. *Diabetes Care*, *29*, 935.
326. Shapiro, A. M., Ricordi, C., Hering, B. J., et al. (2006). International trial of the Edmonton protocol for islet transplantation. *N Eng J Med*, *355*, 1318–1330.
327. Haddad, E. M., McAllister, V. C., Renouf, E., et al. (2006). Cyclosporin versus tacrolimus for liver transplanted patients. *Cochrane Database Syst Rev*, CD005161.
328. Beck, J., Angus, R., Madsen, B., et al. (2007). Islet encapsulation: strategies to enhance islet cell functions. *Tissue Eng*, *13*, 589–599.
329. Trucco, M. (2005). Regeneration of the pancreatic beta cell. *J Clin Invest*, *115*, 5–12.
330. Voltarelli, J. C., Couri, C. E., Stracieri, A. B., et al. (2007). Autologous nonmyeloablative hematopoietic stem cell transplantation in newly diagnosed type 1 diabetes mellitus. *JAMA*, *297*, 1568–1576.

QUESTIONS

1. Complications of DKA may include all except which of the following?
   a. Cerebral edema
   b. Mucormycosis infection
   c. Prerenal failure
   d. ARDS
   e. Venous thrombosis

Answer: b

2. A 13-year-old male with a past history of learning disability is admitted in coma. His weight is 86 kg, BP 110/80, pulse 134, capillary refill 4 seconds. Glucose is 936 mg/dL (52 mM); pH 7.24, HCO_3^- 15 mm, serum Na 140 mEq, K 4.5 mEq, BUN 30 mg/dL, and creatinine 1.2 mg/dL. Urine examination reveals glucose > 2% and trace ketones. After initial rehydration with 0.9% saline with two bolus injections of 10 cc/kg over 3 hours, therapy should continue with which of the following?
   a. Normal saline infusion plus insulin 0.1 U/kg/hr
   b. 0.5 N saline plus insulin 0.1 U/kg/hr
   c. Normal saline plus insulin 0.05 U/kg/hr
   d. 0.5 N saline plus 20 mEq/L KCl
   e. 0.5 N saline plus KCl 20 mEq/L plus insulin 0.05 U/kg/hr

Answer: d

3. A 15-year-old girl who has had type 1 diabetes mellitus for 3 years and confirmed Hashimoto thyroiditis for 2 years experiences repeated episodes of hypoglycemia unrelated to exercise. In addition to reducing the insulin dose, which of the following steps is most appropriate?
   a. Check fT_4, TSH, and thyroid antibodies to ensure compliance with thyroid medication.
   b. Check thyroid function, cortisol, and TTG IgA in a fasting morning sample.
   c. Check TSH, adrenocorticotropic hormone (ACTH) and TTG IgA in a late afternoon (4 to 6 p.m.) blood sample.
   d. Check thyroid function, ACTH, and TTG IgA in a fasting morning sample.
   e. Referral to a psychologist to rule out an eating disorder.

Answer: c

4. The genes predisposing to T_{1a}DM include all except which of the following?
   a. HLA DR
   b. PTPN22
   c. AIRE
   d. PTPN11
   e. CTLA4

Answer: d

5. Which of the following genes is implicated in both T1aDM and T2DM?
   a. KCNJ11
   b. ZnT8
   c. TCF7L2
   d. PPARγ
   e. Insulin

Answer: e

6. HbA1c is commonly used as a means to monitor average blood glucose in the preceding 6 to 8 weeks. An inappropriately low HbA1c may be obtained in all except which of the following conditions?
   a. Hemolytic anemia
   b. Thalassemia major
   c. Sickle cell disease
   d. "Slow glycator" syndrome
   e. Renal failure

Answer: b

CHAPTER 20

Autoimmune Polyglandular Syndromes

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CHAPTER OUTLINE

INTRODUCTION

The autoimmune polyglandular syndromes (APS I and II) are uncommon constellations of organ-specific autoimmune diseases characterized by the occurrence of more than one autoimmune disease in an affected individual (Table 20-1). More commonly, autoimmune disease of endocrine glands occurs in only a single organ, but multiorgan involvement of both endocrine and nonendocrine organs and tissues may be present.

Tolerance is an active state in which the immune system does not mount a reaction against self-antigens.[1-4] If tolerance is not established or is lost, autoimmunity and subsequent disease may result. Although the breakdown in self-tolerance remains mostly unexplained, our improved understanding of the complex interplay between genetics and environment and the resultant aberrant immunologic processes has identified a number of possible mechanisms.[5] To comprehend these mechanisms, a brief overview of how tolerance is maintained is essential.

MECHANISMS UNDERLYING GENERATION OF AUTOIMMUNITY

Introduction

In a normal immune response, the host organism must differentiate self from nonself, initiate an immune response to nonself, and eliminate nonself to protect the host from injury, organ dysfunction, and even death.[1-4] Endogenous antigens represent self, whereas exogenous antigens represent nonself. The adaptive immune system assumes all exogenous antigens are potentially harmful and acts to eliminate nonself. Self-nonself discrimination is carried out by the adaptive (specific) immune system through the use of T- and B-cell surface receptors.[6-8] These receptors recognize distinctive peptides (e.g., the T-cell receptors) or epitopes (e.g., the B-cell antibody receptors) and are the keys to the specificity of the adaptive immune response. Whereas B cells and their receptors recognize soluble antigen or antigens on cell surfaces, T cells and their receptors only perceive short polypeptides presented by specialized cell-surface molecules encoded by the major histocompatibility complex (MHC).[9-11] The human MHC is termed the human leukocyte antigen (HLA) complex. Class I MHC (e.g., HLA-A, HLA-B, and HLA-C molecules) present peptides predominantly derived from the cell cytoplasm to T cells. T cells are classified on the basis of their cluster of differentiation (CD) surface proteins, which bind differentially to antigens presented on class I and class II MHC. For example, CD8 cells, considered "cytotoxic" T cells, typically recognize targets presented on class I MHC. In addition, more "professional" antigen presenting cells known as dendritic cells and defined by a combination of CD markers and the characteristic presence of numerous membrane processes that extend out from the cell body can present peptides of extracellular

TABLE 20-1 **The Autoimmune Polyglandular Syndromes I and II**

	APS I	APS II
Comparative frequency	Less common	More common
Onset	Infancy/early childhood	Late childhood, adulthood
Heredity	Autosomal recessive	Polygenic
Gender	Males = females	Female predominance
Genetics	AIRE gene; no HLA association	HLA associated; DR/DQ
Hypoparathyroidism	77%-89%	None
Mucocutaneous candidiasis	73%-100%	None
Ectodermal dysplasia	77%	None
Addison disease	60%-86%	70%-100%
Type 1 diabetes	4%-18%	41%-52%
Autoimmune thyroid disease	8%-40%	70%
Pernicious anemia	12%-15%	2%-25%
Gonadal failure		
Females	30%-60%	3.5%-10%
Males	7%-17%	5%
Vitiligo	4%-13%	4%-5%
Alopecia	27%	2%
Autoimmune hepatitis	10%-15%	Rare
Malabsorption	10%-18%	Rare

origin via class I MHC molecules. Conversely, CD4 cells typically are activated when peptides derived from the extracellular space are presented via class II MHC (e.g., HLA-DP, HLA-DQ, and HLA-DR molecules).

Regulation of T-cell self-tolerance occurs at two distinct but interdependent levels: centrally and peripherally (described in detail later). Central tolerance occurs in the thymus via positive and negative selection of self-reactive T cells, whereas peripheral tolerance occurs in both lymphoid and nonlymphoid tissues (Figures 20-1 and 20-2). Although many of the mechanisms involved in establishing tolerance remain poorly understood, nearly 15 years of characterizing the autoimmune regulatory gene (AIRE) has improved understanding of positive and negative T-cell selection.[4] The AIRE gene, which codes for a transcription factor found in the medulla of the thymus, plays a critical role in the ectopic expression of tissue-specific antigens, which regulate negative selection of autoreactive T effector cells.[12,13] Deletions in the mouse AIRE homologue result in multiorgan autoimmunity, whereas mutations in the human AIRE gene result in APS I.[14,15]

Tolerance is initially developed in utero. During gestation and early life, tolerance is most easily induced by exposure of the host to a specific antigen. Although the thymus atrophies during puberty, residual thymic tissue provides for T-cell development throughout life. T cells do not require exposure to large doses of antigen to achieve tolerance during their thymic development. However, larger doses of antigen are required to induce B-cell tolerance, and B-cell tolerance is often short lived. B cells and T cells are produced by the bone marrow continuously throughout life. Tolerance is immunologically specific, learned or acquired, most easily induced in immature or developing lymphocytes, and can be induced in mature lymphocytes when costimulatory signals are absent at the time of peptide recognition by the T lymphocyte.

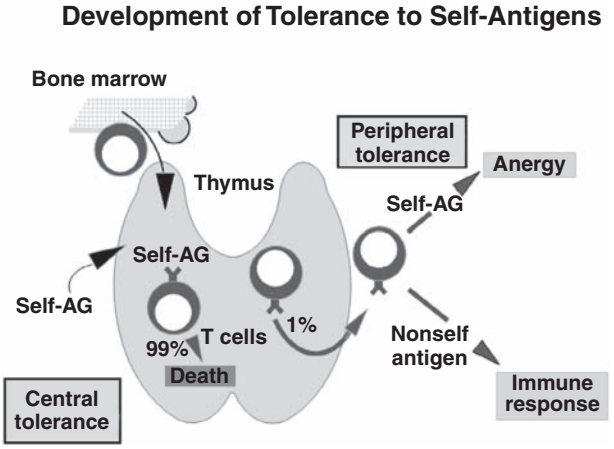


FIGURE 20-1 ■ Normal tolerance pathways. T-cell precursors initially arise in the bone marrow. These progenitors enter the thymus, and developing T cells encounter self-antigens. Strong self-antigen stimulation of developing T cells induces apoptosis, with approximately 99% of all developing T cells dying. This is central immunologic tolerance where strongly antiself reactive T cells are eliminated. Naïve T cells that do leave the thymus can be subsequently tolerized to self-antigens if they encounter self-antigen without the normal costimulatory signals (B7.1/B7.2-CD28, see Figure 20-3). Induction of tolerance outside the thymus is termed *peripheral tolerance* (top right) and is a complementary mechanism to central tolerance. Peripheral tolerance is functionally expressed as anergy: autoreactive cells are present but are inactive (top right). If nonself-antigen is encountered, a normal immune response ensues (bottom right).

Central T-Cell Tolerance

T cells are primarily educated to distinguish self and nonself in the thymus.[16-19] Central T-cell tolerance is the process by which antiself T cells are eliminated in the thymus. As many as 99% of developing thymocytes die in the thymus and never reach the periphery.

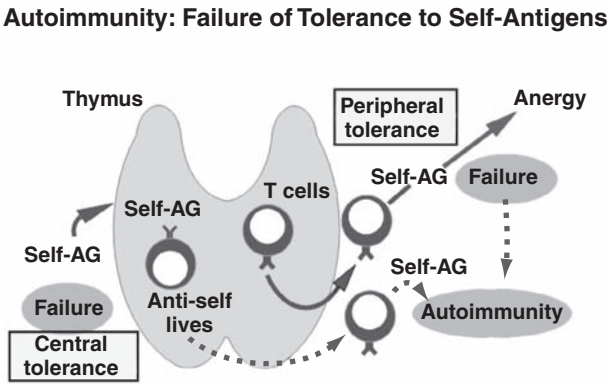


FIGURE 20-2 ■ Autoimmunity: failure of tolerance to self-antigens. With a failure of central tolerance (bottom left), antiself T cells survive that should not normally survive. When these antiself T cells leave the thymus, they are able to produce autoimmunity (dotted arrows). Alternatively, with a failure of peripheral tolerance (top right), if anergy does not occur after contact with self-antigen, an autoimmune response can occur.

T-cell tolerance is a function of the selection of the T-cell receptor (TCR) repertoire that exits the thymus. In the thymic cortex CD4+, CD8+ (double positive) T cells bearing alpha/beta TCRs that bind to self-MHC initially survive (positive selection). In this way the thymus initially chooses for survival T cells that bind to self-MHC as opposed to TCRs that might bind to other self molecules leading to noneffective communication. This positive selection resulting from self peptide-MHC presentation is carried out by thymic nurse epithelial cells in the cortex. At the corticomedullary junction of the thymus, if such saved TCRs bind to self-MHC too tightly, autoreactivity is possible and these T cells then undergo negative selection and suffer apoptotic death. The cells inducing negative selection are macrophages and dendritic cells. This process of positive selection for MHC binding in the thymic cortex and negative selection (at the thymic corticomedullary border) against tight binding to self-peptides accounts for central (thymic) immunologic tolerance.[3,5,13,20]

Peripheral T-Cell Tolerance

Once in the circulation and secondary lymphoid organs (e.g., lymph nodes and spleen), naïve T cells still require multiple signals to become activated.[7] The initial signal is the presentation of antigen-specific peptides to T-cell receptors by MHC molecules. CD4 and CD8 molecules on these T-cell subsets serve as antigen-nonspecific coreceptors binding to nonpolymorphic portions of the class II MHC molecules and class I MHC molecules, respectively. The second signal is antigen nonspecific and is provided by the B7.1 (CD80) and B7.2 (CD86) molecules of the antigen-presenting cell interacting with the CD28 molecule on the T-cell surface.

When the T cell perceives both signals, a cascade of intracellular signaling events occur, leading to T-cell activation. Activated CD4 T cells express numerous cytokines, cytokine receptors, and CTLA-4. Activated CD4 T cells then down-regulate T-cell receptor expression and acquire class II MHC expression. It is unknown why activated human T cells express class II MHC (activated T cells in mice do not express class II MHC). CTLA-4 expression by the activated T cell and its interaction with B7.1/B7.2 provides an immunosuppressive signal to the T cell, thereby down-regulating the T-cell immune responses. Thus, CTLA-4 and CD28 act antithetically: B7.1/B7.2-CD28 turns on T cells, whereas B7.1/B7.2-CTLA-4 down-regulates the T cell (Figure 20-3).

Helper CD4 T cells have classically been subdivided into two distinct lineages: (1) Th1 cells, which activate cell-mediated and some antibody responses, and (2) Th2 cells, which predominantly activate antibody-mediated responses.[21] However, additional T-cell lineages exist (i.e., Th17 cells, T-follicular helper cells, and regulatory T cells) and data have described remarkable plasticity in their cytokine expression, suggesting shifting T-cell functionalities depending on environmental cues.[22] Although overly simplistic, Th1 subsets can be thought of as cells that activate macrophages, natural killer cells, and B cells and secrete predominantly IL-2, interferon gamma (IFN-γ), tumor necrosis factor–beta (TNF-β), and IL-12. Th2 cells elaborate IL-4, IL-5, IL-6, IL-10, and IL-13. Crosstalk between Th1 and Th2 cells occurs; for example, IFN-γ from Th1 cells suppresses Th2 cells and IL-10 from Th2 cells inhibits Th1 cells. Another subset of CD4 T cells has been described as regulatory T cells that secrete the immunomodulatory cytokines IL-10 or transforming growth factor–beta (TGF-β). Regulatory T cells include $CD4^+CD25^+FoxP3^+$ T cells, Tr1, and Th3 cells. Tr1 and Th3 cells express CD4 but do not express CD25 (the IL-2 receptor alpha chain). Tr1 cells secrete IL-10 and TGF-β, whereas Th3 cells secrete IL-4, IL-10, and TGF-β. Although $CD4^+CD25^+FoxP3^+$ T cells can secrete both IL-10 and TGF-β, their regulatory action on autoreactive T cells appears to occur through cell-to-cell contact. Upon activation, CD8 T cells, often with the help of Th1 cells

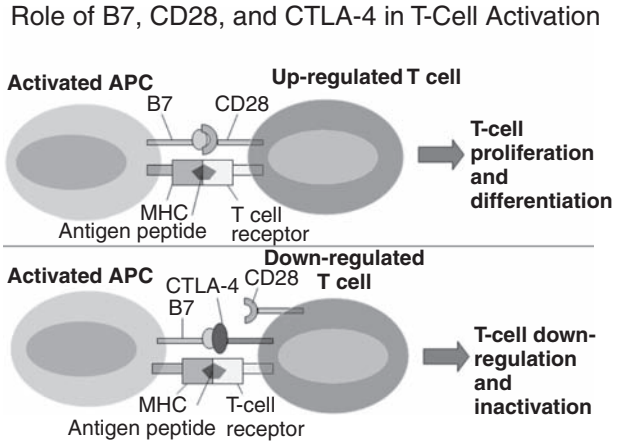


FIGURE 20-3 ■ Role of B7, CD28, and CTLA-4 in T-cell activation. Activated antigen presenting cells (APC) present antigen peptides on the major histocompatibility complex (MHC) molecules and express B7, including B7.1 (CD80) and B7.2 (CD86), costimulators. When B7.1 or B7.2 is bound by CD28 and MHC plus peptide is bound by a T-cell receptor, T-cell proliferation and differentiation of naïve T cells ensues. Conversely, activated T cells express CTLA-4 and bind to B7 (either B7.1 or B7.2] inducing down-regulation and inactivation of T cells.

supplying IFN-γ to up-regulate B7 expression on antigen presenting cells, become functional cytotoxic T killer cells.

The requirement for two signals to activate naïve T cells accounts for peripheral T-cell tolerance. When the naïve T cell perceives antigen peptide presented by MHC molecules without the necessary costimulatory signal (e.g., B7.1/B7.2-CD28), the T cell becomes unresponsive. This state of unresponsiveness is termed anergy. The T cell may also undergo apoptosis (programmed cell death) to be completely removed from the T-cell repertoire. Anergic T cells can generally not be restimulated with antigen peptide displayed by the antigen-presenting cell. Tolerance may also exist because the T-cell receptor does not come into contact with the relevant peptide. This has been termed "T-cell ignorance."

Ongoing characterization of regulatory T cells has improved our understanding of peripheral tolerance. Regulatory T cells play a critical role in suppressing the activity of effector T cells that escape negative selection to self-antigen in the thymus.[23] Functional regulatory T cells are able to anergize previously self-reactive T effector cells, resulting in improved tolerance to self. The expression of the forkhead transcription factor, FoxP3, is specific for identification of the $CD4^+CD25^+$ cell (T_{reg}) population. First identified in the *Scurfy* mouse, a mouse model of immune dysfunction and polyendocrinopathy, abnormal FoxP3 expression is now known to be responsible for the failure in tolerance in humans affected with a similar polyendocrinopathy discussed later.[24-26] The absence of normal FoxP3 expression in humans leads to an extremely rare, X-linked, recessively inherited, and typically fatal autoimmune lymphoproliferative disease known as immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance (IPEX).[27,28] Defects in the forkhead transcription factor FoxP3 that are responsible for IPEX map to Xp11.23-Xq13.3.

B-Cell Tolerance

B cells are partially educated in the bone marrow to be anergized or undergo apoptosis in response to self-antigen when they are at the stage of development of the naïve immature B cell (central B-cell tolerance). In the bone marrow, naïve immature B cells that see multivalent antigens become anergized (unresponsiveness to subsequent stimulation), whereas exposure to highly polyvalent antigens can induce apoptosis.[29]

Naïve immature B cells express IgM on their surface and are not yet IgM and IgD positive as observed in naïve mature B cells. Anergized B cells do not instantly die but will live no longer than unstimulated naïve immature B cells. Naïve mature B cells expressing IgM and IgD on their surface require T-cell help to realize their full potential through affinity maturation and class switching. The absence of T-cell help leads to B-cell tolerance.

Autoimmune Diseases

The organ-specific nature of many autoimmune diseases results from abnormal immune system recognition of tissue-specific self-antigens. In many autoimmune endocrinopathies, the target molecule is either a tissue-specific or tissue-limited (i.e., the protein is not unique to one tissue but is clearly restricted in its distribution) enzyme or cell-surface receptor[30,31] (Table 20-2).

The criteria for classification of a disease as autoimmune are not universally agreed upon.[32] However, major criteria that are generally accepted as strong evidence that the disease is autoimmune include (1) detection of autoantibodies or autoreactive T cells including lymphocytic infiltration of the targeted tissue or organ, (2) disease transfer with antibodies or lymphocytes, (3) disease recurrence in transplanted tissue, and (4) ability to abrogate the disease process with immunosuppression or immunomodulation. Few, if any, human autoimmune diseases meet all four of these criteria. Further information that is supportive of, but not diagnostic for, an autoimmune disease includes (1) increased disease frequency in women compared to men, (2) the presence of other organ-specific autoimmune diseases in affected individuals, and (3) increased frequencies of particular HLA alleles in affected individuals.

Defects in Tolerance that Cause Autoimmune Diseases

Several hypotheses explaining defects in tolerance have been proposed.[33] Theoretically autoimmunity may develop because (1) tolerance never developed to specific self-antigens or (2) established tolerance was lost. If self-antigen is not efficiently presented in the thymus, tolerance may not be established during T-cell education within the thymic cortex.[34] For example, variations in the insulin gene VNTR (variable number of tandem repeats), ~500 base pairs upstream of the insulin gene promoter, influence the extent of insulin gene expression in the thymic cortex. The risk of developing type 1 diabetes is enhanced when certain VNTR alleles are present, which leads to lower mRNA expression of insulin in the thymus. Specifically, certain protective class III alleles are associated with increased thymic expression of insulin and a decreased risk of developing type 1 diabetes, whereas class I alleles are associated with decreased thymic expression of insulin and an increased risk of developing diabetes.[35] Failure to delete specific autoreactive T cells clones predisposes patients to autoimmunity. If autoimmunity does result from defects in thymic tolerance, the defects must be antigen specific because organ-specific autoimmune diseases are usually extremely selective. For example, in type 1 diabetes, whereas the beta cells are attacked and ultimately destroyed by a cell-mediated autoimmune process, the remaining islet cells including alpha cells, delta cells, and pancreatic polypeptide-producing cells are unscathed.

Defects in peripheral tolerance could result from concurrent T-cell stimulation by self-antigen/MHC plus T-cell costimulation (e.g., B7.1/B7.2-CD28) leading to aberrant T-cell activation and an autoimmune response. If tolerance has not been developed because an antigen is sequestered intracellularly or is not expressed in the thymus during T-cell ontogeny, T-cell reactivity in the periphery would not be abrogated. However, several

TABLE 20-2 **Autoantigens in Autoimmune Endocrine and Associated Diseases**

Disease	Autoantigens	Putative Autoantigens
Mucocutaneous candidiasis		IL-17A IL-17F IL-22
Hypoparathyroidism		NALP5 (specific for APS) CaSR
Addison disease	P450c21	P450c17 P450scc
Hashimoto thyroiditis	Thyroperoxidase Thyroglobulin	
Graves disease	Thyrotropin receptor	
Diabetes	Insulin Glutamic acid decarboxylase$_{65}$ IA-2 (ICA 512) IA-2β ZnT8	Proinsulin Carboxypeptidase H ICA69 Glima 38
Premature gonadal failure	P450scc	P450c17 3β hydroxysteroid dehydrogenase
Pernicious anemia	H^+/K^+ ATPase pump Intrinsic factor	
Myasthenia gravis	Acetylcholine receptor α chain	
Vitiligo		Tyrosinase Tyrosinase related protein-2 L-amino acid decarboxylase
Celiac disease	Endomysium transglutaminase	Reticulin Deamidate gliadin
Autoimmune hepatitis	Liver kidney microsome 1	L-amino acid decarboxylase Tryptophan hydroxylase

IL, interleukin; NALP5, NLR family, pyrin domain containing 5; NLR, NACHT, leucine rich; NACHT, nucleotide oligomerization domain-like receptors; CaSR, calcium sensing receptor; IA-2, protein tyrosine phosphatase-like protein; ZnT8, zinc transporter 8; ICA, islet cell autoantigen.

antigens initially thought to be sequestered intracellularly have now been shown to circulate in low concentrations in normal individuals. Thyroglobulin is such a self-antigen in autoimmune thyroid disease. The development of thyroglobulin autoantibodies was believed to follow the release of thyroglobulin from the thyroid gland following viral infection or trauma. "Immunization" with thyroglobulin would hypothetically lead to an antithyroglobulin humoral response and autoimmune thyroid disease would follow. However, we now know that thyroglobulin does circulate in low but appreciable quantities in normal individuals who show no serologic evidence of thyroid autoimmunity. Furthermore, thyroid follicular cell destruction in Hashimoto thyroiditis is cell mediated and not humorally mediated.

If sequestered antigens do play a role in autoimmune disease, viral infections, trauma, ischemia, or irradiation are all mechanisms that could disturb cellular integrity and lead to the release of intracellular antigens.[36] Some self-antigens may never normally come into contact with the immune system unless there is a breakdown of anatomic barriers within the body. An example is the occurrence of autoimmunity to the eye following orbital trauma. Although a rare consequence of orbital damage, initiation of an autoimmune response to eye proteins in adjacent lymph nodes can generate autoreactive T cells that can invade and damage the contralateral eye ("sympathetic ophthalmia").[37,38] Removal of the inciting damaged tissues and immunosuppression may be required to sustain vision in the undamaged eye. Similarly, transient autoantibody reactivity to cardiac myosin following myocardial infarction has also been described.[39]

Tolerance may not develop, hypothetically, if self-antigen expression is delayed during negative selection. When the self-antigen is ultimately expressed, if tolerance has not previously been established, the autoantigen is perceived as foreign and autoreactivity develops. No spontaneous examples of this process have been described. However, in experimental systems where transgenes are placed under control of promoters that can be turned on by exogenous agents such as metals, autoreactivity can be elicited when gene expression is stimulated after the neonatal period.

Alteration of self-antigens as a result of infection or neoplasia is believed to be a plausible theory explaining some types of autoimmunity. As environmental triggers,

viral infections could lead to modification of self-proteins and neoantigen expression (e.g., a new antigen is present on self-cells). Alternatively, a self-antigen may be partially degraded leading to a "new" antigenic target for the adaptive immune system. This new antigen is recognized as foreign by the immune system and the immune response to these new antigens results in autoimmunity.

Some self-cells/tissues may suffer unintended autoimmune damage when substances bind to the cells and elicit an initial immune response. For example, certain drugs bind to red blood cells and result in an immune hemolytic anemia. If an antibody response to the red-cell-bound drug is elicited, the antigen-antibody complex present on the red blood cell can lead to red blood cell destruction. This can occur either through red blood cell phagocytosis by the monocyte-macrophage system or via complement-mediated lysis of the red blood cell. Thus, the red blood cell is an innocent bystander to the antidrug humoral immune response. Theoretically this could also occur with viruses that serendipitously attach to tissues.

Molecular mimicry is one of the most popular explanations for autoimmunity.[36,40] Due to exposure to a dietary, viral, or bacterial antigen (e.g., infection) and molecular mimicry (similarity) between the self-antigen and the foreign antigen, the immune response to the foreign antigen leads to cross reactivity with self-antigen, autoimmunity, and disease.[40-43] For this theory to work, tolerance must not previously exist to the self-antigen. This might be true if the self-antigen is truly sequestered and the immune system has never developed tolerance to the self-antigen. Alternatively, the self-antigen peptides may be present in too low a concentration to elicit an immune response and initial immune system tolerization has not occurred. Only with infection or novel dietary exposure would there be a sufficient degree of self-immunization to develop immune autoreactivity. With immune autoreactivity, self is now recognized as foreign during the response to the cross-reactive pathogen. If the self-antigen is a cell surface antigen, the "pathogen-induced" autoantibodies could fix to self and produce disease via complement fixation, or the antibodies could act as opsonins for fixed or circulating phagocytes (antibody-dependent-cell cytotoxicity). In rheumatic fever, cross-reactivity between Streptococcus M protein and cardiac myosin have been described. In ankylosing spondylitis, cross-reactions between Klebsiella nitrogenase and HLA-B27 have been described. In rheumatoid arthritis there is cross-reactivity between cartilage protein and a mycobacterial proteoglycan wall component.

Aberrant class II MHC expression was a theory in vogue in the mid-1980s. In this hypothesis, cells elicit autoimmune reactions by presenting their own self-peptides via self-expressed class II MHC molecules. Indeed class II MHC expression has been identified on various cells that are targets of autoimmune-mediated cell destruction. Examples include β cells in type 1 diabetes, biliary tract cells in primary biliary cirrhosis, and thyroid follicular cells in Hashimoto thyroiditis. However, there are strong counterarguments to this theory. First, class II MHC molecules do not present intracytoplasmic antigens, which are often targets of attack. Instead, class II MHC molecules present peptides derived from extracellular proteins. Second, accessory molecules are typically needed to activate naïve T cells. If, for example, β cells present peptides via their class II MHC molecules without B7.1 and B7.2, the naïve T cells seeing these peptides in the absence of B7 will actually be tolerized. Indeed, aberrant (or ectopic) class II MHC expression may be a mechanism cells use to actually induce a state of tolerance to down-regulate an immune response. Aberrant class II MHC expression may therefore serve an anti-inflammatory role in modulating an immune response to a lower level of intensity. Costimulator expression (e.g., B7.1/B7.2) is highly regulated even among professional antigen-presenting cells. For example in the basal state, neither macrophages nor B cells express B7. Upon phagocytosis of bacteria species, macrophages will express both class II MHC and B7. Although B cells express class II MHC in their basal state, internalization of bacterial antigen bound to their cell surface receptor antibody molecules will induce B7 expression.

Some cases of autoimmunity may result from superantigens initiating an antiself immune response as part of the polyclonal immune activation process. Superantigens are polyclonal T-cell stimulators that have the ability to cross-link TCR beta chains and MHC molecules. Superantigens have been reported to activate as many as one third of all T cells in the body. In such cases, systemic disease can develop from massive cytokine release (e.g., the systemic inflammatory response syndrome [SIRS]). This is the case in toxic shock syndrome, wherein a staphylococcal exotoxin acts as a superantigen. Mycobacterial antigens have also been proposed as possible superantigens in Crohn disease.[44] This theory presupposes that T cells bearing antiself TCRs have not been deleted or permanently anergized. T cells with antiself receptors may be stimulated, and if they encounter self-antigen, they may further proliferate to develop an autoimmune response.

Similar to polyclonal T-cell activation, polyclonal B-cell stimulation has also been implicated in humoral autoimmunity. Indeed, autoreactive B cells can be found in normal individuals. If an autoreactive clone of B cells encounters self-antigen and a costimulator (which might be nonspecific, e.g., a virus such as Epstein-Barr virus or a bacterial product such as lipopolysaccharide), autoantibodies could be produced, bypassing the need for T-cell help.

However, which of these speculative theories applies to the APS is unknown. Human disease most often results from an interaction of environmental and genetic factors.[45-47] Many environmental factors are implicated in various autoimmune diseases: wheat gliadin ingestion and celiac disease, penicillamine exposure and myasthenia gravis, methimazole and autoimmune hypoglycemia from insulin autoantibodies (seen primarily in Japanese patients), and amiodarone and thyroiditis. Even cancer can be associated with the development of autoimmunity: thymoma and myasthenia gravis,[48] ovarian teratoma and N-methyl-D-aspartate (NDMA) receptor meditated encephalitis,[49] and breast cancer and stiff

person syndrome.[50] Despite remarkable improvements in our understanding of immunology, many achieved through meticulous studies of APS, the mechanisms whereby the complex interaction of genes, environment, and immune system lead to autoimmunity remain to be fully elucidated.

CLASSIFICATION OF THE AUTOIMMUNE POLYGLANDULAR SYNDROMES

APS I, also known as autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), is an autosomal recessive disorder mapped to a single gene (the autoimmune regulator or AIRE gene) on chromosome 21q22.3.[14,15] The presence of two of the following three conditions are prerequisites for the diagnosis of APS I: (1) adrenocortical failure (Addison disease) or serologic evidence of adrenalitis (adrenal autoantibodies), (2) hypoparathyroidism, and (3) chronic mucocutaneous candidiasis.[45,46,51-54] APS II is defined by the coexistence of autoimmune adrenocortical insufficiency or serologic evidence of adrenalitis with autoimmune thyroiditis (Schmidt syndrome) or type 1 diabetes mellitus (Carpenter syndrome: Schmidt syndrome plus type 1 diabetes) or serologic evidence of either thyroid or islet autoimmunity[45,55-58] (Figure 20-4). The presence of thyroiditis without adrenal disease but associated with either type 1 diabetes, pernicious anemia, vitiligo, or alopecia has been referred to by some authors as APS III, whereas additional combinations of autoimmune disease have been referred to as APS IV (i.e., vitiligo plus alopecia, type 1 diabetes plus celiac disease, or type 1 diabetes and vitiligo).[59] However, because APS III and IV differ from APS II only by the presence or absence of adrenocortical disease (and share similar susceptibility genes and immunologic features), we do not recognize APS III or IV as unique syndromes and consider them extensions of the APS II constellation.

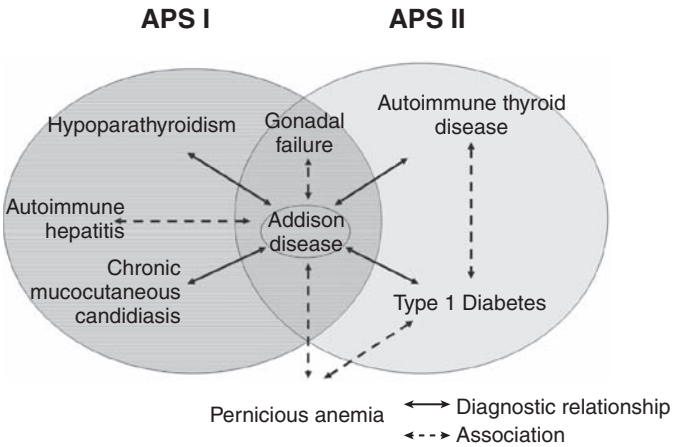


FIGURE 20-4 ■ Diagnostic relationships and common associations in APS I and APS II. The solid lines indicate diagnostic relationships. The dashed lines indicate common associations. The diagnosis of APS I depends on the coexistence of Addison disease (or adrenal autoantibodies) plus either hypoparathyroidism or chronic mucocutaneous candidiasis or both. The diagnosis of APS II depends on the coexistence of Addison disease (or adrenal autoantibodies) plus either autoimmune thyroid disease or type 1 diabetes or both (or their associated autoantibodies).

CLINICAL ASPECTS

APS I

The major disease components, frequencies, and differences between APS I and II are shown in Table 20-1. Although the disease is not common, the largest cohorts of patients have been reported from Finland, the United States, and among Iranian Jews.[51,52,54,60,61] The Finnish cohort of 91 subjects is the largest and most well characterized APS I group of patients in the world.[52]

Persistent mucocutaneous candidiasis is usually the first sign (60% of all APS I patients) and usually appears during the first year or two of life. In the Finnish cohort, 50% developed candidiasis by age 5 years, 94% by age 20 years, and 100% by 40 years of age.[52] All patients with refractory mucocutaneous candidiasis should be thoroughly investigated not only for a T-lymphocyte abnormality (absolute lymphocyte count, enumeration of T-cell subpopulations, assessment of T-cell function) but also for the presence of a polyendocrinopathy. Candidal infections in the diaper area are found early in life, with vulvovaginal candidiasis developing at puberty in females. Colonization of the gut by Candida can lead to intermittent abdominal pain and diarrhea. Infection of the nails with chronic candidiasis may lead to a darkened discoloration, thickening, or erosion. Retrosternal pain in patients with oral candidiasis suggests esophageal candidiasis and can be confirmed by esophagoscopy. Chronic oral mucosal candidiasis must be treated aggressively as candidiasis associated carcinoma of the oral mucosa or esophagus was reported in 7 of the 55 Finnish APS I patients over 25 years of age (note that 5 of the 7 were also smokers).[51,62]

Oral mucous membranes must therefore be protected from exposures that can increase susceptibility to candidal infections. Specifically, patients should be advised to avoid hard, sharp, or spicy foods, as well as whitening toothpastes or abrasives. Dentures or orthodontics can provide additional surfaces for Candidal growth.[51] Data suggest that prolonged therapy with fluconazole, ketoconazole, and miconazole leads to reduced azole susceptibility.[63,64] As such, azole therapy should be limited to two to three courses per year utilizing two polyene antifungals. Specifically, a 1- to 2-minute swish with 2 mL of nystatin followed by an amphotericin B lozenge that is allowed to dissolve without chewing should be given four times daily for 4 to 6 weeks.[51,64] When candidiasis is recurrent, pulse prophylaxis should follow using a 1-week course of either polyene (as described previously) every 3 weeks. Alternatively, 1 week of twice-daily chlorhexidine mouth rinse has also been used as prophylaxis.[51] If topical therapy fails, a 1-week course of high-dose fluconazole (200 to 300 mg in adults) is typically efficacious, though intravenous antifungal therapy may occasionally be required.

Hypoparathyroidism is typically the first endocrinopathy to develop in APS I and eventually occurs in more than 85% of patients. Hypoparathyroidism usually presents after the onset of mucocutaneous candidiasis but before puberty with 33% of APS I patients diagnosed with hypoparathyroidism by 5 years, 66% by 10 years, and nearly 85% by age 30. Severe hypocalcemia as evidenced by seizures, carpopedal spasms, muscle twitching, and laryngospasm may be presenting features of APS I, although these symptoms may be masked in the presence of adrenal insufficiency. Hypocalcemia, hyperphosphatemia and a low intact parathyroid hormone (PTH) level are diagnostic of hypoparathyroidism. Standard therapy consists of calcium salts and activated vitamin D (see Chapter 18 for management). Although data suggest that twice-daily administration of synthetic parathyroid hormone may provide optimal therapy,[65] this approach has not yet been approved by the U.S. Food and Drug Administration (FDA).

Autoimmune adrenocortical insufficiency (Addison disease) is the third major component of APS I and typically occurs after mucocutaneous candidiasis and hypoparathyroidism have been diagnosed. Over 85% of APS I patients will eventually develop adrenal insufficiency. Unfortunately, adrenal insufficiency is often initially missed clinically with the diagnosis commonly made late or at the time of a life-threatening adrenal crisis. In the Finnish cohort of APS I patients, 40% had Addison disease by 10 years and nearly 80% by 30 years.[52] Deficiencies of cortisol, aldosterone, and adrenal androgens may present simultaneously or may evolve over months to years. The initial symptoms of adrenal insufficiency are often nonspecific, mimicking psychiatric or gastrointestinal disease. These include fatigue, weight loss, myalgias, arthralgias, behavioral changes, nausea and vomiting, abdominal pain, and diarrhea. Over time, hyperpigmentation (due to elevated adrenocorticotropic hormone [ACTH]) in non–sun-exposed areas and postural hypotension can usually be found on careful examination. Unexplained hypotonic dehydration should raise the suspicion of Addison disease. Adrenal crises with hyponatremia, hyperkalemia, acidosis, and hypoglycemia may be fatal unless recognized and treated appropriately. As discussed in detail further, adrenal autoantibodies are used to predict adrenal cortical failure. If present, morning cortisol and renin measurements as well as ACTH stimulation testing may be used diagnostically in asymptomatic patients.

Autoimmune gonadal failure occurs in over 50% of women with APS I by age 20 years; less than 25% of males develop testicular insufficiency.[52] Gonadal failure often presents with primary amenorrhea in young women, though menstrual irregularities, polycystic ovaries, or infertility may be presenting features.[52,66] As with autoimmune adrenalitis, gonadal failure can be predicted by the presence of steroidal cell autoantibodies.[67]

Ectodermal dystrophy unrelated to hypoparathyroidism or mucocutaneous candidiasis has been extensively documented in the Finnish cohort. Dental enamel hypoplasia of permanent (but not deciduous) teeth as well as nail dystrophy is commonly found. There may be complete absence of the enamel or transverse hypoplastic bands alternating with zones of well-formed enamel. Dystrophy of nails is manifest by 0.5 to 1 mm pits. Nearly a third of the Finnish patients also had calcification of the tympanic membranes,[68] and 20% to 25% develop keratitis.[52]

As shown in Table 20-1, and in contrast to patients with APS II, type 1 diabetes and thyrogastric autoimmunity (a descriptive term for the combination of autoimmune thyroid disease and atrophic gastritis) are associated with APS I but occur far less frequently than in APS II. When present, thyroiditis is typically atrophic rather than goitrous. Gastric-parietal cell autoimmunity, which leads to atrophic gastritis with resultant achlorhydria and intrinsic factor deficiency, typically presents as iron deficiency anemia or vitamin B_{12}-deficient pernicious anemia. Whereas iron deficiency anemia is microcytic and vitamin B_{12}-deficient anemia is macrocytic, combined iron and vitamin B_{12} deficiency can be normocytic. It is also important to recognize that the spinal cord consequences of vitamin B_{12} deficiency can occur in the absence of anemia. Atrophic gastritis occurs in 15% to 30% of APS I cases with a mean age of onset of 16 years.[52,68]

Nonendocrine organ-specific diseases include alopecia, vitiligo, autoimmune hepatitis, and malabsorption. All types of alopecia may occur. Progression to alopecia totalis (total loss of scalp hair) or universalis (total loss of all body hair including eyelashes, eyebrows, and scalp hair), which are most common, usually occurs before puberty. Vitiligo presents initially as small, pale pigment-lacking skin patches. These may be missed unless specifically sought. Ultraviolet light examination of the skin may be necessary. The appearance of clay-colored stools, dark urine, and jaundice confirms the diagnosis of chronic active autoimmune hepatitis, unrelated to infectious hepatitis. Hepatitis occurs in 10% to 15% of APS I patients and is the leading cause of death. Consequently, all patients suspected of having APS I should have their liver function regularly monitored. Autoimmune hepatitis is typically treated with glucocorticoids until stabilized and with azathioprine if steroids can be successfully weaned.[51] Malabsorption, which may occur intermittently (and typically of fat), has been linked to hypoparathyroidism, bacterial and fungal overgrowth, gluten sensitivity (celiac disease), and IgA deficiency. There have also been rare reports of APS I with diabetes insipidus, growth hormone deficiency, ACTH deficiency, rheumatoid arthritis, Sjögren disease, tubulointerstitial nephritis, autoimmune bronchiolitis, and myopathy.[51,69,70]

APS II

APS II is the most common of the autoimmune polyendocrinopathies (excluding the coincidences of type 1 diabetes and autoimmune thyroid disease). Associated with HLA-DR3 and DR4 as well as HLA-DQA1 and DQB1, APS II does not have a single defining gene mutation like APS I. Unlike APS I, APS II usually has its onset in adulthood, particularly during the third or fourth decades, and is at least three times more common in females than males, whereas APS I is equally common in males and females. In 1926, Schmidt first described the association of adrenocortical and thyroid gland failure,

and in 1964, Carpenter extended this description to include insulin-dependent diabetes mellitus.[55,56] In 1957, the autoimmune nature of these diseases was suggested by Doniach and Roitt's discovery of thyroglobulin autoantibodies in patients with Hashimoto thyroiditis.[71] APS II was originally defined by the occurrence of adrenocortical insufficiency (Addison disease) with autoimmune thyroid disease or type 1 diabetes mellitus. This female bias in APS II is almost certainly related to the coexistence of autoimmune thyroid disease (AITD). Adrenocortical failure is the presentation in approximately 50% of APS II cases. The disease usually has its onset between ages 20 and 50 years, although it is not unusual to find cases before or after these ages.[58,72,73] Several of the disease components may be present at diagnosis. Type 1 diabetes coexists in nearly 50% of patients with Addison disease, whereas AITD coexists in about two thirds of patients with Addison disease. Thus, type 1 diabetes and AITD should be vigorously pursued in any patient presenting with Addison disease.

The most common component of the APS II to occur as an isolated condition is AITD. AITD affects nearly 4.5% of the U.S. population[74] with 80% to 90% of all cases occurring in females. AITD has an increased incidence during the teen years with a peak appearing in the fifth and sixth decades. Chronic lymphocytic thyroiditis (Hashimoto disease) is by far the most common form of AITD, although Graves disease may also occur. Postpartum thyroiditis is best considered to be a transient manifestation of autoimmune thyroiditis following childbirth. Manifestations of postpartum thyroiditis can vary from hypo to hyperthyroidism and discrete periods of hypo- and hyperthyroidism can be seen in individual affected women. Postpartum thyroiditis affects ~5% of all pregnancies with a frequency of ~10% in pregnancies of women with type 1 diabetes. Postpartum thyroiditis usually remits by 1 year following delivery. Several studies have reported on the coexistence of anti-islet immunity (3% to 8%) or even overt type 1 diabetes and AITD.[75,76] Just 1% of patients with otherwise isolated thyroiditis have serologic evidence of adrenal autoimmunity.

Although "polyglandular syndrome" involvement in patients with autoimmune thyroid disease is infrequent, thyroid autoimmunity or a family history of thyroiditis is common in patients with pernicious anemia, vitiligo, alopecia, myasthenia gravis, and Sjögren syndrome.[77-79] More patients with APS I than APS II have vitiligo, but because APS I is far less common, most patients with vitiligo who have another autoimmune disease have APS II. Approximately 20% to 40% of vitiligo patients have another component of APS II, with thyrogastric autoimmunity (concurrent autoimmune thyroid disease and lymphocytic gastritis) being the most common.[80,81] Up to 15% of patients with alopecia (areata, totalis, universalis) and 5% of their first-degree relatives have thyroid disease. Most patients with vitiligo are asymptomatic, and evidence of concurrent autoimmunity can only be ascertained by autoantibody screening. Segmental vitiligo with involvement of dermatomal regions is not associated with autoimmunity.[82]

Nearly 30% of patients with myasthenia gravis (autoimmune disease characterized by muscle weakness worsening during muscular contraction and associated with antiacetylcholine receptor autoantibodies) have AITD. Both Hashimoto thyroiditis and Graves disease may occur in patients with myasthenia.[83,84] Interestingly, patients with concomitant AITD tend to have milder expression of their myasthenia and a lower incidence of thymic disease and acetylcholine receptor α-chain autoantibodies. However, the incidence of ocular myasthenia is higher in patients with Graves disease.

Type 1 diabetes mellitus is a diagnostic component of APS II.[85] The worldwide incidence of type 1 diabetes continues to rise, particularly in children less than 5 years of age. The disease has a peak incidence during the teen years with a smaller but increasing incidence occurring in the preschool years.[86] Nevertheless, the disease may have its onset at any age. Approximately 10% to 15% of "type 2 diabetes" patients with disease onset after 40 years actually have slowly progressive autoimmune disease (latent autoimmune diabetes of adults [LADA]).[87] Unlike APS II, in which there is a female gender bias despite the occurrence of type 1 diabetes, no gender bias is present in patients with isolated type 1 diabetes. AITD (denoted by the presence of thyroperoxidase or thyroglobulin autoantibodies) occurs in 20% to 25% of patients with type 1 diabetes with women representing nearly two thirds of the autoantibody-positive patients.[72] Despite the high prevalence of thyroid autoantibodies, less than 20% of patients with thyroid autoantibodies have evidence of thyroid dysfunction defined as an elevated thyroid-stimulating hormone (TSH) concentration. Gastric parietal cell autoantibodies (PCA) are present in approximately 10% of females and 5% of males with type 1 diabetes.[88] Although pernicious anemia typically affects women after the fifth decade, children with PCA should be closely monitored for the development of pernicious anemia. Atrophic gastritis may lead to the development of a megaloblastic anemia due an inability to produce intrinsic factor with the consequent inability to absorb vitamin B_{12}. Iron deficiency anemia may occur in both adolescents and adults due to an impaired ability to absorb iron consequent to decreased acid production (achlorhydria).[89] Adrenocortical autoimmunity is much less frequent among patients with type 1 diabetes, with serologic evidence reported in 1.5% of cases.[90,91] Tissue transglutaminase or endomysial autoantibodies suggestive of celiac disease are present in 3% to 7% patients with type 1 diabetes.[92] Celiac disease should be suspected in patients with unexplained diarrhea, weight loss, failure to gain weight, or failure to thrive and should be confirmed by intestinal biopsy.[93,94]

Approximately 10% of women less than 40 years of age with APS II develop ovarian failure. Ovarian failure may present as either primary or secondary amenorrhea. In females with biopsy-proven lymphocytic oophoritis, adrenocortical failure or subclinical adrenal autoimmunity is often present.[95] In contrast, progression to gonadal failure is very rare among males with Addison disease.

Pituitary involvement is occasionally seen in APS II.[96,97] Hypophysitis and empty sella syndrome have been described, usually leading to isolated failure of secretion of growth hormone (GH), ACTH, TSH, follicle-stimulating hormone (FSH), or luteinizing hormone (LH).

Several nonendocrinologic conditions have also been reported in association with APS II. These include ulcerative colitis,[98] primary biliary cirrhosis,[99] sarcoidosis,[100,101] achalasia,[102] myositis,[103] and neuropathy.[104]

*I*mmune Dysregulation, *P*olyendocrinopathy, *E*nteropathy, and *X*-linked Inheritance (IPEX)

Neonatal onset type 1 diabetes, dermatitis, enteropathy, thyroiditis, hemolytic anemia, and thrombocytopenia are all part of the rare IPEX constellation. Enhanced understanding of the regulatory T-cell function and the role *FOXP3* has led to its characterization. Patients with IPEX have an absence of FoxP3 expression.[105-108] To date, only long-term immunosuppression or bone marrow transplantation, with the goal of enhancing regulatory T-cell function, have been effective therapies for IPEX. Sustained expression of FoxP3 may reprogram effector T cells to act as regulatory T cells.[109,110] Approaches aimed at enhancing the function of regulatory T cells may potentially be applied to treating all autoimmune diseases.

DIAGNOSTIC APPROACH AND FOLLOW-UP

The approach to diagnosing polyglandular syndromes is threefold (1): autoantibody screening to (i) verify the autoimmune nature of the suspected endocrinopathy and (ii) test for the involvement of other organs and tissues; (2) full assessment of endocrine function in patients with confirmed autoantibodies and autoantibody-negative subjects in whom disease may be suspected clinically; (3) mutation analysis to both confirm the diagnosis and screen siblings and other relatives for their potential carrier status.

Recognition of multiorgan autoimmune diseases prior to their symptomatic phases is vital in minimize associated morbidity and mortality. A thorough history and physical examination should always be performed and a high index of suspicion should be maintained. In addition, family history of multiorgan autoimmune disease should increase the suspicion for a potential APS.

As discussed later, perhaps the greatest single achievement in the early diagnosis of APS I patients since the early 2000s has been the discovery of the association of autoantibodies to type 1 interferons and APS I.[111] *Although not indicative of a tissue-specific immune attack, autoantibodies to interferon (α and ω)* provide a nearly 100% sensitive and specific screening test for APS I.[112-114] The presence of autoantibodies to interferons should be followed by confirmatory testing for AIRE mutations as well as testing for tissue specific autoantibodies (Figure 20-5). These include 21-hydroxylase or adrenal cortex cytoplasmic

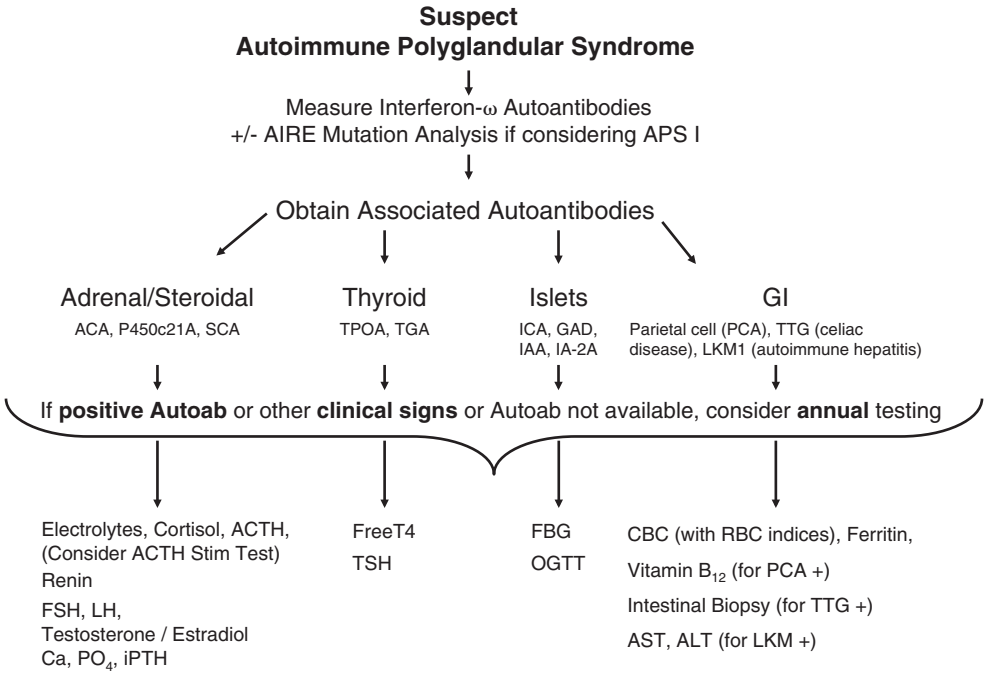


FIGURE 20-5 ■ Antibody and end-organ testing in patients with suspected APS. This flow diagram shows which autoantibodies should be obtained when APS is suspected due to clinical signs and symptoms. Given its sensitivity and specificity, primary screening with interferon-ω antibodies is likely adequate if APS is suspected. That said, we recommend autoantibody testing in subjects with a suspected APS-related disease (adrenalitis, chronic mucocutaneous candidiasis, or autoimmune hypoparathyroidism) at the time of initial diagnosis. We also recommend autoantibody testing in subjects with two or more associated autoimmune diseases (i.e., T1D and autoimmune thyroiditis). Unfortunately, data are lacking to provide firm recommendations regarding the need for repeat autoantibody testing in patients who are initially autoantibody negative. However, if antibodies are negative, we recommend repeat autoantibody testing in early puberty if clinical signs or symptoms of APS persist. For those who are antibody positive, annual testing of end-organ function should be obtained as shown. When autoantibody assays are unavailable, clinicians must use frequent testing of disease-specific end points to identify associated comorbidities.

autoantibodies (for autoimmune Addison disease), GADA, IA-2A, IAA , and more recently ZnT8 autoantibodies (for type 1 diabetes), thyroperoxidase and thyroglobulin autoantibodies (for autoimmune thyroid disease), steroidal cell autoantibodies (for ovarian failure), and transglutaminase or endomysial autoantibodies (for celiac disease). In addition, although not readily available, autoantibodies to 17-hydroxylase, side-chain cleavage enzyme and 3-hydroxysteroid dehydrogenase can be used to detect gonadal and adrenal autoimmunity. Autoantibodies to NALP5 (NLR family, pyrin domain containing 5; NLR = NACHT, leucine rich; NACHT = nucleotide oligomerization domain-like receptors) and the calcium-sensing receptor (putative parathyroid autoantigens) document risk for autoimmune hypoparathyroidism,[115,116] and antibodies to human pituitary enolase may provide evidence of autoimmune hypophysitis.[117] Notably, measurement of certain autoantibodies may not be available to all clinicians and require individualized approaches to screening and follow-up. In addition, although autoantibodies have yet to be identified, data suggest a number of cytokines (IL-17A, IL-17F, and IL-22) may act as autoantigens associated with mucocutaneous candidiasis.[118] Although there is a clear link between the presence of organ-specific autoantibodies and either the presence of preexisting disease or subsequent progression to disease, the number of associated disorders that will develop and their age of appearance are unpredictable. Consequently, long-term follow-up is necessary in both autoantibody-positive and negative subjects.

All patients with a single autoimmune disease must be considered at risk for other autoimmune diseases. Whether and when to screen for other autoantibodies is based on the likelihood of finding another autoimmune disease, cost effectiveness, and the likelihood that screening will prevent morbidity and mortality from other diseases (e.g., diabetic ketoacidosis, Addisonian crisis, or hypocalcemia with seizures) in the future.

Because of the high incidence of AITD in patients with type 1 diabetes, we recommend that such patients have thyroperoxidase and thyroglobulin autoantibodies measured biannually. We prefer this approach over the assessment of thyrotropin (TSH) levels, because autoantibody seroconversion is a much earlier event in the evolution of thyroid disease and their presence would warrant more frequent monitoring (every 6 to 12 months). Measuring both thyroid autoantibodies has close to 90% sensitivity for the detection of AITD. That said, some clinicians prefer to follow at-risk patients by means of TSH given that treatment is typically not initiated until the TSH becomes elevated.

Screening for all components of APS is not recommended in patients with isolated autoimmune thyroid disease. However, several reports have demonstrated an increased incidence of parietal cell autoantibodies in such patients.[89,119] Thus, screening for parietal cell autoantibodies in children with AITD should be considered. In hypothyroid patients with confirmed APS, evidence for adrenal autoimmunity must be sought before starting thyroid hormone replacement therapy because thyroid hormone replacement can precipitate an adrenal crisis in patients with marginal adrenocortical function (by increasing metabolism and catabolism of steroid hormones).

Delayed diagnoses and even preventable deaths still occur in patients with undiagnosed adrenocortical failure. As mentioned previously, the presentation is often vague and nonspecific until an Addisonian crisis ensues. In patients with type 1 diabetes, unexplained hypoglycemia or other unexplained improvement in blood glucose control might be a clue to the diagnosis of Addison disease. Improved sensitivity to injected insulin and improved glycemia may represent the loss of counterregulatory (anti-insulin) activity associated with glucocorticoid deficiency.

All patients with prolonged or unexplained chronic mucocutaneous candidiasis or hypoparathyroidism should be evaluated for APS I. Screening for APS should also be considered in females with premature ovarian failure.

Assessment of end organ function in any patient with positive autoantibodies is recommended annually (see Figure 20-5). Fasting or 2-hour postprandial blood glucose testing, calcium, phosphate, and PTH and TSH levels can effectively assess pancreatic islet, parathyroid, and thyroid function in asymptomatic individuals. Due to its limited sensitivity, we do not recommend the use of HbA1c as a screening tool for T1D. Elevated FSH and LH levels with concomitant low sex steroids confirm gonadal failure. Serial assessments of hemoglobin, hematocrit, and red blood cell indices can assess progression to atrophic gastritis in patients with gastric autoimmunity. The findings of a megaloblastic anemia with an elevated mean corpuscular volume (MCV) suggest vitamin B_{12} deficiency, whereas a microcytic hypochromic anemia suggests iron deficiency. Vitamin B_{12} levels should be followed in all patients with parietal cell autoantibodies as neuropathy can develop without anemia. Before starting therapy, both vitaminB_{12} level and an iron profile should be obtained. Methylmalonic acid levels are not routinely needed in patients with gastric autoimmunity but may be helpful if vitamin B_{12} levels are borderline low.[120] Liver function tests and antimitochondrial autoantibodies should be obtained in patients with APS I. Patients with tissue transglutaminase or endomysial autoantibodies are frequently asymptomatic and should be referred to a gastroenterologist to discuss the need for an intestinal biopsy and potential confirmation of celiac disease.

Low early morning cortisol levels, electrolyte abnormalities (hyponatremia/hyperkalemia), and hypoglycemia represent late changes occurring at or just before the onset of adrenal insufficiency. Just as the natural history of pre–type 1 diabetes is well characterized (see Chapter 19), a similar pattern is seen prior to the development of adrenocortical insufficiency (Figure 20-6). Four stages have been described subsequent to the detection of adrenal autoantibodies: stage 1: increased plasma renin activity with normal to low aldosterone; stage 2: decreased cortisol response after

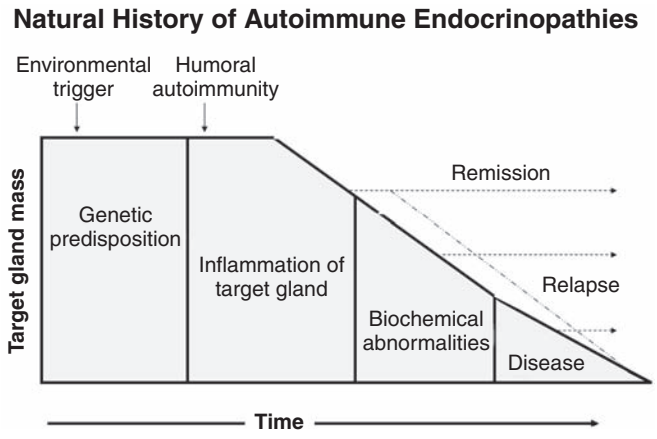


FIGURE 20-6 ■ Proposed model of the natural history of an organ-specific autoimmune disease. In genetically susceptible individuals, the onset of autoimmunity (identified by the presence of serum autoantibodies) is thought to be triggered by environmental agents. These organ-specific autoantibodies can be identified months to years before the disease becomes clinically manifest, allowing for the prediction and early diagnosis of the disease before the clinical manifestations or metabolic abnormalities can usually be detected.

parenteral ACTH administration; stage 3: elevated basal ACTH; and stage 4: low basal cortisol (Table 20-3).[121,122] In individuals with adrenocortical autoantibodies, screening with a measurement of the morning cortisol concentration should be done. If normal, mid-afternoon ACTH and supine renin levels should be measured. Complete assessment of adrenocortical function should be carried out in those with ACTH levels > 55 pg/mL or in those with elevated renin concentrations.

TREATMENT

Hormone replacement or other therapies for the component diseases of APS I and APS II are similar whether the ailments occur in isolation or in association with other conditions. Specific therapies are described in the individual chapters.

GENETICS OF APS I

APS I is inherited in an autosomal recessive fashion.[123] Unlike APS II, specific HLA alleles are not associated with APS I.[124] APS I has been mapped to chromosome 21q22.1 and the AIRE gene subsequently cloned.[14] Patients with APS I are homozygous (possess two mutated copies) for the AIRE gene with their parents being heterozygous for the mutated gene. AIRE is expressed in the thymus, lymph nodes, and fetal liver as well as in pancreas, adrenal cortex, and testes. The gene spans 11.9 kb, contains 14 exons, and the protein is 545 amino acids.[15]

Initially, five AIRE mutations were described: one nonsense mutation and four frameshift mutations.[14,124] Now, more than 70 mutations in the AIRE gene have been reported; mutations are present in over 98% of patients with APS I.[53,125-127] Multiple shared and unique mutations have been defined in Sardinians, Britons, Italians, Finns, Japanese, and North Americans.[54,128,129] Sequence analysis demonstrates that the AIRE transcribed protein displays features common to many transcription factors.[3,130] Further understanding of the AIRE protein's function may provide fundamental insights into the nature of autoimmunity.

GENETICS OF APS II

Whereas APS I displays an autosomal recessive Mendelian pattern of inheritance, APS II is not inherited as a single gene mutation. APS II is much more typical of other autoimmune endocrinopathies where cases can occur either sporadically or within families with APS II.

Overall, like patients with type 1 diabetes, patients with APS II have similar HLA associations, especially having increased frequencies of HLA-DR3 (DQB1*0201), DR4 (DQB1*0302), and DQA1*0301.[85,131] This is not surprising, as type 1 diabetes, a frequent component of APS II, is strongly HLA associated. However, when patients with type 1 diabetes are removed from the APS II subject group, the association between HLA-DR alleles and APS II disappears. Thus HLA-DR, in the absence of type 1 diabetes, is not a major locus for the development of APS II. Previous studies have shown that Addison disease and type 1 diabetes not only share the major risk DR3/DR4 genotype but also that Addison disease is particularly associated with DRB1*0404 and DRB1*0301 haplotypes.[132] It is estimated that 1 in 20 patients with type 1 diabetes with this haplotype will have adrenal autoantibodies.[133,134] Another high-risk allele for Addison disease is DRB5 (DQB1*0301). Graves disease is more

TABLE 20-3 **Stages in the Development of Autoimmune Addison Disease**

Stage	Renin	Aldosterone	Basal Cortisol	Cortisol Post ACTH	Basal ACTH
1	Elevated	N or Low	N	N	N
2	Elevated	N or Low	N	Low	N
3	Elevated	N or Low	N	Low	Elevated
4	Elevated	Low	Low*	Low	Elevated

*Clinical Addison disease.
ACTH, adrenocorticotropic hormone; N, normal.

often associated with HLA-DR3 as opposed to Hashimoto thyroiditis, which is associated with HLA-DR4 or DR5.[135,136] Autoimmune hypoparathyroidism and mucocutaneous candidiasis do not display associations with specific HLA alleles.

Because the association of APS II with specific HLA alleles is only modest, other genes must be important in providing susceptibility. To identify non-HLA genes that influence susceptibility to APS II, genome-wide scan or linkage studies are necessary. The G allele of the CTLA-4 exon 1 A/G diallelic polymorphism has been associated with Addison disease in APS II and less strongly associated with isolated Addison disease.[137]

AUTOANTIBODIES IN AUTOIMMUNE POLYGLANDULAR SYNDROMES

Autoantibodies are antibodies, predominantly IgG, that bind to self (auto)antigens. Autoantibodies may be pathogenic as observed in Graves disease or myasthenia gravis. In Graves disease, agonistic autoantibodies directed against the thyroid follicular cell TSH receptor stimulate overproduction of thyroid hormone causing hyperthyroidism.[138] In myasthenia gravis, autoantibodies directed against the motor end plate acetylcholine receptor located on myocytes stimulate internalization of the acetylcholine receptor producing muscular weakness. Usually, however, autoantibodies serve solely as serologic markers of autoimmunity such as in type 1 diabetes where islet cell autoantibodies (ICA), insulin autoantibodies (IAA), glutamic acid decarboxylase autoantibodies (GADA), insulinoma associated autoantibodies (IA-2A), and autoantibodies to the zinc transporter 8 (ZnT8A) are indicators of ongoing autoimmunity.[139,140] Interestingly, in patients with APS I, GADA are not as predictive of the subsequent development of type 1 diabetes.[51]

Detection of autoantibodies in the autoimmune polyglandular syndromes serves several important functions. First, detection of autoantibodies allows for a specific autoimmune diagnosis to be established.[141] Second, autoantibody detection in asymptomatic individuals indicates an increased risk for the later development of clinical disease.[90] Third, the presence of an autoantibody or one autoimmune disease in an individual may suggest an increased risk for other associated autoimmune diseases.

Non–Organ-Specific Autoantibodies

Anti-Interferon Autoantibodies (AIA)

Autoantibodies to interferons (α and ω) provide a highly sensitive screening test for APS I. First used clinically as indicators of risk for myasthenia gravis and thymoma, high-titer AIA were unexpectedly found in 60/60 Finnish APS I patients and 16/16 Norwegian APS I patients.[111] Notably, AIA were typically detected (using stored serum samples) in known APS I patients prior to the onset of organ specific autoantibodies. In fact, several APS I patients developed AIA prior to the onset of mucocutaneous candidiasis. As further evidence of the specificity of AIA, subjects with AIRE mutations but lacking classic features of APS I (mucocutaneous candidiasis, hypoparathyroidism, or adrenal insufficiency) have also demonstrated high titers to AIA. As such, AIA are likely to become the primary screening test for APS I. The specificity of AIA for APS I suggests that interferon-γ (an interferon without detectable autoantibodies in APS I patients) could provide effective immunotherapy in APS I patients.[111] Finally, AIA can be detected by a competitive immunoassay.[114]

Autoantibodies to IL-17A, IL-17F, and IL-22

Mucocutaneous candidiasis is often the presenting clinical abnormality in APS I. Although anti-interferon antibodies can predict APS I, they do not predict the order or severity of the known APS I components. Disease or organ specific autoantibodies are therefore needed to provide patients and physicians with optimal anticipatory guidance. Improvements in our understanding of host defense to Candida led several groups to hypothesize that autoantibodies to specific cytokines, IL-17A, IL-17F, and IL-22, may both predict and explain the mucocutaneous candidiasis associated with APS I. In one study, 33/33 patients with APS I and mucocutanous candidiasis demonstrated high titer autoantibodies to IL-17A, IL-17F, and IL-22 (versus 0/37 controls), and 0/103 patients with isolated autoimmune diseases were positive.[118] These observations have subsequently been confirmed in other cohorts and indeed suggest that autoantibodies to IL-17A, IL-17F, and IL-22 may be a useful tool in predicting mucocutaneous candidiasis.[142,143] Currently these autoantibodies are only used in the research setting. Recall that under normal circumstances IL-17 and IL-22 are involved in the activities of Th17 cells that enhance local cellular innate immunity.

Organ-Specific Autoantibodies

Adrenal Cytoplasmic Autoantibodies (ACA)

Adrenal cytoplasmic autoantibodies (ACA) were first detected using a complement-fixation technique with saline extracts of adrenal tissue and soon afterward by indirect immunofluorescence.[144] Usually all layers of the adrenal cortex (but not the medulla) fluoresce.[121] The microsomal localization of autoantigens has been confirmed using ultracentrifuged cellular components.[145] Other assays for adrenal autoantibodies include solid-phase radioimmunoassays and nonradioactive enzyme-linked immunoabsorbent assays.[122,146]

Up to 75% to 80% of subjects with new-onset Addison disease exhibit ACA.[121] Approximately 50% of asymptomatic ACA-positive individuals develop Addison disease in less than 3 years. In a follow-up of 20 ACA-positive children followed for up to 11 years, the cumulative risk for developing Addison disease was 100%.[121] ACA are also predictive of the subsequent development of Addison disease in adults, although less frequently than in children. Higher titers of ACA and complement fixing ACA have been associated with an increased risk of displaying clinical disease.[147]

Autoantibodies to the surface of adrenal cortical cells have been described but are not detected routinely because of the difficulty in obtaining fresh human or animal adrenal tissue that can be used for such assays. However, almost 90% of individuals with Addison disease were reported to exhibit such autoantibodies.[148]

ACA are detected in all forms of autoimmune Addison disease, be it isolated Addison disease or as part of APS I or APS II. As stated previously, subjects with other forms of organ-specific autoimmune disease exhibit increased frequencies of ACA, which are highly predictive of the subsequent development of adrenal insufficiency.

Adrenal Enzyme Autoantibodies

Typical of many organ-specific autoimmune diseases, major autoantigens that serve as targets of autoantibodies in APS are enzymes. Such enzymes are expressed in the tissues that are being targeted for humoral or cell-mediated autoimmune attack. Examples of various autoimmune diseases where enzymes are targeted are illustrated in Table 20-2. A discussion of adrenal hormone synthesis can be found in Chapter 13.

21-hydroxylase (P450c21) is a major autoantigen recognized by sera from patients with Addison disease.[149] There is a strong correlation between positivity for ACA and P450c21 autoantibodies, and P450c21 autoantibodies appear to be an even more sensitive indicator of disease.[150] Other enzymes have been identified as autoantigens in patients with either isolated autoimmune Addison disease or APS including P450 cholesterol side chain cleavage enzyme (P450scc), 17α-hydroxylase (P450c17), and 3β-hydroxysteroid dehydrogenase (which is not a P450 enzyme).[150]

Adrenal Enzyme Autoantibodies in APS I

Although autoantibodies to P450c21 (21-hydroxylase), P450ssc (side-chain cleavage enzyme), and P450c17 (17-hydroxylase) have been reported, autoantibodies to P450c21 are most commonly identified in patients with adrenal autoimmunity. Nearly 75% of APS I and APS II patients have P450c21 autoantibodies present.[121]

The autoantigenic epitopes of the P450c21 enzyme are located in the C-terminal end and in a central region of the enzyme.[151] It has been reported that two of four epitopes recognized by P450c17 autoantibodies cross-reacted with P450c21, indicating that reactivity to one of these autoantigens could actually reflect molecular mimicry between such epitopes.[152] Except for the N-terminal amino acids 1-40 and the C-terminal amino acids 456-521, immunoreactive epitopes have been described throughout P450c21.[153]

Higher titers of both ACA and P450c21 autoantibodies appear to correlate with both greater impairment of adrenocortical function and the predicted development of Addison disease.[121] As with many autoimmune diseases, an inverse correlation exists between autoantibody titer and duration of disease in patients with Addison disease.[154] This is consistent with the concept that once an autoantigen is completely destroyed, the immune system is no longer stimulated to produce autoantibodies.

Adrenal Enzyme Autoantibodies in APS II

When clinical or preclinical Addison disease is present, there is no unique combination of adrenal or gonadal antibodies that separate APS I from APS II.[141,155,156] The differentiation of APS I from APS II is made either on clinical grounds or, in asymptomatic patients, by detecting concomitant autoantibodies associated with APS II. Furthermore, there are no unique epitopes recognized by P450c21 autoantibodies that allow differentiation of isolated Addison disease versus APS I or APS II.[157] However, APS I can be distinguished from APS II via molecular diagnostic tests for mutation in the AIRE gene.

Steroidal Cell/Gonadal Autoantibodies (SCA)

Some individuals with ACA have sera that cross-react with reproductive-steroid-producing tissues including the theca interna of the graafian follicle, Leydig cells of the testis, or syncytiotrophoblastic layer of the placenta.[134,158] Sera that recognize antigens in both adrenal and reproductive-steroid–producing tissues whose immunoreactivity cannot be absorbed with adrenal extracts are termed SCA. Such variability in immunoreactivity likely represents differences in autoantigen density or epitope availability differences among the tissues.

In asymptomatic patients, SCA are associated with an increased risk of developing primary autoimmune gonadal failure. In women, this usually manifests as either primary amenorrhea or premature menopause. Men are usually asymptomatic. When ACA are present in the absence of SCA, gonadal failure is rare.[134] Overall, approximately one third of individuals with ACA have SCA (using ovary, testis, or placenta as the antigen source). SCA are more common in APS I than in APS II patients. It is estimated that 60% of subjects with APS I and Addison disease express SCA, compared to 30% of patients with APS II.

Premature ovarian failure independent of APS may can also occur as a consequence of autoimmunity.[159] However, Addison disease coexists in approximately 2% to 10% of women with autoimmune-mediated premature ovarian failure.[160] Like other organ-specific autoimmune diseases, autoimmune ovarian failure is characterized histologically by ovarian infiltration with inflammatory cells.[161] Many patients with either isolated premature ovarian failure or premature ovarian failure associated with APS express SCA.[162] Five of 5 women with SCA, all of whom also had ACA consistent with an autoimmune polyglandular syndrome, manifested ovarian failure.[163]

SCA, depending on the assay used, have been reported in 4% to 87% of women with premature ovarian failure.[158,164,165] SCA are also predictive of later gonadal failure in APS women with normal menses at the time of initial study. It has been reported that 100% (11 of 11) of APS I patients who were positive for SCA developed primary ovarian failure during a follow-up period of up to 12 years.[163] Autoantibodies reacting strongly to the pig zona pellucida as determined by indirect immunofluorescence were observed in 6 of 22 women with infertility.[166]

The nature of the ovarian autoantigens in premature ovarian failure is controversial. Because the adrenal cortex and gonad share several synthetic pathways common to cells producing both adrenocortical steroids and sex steroids, humoral autoimmunity to shared autoantigens might be expected in patients with both Addison disease and gonadal failure. The synthesis of sex and adrenal steroids requires P450scc, 3β-hydroxysteroid dehydrogenase, and P450c17. Data are, however, contradictory. In one study, SCA activity was removed by preabsorption with recombinant human 3β-hydroxysteroid dehydrogenase, suggesting that this enzyme was a major autoantigen detected by SCA-positive sera.[167] Another study suggested that SCA correlated best with reactivity to P450scc and a 51-kilodalton autoantigen that binds to the aromatic L-amino acid decarboxylase present in granulosa cells, placenta, liver, and pancreatic beta cells.[168]

Autoantibodies in Hypoparathyroidism

Autoimmune hypoparathyroidism is a characteristic disorder essentially unique to APS I. Hypoparathyroidism is absent in subjects with APS II. In the original report of parathyroid autoantibodies detected using indirect immunofluorescence, nearly 40% of patients with autoimmune hypoparathyroidism were found to have parathyroid cytoplasmic autoantibodies versus 6% of controls.[169,170] However, other laboratories did not confirm the initial reports of the existence of such parathyroid cytoplasmic autoantibodies.[171,172] It was shown that autoantibodies detected by indirect immunofluorescence directed against the parathyroid gland could be preabsorbed with human mitochondria, indicating that such autoantibodies were not tissue specific.[172]

Several different antiparathyroid autoantibodies have been reported in patients with hypoparathyroidism. These include autoantibodies that may bind to cultured bovine endothelial cells.[173,174] Unrelated to APS I or APS II, autoantibodies that bind to anti-PTH antibodies (employed in a PTH immunoassay—i.e., anti-idiotypic PTH autoantibodies) have been described.[175] Autoantibodies to the extracellular domain of the calcium receptor have also been described.[116,176] In contrast, autoantibodies that block the calcium receptor have been described that cause hyperparathyroidism (e.g., "autoimmune hypercalcemia").[177] More recently, screening of human parathyroid complementary DNA libraries with sera from patients with known APS I and hypoparathyroidism identified NACHT leucine-rich repeat protein 5 (NALP5) as an important parathyroid autoantigen. Autoantibodies were detected in 49% of patients with APS I and hypoparathyroidism but were absent in all 293 controls.[115] Notably, NALP5 autoantibodies appear to be specific for hypoparathyroidism related to APS I, as they are rarely (0.69%) seen is patients with idiopathic hypoparathyroidism.[178]

Other Autoantibodies in APS I and II

In addition to the autoantibodies discussed in detail earlier, autoantibodies to tyrosine hydroxylase have been reported in ~40% of patients with APS I and correlate with alopecia areata.[179] Antibodies to liver/kidney microsome type 1 (LKM1) are found in nearly 100% of patients with type 2 autoimmune hepatitis.[180] Forty percent of such patients have associated autoimmune disease commonly seen in APS I. The hepatic autoantigens P450IA2 and P4502A6 have been reported as targets of these antibodies. Autoantibodies to aromatic L-amino acid decarboxylase have also been recognized in APS I and isolated cases of Addison disease.[168] Finally, pteridine-dependent hydroxylases have also been proposed as autoantigens in APS I.[181]

SUMMARY

The autoimmune polyglandular syndromes result from a loss of tolerance to self-antigens. A thorough understanding of tolerance induction and defects in tolerance is required to fully understand the immunopathogenesis of APS. APS I, an autosomal recessive disorder mapped to the AIRE gene, is defined by the presence of at least two of the following three findings: (1) adrenocortical autoimmunity, (2) hypoparathyroidism, or (3) mucocutaneous candidiasis. APS II is defined by the coexistence of autoimmune adrenocortical insufficiency or serologic evidence of adrenalitis with autoimmune thyroiditis or type 1 diabetes mellitus. The occurrence of any one component disease of an APS may be linked to the occurrence of others through shared autoimmunity background genes that lead to a loss of tolerance. As such, a high index of suspicion should be maintained whenever one autoimmune disorder is diagnosed. For example, in type 1 diabetes, it is routine to screen for thyroid autoimmunity and celiac disease.

Treatment of APS should be aimed at optimal management of the specific underlying diseases. Screening for the presence of associated autoimmune disorders should be performed regularly. An improved understanding of the interaction among susceptibility genes, environmental triggers, and the development of impaired immune tolerance should prove to be the best path to improved diagnostic and therapeutic modalities in the care of patients with APS.

REFERENCES

1. Ballotti, S., Chiarelli, F., & de Martino, M. (2006). Autoimmunity: basic mechanisms and implications in endocrine diseases. Part I. *Horm Res*, *66*, 132–141.
2. Ballotti, S., Chiarelli, F., & de Martino, M. (2006). Autoimmunity: basic mechanisms and implications in endocrine diseases. Part II. *Horm Res*, *66*, 142–152.
3. Anderson, M. S., Venanzi, E. S., Klein, L., et al. (2002). Projection of an immunological self shadow within the thymus by the Aire protein. *Science*, *298*, 1395–1401.
4. Mathis, D., & Benoist, C. (2007). A decade of AIRE. *Nat Rev Immunol*, *7*, 645–650.
5. Cho, J. H., & Gregersen, P. K. (2011). Genomics and the multifactorial nature of human autoimmune disease. *N Engl J Med*, *365*, 1612–1623.
6. Delves, P. J., & Roitt, I. M. (2000). The immune system. First of two parts. *N Engl J Med*, *343*, 37–49.
7. Delves, P. J., & Roitt, I. M. (2000). The immune system. Second of two parts. *N Engl J Med*, *343*, 108–117.

8. Unanue, E. R. (2002). Perspective on antigen processing and presentation. *Immunol Rev*, *185*, 86–102.
9. Cresswell, P, Ackerman, A. L., Giodini, A, et al. (2005). Mechanisms of MHC class I-restricted antigen processing and cross-presentation. *Immunol Rev*, *207*, 145–157.
10. Holling, T. M., Schooten, E., & van Den Elsen, P. J. (2004). Function and regulation of MHC class II molecules in T-lymphocytes: of mice and men. *Human Immunology*, *65*, 282–290.
11. Neefjes, J., Jongsma, M. L., Paul, P., & Bakke, O. (2011). Towards a systems understanding of MHC class I and MHC class II antigen presentation. *Nat Rev Immunol*, *11*, 823–836.
12. Ruan, Q. G., Tung, K., Eisenman, D., et al. (2007). The autoimmune regulator directly controls the expression of genes critical for thymic epithelial function. *J Immunol*, *178*, 7173–7180.
13. Anderson, M. S., Venanzi, E. S., Chen, Z., et al. (2005). The cellular mechanism of Aire control of T cell tolerance. *Immunity*, *23*, 227–239.
14. Nagamine, K., Peterson, P., Scott, H. S., et al. (1997). Positional cloning of the APECED gene. *Nat Genet*, *17*, 393–398.
15. An autoimmune disease, APECED, caused by mutations in a novel gene featuring two PHD-type zinc-finger domains. (1997). The Finnish-German APECED Consortium. Autoimmune Polyendocrinopathy-Candidiasis-Ectodermal Dystrophy. *Nat Genet*, *17*, 399–403.
16. Edelmann, S. L., Marconi, P., & Brocker, T. (2011). Peripheral T cells re-enter the thymus and interfere with central tolerance induction. *J Immunol*, *186*, 5612–5619.
17. von Boehmer, H., Aifantis, I., Gounari, F., et al. (2003). Thymic selection revisited: how essential is it? *Immunol Rev*, *191*, 62–78.
18. von Boehmer, H., & Kisielow, P. (1990). Self-nonself discrimination by T cells. *Science*, *248*, 1369–1373.
19. Ramsdell, F., & Fowlkes, B. J. (1990). Clonal deletion versus clonal anergy: the role of the thymus in inducing self tolerance. *Science*, *248*, 1342–1348.
20. Mathis, D., & Benoist, C. (2004). Back to central tolerance. *Immunity*, *20*, 509–516.
21. Abbas, A. K., Murphy, K. M., & Sher, A. (1996). Functional diversity of helper T lymphocytes. *Nature*, *383*, 787–793.
22. O'Shea, J. J., & Paul, W. E. (2010). Mechanisms underlying lineage commitment and plasticity of helper CD4+ T cells. *Science*, *327*, 1098–1102.
23. Lan, R. Y., Ansari, A. A., Lian, Z. X., & Gershwin, M. E. (2005). Regulatory T cells: development, function and role in autoimmunity. *Autoimmun Rev*, *4*, 351–363.
24. Ziegler, S. F. (2006). FOXP3: of mice and men. *Annu Rev Immunol*, *24*, 209–226.
25. Kyewski, B., & Klein, L. (2006). A central role for central tolerance. *Annu Rev Immunol*, *24*, 571–606.
26. Fuchizawa, T., Adachi, Y., Ito, Y., et al. (2006). Defective regulatory and effector T cell functions in patients with FOXP3 mutations. *J Clin Invest*, *116*, 1713–1722.
28. Le Bras, S., & Geha, R. S. (2006). IPEX and the role of Foxp3 in the development and function of human Tregs. *J Clin Invest*, *116*, 1473–1475.
29. Meffre, E. (2012). The establishment of early B cell tolerance in humans: lessons from primary immunodeficiency diseases. *Ann NY Acad Sci*, *1246*, 1–10.
30. Kyewski, B., & Derbinski, J. (2004). Self-representation in the thymus: an extended view. *Nat Rev Immunol*, *4*, 688–698.
31. Chiovato, L., Latrofa, F., Braverman, L. E., et al. (2003). Disappearance of humoral thyroid autoimmunity after complete removal of thyroid antigens. *Ann Intern Med*, *139*, 346–351.
32. Pisetsky, D. S. (2006). Fulfilling Koch's postulates of autoimmunity: anti-NR2 antibodies in mice and men. *Arthritis Rheum*, *54*, 2349–2352.
33. Goodnow, C. C., Sprent, J., de St Groth, B. F., & Vinuesa, C. G. (2005). Cellular and genetic mechanisms of self tolerance and autoimmunity. *Nature*, *435*, 590–597.
34. Viret, C., Sant'Angelo, D. B., He, X., et al. (2001). A role for accessibility to self-peptide-self-MHC complexes in intrathymic negative selection. *J Immunol*, *166*, 4429–4437.
35. Vafiadis, P., Ounissi-Benkalha, H., Palumbo, M., et al. (2001). Class III alleles of the variable number of tandem repeat insulin polymorphism associated with silencing of thymic insulin predispose to type 1 diabetes 10.1210/jc.86.8.3705. *J Clin Endocrinol Metab*, *86*, 3705–3710.
36. Cohen, I. R. (2001). Antigenic mimicry, clonal selection and autoimmunity. *J Autoimmun*, *16*, 337–340.
37. Damico, F. M., Kiss, S., & Young, L. H. (2005). Sympathetic ophthalmia. *Semin Ophthalmol*, *20*, 191–197.
38. Chang, G. C., & Young, L. H. (2011). Sympathetic ophthalmia. *Semin Ophthalmol*, *26*, 316–320.
39. Zhang, M., Alicot, E. M., Chiu, I., et al. (2006). Identification of the target self-antigens in reperfusion injury. *J Exp Med*, *203*, 141–152.
40. Cusick, M. F., Libbey, J. E., & Fujinami, R. S. (2012). Molecular mimicry as a mechanism of autoimmune disease. *Clin Rev Allergy Immunol*, *42*, 102–111.
41. Soulas, P., Woods, A., Jaulhac, B., et al. (2005). Autoantigen, innate immunity, and T cells cooperate to break B cell tolerance during bacterial infection. *J Clin Invest*, *115*, 2257–2267.
42. Olson, J. K., Croxford, J. L., & Miller, S. D. (2001). Virus-induced autoimmunity: potential role of viruses in initiation, perpetuation, and progression of T-cell-mediated autoimmune disease. *Viral Immunol*, *14*, 227–250.
43. Ziegler, A-. G., Schmid, S., Huber, D., et al. (2003). Early infant feeding and risk of developing type 1 diabetes-associated autoantibodies. *JAMA*, *290*, 1721–1728.
44. McKay, D. M. (2001). Bacterial superantigens: provocateurs of gut dysfunction and inflammation? *Trends Immunol*, *22*, 497–501.
45. Michels, A. W., & Gottlieb, P. A. (2010). Autoimmune polyglandular syndromes. *Nat Rev Endocrinol*, *6*, 270–277.
46. Owen, C. J., & Cheetham, T. D. (2009). Diagnosis and management of polyendocrinopathy syndromes. *Endocrinol Metab Clin North Am*, *38*, 419–436.
47. Tait, K. F., & Gough, S. C. L. (2003). The genetics of autoimmune endocrine disease. *Clin Endocrinol*, *59*, 1–11.
48. Miyazaki, Y., Hirayama, M., Watanabe, H., et al. (2012). Paraneoplastic encephalitis associated with myasthenia gravis and malignant thymoma. *J Clin Neurosci*, *19*, 336–338.
49. Naoura, I., Didelot, A., Walker, F., et al. (2011). Anti-N-methyl-D-aspartate receptor encephalitis complicating ovarian teratomas: a case report. *Am J Obstet Gynecol*, *205*, e6–e8.
50. Rosin, L., DeCamilli, P., Butler, M., et al. (1998). Stiff-man syndrome in a woman with breast cancer: an uncommon central nervous system paraneoplastic syndrome. *Neurology*, *50*, 94–98.
51. Husebye, E. S., Perheentupa, J., Rautemaa, R., & Kampe, O. (2009). Clinical manifestations and management of patients with autoimmune polyendocrine syndrome type I. *J Intern Med*, *265*, 514–529.
52. Perheentupa, J. (2006). Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. *J Clin Endocrinol Metab*, *91*, 2843–2850.
53. Buzi, F., Badolato, R., Mazza, C., et al. (2003). Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy syndrome: time to review diagnostic criteria? *J Clin Endocrinol Metab*, *88*, 3146–3148.
54. Peterson, P., & Peltonen, L. (2005). Autoimmune polyendocrinopathy syndrome type 1 (APS1) and AIRE gene: New views on molecular basis of autoimmunity. *J Autoimmun*, *25*, 49–55.
55. Schmidt, M. (1926). Eine biglandulare Erkrankung (Nebennieren und Schilddrusse) bei Morbus Addisonii. *Verh Dtsch Ges Pathol Ges*, *21*, 212–221 (in German).
56. Carpenter, C. C., Solomon, N., Silverberg, S. G., et al. (1964). Schmidt's syndrome (thyroid and adrenal insufficiency): a review of the literature and a report of fifteen new cases including ten instances of coexistent diabetes mellitus. *Medicine* (Baltimore), *43*, 153–180.
57. Eisenbarth, G. S., & Gottlieb, P. A. (2004). Autoimmune polyendocrine syndromes. *N Engl J Med*, *350*, 2068–2079.
58. Betterle, C., Dal Pra, C., Mantero, F., & Zanchetta, R. (2002). Autoimmune adrenal insufficiency and autoimmune polyendocrine syndromes: autoantibodies, autoantigens, and their applicability in diagnosis and disease prediction. *Endocr Rev*, *23*, 327–364.
59. Amerio, P., Tracanna, M., De Remigis, P., et al.(2006). Vitiligo associated with other autoimmune diseases: polyglandular autoimmune syndrome types 3B+C and 4. *Clin Exp Dermatol*, *31*, 746–749.
60. Kumar, P. G., Laloraya, M., Wang, C. Y., et al. (2001). The autoimmune regulator (AIRE) is a DNA-binding protein. *J Biol Chem*, *276*, 41357–41364.
61. Wolff, A. S., Erichsen, M. M., Meager, A., et al. (2007). Autoimmune polyendocrine syndrome type 1 in Norway: phenotypic

variation, autoantibodies, and novel mutations in the autoimmune regulator gene. *J Clin Endocrinol Metab*, *92*, 595–603.
62. Rautemaa, R., Hietanen, J., Niissalo, S., et al. (2007). Oral and oesophageal squamous cell carcinoma—a complication or component of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED, APS-I). *Oral Oncol*, *43*, 607–613.
63. Siikala, E., Richardson, M., Pfaller, M. A., et al. (2009). Candida albicans isolates from APECED patients show decreased susceptibility to miconazole. *Int J Antimicrob Agents*, *34*, 607–609.
64. Rautemaa, R., Richardson, M., Pfaller, M., et al. (2008). Reduction of fluconazole susceptibility of Candida albicans in APECED patients due to long-term use of ketoconazole and miconazole. *Scand J Infect Dis*, *40*, 904–907.
65. Winer, K. K., Sinaii, N., Peterson, D., et al. (2008). Effects of once versus twice-daily parathyroid hormone 1–34 therapy in children with hypoparathyroidism. *J Clin Endocrinol Metab*, *93*, 3389–3395.
66. Maclaren, N., Chen, Q-. Y., Kukreja, A., et al. (2001). Autoimmune hypogonadism as part of an autoimmune polyglandular syndrome. *J Soc Gynecol Investig*, *8*, S52–S54.
67. Soderbergh, A., Myhre, A. G., Ekwall, O., et al. (2004). Prevalence and clinical associations of 10 defined autoantibodies in autoimmune polyendocrine syndrome type I. *J Clin Endocrinol Metab*, *89*, 557–562.
68. Ahonen, P., Myllarniemi, S., Sipila, I., & Perheentupa, J. (1990). Clinical variation of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) in a series of 68 patients. *N Engl J Med*, *322*, 1829–1836.
69. Gazulla Abio, J., Benavente Aguilar, I., Ricoy Campo, J. R., & Madero Barrajon, P. (2005). [Myopathy with trabecular fibers associated with familiar autoimmune polyglandular syndrome type 1]. *Neurologia*, *20*, 702–708.
70. De Luca, F., Valenzise, M., Alaggio, R., et al. (2008). Sicilian family with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) and lethal lung disease in one of the affected brothers. *Eur J Pediatr*, *167*, 1283–1288.
71. Doniach, D., & Roitt, I. M. (1957). Auto-immunity in Hashimoto's disease and its implications. *J Clin Endocrinol Metab*, *17*, 1293–1304.
72. Betterle, C., Lazzarotto, F., & Presotto, F. (2004). Autoimmune polyglandular syndrome type 2: the tip of an iceberg? *Clin Exp Immunol*, *137*, 225–233.
73. Hugle, B., Dollmann, R., Keller, E., & Kiess, W. (2004). Addison's crisis in adolescent patients with previously diagnosed diabetes mellitus as manifestation of polyglandular autoimmune syndrome type II—report of two patients. *J Pediatr Endocrinol Metab*, *17*, 93–97.
74. Hollowell, J. G., Staehling, N. W., Flanders, W. D., et al. (2002). Serum TSH, T4, and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). *J Clin Endocrinol Metab*, *87*, 489–499.
75. Jaeger, C., Hatziagelaki, E., Petzoldt, R., & Bretzel, R. G. (2001). Comparative analysis of organ-specific autoantibodies and celiac disease—associated antibodies in type 1 diabetic patients, their first-degree relatives, and healthy control subjects. *Diabetes Care*, *24*, 27–32.
76. Kordonouri, O., Klinghammer, A., Lang, E. B., et al. (2002). Thyroid autoimmunity in children and adolescents with type 1 diabetes: a multicenter survey. *Diabetes Care*, *25*, 1346–1350.
77. Alkhateeb, A., Fain, P. R., Thody, A., et al. (2003). Epidemiology of vitiligo and associated autoimmune diseases in caucasian probands and their families pigment cell research. *Pigment Cell Research*, *16*, 208–214.
78. Ruggeri, R. M., Galletti, M., Mandolfino, M. G., et al. (2002). Thyroid hormone autoantibodies in primary Sjogren syndrome and rheumatoid arthritis are more prevalent than in autoimmune thyroid disease, becoming progressively more frequent in these diseases. *J Endocrinol Invest*, *25*, 447–454.
79. Tsao, C. Y., Mendell, J. R., Lo, W. D., et al. (2000). Myasthenia gravis and associated autoimmune diseases in children. *J Child Neurol*, *15*, 767–769.
80. Zetting, G., Tanew, A., Fischer, G., et al. (2003). Autoimmune diseases in vitiligo: do anti-nuclear antibodies decrease thyroid volume? *Clin Exp Immunol*, *131*, 347–354.
81. Kakourou, T., Kanaka-Gantenbein, C., Papadopoulou, A., et al. (2005). Increased prevalence of chronic autoimmune (Hashimoto's) thyroiditis in children and adolescents with vitiligo. *J Am Acad Dermatol*, *53*, 220–223.
82. Hann, S. K., & Lee, H. J. (1996). Segmental vitiligo: clinical findings in 208 patients. *J Am Acad Dermatol*, *35*, 671–674.
83. Tanwani, L. K., Lohano, V., Ewart, R., et al. (2001). Myasthenia gravis in conjunction with Graves' disease: a diagnostic challenge. *Endocr Pract*, *7*, 275–278.
84. Weetman, A. P. (2005). Non-thyroid autoantibodies in autoimmune thyroid disease. *Best Pract Res Clin Endocrinol Metab*, *19*, 17–32.
85. Weinstock, C., Matheis, N., Barkia, S., et al. (2011). Autoimmune polyglandular syndrome type 2 shows the same HLA class II pattern as type 1 diabetes. *Tissue Antigens*, *77*, 317–324.
86. Karvonen, M., Viik-Kajander, M., Moltchanova, E., et al. (2000). Incidence of childhood type 1 diabetes worldwide. Diabetes Mondiale (DiaMond) Project Group. *Diabetes Care*, *23*, 1516–1526.
87. Stenstrom, G., Gottsater, A., Bakhtadze, E., et al. (2005). Latent autoimmune diabetes in adults: definition, prevalence, {beta}-cell function, and treatment. *Diabetes*, *54*, S68–S72.
88. Alonso, N., Granada, M. L., Salinas, I., et al. (2005). An early marker of pernicious anemia in patients with type 1 diabetes 10.1210/jc.2005-0580. *J Clin Endocrinol Metab*, *90*, 5254–5258.
89. Segni, M., Borrelli, O., Pucarelli, I., et al. (2004). Early manifestations of gastric autoimmunity in patients with juvenile autoimmune thyroid diseases. *J Clin Endocrinol Metab*, *89*, 4944–4948.
90. Barker, J. M. (2006). Type 1 diabetes-associated autoimmunity: natural history, genetic associations, and screening. *J Clin Endocrinol Metab*, *91*, 1210–1217.
91. Barker, J. M., Ide, A., Hostetler, C., et al. (2005). Endocrine and immunogenetic testing in individuals with type 1 diabetes and 21-hydroxylase autoantibodies: Addison's disease in a high-risk population. *J Clin Endocrinol Metab*, *90*, 128–134.
92. Hansson, T., Dahlbom, I., Rogberg, S., et al. (2002). Recombinant human tissue transglutaminase for diagnosis and follow-up of childhood coeliac disease. *Pediatr Res*, *51*, 700–705.
93. Smith, C. M., Clarke, C. F., Porteous, L. E., et al. (2000). Prevalence of coeliac disease and longitudinal follow-up of antigliadin antibody status in children and adolescents with type 1 diabetes mellitus. *Pediatr Diabetes*, *1*, 199–203.
94. Not, T., Tommasini, A., Tonini, G., et al. (2001). Undiagnosed coeliac disease and risk of autoimmune disorders in subjects with type I diabetes mellitus. *Diabetologia*, *44*, 151–155.
95. Welt, C. K., Falorni, A., Taylor, A. E., et al. (2005). Selective theca cell dysfunction in autoimmune oophoritis results in multifollicular development, decreased estradiol, and elevated inhibin B levels. *J Clin Endocrinol Metab*, *90*, 3069–3076.
96. Cemeroglu, A. P., Bober, E., Dundar, B., & Buyukgebiz, A. (2001). Autoimmune polyglandular endocrinopathy and anterior hypophysitis in a 14 year-old girl presenting with delayed puberty. *J Pediatr Endocrinol Metab*, *14*, 909–914.
97. Zung, A., Andrews-Murray, G., Winqvist, O., & Chalew, S. A. (1997). Growth hormone deficiency in autoimmune polyglandular syndrome. *J Pediatr Endocrinol Metab*, *10*, 69–72.
98. Govindarajan, R., & Galpin, O. P. (1992). Coexistence of Addison's disease, ulcerative colitis, hypothyroidism and pernicious anemia. *J Clin Gastroenterol*, *15*, 82–83.
99. Ko, G. T., Szeto, C. C., Yeung, V. T., et al. (1996). Autoimmune polyglandular syndrome and primary biliary cirrhosis. *Br J Clin Pract*, *50*, 344–346.
100. Papadopoulos, K. I., Hornblad, Y., Liljebladh, H., & Hallengren, B. (1996). High frequency of endocrine autoimmunity in patients with sarcoidosis. *Eur J Endocrinol*, *134*, 331–336.
101. Watson, J. P., & Lewis, R. A. (1996). Schmidt's syndrome associated with sarcoidosis. *Postgrad Med J*, *72*, 435–436.
102. Fritzen, R., Bornstein, S. R., & Scherbaum, W. A. (1996). Megaoesophagus in a patient with autoimmune polyglandular syndrome type II. *Clin Endocrinol* (Oxf), *45*, 493–498.
103. Heuss, D., Engelhardt, A., Gobel, H., & Neundorfer, B. (1995). Myopathological findings in interstitial myositis in type II polyendocrine autoimmune syndrome (Schmidt's syndrome). *Neurol Res*, *17*, 233–237.
104. Watkins, P. J., Gayle, C., Alsanjari, N., et al. (1995). Severe sensory-autonomic neuropathy and endocrinopathy in insulin-dependent diabetes. *Qjm*, *88*, 795–804.
105. Wildin, R. S., & Freitas, A. (2005). IPEX and FOXP3: Clinical and research perspectives. *J Autoimmun*, *25*, 56–62.
106. Bennett, C. L., Christie, J., Ramsdell, F., et al. (2001). The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. *Nat Genet*, *27*, 20–21.

107. Wildin, R. S., Ramsdell, F., Peake, J., et al. (2001). X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. *Nat Genet*, *27*, 18–20.
108. Zavattari, P., Deidda, E., Pitzalis, M., et al. (2004). No association between variation of the FOXP3 gene and common type 1 diabetes in the Sardinian population. *Diabetes*, *53*, 1911–1914.
109. Hori, S., Nomura, T., & Sakaguchi, S. (2003). Control of regulatory T cell development by the transcription factor Foxp3. *Science*, *299*, 1057–1061.
110. Hori, S., Takahashi, T., & Sakaguchi, S. (2003). Control of autoimmunity by naturally arising regulatory CD4+ T cells. *Adv Immunol*, *81*, 331–371.
111. Meager, A., Visvalingam, K., Peterson, P., et al. (2006). Anti-interferon autoantibodies in autoimmune polyendocrinopathy syndrome type 1. *PLoS Med*, *3*, e289.
112. Meloni, A., Furcas, M., Cetani, F., et al. (2008). Autoantibodies against type I interferons as an additional diagnostic criterion for autoimmune polyendocrine syndrome type I. *J Clin Endocrinol Metab*, *93*, 4389–4397.
113. Oftedal, B. E., Wolff, A. S., Bratland, E., et al. (2008). Radioimmunoassay for autoantibodies against interferon omega; its use in the diagnosis of autoimmune polyendocrine syndrome type I. *Clin Immunol*, *129*, 163–169.
114. Zhang, L., Barker, J. M., Babu, S., et al. (2007). A robust immunoassay for anti-interferon autoantibodies that is highly specific for patients with autoimmune polyglandular syndrome type 1. *Clin Immunol*, *125*, 131–137.
115. Alimohammadi, M., Bjorklund, P., Hallgren, A., et al. (2008). Autoimmune polyendocrine syndrome type 1 and NALP5, a parathyroid autoantigen. *N Engl J Med*, *358*, 1018–1028.
116. Gavalas, N. G., Kemp, E. H., Krohn, K. J., et al. (2007). The calcium-sensing receptor is a target of autoantibodies in patients with autoimmune polyendocrine syndrome type 1. *J Clin Endocrinol Metab*, *92*, 2107–2114.
117. O'Dwyer, D. T., McElduff, P., Peterson, P., et al. (2007). Pituitary autoantibodies in autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED). *Acta Biomed*, *78*(Suppl. 1), 248–254.
118. Puel, A., Doffinger, R., Natividad, A., et al. (2010). Autoantibodies against IL-17A, IL-17F, and IL-22 in patients with chronic mucocutaneous candidiasis and autoimmune polyendocrine syndrome type I. *J Exp Med*, *207*, 291–297.
119. Bright, G. M., Blizzard, R. M., Kaiser, D. L., & Clarke, W. L. (1982). Organ-specific autoantibodies in children with common endocrine diseases. *J Pediatr*, *100*, 8–14.
120. Elin, R. J., & Winter, W. E. (2001). Methylmalonic acid: a test whose time has come? *Arch Pathol Lab Med*, *125*, 824–827.
121. Coco, G., Dal Pra, C., Presotto, F., et al. (2006). Estimated risk for developing autoimmune Addison's disease in patients with adrenal cortex autoantibodies. *J Clin Endocrinol Metab*, *91*, 1637–1645.
122. Betterle, C., Coco, G., & Zanchetta, R. (2005). Adrenal cortex autoantibodies in subjects with normal adrenal function. *Best Pract Res Clin Endocrinol Metab*, *19*, 85–99.
123. Ahonen, P. (1985). Autoimmune polyendocrinopathy-candidosis-ectodermal dystrophy (APECED): autosomal recessive inheritance. *Clin Genet*, *27*, 535–542.
124. Aaltonen, J., Bjorses, P., Sandkuijl, L., et al. (1994). An autosomal locus causing autoimmune disease: autoimmune polyglandular disease type I assigned to chromosome 21. *Nat Genet*, *8*, 83–87.
125. Trebusak Podkrajsek, K., Bratanic, N., Krzisnik, C., & Battelino, T. (2005). Autoimmune regulator-1 messenger ribonucleic acid analysis in a novel intronic mutation and two additional novel AIRE gene mutations in a cohort of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy patients. *J Clin Endocrinol Metab*, *90*, 4930–4935.
126. Meloni, A., Fiorillo, E., Corda, D., et al. (2005). Two novel mutations of the AIRE protein affecting its homodimerization properties. *Hum Mutat*, *25*, 319.
127. Ulinski, T., Perrin, L., Morris, M., et al. (2006). Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy syndrome with renal failure: impact of posttransplant immunosuppression on disease activity. *J Clin Endocrinol Metab*, *91*, 192–195.
128. Cervato, S., Mariniello, B., Lazzarotto, F., et al. (2009). Evaluation of the autoimmune regulator (AIRE) gene mutations in a cohort of Italian patients with autoimmune-polyendocrinopathy-candidiasis-ectodermal-dystrophy (APECED) and in their relatives. *Clin Endocrinol* (Oxf), *70*, 421–428.
129. F Magitta, N., Pura, M., S Bøe Wolff, A., et al. (2008). Autoimmune polyendocrine syndrome type I in Slovakia: relevance of screening patients with autoimmune Addison's disease. *Eur J Endocrinol*, *158*, 705–709.
130. Zuklys, S., Balciunaite, G., Agarwal, A., et al. (2000). Normal thymic architecture and negative selection are associated with Aire Expression, the gene defective in the autoimmune-polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED). *J Immunol*, *165*, 1976–1983.
131. Wallaschofski, H., Meyer, A., Tuschy, U., & Lohmann, T. (2003). HLA-DQA1*0301-associated susceptibility for autoimmune polyglandular syndrome type II and III. *Horm Metab Res*, *35*, 120–124.
132. Robles, D. T., Fain, P. R., Gottlieb, P. A., & Eisenbarth, G. S. (2002). The genetics of autoimmune polyendocrine syndrome type II. *Endocrinol Metab Clin North Am*, *31*, 353–368, vi-vii.
133. Brewer, K. W., Parziale, V. S., & Eisenbarth, G. S. (1997). Screening patients with insulin-dependent diabetes mellitus for adrenal insufficiency. *N Engl J Med*, *337*, 202.
134. Myhre, A. G., Undlien, D. E., Lovas, K., et al. (2002). Autoimmune adrenocortical failure in norway autoantibodies and human leukocyte antigen class ii associations related to clinical features. *J Clin Endocrinol Metab*, *87*, 618–623.
135. Hunt, P. J., Marshall, S. E., Weetman, A. P., et al. (2001). Histocompatibility leucocyte antigens and closely linked immunomodulatory genes in autoimmune thyroid disease. *Clinical Endocrinology*, *55*, 491–499.
136. Levin, L., Ban, Y., Concepcion, E., et al. (2004). Analysis of HLA genes in families with autoimmune diabetes and thyroiditis. *Hum Immunol*, *65*, 640–647.
137. Vaidya, B., Imrie, H., Geatch, D. R., et al. (2000). Association analysis of the cytotoxic T lymphocyte antigen-4 (CTLA-4) and autoimmune regulator-1 (AIRE-1) genes in sporadic autoimmune Addison's disease. *J Clin Endocrinol Metab*, *85*, 688–691.
138. Schott, M., Minich, W. B., Willenberg, H. S., et al. (2005). Relevance of TSH receptor stimulating and blocking autoantibody measurement for the prediction of relapse in Graves disease. *Horm Metab Res*, *37*, 741–744.
139. Winter, W. E., Harris, N., & Schatz, D. (2002). Immunological markers in the diagnosis and prediction of autoimmune type 1a diabetes. *Clin Diabetes*, *20*, 183–191.
140. Winter, W. E., Harris, N., & Schatz, D. (2002). Type 1 diabetes islet autoantibody markers. *Diabetes Technol Ther*, *4*, 817–839.
141. Soderbergh, A., Myhre, A. G., Ekwall, O., et al. (2004). Prevalence and clinical associations of 10 defined autoantibodies in autoimmune polyendocrine syndrome type I. *J Clin Endocrinol Metab*, *89*, 557–562.
142. Ng, W. F., von Delwig, A., Carmichael, A. J., et al. (2010). Impaired T(H)17 responses in patients with chronic mucocutaneous candidiasis with and without autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. *J Allergy Clin Immunol*, *126*, 1006–1015, e1001–e1004.
143. Browne, S. K., & Holland, S. M. (2010). Immunodeficiency secondary to anticytokine autoantibodies. *Curr Opin Allergy Clin Immunol*, *10*, 534–541.
144. Blizzard, R. M., & Kyle, M. (1963). Studies of the adrenal antigens and antibodies in Addison's disease. *J Clin Invest*, *42*, 1653–1660.
145. Drexhage, H. A., Bottazzo, G. F., Bitensky, L., et al. (1981). Thyroid growth-blocking antibodies in primary myxoedema. *Nature*, *289*, 594–596.
146. Betterle, C., Volpato, M., Pedini, B., et al. (1999). Adrenal-cortex autoantibodies and steroid-producing cells autoantibodies in patients with Addison's disease: comparison of immunofluorescence and immunoprecipitation assays. *J Clin Endocrinol Metab*, *84*, 618–622.
147. Betterle, C., Volpato, M., Smith, B. R., et al. (1997). I. Adrenal cortex and steroid 21-hydroxylase autoantibodies in adult patients with organ-specific autoimmune diseases: markers of low progression to clinical Addison's disease. *J Clin Endocrinol Metab*, *82*, 932–938.

148. Khoury, E. L., Hammond, L., Bottazzo, G. F., & Doniach, D. (1981). Surface-reactive antibodies to human adrenal cells in Addison's disease. *Clin Exp Immunol*, *45*, 48–55.
149. Husebye, E. S., Bratland, E., Bredholt, G., et al. (2006). The substrate-binding domain of 21-hydroxylase, the main autoantigen in autoimmune Addison's disease, is an immunodominant T cell epitope. *Endocrinology*, *147*, 2411–2416.
150. de Carmo Silva, R., Kater, C., Dib, S. A., et al. (2000). Autoantibodies against recombinant human steroidogenic enzymes 21-hydroxylase, side-chain cleavage and 17alpha-hydroxylase in Addison's disease and autoimmune polyendocrine syndrome type III. *Eur J Endocrinol*, *142*, 187–194.
151. Nikoshkov, A., Falorni, A., Lajic, S., et al. (1999). A conformation-dependent epitope in Addison's disease and other endocrinological autoimmune diseases maps to a carboxyl-terminal functional domain of human steroid 21-hydroxylase. *J Immunol*, *162*, 2422–2426.
152. Peterson, P., & Krohn, K. J. (1994). Mapping of B cell epitopes on steroid 17 alpha-hydroxylase, an autoantigen in autoimmune polyglandular syndrome type I. *Clin Exp Immunol*, *98*, 104–109.
153. Liiv, I., Teesalu, K., Peterson, P., et al. (2002). Epitope mapping of cytochrome P450 cholesterol side-chain cleavage enzyme by sera from patients with autoimmune polyglandular syndrome type 1. *Eur J Endocrinol*, *146*, 113–119.
154. Ross, I., Boulle, A., Soule, S., et al. (2010). Autoimmunity predominates in a large South African cohort with Addison's disease of mainly European descent despite long-standing disease and is associated with HLA DQB*0201. *Clin Endocrinol* (Oxf), *73*, 291–298.
155. Falorni, A., Laureti, S., & Santeusanio, F. (2002). Autoantibodies in autoimmune polyendocrine syndrome type II. *Endocrinol Metab Clin North Am*, *31*, 369–389, vii.
156. Betterle, C., Lazzarotto, F., & Presotto, F. (2004). Autoimmune polyglandular syndrome type 2: the tip of an iceberg? *Clin Exp Immunol*, *137*, 225–233.
157. Nikoshkov, A., Falorni, A., Lajic, S., et al. (1999). A conformation-dependent epitope in Addison's disease and other endocrinological autoimmune diseases maps to a carboxyl-terminal functional domain of human steroid 21-hydroxylase. *J Immunol*, *162*, 2422–2426.
158. Dal Pra, C., Chen, S., Furmaniak, J., et al. (2003). Autoantibodies to steroidogenic enzymes in patients with premature ovarian failure with and without Addison's disease. *Eur J Endocrinol*, *148*, 565–570.
159. Goswami, D., & Conway, G. S. (2005). Premature ovarian failure. *Hum Reprod Update*, *11*, 391–410.
160. Bakalov, V. K., Vanderhoof, V. H., Bondy, C. A., & Nelson, L. M. (2002). Adrenal antibodies detect asymptomatic autoimmune adrenal insufficiency in young women with spontaneous premature ovarian failure. *Hum Reprod*, *17*, 2096–2100.
161. Santoro, N. (2001). Research on the mechanisms of premature ovarian failure. *J Soc Gynecol Investig*, *8*, S10–S12.
162. Irvine, W. J., Chan, M. M., Scarth, L., et al. (1968). Immunological aspects of premature ovarian failure associated with idiopathic Addison's disease. *Lancet*, *2*, 883–887.
163. Ahonen, P., Miettinen, A., & Perheentupa, J. (1987). Adrenal and steroidal cell antibodies in patients with autoimmune polyglandular disease type I and risk of adrenocortical and ovarian failure. *J Clin Endocrinol Metab*, *64*, 494–500.
164. Falorni, A., Laureti, S., Candeloro, P., et al. (2002). Steroid-cell autoantibodies are preferentially expressed in women with premature ovarian failure who have adrenal autoimmunity. *Fertil Steril*, *78*, 270–279.
165. Reimand, K., Peterson, P., Hyoty, H., et al. (2000). 3{beta}-Hydroxysteroid dehydrogenase autoantibodies are rare in premature ovarian failure. *J Clin Endocrinol Metab*, *85*, 2324–2326.
166. Shivers, C. A., & Dunbar, B. S. (1977). Autoantibodies to zona pellucida: a possible cause for infertility in women. *Science*, *197*, 1082–1084.
167. Uibo, R., Aavik, E., Peterson, P., et al. (1994). Autoantibodies to cytochrome P450 enzymes P450scc, P450c17, and P450c21 in autoimmune polyglandular disease types I and II and in isolated Addison's disease. *J Clin Endocrinol Metab*, *78*, 323–328.
168. Soderbergh, A., Rorsman, F., Halonen, M., et al. (2000). Autoantibodies against aromatic L-amino acid decarboxylase identifies a subgroup of patients with Addison's disease. *J Clin Endocrinol Metab*, *85*, 460–463.
169. Blizzard, R. M., Chee, D., & Davis, W. (1966). The incidence of parathyroid and other antibodies in the sera of patients with idiopathic hypoparathyroidism. *Clin Exp Immunol*, *1*, 119–128.
170. Irvine, W. J., & Scarth, L. (1969). Antibody to the oxyphil cells of the human parathyroid in idiopathic hypoparathyroidism. *Clin Exp Immunol*, *4*, 505–510.
171. Chapman, C. K., Bradwell, A. R., & Dykks, P. W. (1986). Do parathyroid and adrenal autoantibodies coexist? *J Clin Pathol*, *39*, 813–814.
172. Betterle, C., Caretto, A., Zeviani, M., et al. (1985). Demonstration and characterization of anti-human mitochondria autoantibodies in idiopathic hypoparathyroidism and in other conditions. *Clin Exp Immunol*, *62*, 353–360.
173. Brandi, M. L., Aurbach, G. D., Fattorossi, A., et al. (1986). Antibodies cytotoxic to bovine parathyroid cells in autoimmune hypoparathyroidism. *Proc Natl Acad Sci USA*, *83*, 8366–8369.
174. Fattorossi, A., Aurbach, G. D., Sakaguchi, K., et al. (1988). Anti-endothelial cell antibodies: detection and characterization in sera from patients with autoimmune hypoparathyroidism. *Proc Natl Acad Sci USA*, *85*, 4015–4019.
175. McElduff, A., Lackmann, M., & Wilkinson, M. (1992). Anti-idiotypic PTH antibodies as a cause of elevated immunoreactive parathyroid hormone in idiopathic hypoparathyroidism, a second case: another manifestation of autoimmune endocrine disease? *Calcif Tissue Int*, *51*, 121–126.
176. Kifor, O., McElduff, A., LeBoff, M. S., et al. (2004). Activating antibodies to the calcium-sensing receptor in two patients with autoimmune hypoparathyroidism. *J Clin Endocrinol Metab*, *89*, 548–556.
177. Kifor, O., Moore, F. D., Jr., Delaney, M., et al. (2003). A syndrome of hypocalciuric hypercalcemia caused by autoantibodies directed at the calcium-sensing receptor. *J Clin Endocrinol Metab*, *88*, 60–72.
178. Tomar, N., Kaushal, E., Das, M., et al. (2012). Prevalence and significance of NALP5 autoantibodies in patients with idiopathic hypoparathyroidism. *J Clin Endocrinol Metab*, *97*, 1219–1226.
179. Hedstrand, H., Ekwall, O., Haavik, J., et al. (2000). Identification of tyrosine hydroxylase as an autoantigen in autoimmune polyendocrine syndrome type I. *Biochem Biophys Res Commun*, *267*, 456–461.
180. Diamantis, I., & Boumpas, D. T. (2004). Autoimmune hepatitis: evolving concepts. *Autoimmun Rev*, *3*, 207–214.
181. Ekwall, O., Hedstrand, H., Haavik, J., et al. (2000). Pteridin-dependent hydroxylases as autoantigens in autoimmune polyendocrine syndrome type I. *J Clin Endocrinol Metab*, *85*, 2944–2950.

QUESTIONS

1. Mutations in which one of the following genes causes autoimmune polyglandular syndrome type 1?
   a. Menin
   b. Ret proto-oncogene
   c. Autoimmune regulator
   d. Hepatic nuclear factor-1
   e. Nuclear factor kappa B

Answer: c

2. Genetic predisposition to autoimmune polyglandular syndrome type 2 (especially when type 1 diabetes is present) is most strongly associated with which one of the following loci?
   a. MHC class I genes
   b. MHC class II genes
   c. MHC class III genes
   d. CTLA-4
   e. Insulin gene

Answer: b

3. Which one of the following autoantibodies is the strongest predictor of the development of pernicious anemia?
   a. Autoantibodies to thyroperoxidase
   b. Autoantibodies to the acetylcholine receptor alpha chain
   c. Autoantibodies to voltage-gated Ca^{2+} channel
   d. Autoantibodies to extrinsic factor
   e. Autoantibodies to the Na^+/H^+ ATPase pump

Answer: e

4. Which one of the following autoantibodies is the strongest predictor of the development of Hashimoto thyroiditis?
   a. Autoantibodies to thyroperoxidase
   b. Autoantibodies to thyroglobulin
   c. Autoantibodies to the Na^+/I^- symporter
   d. Autoantibodies to intrinsic factor
   e. Autoantibodies to the TSH receptor

Answer: a

5. Which one of the following autoantibodies is most specific for Graves disease?
   a. Autoantibodies to thyroperoxidase
   b. Autoantibodies to thyroglobulin
   c. Autoantibodies to the Na^+/I^- symporter
   d. Autoantibodies to intrinsic factor
   e. Autoantibodies to the TSH receptor

Answer: e

6. What is the major target autoantigen in autoimmune Addison disease?
   a. Galactose-1-phosphate uridylyltransferase
   b. 21-hydroxylase
   c. Glutamic acid decarboxylase
   d. Phenylalanine hydroxylase
   e. 11 beta-hydroxylase

Answer: b

7. Which one of the following problems occurs in autoimmune polyglandular syndrome type 1 but not in autoimmune polyglandular syndrome type 2?
   a. Ectodermal dysplasia
   b. Pernicious anemia
   c. Vitiligo
   d. Addison disease
   e. Autoimmune thyroid disease

Answer: a

8. A child presents with a hypocalcemia seizure and extensive candidal infection of the nails. What is the most likely diagnosis?
   a. Addison disease
   b. Autoimmune thyroid disease
   c. Autoimmune polyglandular syndrome type 1
   d. Autoimmune polyglandular syndrome type 2
   e. Multiple endocrine neoplasia syndrome type 2B

Answer: c

9. What is the risk of a sibling of the above child being similarly affected?
   a. $< 1\%$
   b. $\sim 5\%$
   c. $\sim 25\%$
   d. $\sim 50\%$
   e. $\sim 100\%$

Answer: c

10. Which one of the following findings is the earliest biochemical evidence of Addison disease?
    a. Low AM cortisol
    b. Low cortisol response to cosyntropin
    c. Elevated basal ACTH
    d. Elevated renin

Answer: d

CHAPTER 21

Hypoglycemia in the Toddler and Child

David R. Langdon, MD • Charles A. Stanley, MD • Mark A. Sperling, MD

CHAPTER OUTLINE

INTRODUCTION

Hypoglycemia is less common than hyperglycemia after early infancy, but it can be caused by a greater variety of problems and conditions and can be especially harmful to a developing brain. As the growing infant goes longer between feedings, a variety of endocrine and metabolic adaptations maintain a steady fuel supply. Depending on inherent severity, congenital defects of these systems can first manifest hypoglycemia later in infancy, and acquired defects can produce hypoglycemia in older children and adults. However, throughout life the many forms of hyperinsulinism are among the most important and dangerous causes. This chapter describes an approach to diagnosis based on identifying which system of endocrine/metabolic adaptation is responsible for the failure to maintain normal fuel homeostasis. Key diagnostic information is often best derived from specimens obtained immediately before reversing hypoglycemia (the so-called critical samples).

Different definitions of hypoglycemia serve different purposes,[1,2] and the effects of specific plasma glucose levels may vary among patients, especially those with previous hypo- and hyperglycemia. As there is no exact correspondence between risk of harm and either severity of symptoms or specific plasma glucose level, hypoglycemia should be treated as an emergency. Defensive hormonal and autonomic responses are triggered at glucose levels between 55 mg/dL and 68 mg/dL,[3-6] and major neuroglycopenic manifestations can occur below 50 mg/dL.[7,8] Thus a plasma glucose level of 55 serves as a reasonable threshold for diagnostic investigation and therapeutic reversal. A plasma glucose concentration between 70 and 100 mg/dL should be the target range for acute and ongoing management of hypoglycemia.

PHYSIOLOGIC DEVELOPMENT OF GLUCOSE METABOLISM DURING INFANCY AND CHILDHOOD

Glucose Utilization and Production

As a meal is digested, glucose is in ample supply, but during postabsorptive fasting, glucose production must match glucose utilization rates that are markedly higher per kilogram of body weight in infants than in adults, due to their larger brain size relative to body weight.[9] Bier and coworkers showed by stable isotope measurements of glucose turnover rates that the brains of infants and children

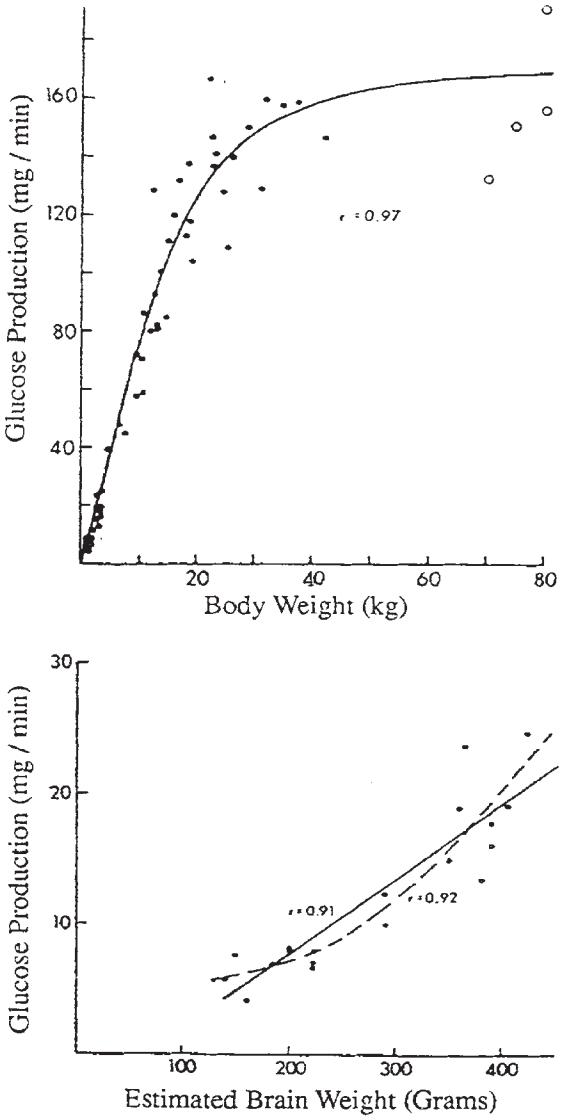


FIGURE 21-1 ■ Glucose production as a function of body weight (top) and estimated brain weight (bottom). Note the change in slope at approximately 40 kg of body weight when brain growth is complete. (From Bier, D. M., Leake, R. D., Haymond, M. W., et al. (1977). Measurement of "true" glucose production rates in infancy and childhood, with 6,6-dideuteroglucose. *Diabetes, 20,* 1016.)

use glucose at rates of 4 to 6 mg/kg/min, equivalent to almost all endogenous glucose production during fasting.[10] (Figure 21-1) The rate of glucose production by the liver is linearly correlated with estimated brain weight at all ages. Muscle amino acids are the principal source of gluconeogenic precursors during fasting, but the substantially smaller muscle mass of infants relative to body mass limits the duration of fasting.[11]

Because brain growth is nearly complete as the body reaches 40 kg at 10 to 12 years of age, little additional glucose production is needed in adolescents and adults, and glucose can be maintained above 70 mg/dL for progressively longer durations. Infants from 1 week to 1 year of age should be able to tolerate 15 to 18 hours of fasting before plasma glucose concentrations fall below 70.[12,13] By 1 year of age, a normal child should be able to fast up to 24 hours.[14-16] By 5 years of age, a fast of up to 36 hours may be tolerated, whereas most adults can maintain fasting glucose above 70 mg/dL for 48 to 72 hours.[17] Hypoglycemia induced by fasting of shorter duration than expected for age should, therefore, alert the clinician to the possibility of an underlying disorder.[18]

Fasting Adaptation to Longer Feeding Intervals

With feeding, plasma insulin concentrations rise from values of 3 to 10 μU/mL to peaks of 20 to 50 μU/mL and stimulate glycogen synthesis, inhibit gluconeogenesis, and enhance peripheral (muscle) glucose uptake (Table 21-1). Simultaneously, triglyceride synthesis is activated and lipolysis and ketogenesis are curtailed. In the postabsorptive state, plasma glucose levels decline, and at an average threshold of 81 mg/dL (4.5 mmol/L) insulin secretion is reduced.[7] Coupled with a rise in counter-regulatory hormones (glucagon and epinephrine) that begins to occur as the glucose reaches 68 mg/dL (3.8 mmol/L) and sympathetic activation at 55 (3 mmol/L), the fall in insulin levels reverses the anabolic pathways to ensure adequate supplies of glucose, fatty acids, and ketones (Box 21-1).[19,20] The fatty acids mobilized from adipose tissue serve as alternative fuels for muscle, including cardiac muscle, thereby sparing glucose for brain metabolism. Glucose utilization is further spared by partial oxidation of fatty acids to ketones in the liver, which are then released to serve as substrate for the brain.[21]

The first phase in the metabolic defense against hypoglycemia is hepatic glycogenolysis (Figure 21-2). In infants, liver glycogen stores may provide glucose for up to 4 hours. As the child grows, glycogen reserves relative to brain glucose utilization are greater and may provide glucose for up to 8 hours of fasting. Glucagon and epinephrine, as insulin levels are suppressed, trigger glycogenolysis. Deficiency of these two hormones is unusual except in children on beta-blocker drugs. Therefore, hypoglycemia occurring early in fasting suggests either excess insulin secretion or a primary disorder in glycogenolysis.

TABLE 21-1 **Hormonal Regulation of Fasting Metabolic Systems**

Hormone	Hepatic Glycogenolysis	Hepatic Gluconeogenesis	Adipose Tissue Lipolysis	Hepatic Ketogenesis
Insulin	Inhibits	Inhibits	Inhibits	Inhibits
Glucagon	Stimulates			Stimulates
Epinephrine	Stimulates	Stimulates	Stimulates	Stimulates
Growth hormone		Stimulates	Stimulates	
Cortisol		Stimulates		

BOX 21-1 Symptoms of Hypoglycemia

Neurogenic Symptoms Due to Activation of Autonomic Nervous System

- Sweating
- Shakiness, trembling
- Tachycardia
- Anxiety, nervousness
- Weakness
- Hunger
- Nausea, vomiting
- Pallor
- Hypothermia

Neuroglycopenic Symptoms Due to Decreased Cerebral Glucose Use

- Headache
- Visual disturbances
- Lethargy, lassitude
- Restlessness, irritability
- Difficulty with speech and thinking, inability to concentrate
- Mental confusion
- Somnolence, stupor, prolonged sleep
- Loss of consciousness, coma
- Hypothermia
- Twitching, convulsions, "epilepsy"
- Bizarre neurologic signs
- Motor disturbances
- Sensory disturbances
- Loss of intellectual ability
- Personality changes
- Bizarre behavior
- Outburst of temper
- Psychological disintegration
- Manic behavior
- Depression
- Psychoses
- Permanent mental or neurologic damage

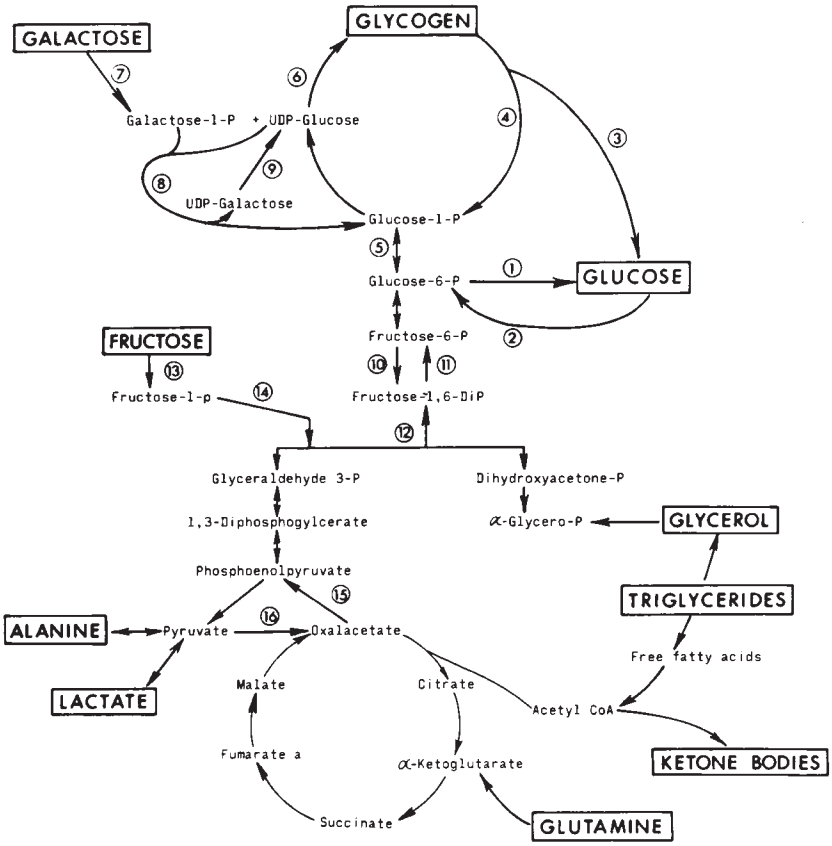


FIGURE 21-2 ■ Contribution of major fasting systems to brain metabolism over time. Key metabolic pathways of intermediary metabolism. Disruption of the elements of these pathways may be pathogenetic in the development of hypoglycemia. Not shown is the hormonal control of these pathways. Indicated are (1) glucose 6-phosphatase, (2) glucokinase, (3) amylo-1,6-glucosidase, (4) phosphorylase, (5) phosphoglucomutase, (6) glycogen synthetase, (7) galactokinase, (8) galactose 1-phosphate uridyl transferase, (9) uridine diphosphogalactose-4-epimerase, (10) phosphofructokinase, (11) fructose 1,6- diphosphatase, (12) fructose 1,6-diphosphate aldolase, (13) fructokinase, (14) fructose 1-phosphate aldolase, (15) phosphoenolpyruvate carboxykinase, and (16) pyruvate carboxylase. UDP, uridine diphosphate. (From Pagliara, A. S., Karl, I. E., Haymond, M., & Kipnis, D. M. (1973). Hypoglycemia in infancy and childhood. *J Pediatr*, *82*, 365–379, 558–577.)

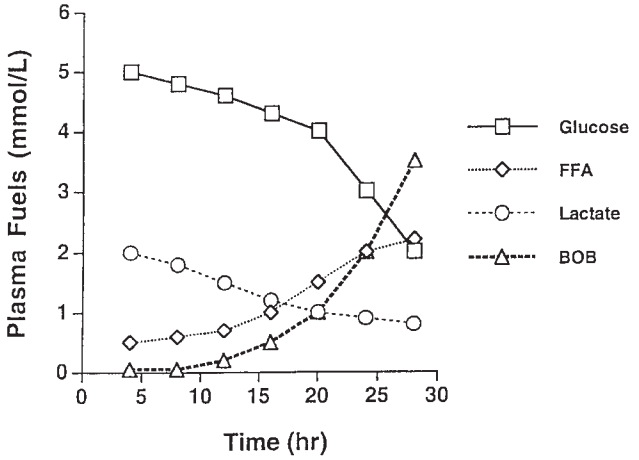


FIGURE 21-3 ■ Changes in major metabolic fuels during fasting in a normal infant. Note that plasma glucose declines toward hypoglycemic values by 24 hours as hepatic glycogen reserves are depleted. The level of lactate, a representative gluconeogenic substrate, declines gradually during the fast. Late in fasting, levels of plasma free fatty acids (FFA) increase as lipolysis is activated—followed by an increase in β-hydroxybutyrate as rates of hepatic fatty acid oxidation and ketogenesis increase.

As glycogen stores become depleted, there is a greater reliance on gluconeogenesis to maintain plasma glucose levels. The main gluconeogenic precursors are amino acids, especially alanine, most of which is generated from skeletal muscle. To prevent excessive breakdown of muscle protein, adipose tissue provides an additional fuel source in the form of triglycerides hydrolyzed to glycerol, which the liver can use for gluconeogenesis, and free fatty acids, which become the major fuel source for the body at later stages of fasting. Mitochondrial fatty acid oxidation (FAO) in the liver produces ketone bodies (β-hydroxybutyrate and acetoacetate) that can be used especially by the brain but also by muscle, including cardiac muscle, for energy production (Figure 21-3). This decreases glucose utilization by these organs and helps ensure an adequate supply of glucose to the brain and to tissues that use only glucose as fuel (e.g., red blood cells). Breakdown of adipose tissue triglycerides (lipolysis) is triggered by secretion of the counter-regulatory hormones (epinephrine and growth hormone [GH]) and by declining levels of insulin. In infants, elevation of plasma ketones begins by 12 to 18 hours into fasting. In older children, ketonemia may not appear until 18 to 24 hours of fasting.[22] Cortisol, produced during stress, can further accelerate gluconeogenesis.

Defects in gluconeogenesis (e.g., fructose-1,6-bisphosphatase deficiency) will usually become manifest only after glycogen stores have been depleted and will thus not occur in the recently fed state. FAO disorders can be triggered by more prolonged fasting. In the first months of life, feeding intervals gradually increase from the typical 2 to 3 hours for the on-demand breastfed infant to 4 hours, and eventually to 8 to 12 hours as nighttime feedings are omitted. For this reason, disorders of gluconeogenesis and FAO rarely present as hypoglycemia in the newborn period when feeding is frequent, but rather later in infancy as fasting is prolonged (or, rarely, immediately after birth before lactation is established). Congenital or acquired deficiencies of the counter-regulatory hormones that facilitate these processes (cortisol and growth hormone) may also result in hypoglycemia—in the newborn period if congenital and severe or later in infancy when longer periods of fasting occur. Combined deficiency of cortisol (adrenocorticotropic hormone [ACTH]) and GH in hypopituitarism may produce earlier onset and more severe hypoglycemia than occurs with isolated hormone deficiencies.

SYMPTOMS, SIGNS, AND EFFECTS OF HYPOGLYCEMIA

Neuroendocrine defenses against hypoglycemia consist of the counter-regulatory hormones that shift metabolic processes toward glucose production and the autonomic responses that provide most of the recognizable symptoms. Counter-regulatory hormones rise as the plasma glucose drops below an average of 68 mg/dL, though the rise of these hormones is not usually clinically detectable nor rapidly measurable (Table 21-2).[5,19,20,23] Of the

TABLE 21-2 **Mean Thresholds of Hypoglycemic Defenses and Effects**

	Plasma Glucose		References
	MG/DL	MMOL/L	
Reduction of insulin secretion	80-85	4.4-4.7	20
Slight slowing of auditory response time	72	4	44
Increased glucagon and epinephrine release	68	3.8	5, 20, 42, 486
Increased cortisol and growth hormone release	60	3.3	5, 20, 42, 486
Autonomic responses and awareness of hypoglycemia	55	3	5, 42, 486
Subtle EEG changes	55	3	45
Insulin secretion minimal	50	2.8	487
Obviously impaired cognitive function	49	2.7	5, 486
Reduced level of consciousness, seizures	30-40	1.7-2.2	7
Neuronal death and brain damage	< 18	< 1	7

counter-regulatory hormones, glucagon and epinephrine exert the largest immediate effects on glucose metabolism cortisol and growth hormone effects are slower, and the many other hormones (e.g., prolactin, vasopressin) that rise in response to hypoglycemia are less important still. Control of the counter-regulatory responses is at least partially local, with intra-islet control of glucagon release by release of insulin suppression, and multiple effects on glucose metabolism and eating behavior are influenced by glucose sensing in the portal vein.[24-26] However, the primary site of glucose sensing and response is in the ventromedial nucleus of the hypothalamus.[27]

Clinically recognizable signs and symptoms of hypoglycemia occur at slightly lower plasma glucose levels and most fall into one of two categories: autonomic or neuroglycopenic (see Box 21-1). Autonomic manifestations resulting from activation of the sympathetic nervous system, typically at a plasma glucose threshold of 55 mg/dL (3 mmo/L), provide the most recognizable signal to a person that glucose is falling and food is needed.[7,28] Adrenergic symptoms such as tachycardia, tremor, and anxiety are produced both by local sympathetic neural effects and by the peripheral effects of epinephrine released from the adrenals. Cholinergic symptoms include sweating, hunger, and paresthesias.[28] Hypothermia often occurs with prolonged hypoglycemia in older children and adults; evidence suggests a neurogenic mechanism.[29,30]

The trigger for counter-regulatory responses is the plasma glucose level itself. The rate of glucose fall and the level of insulin have little effect.[5,31] Activation thresholds for these neuroendocrine responses (both the counter-regulatory hormones and sympathetic activation) have been best established for healthy young adults and vary only slightly by sex,[32] age,[33] exercise,[34] sleep,[35] and nutritional status (Figure 21-4).[36] Certain drugs can dampen (e.g., beta-blockers) or amplify (e.g., caffeine) the responses.[37]

The most clinically important alterations of neuroendocrine response thresholds result from prior hypo- and hyperglycemia. Even a single episode of moderately severe hypoglycemia can blunt or lower the activation thresholds for 24 hours or more.[38-40] Prolonged or recurrent hypoglycemia can so reduce the autonomic responses (termed *hypoglycemia-associated autonomic failure* [HAAF]) that neuroglycopenic effects may be the sole clinical manifestation of severe hypoglycemia.[41] The effect can be demonstrated not just in adults and children with diabetes but also in nondiabetic adults and in infants with recurrent hypoglycemia due to hyperinsulinism.[40] Conversely, chronic hyperglycemia is associated with higher glucose thresholds for counter-regulatory responses.[42,43]

Neuroglycopenic effects become progressively more severe as glucose deprivation affects brain function. For the affected person, neuroglycopenic effects are less recognizable than the autonomic symptoms and are better characterized as a continuum of increasing impairment than a single "threshold." However, prior hypoglycemia does not lower the threshold levels for the objectively measurable neuroglycopenic effects.[20] A plasma glucose decline from 87 to 72 mg/dL increases the latency of

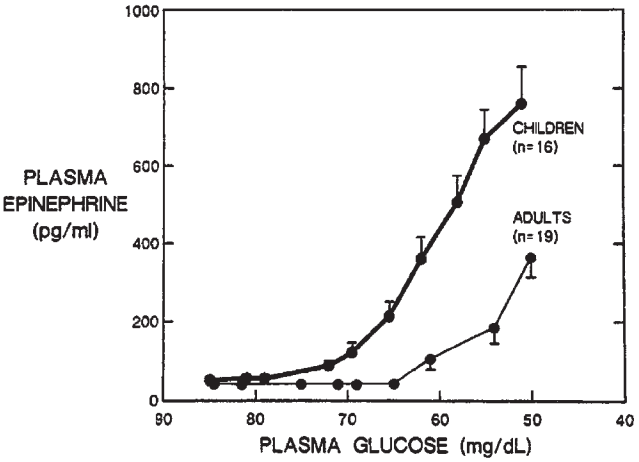


FIGURE 21-4 ■ Plasma epinephrine response of children and adults to step decreases in blood glucose concentration during a hyperinsulinemic clamp procedure. Note that the increase in epinephrine concentration occurs at a significantly higher glucose concentration in children than in adults. In addition, at comparable glycemia of less than 60 mg/dL, epinephrine responses in children are approximately threefold higher than in adults. (From Jones, T. W., Borg, W. P., Boulware, S. D., et al. (1995). Enhanced adrenomedullary response and increased susceptibility to neuroglycopenia: mechanisms underlying the adverse effects of sugar ingestion in healthy children. *J Pediatr, 126,* 171.)

P300 waves, an auditory event-related potential said to be a sensitive and specific test of cognitive function that occurs at glucose levels above the neurogenic symptom threshold.[44] Subclinical epileptiform electroencephalographic changes occur in most children at glucose levels approaching 55 mg/dL (3.1 mmol/L).[45] More obvious cognitive effects, such as slower response times and impaired judgment, occur at an average threshold of 49 mg/dL (2.7 mmol/L).[7,46,47] At even mildly lower plasma glucose levels, lethargy or confusion become evident, followed by seizures and coma. In infants and young children, neuroglycopenic manifestations may involve twitching, poor feeding, irritability, high-pitched crying, or even vomiting.[48] With prolonged hypoglycemia in older children and adults, bizarre, uncharacteristic, or "automatic" behavior (including physical aggression or, rarely, criminal offenses) can occur.[49-51] All of these cognitive, behavioral, and consciousness effects are typically completely reversed when the glucose level is raised, though subtle neuropsychological impairment may be measurable days later.[52]

More severe and prolonged glucose deprivation produces brain damage from neuronal death.[7,53,54] In experiments with primates, 5 to 6 hours of glucose levels below 20 mg/dL (1.1 mmol/L) reliably produced severe damage.[7] Severe hypoglycemia causes characteristic pathologic changes in cortical tissue and white matter, though the cerebellum and brainstem are usually spared.[55] Magnetic resonance imaging changes characteristic of hypoglycemic damage can be seen in both infants and adults.[56,57] Permanent cognitive impairment is measurable in many children and adults with a history of recurrent, severe hypoglycemia.[58-62]

Facilitated glucose transport across the blood-brain barrier, mediated by the glucose transporter-1, is dependent on the arterial plasma glucose concentration and

independent of insulin. Reduction of neurogenic symptoms with repeated hypoglycemia does not depend on increased glucose transport.[63] The conditions in which ketones or lactate can be used as alternate fuels during hypoglycemia are uncertain, but the ability to replace glucose is limited.[64-67] A final intracerebral defense during hypoglycemia is afforded by small stores of astrocyte glycogen that can be converted to lactate and may provide up to 20 minutes of fuel support for neuronal function.[68,69]

Other signs and symptoms of hypoglycemia are not as clearly related to the counter-regulatory or neurogenic defenses, or to the neuroglycopenic effects. The younger the child, the more nonspecific the manifestations may be, including cyanosis, bradycardia, apnea, and apparent respiratory distress.[48,70]

DEFINITION OF HYPOGLYCEMIA

A clinically useful definition of hypoglycemia cannot be based simply on a single value of plasma glucose concentration.[1,71] For children and adults, hypoglycemia is best defined as a plasma glucose concentration low enough to elicit defensive neuroendocrine responses or to impair brain function. Counter-regulatory hormones rise at an average threshold of 68 mg/dL, and autonomic effects and the most recognizable symptoms of hypoglycemia occur at an average threshold of 55, but, as noted previously, these responses can be blunted or the thresholds shifted by prior hypo- and hyperglycemia. Brain function is slightly affected at plasma glucose levels as high as 70, and more clearly at an average threshold of 49 but these effects may be less obvious in young children.

The Endocrine Society has developed guidelines for the evaluation and management of adult hypoglycemia, which emphasize the value of Whipple's triad for confirming hypoglycemia in adults: (1) symptoms or signs of hypoglycemia occurring with (2) a low measured blood glucose and (3) resolving when glucose is raised.[71] Although this is also a useful approach for older children, Whipple's triad may be less applicable to infants and young children, in whom the observable effects of hypoglycemia can be less specific, who are too young to report symptoms, and for whom establishing a threshold definition of hypoglycemia has been difficult.[72-75] Despite the frequency with which hypoglycemia is not accompanied by obvious symptoms in infants, evidence of harm to the brain from prolonged or recurrent hypoglycemia suggests the same clinical thresholds and treatment goals are applicable to young and old alike.

For the purposes of this chapter and for clinical practice, two hypoglycemia thresholds are used: a level sufficiently low for obtaining diagnostic tests and a level that is the goal for treatment. For diagnostic purposes, a plasma glucose value of less than 55 mg/dL (3 mmol/L) is sufficiently low for obtaining samples for determining the etiology of the hypoglycemia and for terminating provocative tests. In contrast, the lower limit of the plasma glucose range for therapeutic purposes should be 70 mg/dL (3.9 mmol/L), which is also the lower end of the "target range" for therapy in diabetic patients. Setting $\geq$ 70 mg/dL as the therapeutic goal is especially important to avoid periods of low glucose that may blunt the neuroendocrine and symptomatic responses to hypoglycemia and lead to greater susceptibility to subsequent episodes of hypoglycemia.

Plasma glucose values must be interpreted with an awareness of numerous physiologic, technical, and artifactual sources of variation. Because whole-blood glucose levels are 10% to 15% less than plasma concentrations, it is preferable to consistently refer to plasma glucose concentrations.[76] As blood from large veins will have lower glucose values than simultaneous blood from arteries, especially postprandially, it is important of refer to arterialized venous plasma glucose concentrations as the standard.[77] When blood is drawn but not immediately separated, glucose levels decline due to red cell glycolysis; this is a common cause of artifactually low glucose levels reported in metabolic panels run by commercial labs.[78] Glucose meters originally designed for diabetes management are useful for screening purposes, but none of the currently available meters is sufficiently accurate for a diagnosis of hypoglycemia without laboratory corroboration.[79] Any meter plasma glucose level $<$ 60 mg/dL should be confirmed by an accurate laboratory determination of plasma glucose.[80]

MAJOR CAUSES OF HYPOGLYCEMIA IN THE INFANT, CHILD, AND YOUNG ADULT

Hyperinsulinism

Hyperinsulinism is the most common and the most severe form of hypoglycemia in infancy.[81-83] The hypoglycemia of hyperinsulinism is particularly dangerous to the brain because it is associated with inadequate amounts of all brain fuels (low plasma ketones and glucose).[62] Although 60% of patients with congenital hyperinsulinism present in the first week of life, milder forms continue to be a significant cause of hypoglycemia throughout infancy and later. Despite a lengthening list of known genetic causes of congenital hyperinsulinism, many infants have forms for which the underlying mechanism remains unknown.[84,85]

Congenital hyperinsulinism often becomes evident after the first months of life, when the feeding intervals lengthen and night feedings are omitted. Such infants may present with early morning seizures or lethargy. Many will have a history of hypoglycemia in the newborn period that was not fully appreciated or will have a history of previous seizures.[86] Some of these children may have previously unexplained developmental delay. In dominant forms of hyperinsulinism, a parent or other relative may report a more subtle history of hypoglycemia.[87] Defects of adenosine triphosphate (ATP)-sensitive potassium channel (KATP channel) are the most common form of congenital hyperinsulinism, but a growing number of other defects in insulin secretion are being identified. As outlined in Chapter 5, all of the genetic causes of hyperinsulinism may initially present in the older infant and child.[88-90]

Hypoglycemia due to hyperinsulinism after the neonatal period is likely to present as recurrent episodes of neuroglycopenic symptoms in an otherwise well child. The hypoglycemia may be mild or severe, occurring after overnight fasting or in a "reactive" pattern 2 to 3 hours

after a meal. Obvious adrenergic signs (pallor, perspiration) are common but not invariably present; seizures are frequent. The diagnosis of hyperinsulinism is suggested when hypoglycemia is accompanied by the absence of hyperketonemia and injection of glucagon is followed by a large increase in plasma glucose. A critical specimen obtained during hypoglycemia that demonstrates measurable amounts of insulin, C peptide, or proinsulin (Table 21-3) can be conclusive. However, it is not always easy to demonstrate elevated insulin levels.[91]

Standard insulin assays may not detect exogenous insulin analogs such as lispro, aspart, glargine, glulisine, and detemir.[92] Plasma free fatty acids and beta-hydroxybutyrate (βOHB) are inappropriately low during hypoglycemia due to hyperinsulinism, as is insulin-like growth-factor-binding protein-1 (IGFBP1).[93] A glycemic response to glucagon at the time of hypoglycemia is strong evidence of hyperinsulinism as well.[12,94,95] Because of the increased glucose utilization of hyperinsulinism, a glucose infusion rate above 8 mg/kg/min may be needed to maintain glucose above 70 mg/dL in young infants. However, this is usually more difficult to demonstrate or is inconsistently present in older children.

KATP Channel Hyperinsulinism

Two genes, SUR1 and Kir6.2, encode the two subunits of the adenosine triphosphate KATP channel in the plasma membrane of the beta cell. Potassium efflux through this channel hyperpolarizes the beta cell plasma membrane and is a key negative regulator of insulin secretion; defects that impede potassium efflux or inhibit KATP channel activity cause excessive insulin secretion. As described in Chapter 5, three distinct defects of these genes are known to cause hyperinsulinism:

- Autosomal-recessive inheritance of two KATP mutations produces a severe neonatal onset of disease that is unresponsive to diazoxide. These mutations are associated with diffuse histologic changes in the pancreatic islets.[96]
- A clone of beta cells possessing a single recessive mutation on the paternal chromosome may lose the normal maternal allele, resulting in homozygosity for a recessive KATP channel mutation.[89] This "two-hit defect" produces a focal area of adenomatosis in the pancreas.
- Rarer, dominantly inherited autosomal mutations usually cause a milder form of KATP hyperinsulinism that often presents after infancy and responds to diazoxide. Dominant KATP mutations that produce severe, diazoxide-unresponsive disease have also been reported.[97,98]

The diffuse and focal forms of KATP hyperinsulinism account for more than 90% of cases of congenital hyperinsulinism in infancy and are similar in their clinical manifestations. Both may require surgery to maintain safe glucose levels. After confirmation of hyperinsulinemic hypoglycemia by the criteria presented in Table 21-3 and exclusion of the more rare forms described in the following discussion, a KATP channel defect can be presumed the most likely diagnosis. While blood is being tested for specific mutations (e.g., www.genetests.org), management may proceed without molecular confirmation. Treatment measures able to maintain blood glucose levels above 70 mg/dL without surgery are referred to as "medical management" (Table 21-4).

Diazoxide (5 to 15 mg/kg/day) is the first-line drug, but it is often ineffective for KATP hyperinsulinism. Octreotide may be given by subcutaneous injection or infusion at 15 to 20 μg/kg/day.[99] Calcium channel blockers have occasionally been tried but are generally not considered effective.[100] If medical management does not maintain the plasma glucose concentration > 70 mg/dL with a normal feeding schedule, surgery may be necessary.

TABLE 21-3 **Fasting Test Results Consistent with Insulinoma**

	Normal	Indicative of Hyperinsulinism	Typical of Insulinoma
Time to glucose nadir, hours	> 48	< 48	2-48
Glucose, mmol/L	> 2.2	< 2.5	1.4-3
Glucose, mg/dL	> 40	< 45	25-55
Insulin, pmol/L	< 18	> 18	6-200
Insulin, μU/mL	< 2.5	> 2.5	3-42
Proinsulin, pmol/L	< 5	> 5	10-800
Proinsulin, ng/mL	< 0.2	> 0.2	0.1-9
C-peptide, nmol/L	< 0.2	> 0.2	0.2-1.8
C-peptide, ng/mL	< 1	> 1	1-9
βOHB, mmol/L	> 2.7	< 2.7	< 2
Glucose peak after glucagon	< 1.4 < 25	> 1.4 > 25	1.5-5.4 27-90

Values are compiled from several large series of mostly adult patients.[193,195,198,488] Levels were obtained at time of symptomatic hypoglycemia or when glucose fell below 2.5. Levels for normal patients are at the end of a 48- or 72-hour fast. In each series, up to 3% of patients were 12 to 20 years old, though fasting longer than 24 hours is rarely necessary for the diagnosis of hypoglycemia in children. Normal ranges are somewhat assay specific and not precisely arithmetically convertible. In each series, a few patients in each category fell outside these ranges for single parameters, so diagnoses should not be based on single values near the edges of expected ranges.

TABLE 21-4 **Disorders of Glycogen Storage Causing Hypoglycemia**

GSD		Protein	Gene	Inheritance	Relative Incidence	Fasting Hypoglycemia
0	Glycogen synthase deficiency	Glycogen synthase	*GYS2* on 12p12.2	AR	U	Usually mild
Ia	von Gierke disease	Glucose 6-phosphatase	*G6PC* on 17q21	AR	C	Universal, severe
Ib		Glucose 6-phosphate translocase	*SLC37A4* on 11q23	AR	R	Universal, severe
IIIa	Cori or Forbes disease, debrancher deficiency, limit dextrinosis	Amylo-1,6-glucosidase in liver and muscle	*AGL* on 1p21	AR	C	Mild to moderate
IIIb	Cori or Forbes disease, debrancher deficiency, limit dextrinosis	Amylo-1,6-glucosidase in liver	*AGL* on 1p21	AR	R	Mild to moderate
IV	Andersen disease, amylopectinosis	Branching enzyme	*GBE* on 3p12.2	AR	U	With advanced liver failure
VI	Hers disease	Glycogen phosphorylase	*PYGL* on 14q22.2	AR	U	Mild
IXa	Hug or Huijing disease	α subunit of phos b kinase	*PHKA2* on Xp22.13	XL	C	Mild to moderate
IXb		β subunit of phos b kinase	*PHKB* on 16q12.1	AR	R	Mild to moderate
IXc		γ subunit of phos b kinase	*PHKG2* on 16p11.2	AR	R	Mild
XI	Fanconi-Bickel disease	Glucose transporter 2	*GLUT2* on 3q26.2	AR	R	Mild

GSD, glycogen storage disease; AR, autosomal recessive; XL, X-linked. Relative incidence compared to other GSDs: C, common; U, uncommon; R, rare.

Surgical treatment for focal KATP hyperinsulinism is curative, whereas for diffuse disease even a 95% to 99% pancreatectomy may not cure the hypoglycemia (and diabetes is a frequent sequela).[95] ^{18}F-DOPA PET scanning may localize a focal lesion preoperatively, but definitive differentiation of focal and diffuse disease is made intraoperatively by repeated examination of frozen sections.[94] This difficult procedure requires a patient surgeon and a dedicated team of histopathologists.[101-103]

Glutamate Dehydrogenase Hyperinsulinism

Hyperinsulinism can also be caused by activating mutations in the gene (GLUD1 on 10q) for glutamate dehydrogenase (GDH).[90] This disorder, also known as the hyperinsulinism-hyperammonemia syndrome, is a milder form of hyperinsulinism than KATP hyperinsulinism and is more likely to present in late infancy and early childhood than in the newborn period.[104,105] Mutations in GDH cause disease in an autosomal-dominant fashion, but up to 80% cases may be de novo mutations. Severity may vary within a family from seizures in infancy to mild postprandial hypoglycemia in adults. Ingestion of protein without carbohydrate may be especially likely to depress the blood glucose.

Activating mutations of GDH in the beta cell amplify leucine-triggered production of ATP, independent of glucose levels, which then causes closure of KATP channels and insulin release. In the kidney, the same mutation increases oxidation of glutamate to α-ketoglutarate with the production of ammonia. In addition to increased renal ammonia production, the low levels of glutamate in the liver may impair the production of N-acetylglutamate, an important allosteric activator of the urea cycle. Thus, the same mutation causes excessive insulin production in the pancreas, excessive ammonia production by the kidneys, and, possibly, impaired urea synthesis in the liver.[106]

The diagnosis of GDH hyperinsulinism is similar to that of other forms of hyperinsulinism. In addition to the usual laboratory findings of hyperinsulinism, however, persistently elevated ammonia levels (typically 80 to 120 μmol/L) are diagnostic of this disorder.[107] Most patients respond to diazoxide, and surgery is never necessary.[104,108] High ammonia levels do not appear to cause problems in GDH hyperinsulinism because the hyperammonemia is not associated with elevated neuronal glutamine, the substance thought to be principally responsible for the central nervous system (CNS) toxicity evident with other forms of hyperammonemia.[109]

Glucokinase Hyperinsulinism

Glucokinase is the enzyme that serves as the glucose sensor in the beta cells of the pancreas.[110] Gain-of-function mutations can result in a lower glucose threshold for insulin secretion, leading to persistent hypoglycemia, just as loss-of-function mutations produce a higher glucose secretion threshold, causing a common form of mild monogenic diabetes (MODY2).[111] At least 15 different dominantly expressed mutations have been reported.[112] Enzyme activities, glucose thresholds, and clinical severity of these mutations can vary. The more severe mutations tend to be de novo rather than inherited. The in vitro enzyme kinetics of each mutation is of limited value for predicting clinical severity and course.[112]

In some cases, glucokinase mutations have produced neonatal hypoglycemia severe enough to require pancreatic surgery to stabilize glucose levels. Other cases have presented as hypoglycemic seizures in childhood or even reactive hypoglycemia in older relatives. Like the KATP forms of hyperinsulinism, glucokinase mutations can cause apparently transient neonatal hypoglycemia that becomes asymptomatic for many years, only to recur later in life.[113,114] In some cases, older relatives with the same mutations have had milder degrees of severity.[115]

Glucokinase hyperinsulinism (GCK-HI) presents some challenges to diagnosis: when glucose falls below the insulin secretion threshold, free fatty acids and βOHB can rise. In the two GCK-HI patients in whom it was tested, the acute insulin response to intravenous glucose was greater than is usually seen with KATP hyperinsulinism.[86] Ammonia levels are normal, and GCK-HI patients do not have protein-sensitive hypoglycemia. The response to diazoxide has been partial in most patients. Severe cases could not be controlled on diazoxide and required pancreatectomy.[116]

SCHAD (or HADH) Hyperinsulinism

Short chain 3-hydroxyacyl-CoA dehydrogenase (SCHAD or HADH) is an enzyme of mitochondrial fatty acid oxidation that catalyzes oxidation of straight-chain 3-hydroxyacyl-CoAs, and is involved in multipathway complexes with other enzymes in several tissues.[117] Pancreatic beta cells have relatively high levels of SCHAD activity, where it is associated with, and helps negatively regulate, GDH, the enzyme of hyperammonemic hyperinsulinism.[118] Homozygous mutations of the *HADH* gene on chromosome 4 at q22-26 lead to an absence of SCHAD protein, deficient enzyme activity, an elevation of plasma short chain acyl-carnitine metabolites, and an autosomal recessive form of congenital hyperinsulinism.[119]

More than 20 patients, of at least 8 kindreds and with 12 different *HADH* mutations, have been reported with varying degrees of hyperinsulinemic hypoglycemia.[120] Studies of the mouse model of SCHAD-HI suggest that a deficiency of HADH allows amplified activity of glutamate dehydrogenase (GDH), the same enzyme involved in hyperammonemic hyperinsulinism.[118] Some cases have displayed severe hypoglycemia as newborn infants, but some showed no signs of hypoglycemia until several months of age.[121,122] HADH hyperinsulinism resembles other forms of hyperinsulinism, with elevated insulin levels during hypoketotic hypoglycemia. Elevated levels of 3-hydroxybutyryl-carnitine in plasma and 3-hydroxyglutarate in urine have suggested the diagnosis in some patients. Affected patients share some features of hyperammonemic hyperinsulinism, such as a marked sensitivity to protein-induced hypoglycemia, but without the hyperammonemia characteristic of GDH-HI. Unlike other fatty acid oxidation disorders, muscle and liver function have not been clinically affected. Hypoglycemia has been severe enough in some patients to cause brain damage, but treatment with diazoxide was effective in all cases so far described.[120,123,124]

The oldest patient with HADH hypoglycemia was originally reported in 1977 as an instance of glucagon deficiency, in part because of a brisk rise of glucose after glucagon was given. This case was cited as an example of glucagon deficiency for years, before it was recognized that a glycemic response to glucagon was a characteristic feature of hyperinsulinemic hypoglycemia.[125] Reinvestigation of the proband and other family members discovered the *HADH* mutation.[126,127] Although this condition is usually (maybe irreversibly) referred to as a SCHAD deficiency in the endocrine literature, this nomenclature has been criticized as misleading, as another enzyme—a member of the short chain dehydrogenase/reductase superfamily with a role in brain development and perhaps Alzheimer and Parkinson diseases—has been officially and more accurately termed short chain 3-hydroxyacyl-CoA dehydrogenase (SCHAD) for years.[128]

HNF4A Hyperinsulinism

Hepatocyte nuclear factor 4α is a transcription factor important for pancreatic beta cell development and insulin secretion. Heterozygous inactivating mutations of the HNF4A gene are a well-recognized cause of monogenic diabetes (MODY1). Recently it was recognized that these mutations also cause excessive insulin secretion in early life, manifested by fetal macrosomia and persistent neonatal hypoglycemia. Although the diazoxide-responsive hyperinsulinemic hypoglycemia is usually transient, it may persist for several years into childhood.[129] In most cases of HNF4A, the birthweight has been above average, hypoglycemia occurred in the first days of life, and a parent has had a history of monogenic diabetes.[130,131] In one case, diazoxide-responsive hyperinsulinism was recognized on the first day of life, but the infant also developed hepatomegaly and renal Fanconi syndrome in the first year, suggesting that expression of the GLUT2 transporter was impaired by the defect in HNF4A.[132]

HNF1A Hyperinsulinism

Hepatocyte nuclear factor 1 is another transcription factor important for beta cell development. Mutations can cause a common form of monogenic diabetes known as MODY 3. Two cases of mutations causing transient, diazoxide-responsive hyperinsulinemic hypoglycemia presenting at 3 and 20 months of age have been described. In both cases, the specific mutations have been reported in older patients with MODY3 and were inherited from the fathers.[132]

Exercise-Induced Hyperinsulinemic Hypoglycemia

A small number of children (infants to adolescents) have had hypoglycemic seizures or syncope after intense anaerobic exercise such as soccer or swimming.[133] So far, 13 patients belonging to three families have been found with exercise-induced hyperinsulinemic hypoglycemia (EIHI).[134] Clinical presentations have varied, with some having severe hypoglycemic episodes from infancy, some presenting with hypoglycemic syncope after exercise as adolescents, and some only mildly affected as adults. Lab tests in severe episodes have indicated hyperinsulinism, with low ketones

and free fatty acids, and inappropriately measurable insulin.[134] Most patients with EIHI maintained normal glucoses during prolonged fasting. Provocative testing with brief, intense bicycle exercise induced the normal rise in plasma lactate and pyruvate levels, but affected patients' insulin levels rose markedly for about 10 minutes after the exercise, causing hypoglycemia within the next 45 minutes.[135] Intravenous infusion of 300 mmol pyruvate over 3 minutes produced a fivefold elevation of insulin within 3 minutes in patients with EIHI and has been proposed as an alternative diagnostic test. Recurrent hypoglycemia of those more severely affected was only partially preventable with diazoxide treatment.

Excessive insulin secretion in EIHI occurs because the beta cells express abnormal amounts of the MCT1 monocarboxylate transporter due to a mutation of the gene (*SLC16A1*) that normally prevents expression on beta cells. Although pyruvate makes an excellent substrate for ATP production, normal beta cells do not have the MCT1 transporter and hence remain unaffected by high postexercise levels of lactate and pyruvate. EIHI is inherited as an autosomal dominant condition in three of the affected families.[134,136]

This mechanism seems confirmed by the report of a 16-year-old boy with an insulinoma characterized by expression of MCT1 and hyperinsulinemic hypoglycemia induced by exercise and cured by resection of the insulinoma.[137]

Carbohydrate-Deficient Glycoprotein Hyperinsulinism

Congenital disorders of glycosylation (CDG) are caused by deficient glycosylation of proteins or lipids.[138] Many CDGs are known and more are being identified each year. Whole exome sequencing is allowing rapid identification of responsible genes,[139] and a newer gene-based nomenclature system is replacing an older categorization based on transferrin isoelectrofocusing patterns.[140] Hypoglycemia due to hyperinsulinism has been reported in children with three of the earliest identified defects of N-glycosylation: CDG Ia, Ib, and Id (OMIM IDs: 601785, 602579, and 601110). The initial confirmatory test for all of these forms of CDG is abnormal isoelectric focusing of transferrin.

Most cases of CDG hyperinsulinism have been identified in the newborn period, but many have been diagnosed later in infancy or early childhood. CDG hyperinsulinism is usually accompanied by manifestations affecting other organ systems—especially the brain, liver, gut, and skeleton—but cases of all three types have been reported in which hyperinsulinemic hypoglycemia was the presenting or dominant problem.[141-143] The mechanism of excessive insulin production has not been determined.

PMM2-CDG (CDG-Ia) is the most common type of CDG and involves deficient activity of phosphomannomutase 2 resulting from mutations of *PMM2*. Many organ systems can be variably affected.[144] Most patients have severe developmental delay, cerebellar hypoplasia, hypotonia, and seizures. Protein-losing enteropathy and liver disease contribute to failure to thrive. Deficient levels of antithrombin III can cause thromboses. Dysmorphic features can be subtle or obvious, including unusual fat distribution and inverted nipples. Hyperinsulinemic hypoglycemia occurs in only a minority of patients but can be mild or severe enough to warrant pancreatectomy.[141,145]

MPI-CDG (CDG-Ib) involves deficient activity of phosphomannose isomerase resulting from mutations of *MPI*. Although the sialotransferrin pattern resembles that of PMM2-CDG, the central nervous system is spared, and hyperinsulinemic hypoglycemia is a common feature, presenting in the first days of life or later in the first year.[142,146] Hepatic disease and protein-losing enteropathy are usually the dominant clinical problems.[147] Some children have had cyclic vomiting. Clinical severity is variable, and mildly affected adults have been diagnosed. Oral mannose in doses of up to 150 mg/kg/day correct most of the clinical abnormalities, making MPI-CDG the only CGD with a specific treatment.[148]

ALD3-CDG (CDG-Id) involves deficient activity of mannosyltransferase 6 resulting from mutations of *ALD3*. Clinical features resemble those of PMM2-CDG, with severe central nervous system damage. One case with severe neonatal hyperinsulinemic hypoglycemia has been reported; modest beta cell hypertrophy was observed at autopsy.

Uncoupling Protein 2 Hyperinsulinism

Uncoupling protein 2 plays a role in the modulation of oxidative metabolism in mitochondria. Increased UCP2 activity reduces ATP generation. A loss-of-function mutation of *UCP2* has been found to cause enhanced ATP generation and consequently amplified insulin secretion severe enough to cause hyperinsulinemic hypoglycemia. Of the two initial cases, one had developed hypoglycemia shortly after birth, but the other presented with a hypoglycemic seizure at 8 months of age.[149,150] Both cases responded to treatment with diazoxide.

Hyperinsulinism in Tyrosinemia

Hyperinsulinemic hypoglycemia occurs in many infants and young children with type 1 (hepatorenal) tyrosinemia due to deficient fumarylacetoacetate hydrolase activity (OMIM ID: 276700).[151] Problems may include acute liver failure, cirrhosis, hepatomegaly, glomerulosclerosis, renal Fanconi syndrome with rickets, and acute crises with painful neuropathy. Most expanded newborn screening programs detect tyrosinemia. Plasma amino acid screening reveals markedly elevated tyrosine, and urine organic acid screening contains increased succinylacetone. Current treatment options include liver transplant or NTBC [2-(2-Nitro-4-Trifluoromethylbenzoyl)-1,3-Cyclohexanedione]. The mechanism of excessive insulin secretion is not known, but beta cell hyperplasia has been seen in the pancreas. Some infants have been born with macrosomia and cardiac hypertrophy suggestive of fetal insulin excess. Hypoglycemia can be severe enough to threaten the brain, but it responds to diazoxide and improves with age.[152]

AKT2 Hypoglycemia

Hypoglycemia with the characteristics of hyperinsulinism (except excessive insulin secretion) could be expected

to result from excessive activity of insulin receptors or amplification of postreceptor signals. Among the major effects of insulin receptor activation is amplification of intracellular AKT serine/threonine kinase activity; loss of AKT kinase activity produces insulin resistance. Three children with hypoglycemia due to gain of function mutations of AKT2 have been described.[153] Beginning around 6 months of age, all had episodes of severe, symptomatic hypoketotic hypoglycemia characterized by low levels of insulin, proinsulin, and free fatty acids. Other clinical features included large birth weight and postnatal left-sided overgrowth of body or face. The three patients were unrelated and each had the same de novo mutation of *AKT2*. The mutations were postzygotic and mosaic, which probably explains the localized nature of the overgrowth.

Hyperinsulinemic Hypoglycemia Associated with Insulin Resistance

Hypoglycemia accompanying insulin resistance might seem paradoxical, but several forms occur. Insulin resistance can result from defects of the insulin receptor, of downstream signaling, or secondary effects. Recessive mutations of the insulin receptor gene *INSR* at 19p13.2 produce three of the most severe forms of insulin resistance known: Donohue syndrome (OMIM ID: 246200), Rabson-Mendenhall syndrome (OMIM ID: 262190), and type A insulin resistance (OMIM ID: 610549).[154] Clinical features and specific mutations distinguish the three syndromes. Insulin secretion and insulin levels are increased manyfold, and the beta cells are often hyperplastic. Hypoglycemia can occur with all three by mechanisms not entirely understood. Chronic beta cell insulin overproduction may impair the ability to reduce secretion when glucose is falling or may be associated with reduced clearance of circulating insulin.

Infants with Donohue syndrome are diagnosable in the newborn period and most die of infection in the first years of life. Features include intrauterine growth retardation, diminished subcutaneous fat, an unusual face ("leprechaunism"), enlargement of breasts or clitoris, hirsutism, acanthosis, and postprandial hyperglycemia. Hypoglycemia after a few hours of fasting typically begins in the first days after birth and persists throughout life. Despite high insulin levels, and unlike most forms of hyperinsulinemic hypoglycemia, some of the best-studied infants have demonstrated shortened fasting tolerance and accelerated ketosis suggestive of inadequate glycogen stores.[155] Frequent feedings have been the mainstay of management, but diazoxide has been tried in some with variable responses.[156,157]

Children with Rabson-Mendenhall syndrome lack the extreme lipodystrophy and dysmorphic features of Donohue syndrome and typically develop severe, insulin-resistant diabetes mellitus.[158] Additional features of the originally described syndrome include abnormal teeth and skin, hypertrichosis, acanthosis, and pineal hyperplasia. Many have been reported to have hypoglycemia, but more often reactive than fasting. Death in childhood from diabetic ketoacidosis is common.

Postprandial (reactive) hypoglycemia has also been reported in adolescents with type A insulin resistance (acanthosis, polycystic ovary syndrome), in which diabetes mellitus develops more gradually, sometimes as the adolescent reaches adulthood.[154] One unusual familial mutation of the insulin receptor has been described with episodes of postprandial hypoglycemia severe enough to cause seizures, attributed to slower insulin clearance.[159]

Reactive hypoglycemia has also been reported in insulin-resistant conditions due to post-receptor disorders.[160]

Factitious Hyperinsulinism

Instances of factitious hyperinsulinism have been reported in infants and children, typically the result of a caregiver administering insulin or an insulin secretogogue.[161] This is a form of child abuse referred to as Munchausen by proxy or Meadow syndrome.[162] In most reported cases, the parent was a nurse or other medical professional—or family members used insulin or sulfonylureas for diabetes.[162-165] This type of child abuse may be lethal.[166]

Insulin administration may be difficult to detect, particularly because conventional insulin assays vary as to sensitivity for insulin analogues (lispro, aspart, glargine, glulisine, detemir, degludec).[92,167,168] Typically, classic symptoms of hyperinsulinism are present on an irregular basis. Fasting studies in the absence of the caregivers will be normal. As in other forms of excessive insulin, hypoglycemia is accompanied by a suppression of free fatty acids, ketones, and a positive response to glucagon—indicating that glycogen deposits are abundant and their release inhibited by insulin.

In cases in which regular or NPH insulin have been given, insulin levels may be remarkably high at the times of hypoglycemia. The most conclusive evidence of exogenous insulin is the suppression of C peptide to undetectable levels at the time other evidence indicates insulin excess, indicating that endogenous insulin production has been suppressed.[169] Insulinomas may secrete high insulin levels, but proinsulin levels will also be persistently elevated.[170]

Oral hypoglycemic agents that induce insulin secretion, especially sulfonylureas, will cause elevation of both insulin and C peptide.[171-173] Many cases of hypoglycemia occurring or persisting up to 24 hours after single-dose ingestions of sulfonylureas in toddlers and adolescents have been reported.[174,175] Because they induce endogenous insulin secretion, its use is difficult to detect unless suspected because the age suggests ingestion. Routine toxicology screens on blood and urine may not detect sulfonylureas, but if a sample of the suspected drug can be supplied, specific testing can be arranged. Recovery with intravenous glucose support is usual; diazoxide and octreotide have been used in severe cases.

Autoimmune Hypoglycemia

Three types of autoimmune hypoglycemia have been reported, mediated by antibodies to insulin, to insulin receptors, and to beta cells. Hypoglycemia due to anti-insulin antibodies has been described most often in Japanese women ("Hirata disease"[176]), but cases have been reported in both sexes, all ages, and from many regions.[177] Nearly all autoimmune hypoglycemia in infants and children has been of this type.[178-181] The presumed mechanism of hypoglycemia is slow dissociation of

insulin from antibodies during the postprandial or postabsorptive periods. The hypoglycemia is most often reactive, but may be fasting, and has sometimes been severe enough to cause seizures or coma.[182] Metabolic features are those of hyperinsulinism, with low ketone and fatty acid levels, but intermittent ketosis accompanying severe hypoglycemia was reported in two affected children. Measured levels of insulin can be high, but because much of it is not freshly secreted, C-peptide levels are lower than typical of insulinoma or most endogenous hyperinsulinism, though not as undetectable as is usual with exogenous hyperinsulinism.[177] Most older patients with this condition have other autoimmune diseases, and a number of drugs (especially methimazole) have been apparent precipitants.[183] Among treatments reported to improve the hypoglycemia have been courses of glucocorticoids, plasmapheresis, and intravenous immunoglobulin infusions.

Autoimmune hypoglycemia due to antibodies that bind to and activate insulin receptors rather than insulin has occurred almost exclusively in adults, usually in association with acanthosis and type B insulin resistance, or a malignancy or severe inflammatory disorder. Some patients have had both anti-insulin and antireceptor antibodies.[184] A case in an infant has been described.[181] C-peptide and insulin levels (with some exceptions) are typically undetectable. An even rarer form of autoimmune hypoglycemia has been postulated to involve antibodies directed against surface antigens on beta cells, resulting in stimulation of inappropriate insulin release.[185]

Insulinoma

Insulin-secreting adenomas of the pancreas are rare tumors but are the most common cause of new, severe hypoglycemia due to endogenous insulin in otherwise well adolescents and adults. Fewer than 100 childhood cases have been reported since the 1960s, but a few have occurred even in the first year of life.[186,187] Most insulinomas are sporadic, solitary tumors 0.5 to 2 cm in size, but a minority are multiple.[188] About 10% of adult cases are malignant (i.e., metastatic or locally invasive), but childhood insulinomas are only rarely malignant.[189] Surgical excision is usually curative, except in cases with multiple lesions, as in common with MEN1. Diazoxide can temporarily control the hypoglycemia in most.[190,191]

Insulinomas in older children and adults usually present as episodes of neuroglycopenic and autonomic symptoms (e.g., confusion and diaphoresis), often recurring for months before the diagnosis is made.[192,193] Autonomic symptoms may diminish as hypoglycemia recurs (hypoglycemia unawareness).[194] Although some patients have had recurrent seizures or other symptoms for years before diagnosis, there have been no detailed reports of cases in which only autonomic symptoms without neuroglycopenic effects led to the diagnosis of an insulinoma. The hypoglycemic episodes occur most commonly with fasting, but they may be reactive (postprandial) in some patients, and sometimes can be associated with exercise.[193,195,196] Accelerated weight gain in the year prior to diagnosis is common.[192] In many cases the evaluation is initiated by a new hypoglycemic seizure. When symptoms are milder, the initial step of the evaluation is directed toward determining whether the Whipple criteria are fulfilled: measurably low glucose levels at the time of characteristic neuroglycopenic and autonomic symptoms, with relief by carbohydrates.[197] A diagnostic fast may be necessary to confirm a hyperinsulinemic form of hypoglycemia.[191,198] In most cases glucose will fall below 50 mg/dL (2.8 mmol/L) well before 24 hours, and insulin, proinsulin, and C-peptide concentrations will be elevated while βOHB levels remain suppressed. Despite attempts to identify test results or ratios (e.g., insulin:glucose, proinsulin:insulin, C-peptide ratio) that would specifically confirm the presence of an insulinoma, results are characteristic of hyperinsulinism. Typical results reported in large series of adult patients are listed in Table 21-3[199]; results from a much smaller number of pediatric insulinomas have been comparable.[200] When symptoms have been primarily postprandial, a mixed meal tolerance test may be more likely confirmative than a fast.[196]

As no hormone testing distinguishes a solitary, resectable insulinoma from other forms of diffuse endogenous hyperinsulinism or multiple insulinomas, it is helpful to visualize the tumor before surgery.[191] Routine imaging of the pancreas by ultrasound, spiral computed tomography, or magnetic resonance may fail to reveal the tumor in 20% to 40% of patients, especially when it is smaller than 2 cm in size, and many enhanced imaging procedures have been devised.[201,202] Endoscopic ultrasonography has been preferred at many institutions.[203] Positron emission tomography (PET) scanning with various labels may show multicentric or extrapancreatic tumors.[204,205] When imaging fails to reveal a tumor, attempts have been made to localize the tumor to a region of the pancreas by pancreatic arterial stimulation with calcium and venous sampling (PASVS) of insulin levels.[206] Calcium-stimulated venous sampling, like many other localization techniques, has the highest success rates in experienced hands. Intraoperative ultrasonography can reveal most insulinomas not localized preoperatively.[191]

About 5% to 10% of adults with insulinomas, and a higher percentage of children, have multiple endocrine neoplasia syndrome 1 (MEN1, Wermer syndrome).[188,207] MEN1 insulinomas are usually multiple and can occur in childhood.[191] This disorder is characterized by functioning or nonfunctioning adenomas of the parathyroids, pituitary, and pancreas and is inherited in an autosomal-dominant fashion. MEN1 is caused by inactivating mutations of the *MENIN* gene on chromosome 11.[208] Gene testing in affected families can identify children at risk for insulinoma; annual fasting glucose, insulin, and proinsulin are recommended from the age of 5 years.[209]

Hyperinsulinism after Gastrointestinal Surgery

Gastrointestinal hormones and signals—the enteroinsular axis—play an important role in regulating both insulin secretion and sensitivity. Disruption of this system by gastric surgery can result in hypoglycemia from intermittently excessive postprandial insulin release.[210] This "alimentary hypoglycemia" is usually postprandial (i.e., reactive) rather than fasting and is due in large part to an amplified release of glucagon-like peptide 1 (GLP-1).[211]

In young children, postprandial hypoglycemia is common after Nissen fundoplications for gastroesophageal reflux, sometimes accompanying bowel symptoms as part of the "dumping syndrome," and sometimes as isolated neuroglycopenic and autonomic symptoms 1 to 3 hours after meals.[212,213] Hypoglycemia can usually be confirmed during symptoms after a typical feeding or a mixed meal tolerance test. Hypoglycemia can sometimes be abated by dietary measures that slow digestion, such as avoiding simple sugars, providing fat and protein with carbohydrates, or adding pectin or uncooked cornstarch.[214] In more severe cases, hypoglycemia can be prevented with acarbose, an alpha-glucosidase inhibitor that slows starch digestion, or a long-acting somatostatin analog such as octreotide.[215] Acarbose doses between 25 and 100 mg per feeding have been reported to be effective.[216]

Bariatric surgery of older patients can produce a similar phenomenon.[217] Amplified insulin release or improved insulin sensitivity after gastric bypass, banding, or sleeves can produce immediate improvement in patients with type 2 diabetes. However, in some nondiabetic patients, the insulin effect has been severe enough to cause symptomatic hypoglycemia.[218] Dietary measures for slowing digestion are sometimes effective, and both acarbose and octreotide have been used.[210] In a few, continuous feeding or subtotal pancreatectomy has been required to control the hypoglycemia.

Hyperinsulinemic Hypoglycemia Prodromal to Diabetes Mellitus

Although the most common and familiar type of hypoglycemia occurs during treatment of diabetes with insulin or insulin secretagogues, hypoglycemia preceding or accompanying the development of diabetes has been documented in a variety of circumstances. A relationship, "dysinsulinism," between reactive hypoglycemia and common type 2 diabetes was postulated as early as 1930[219] and widely recognized among diabetologists in the middle of the 20th century.[220-224] Pediatric cases were reported.[225] Early diabetes is often characterized by loss of the first-phase insulin response to food, resulting in higher glucose excursions but followed by lower glucose nadirs. In most cases the hypoglycemia does not reach levels low enough to cause neuroglycopenic symptoms. Over subsequent decades, research demonstrated the unreliability of oral glucose tolerance tests (OGTTs) for pursuing purely autonomic postprandial symptoms, which led to diminished interest in this phenomenon, though many laypeople have heard of the relationship.[226]

Instances of both fasting and postprandial hypoglycemia occurring prior to the onset of type 1 diabetes, or during an insulin-free "honeymoon," have also been reported, and mechanisms involving insulin antibodies, excessive second-phase insulin release after defective first-phase insulin release, or insulin release during inflammatory destruction of beta cells are plausible.[227-231]

Although the pathophysiology of cystic fibrosis–related diabetes (CFRD) is distinct from both type 1 and type 2, spontaneous fasting hypoglycemia and reactive hypoglycemia associated with early impairment of glucose tolerance occur, though whether either predicts the progression of CFRD is controversial.[232,233]

Nonislet Tumor Hypoglycemia

Certain non–insulin-secreting tumors are sometimes associated with paraneoplastic hypoglycemia.[234] Most cases have involved large, malignant tumors of mesenchymal, epithelial, or hematopoietic origin, referred to as the Doege-Potter syndrome.[235,236] Cases in children are rarer but have been reported with neuroblastoma and Wilms tumor.[237] The hypoglycemic episodes typically occur while fasting, with inappropriately low free fatty acids, increased glucose utilization, and suppressed hepatic glucose output suggestive of insulin excess. Proinsulin, insulin, and C-peptide levels are low, and diazoxide and somatostatin are ineffective, suggesting activation of insulin receptors by other circulating factors.[238] In most cases, levels of insulin-like growth factor 2 (IGF-2) have been found that are high enough to cross-activate both insulin and insulin-like growth factor 1 (IGF-1) receptors, producing hypoglycemia with the metabolic characteristics of insulin excess.[239] IGF-2 is normally produced in the liver and limited amounts are secreted in blood, bound to IGF-binding protein (IGF-BP3) and the acid-labile subunit (ALS). Tumor-produced IGF-2 can be incompletely processed and bound, hence more active, or structurally abnormal—a large molecular form called "big IGF-2." Stimulation of the insulin receptor causes hypoglycemia, whereas stimulation of the IGF-2 receptor causes down-regulation of growth hormone secretion, resulting in low levels of IGF-1 and IGF-BP3.[240]

The combination of elevated IGF-2 with suppressed IGF-1 and insulin confirms tumor hypoglycemia of this variety and suggests an effective intervention if the tumor cannot be completely removed. Treatment with growth hormone raises IGF-BP3 levels, which reduces the free IGF pool and thereby ameliorates hypoglycemia.[237]

Not all paraneoplastic hypoglycemia is mediated by IGF-2. Hypoglycemia attributed to increased anaerobic glucose metabolism has been reported in lymphomas and leukemias, in association with lactic acidosis.[241,242] Paraneoplastic autoimmune hypoglycemia was described earlier.

Glycogen Storage Diseases

Most glycogen occurs in hepatocytes, where it serves as a glucose reservoir for postabsorptive periods. Glycogenolysis provides a large portion of plasma glucose beginning a few hours after each meal and continuing until it is largely depleted 16 to 24 hours later. Glycogen is resynthesized after meals when glucose and insulin levels rise. Approximately a dozen enzyme systems are involved in movement of glucose into and out of liver cells, glycogen synthesis and glycogenolysis, and defects of these enzymes impair a variety of organs.[243] Traditionally the glycogen storage diseases (GSDs) are numbered and described as defects of specific enzyme activities, but the discovery that multiple proteins and genes are involved in some of the enzyme activities explains the multiple forms and inheritances of some of the specific GSDs. Several of them can interfere with the maintenance of plasma glucose and cause postabsorptive hypoglycemia (see Table 21-4).

Glucose 6-Phosphatase Deficiency (Types 1a and 1b GSD)

Hydrolysis of glucose 6-phosphate to free glucose, catalyzed by glucose 6-phosphatase, is the final step in both the glycogenolytic and gluconeogenic pathways, and impairment of this activity results in severe postabsorptive hypoglycemia. Although hypoglycemia is present from birth, GSD 1 in its classic form (type 1a, with an estimated incidence of 1:100,000),[244] is more often recognized later in the first or second year of life as the hepatomegaly produces an increasingly protuberant abdomen and the child fails to grow. Metabolic acidosis is common and is caused by marked lactic acidosis and mild ketosis. Levels of triglycerides and cholesterol may be high enough to cause lipemic serum and xanthomas.[245] Hypophosphatemia, hyperuricemia, and anomalies of platelet adhesiveness also characterize GSD 1.

Affected children display a remarkable tolerance to their chronic hypoglycemia. Blood glucose values in the range of 20 to 50 mg/dL are associated with few autonomic or neuroglycopenic symptoms of hypoglycemia, reflecting the adaptation of the CNS to alternative sources of fuel (lactate), and the down-regulation of counter-regulatory autonomic responses with prolonged hypoglycemia. However, insulin is appropriately suppressed, and the counter-regulatory hormones—GH, glucagon, cortisol, and catecholamines—are elevated. These hormonal changes stimulate glycogenolysis and gluconeogenesis to produce glucose 6-phosphate, with lactic acidosis reflecting increased formation and decreased utilization of lactate. The hormonal changes also promote exaggerated lipolysis, resulting in elevations of triglycerides and fatty liver.[245]

Depletion of hepatic ATP and inorganic phosphate increases the rate of uric acid production by stimulating the degradation of preformed nucleotides, and reduced clearance of uric acid due to competition with lactic acid for common shared renal tubular secretory sites results in hyperuricemia severe enough to cause damage to kidneys and joints. The defect in platelet adhesiveness and resulting clotting abnormalities are consequences of the hypertriglyceridemia and may be the consequence of ATP depletion. Although the liver in GSD 1 is laden with glycogen and triglycerides, results of liver function tests remain essentially normal other than mild elevations of serum aspartate aminotransferase levels.[243]

Renal tubules and intestinal mucosa also express the enzyme glucose 6-phosphatase. Renal biopsy reveals excessive glycogen deposition. Long-term renal dysfunction can occur with histologic findings remarkably similar to those found in diabetes (focal glomerulosclerosis). Renal manifestations include kidney enlargement, glomerular hyperfiltration, hypercalciuria, and a mild tubular acidosis.[246] Late renal complications may include Fanconi syndrome, nephrocalcinosis, progressive proteinuria, and eventually end-stage renal failure.[247] Intestinal effects are uncommon but can include diarrhea. Nodular liver enlargement and adenomas often develop after the first decade of life.[248]

The clinical heterogeneity reflects the complexity of hepatic glucose 6-phosphatase, a multicomponent system whose enzyme activity is tightly linked to the inner aspect of the endoplasmic reticular membrane. Glucose 6-phosphatase activity depends on three translocase systems that allow entry of glucose 6-phosphate (T1), exit of phosphate (T2), and exit of glucose (T3) from the lumen of the endoplasmic reticulum. Classic deficiency of enzyme activity is termed *type 1a*, whereas deficiency of translocase T1 is termed *type 1b*. Deficiencies of the T2 and T3 transporters might cause GSD and have been tentatively termed GSD 1c and 1d but have not been definitively documented.[243]

Type 1b occurs in children with biochemical features that are similar to those of type 1a but with the addition of neutrophil deficiency, oral lesions, perianal abscesses attributed to the neutropenia, and chronic enteritis indistinguishable from Crohn disease.[243,249] Treatment with granulocyte-macrophage colony-stimulating factor to increase the neutrophil count ameliorates the enteritis.

GSD 1 should be suspected in children with growth failure, protuberant abdomen due to massive hepatomegaly, or hypoglycemia with lactic acidosis. A finding of elevated lactate levels with hypoglycemia combined with an abnormal fed glucagon stimulation test clinches the diagnosis. In the normal child, a 2-hour postprandial (fed) glucagon stimulation test will cause a rise in glucose of 30 mg/dL within 30 minutes and no change in lactate. In a patient with GSD 1, lactate rises (but not glucose). Fructose 1,6-diphosphatase deficiency resembles GSD 1, but the fed glucagon stimulation test will be normal. Liver biopsy with estimation of glucose-6-phosphatase activity was the classic means of definitive diagnosis, but genetic testing for GSD1a and 1b1 is now commercially available.[250]

Treatment regimens have transformed the prognostic outlook for duration and quality of life and have resulted in the reversal of most of the metabolic disturbances in GSD1.[251,252] Acceptance of these regimens, including passage of a nasogastric tube for nocturnal provision of glucose, is excellent, and the long-term outcome of this innovative approach is promising.[253] Patients are fed every 2 hours during the day and have a continuous supply of glucose through a nasogastric tube overnight. The rate of glucose infusion is tailored to the individual but is usually 6 to 8 mg/kg/min (slightly greater than normal hepatic glucose production).[254] Intermittent cornstarch therapy has been successfully used in patients older than 1 year. Uncooked cornstarch is given in doses of 1 to 2 g/kg every 4 hours, and in some children it is given every 6 hours overnight.[255]

Long-term therapy with uncooked cornstarch and overnight tube feedings improves linear growth and diminishes osteoporosis. Most patients require treatment with allopurinol for hyperuricemia. Although there are no controlled trials of the effect of good metabolic control of GSD on renal disease, some evidence supports the hypothesis that good control lessens the degree of renal impairment.[256] This is assumed from an apparent trend toward later onset of renal disease since the adoption of more effective current treatments.[257] Liver transplantation is not routinely recommended but offers the promise of a long-term cure for patients who develop liver nodules that become malignant.[258]

Amylo-1,6-Glucosidase Deficiency (Debrancher Deficiency, GSD Type 3)

A deficiency of debrancher enzyme activity results in GSD type 3, often affecting both liver and muscle. Children with this disease usually present with hepatomegaly and poor growth. Liver function tests (especially transaminases) may be markedly increased. The *AGL* gene locus is on 1p21, and mutation analysis (at least for common mutations) is available from commercial labs.

These patients have an inability to degrade glycogen beyond the 1:4/1:6 branch point, with the result that only the outer 5% to 10% of glucose residues can be released by phosphorylase and the remaining glycogen accumulates as a limit dextrin. In the fed state, glucagon elicits a normal glycemic response but after liver glycogen reaches the limit dextrin stage during fasting, glucagon no longer raises plasma glucose. This partial defect of glycogenolysis results in the development of hypoglycemia and ketosis during fasting, though postabsorptive hypoglycemia is less common and less severe than in GSD type 1. Because gluconeogenic pathways are intact, lactic acidosis, hyperuricemia, and severe lipemia do not occur. Both hypoglycemia and liver disease tend to improve substantially after puberty.

However, debrancher enzyme is a large enzyme with two active sites, with the various mutations resulting in a range of possible clinical effects in muscle and other tissue. Creatinine phosphokinase is often elevated in children with GSD 3 but does not always predict severe myopathy. Children with GSD 3 often have subclinical muscle weakness with subtle signs, but significant proportion will develop more severe and debilitating myopathies in the third decade of life. Severe cases may involve life-threatening cardiomyopathy.

Two dietary regimens have been advocated for GSD type 3.[259] For those with liver involvement alone, frequent feedings and uncooked cornstarch have been recommended in the expectation that an avoidance of prolonged fasting will be sufficient to prevent hypoglycemia and improve growth.[260] However, in patients with liver and muscle involvement, the use of a low-carbohydrate, high-protein diet has been advocated—with the argument that providing glucose by gluconeogenesis from protein precursors reduces the need to deplete muscle alanine stores. There are no large controlled trials comparing the efficacies of the two approaches. Nasogastric or intravenous glucose infusions may occasionally be indicated if caloric intake cannot be maintained during intercurrent illness.

Liver Phosphorylase and Phosphorylase Kinase Deficiency (GSD Types 6 and 9)

Phosphorylase is the key enzyme of glycogenolysis. Deficient activity results in several forms of glycogen storage disease with hepatomegaly and postabsorptive or fasting hypoglycemia. Phosphorylase requires activation by phosphorylase kinase, and similar clinical problems can result from genetic defects of phosphorylase kinase. Disease resulting from mutations of the *PYGL* gene for phosphorylase itself is referred to as GSD 6.[261] Phosphorylase kinase is a large protein consisting of four copies of four different subunits, encoded by four genes.[243] Liver disease resulting from mutations of the genes for phosphorylase kinase is referred to as GSD 9, and three forms have been found to cause hypoglycemia (see Table 21-4).[262,263] Several attempts to number and distinguish these forms of GSD before the underlying gene defects were entirely understood have left a history of contradictory numbering systems and nomenclature.[243]

Although the different forms of GSD 6 and 9 are clinically similar, there is some heterogeneity of features and severity.[262] Hepatomegaly from excessive deposition of glycogen, some growth retardation, and occasional symptomatic hypoglycemia occur. The hypoglycemia is milder than that of GSD 1, and it is not accompanied by marked lactic acidosis or hyperuricemia. Patients with GSD 9b gradually develop a myopathy as well as the liver disease. Gene tests are commercially available. Frequent feedings with a diet low in sugar and higher in complex carbohydrates and protein will suffice for most. Some will need parenteral glucose support for illness or surgery. Patients with GSD 6 and 9 generally do very well, but a few have more severe hypoglycemia with intermittent elevations of lactic acid, and there has been a report of liver cirrhosis in a patient with GSD type 9.[263,264]

Glycogen Synthase Deficiency

Impairment of glycogen synthesis results in reduced rather than excessive glycogen storage, and it does not result in liver enlargement but can still cause intermittent hypoglycemia. Although less common than some of the other GSDs, enough cases of glycogen synthase deficiency have been reported to describe the phenotype.[265,266] Glycogen synthase activity is markedly reduced in liver but normal in muscle. Infants and children with GSD 0 may have symptoms of fasting-induced hypoglycemia and hyperketonemia from infancy. However, a high-carbohydrate meal may result in transient hyperglycemia with glucosuria after meals.[267]

During fasting hypoglycemia, levels of the counterregulatory hormones, including catecholamines, are appropriately elevated or normal—whereas insulin levels are appropriately low. Exogenously administered glucagon produces a glycemic response soon after meals but no response after a 12-hour fast because glycogen synthase activity is markedly reduced in liver but normal in muscle so that the glycemic response soon after meals represents the release of glucose from muscle glycogen stores. Gluconeogenic capacity is intact. In the index case, protein-rich feedings at frequent intervals resulted in dramatic clinical improvement, including improved growth. Since the initial case reports, more patients have been described. Treatment with uncooked cornstarch has been helpful. Although the majority of cases are recessive mutations, one pedigree has been reported in whom the mother of two affected children had some hypoglycemia. This condition mimics the syndrome of ketotic hypoglycemia and should be considered in the differential diagnosis of that syndrome.

Glycogen Branching Enzyme Deficiency (Type 4 GSD)

Deficiency of glycogen branching enzyme results in Andersen disease. The impairment of glycogenolysis is minimal and gluconeogenesis is normal, so hypoglycemia

is a rare problem in infancy. However the progressive liver damage is more severe than in other GSDs, and fasting hypoglycemia has been reported with advanced liver disease in GSD 4.[243]

Fanconi-Bickel Syndrome

Fanconi-Bickel syndrome is an autosomal recessive disease caused by mutations of the *GLUT2* gene that encodes the principal glucose transporter of the hepatocyte membranes.[268] Movement of glucose across the membrane of liver cells is necessary for glucose homeostasis and defects of the membrane glucose transporter can result in both hypoglycemia and glycogen accumulation in the liver. The hypoglycemia is fasting and often accompanied by ketosis. It is attributed to a combination of impaired transport of glucose from the hepatocyte cytoplasm to the blood, and to excessive urinary glucose loss. Postprandial hyperglycemia can occur. The principal renal manifestation is defective proximal tubular reabsorption (Fanconi syndrome), with glucosuria and phosphaturia. Phosphaturia can be severe enough to cause hypophosphatemic rickets. Growth in childhood is poor. Treatment consists of avoidance of excessive sugar, and cornstarch if necessary to support adequate glucose and growth.

Ketotic Hypoglycemia

"Hypoglycemia with ketosis" is a diagnostic category and "ketotic hypoglycemia" a specific diagnosis. Ketosis accompanying new hypoglycemia suggests it has not been caused by any of the many varieties of hyperinsulinemic and reactive hypoglycemia, though not infallibly.[269] A single episode of hypoglycemia and ketosis can occur during the stress and anorexia of an illness like gastroenteritis.[270] Hypoglycemia with ketosis can also occur, sometimes repeatedly until diagnosed, from a variety of inborn errors of carbohydrate metabolism, hormone deficiencies, ingestions, and prolonged starvation. However, most otherwise healthy young children who suffer repeated episodes of morning hypoglycemia and ketosis will be diagnosed with *ketotic hypoglycemia* with no other underlying disease.[271]

Ketotic hypoglycemia has been recognized for nearly a century as a common type of childhood hypoglycemia,[272] with a well-characterized presentation and course but an incompletely understood pathophysiology.[273-275] Usually this condition presents as recurrent episodes of morning hypoglycemia in the second or third year of life—but onset as early as 6 months has been reported. The condition usually remits spontaneously by the age of 8 to 9 years. The classic history is of a child who has eaten poorly or misses an evening meal, is difficult to rouse from sleep the next morning, and displays neuroglycopenic symptoms that may range from lethargy to seizure. Hypoglycemic episodes are especially likely to occur during an illness, when food intake is limited.

At the time of documented hypoglycemia, high levels of ketones are found in plasma and urine and plasma insulin concentrations are low (typically under 2 μU/mL using a high-sensitivity assay). It remains unsettled whether these children display an accelerated, but qualitatively normal, metabolic response to fasting (i.e., one tail of the normal distribution) or whether ketotic hypoglycemia is a heterogeneous group of metabolic disorders of limited substrate availability awaiting further delineation.[276,277]

Several studies have shown that ketotic hypoglycemia reflects underproduction rather than overutilization of glucose.[278] Children with ketotic hypoglycemia have plasma alanine concentrations that are reduced in the basal state after an overnight fast and fall still farther with prolonged fasting.[21,279] Infusions of alanine, fructose, or glycerol produce a rise in plasma glucose concentration without significant changes in blood lactate or pyruvate levels, indicating that the entire gluconeogenic pathway from the level of pyruvate is intact, and suggesting that a deficiency of substrate rather than a defect in gluconeogenesis is involved.[280]

Glycogenolytic pathways are also intact because glucagon induces a normal glycemic response in affected children during the fed state but not at the time of hypoglycemia. Plasma glycerol levels are normal in these children, in both the fed and fasted states. The metabolic response to infusion of β-hydroxybutyrate does not differ from that of normal children. Finally, the levels of hormones that counter hypoglycemia are appropriately elevated, whereas insulin levels are appropriately low.[275]

Alanine is the major amino acid used for gluconeogenesis. Its formation and release from muscle during periods of caloric restriction is enhanced by the presence of a glucose-alanine cycle, as well as by de novo formation from other substrates such as branched chain amino acids. Although defects in any of the complex steps involved in protein catabolism, oxidative deamination of amino acids, transamination, alanine synthesis, or alanine or glutamine efflux from muscle could contribute to ketotic hypoglycemia, no specific defects can be demonstrated in the majority of children with ketotic hypoglycemia. It was pointed out in the original description of this syndrome that children with ketotic hypoglycemia are frequently smaller than age-matched controls and often have a history of transient neonatal hypoglycemia. Thus, a compromised supply of gluconeogenic substrate may simply reflect the reduced reserve of a small muscle mass at an age when glucose demands per unit of body weight to support brain metabolism are relatively high.

Ketosis represents the attempt at switching to an alternate fuel supply. Those with ketotic hypoglycemia may represent the low end of the spectrum of capacity to tolerate fasting.[277] Similar relative intolerance to fasting is present in normal children, who cannot maintain normal blood glucose levels after 20 to 36 hours of fasting (compared with the capacity of adults for prolonged fasting). Spontaneous remission by age 8 to 9 years might be explained by the increase in muscle bulk relative to brain size, with a resultant increase in the supply of endogenous substrate and the relative decrease in glucose requirement per unit of body mass with increasing age.

Ketotic hypoglycemia should be considered only a diagnosis of exclusion, as episodic hypoglycemia with ketosis can occur with deficiencies of several hormones or a variety of defects of gluconeogenesis or glycogen metabolism, especially hypopituitarism and glycogen storage diseases.[281,282] The diagnosis of ketotic hypoglycemia is confirmed by a

supervised fast. Hypoglycemia with elevated plasma free fatty acids, β-hydroxybutyrate, and acetoacetate develops within 14 to 24 hours in most of these children, whereas normal children of similar age can withstand fasting without developing hypoglycemia for at least 24 hours.

Episodes of ketotic hypoglycemia can be prevented or minimized by avoidance of prolonged fasting. The overnight fast should be shortened to less than 10 to 12 hours with a carbohydrate bedtime snack and prompt breakfast. When episodes have been triggered by illness, parents may test the child's urine for ketones—the appearance of which precedes the hypoglycemia by several hours and indicates a need for high-carbohydrate liquids. If these cannot be tolerated, the child should be taken to the emergency department for intravenous glucose. A letter of explanation and treatment recommendation may expedite the emergency department response.

Hormone Deficiency

Four primary counter-regulatory hormones—glucagon, epinephrine, cortisol, and growth hormone—help maintain blood glucose levels and can produce hyperglycemia in excess. In experimental circumstances, glucagon and epinephrine are the primary hormonal defenses against hypoglycemia, yet spontaneous deficiencies of only cortisol and GH are clearly demonstrable causes of clinically significant hypoglycemia in childhood.

Growth Hormone Deficiency and Hypopituitarism

Although growth hormone has been shown to play only a minor role in defending glucose levels against acute insulin-induced hypoglycemia, it is more important in supporting glucose during prolonged fasting by limiting insulin sensitivity, reducing glucose utilization, and inducing lipolysis.[283-285] The occurrence of symptomatic and asymptomatic hypoglycemia in both isolated growth hormone deficiency and in multiple pituitary deficiencies was recognized as soon as growth hormone was measurable and the clinical features of hypopituitarism were delineated.[286,287-289]

In the newborn period, hypopituitarism can produce persistent or recurrent hypoglycemia.[290] Some infants may require a glucose infusion rate high enough to resemble congenital hyperinsulinism.[291] In males, a microphallus may provide a clue to coexistent pituitary gonadotropin deficiency. Neonatal jaundice is a common accompaniment of congenital hypopituitarism and may result from either indirect or combined hyperbilirubinemia. Cholestatic jaundice with liver enzyme elevation may suggest neonatal hepatitis but can occur solely from congenital hypopituitarism.[292] The diagnosis of congenital growth hormone deficiency cannot be based on a single growth hormone obtained during hypoglycemia[293] but is usually based on a combination of clinical evidence, including pituitary or other midline defects by imaging.[294]

Morning hypoglycemia with ketosis is a potential presenting indicator of isolated idiopathic GH deficiency in young children.[295,296] Insulin levels are quite low, and glycemic response to glucagon during spontaneous hypoglycemia is poor. However, the degree of ketosis at time of hypoglycemia may be lower than in the more common type of ketotic hypoglycemia, perhaps reflecting the increased insulin sensitivity, and this relative deficiency of alternate fuel generation may make the hypoglycemia of GH deficiency more dangerous to the brain.[297] Low GH levels at the time of spontaneous or fasting-induced hypoglycemia are suggestive of deficiency, though this is an inconsistent and often unreliable finding.[293,298] Poor growth and low IGF-1 levels may be more reliable clues after early infancy, and the diagnosis of GH deficiency severe enough to cause hypoglycemia can be confirmed by low GH responses to standard provocative tests. Hypoglycemia has been reported in older children and adults with either isolated GH deficiency or multiple pituitary deficiencies, usually precipitated by stress, starvation, or exercise.[288,299]

Growth Hormone Resistance and IGF-1 Deficiency

Recurrent fasting hypoglycemia is common in the most extreme form of growth hormone resistance due to genetic absence of the GH receptor (Laron dwarfism).[300,301] The hypoglycemia tends to improve in adolescence, though still can occur with prolonged fasting. Similar frequencies of hypoglycemia (45% or more) have been reported in the two largest populations with this condition in Israel and Ecuador, suggesting it may not be the entire explanation for the lower intelligence levels described in the Israeli patients. The vulnerability to fasting hypoglycemia improves with treatment with synthetic insulin-like growth factor 1 (mecasermin), though one of the insulin-like effects of the treatment can be hypoglycemia within an hour of the injection if the child has not eaten.

Cortisol and Adrenocorticotropin Deficiencies

Although cortisol seems to play a secondary role in counter-regulatory responses to acute hypoglycemia, it supports glucose production during fasting and stress by increasing gluconeogenic enzyme activities and by mobilizing precursors.[4,302] Cortisol also plays an indirect role in hypoglycemia defense by enabling epinephrine release from the adrenal medullas: activity of phenylethanolamine N-methyl transferase (PNMT), the enzyme that converts norepinephrine to epinephrine in the adrenal medulla, depends on cortisol.[303] Cortisol deficiency amplifies insulin sensitivity, so the diminishing insulin requirement and increasing hypoglycemia is a long-recognized presentation of Addison's disease in developing in insulin-dependent diabetes.[304]

Fasting or postabsorptive hypoglycemia can occur in all forms of glucocorticoid deficiency, but it is more common in some patients and circumstances than others. Younger patients, especially infants, are more vulnerable than adolescents and adults.[305] Hypoglycemia is most likely to occur after prolonged fasting or during the stress of illness, especially if the usual daily glucocorticoid replacement has been interrupted by illness.[306,307]

ACTH deficiency or unresponsiveness is more likely to produce hypoglycemia than primary adrenal failure, especially as part of congenital hypopituitarism with growth

hormone deficiency. Congenital ACTH deficiency presenting with early hypoglycemia has been reported with mutations of several genes involved in pituitary development (POU1F1, PROP1, TPIT).[294,308,309] Some of the patients have had other pituitary deficiencies. In some of them, ACTH insufficiency was not clinically apparent in early infancy when initial testing was performed, but it became clinically apparent later in childhood.

An increasing number of specific genetic defects resulting in isolated ACTH deficiency or resistance have been identified as well. Red hair and early-onset obesity are better known than ACTH deficiency in POMC mutations, but at least one death has been described.[310] Hypoglycemia in an infant due to ACTH deficiency has been attributed to a defect of prohormone cleavage.[311] Idiopathic ACTH deficiency can be acquired in childhood, adolescence, or later adult life.[312] Circumstantial evidence such as an association with autoimmune thyroiditis suggests autoimmune hypophysitis as a common cause. In some instances, antipituitary antibodies have been demonstrated.[313] Hyperpigmentation accompanies other manifestations of adrenal insufficiency in ACTH resistance.[314]

Iatrogenic suppression of adrenal function is one of the most common causes of glucocorticoid insufficiency, but it may be overlooked because the obvious electrolyte abnormalities of mineralocorticoid deficiency are absent. An adrenal crisis with prostration, vomiting, hypotension, and hypoglycemia can be triggered by a stressful event in a child recently weaned from high-dose glucocorticoid therapy and has been reported even on alternate day low-dose therapy.[232] More often, hypoglycemia and milder manifestations may be caused by the inhaled and nasal glucocorticoid preparations that have become the mainstay of allergy treatment for millions of otherwise healthy children.[233] Although most tolerate it well, ongoing combined use of topical and inhaled glucocorticoid preparations can cause enough adrenal suppression to produce episodic hypoglycemia and interference with growth in older children.[315] Absence of the 0800 cortisol peak in a child receiving topical or inhaled glucocorticoids suggests this possibility.

Hypoglycemia is a less common presentation of primary adrenal insufficiency, but it often occurs during illness or starvation in patients already being treated with replacement glucocorticoids.[305,306] Primary adrenal insufficiency often accompanied by deficient epinephrine responses to hypoglycemia, and hypoglycemia manifested by neuroglycopenic rather than adrenergic symptoms may be less recognizable.[316] The most common form of primary adrenal insufficiency is congenital adrenal hyperplasia, and surveys and case reports document hypoglycemia as a relatively frequent manifestation during treatment.[305,317,318]

Diagnosis of cortisol or ACTH deficiency as a cause of a child's hypoglycemia cannot be based on a single low cortisol during hypoglycemia, but on other clinical evidence or standard adrenal stimulation tests.[293] Primary adrenal insufficiency is best sought by measurement of ACTH concentrations, which will be elevated even when the cortisol concentration itself remains in the normal range, similar to the elevation of TSH that occurs in early (subclinical) hypothyroidism while T_4 or fT_4 is still within the normal range.

Epinephrine and Glucagon Deficiencies

The importance of epinephrine and glucagon in the counter-regulatory responses to hypoglycemia suggests that isolated deficiency of either hormone would be likely to cause hypoglycemia, but no instances of hypoglycemia caused by isolated, primary deficiency of either hormone have been well-demonstrated in children.

Although there have been case reports of reduced epinephrine excretion in patients with hypoglycemia, evidence from patients with diabetes and insulinomas suggests that repeated hypoglycemia is more likely to cause diminished epinephrine excretion than vice versa. A reduction in insulin-induced hypoglycemia restores normal catecholamine responses. Much attention was paid to below average epinephrine responses in ketotic hypoglycemia in the early investigations, but no primary defects of epinephrine were demonstrated.[319,320] Defective epinephrine responses accompany adrenal insufficiency but can be at least partly restored by adequate glucocorticoid replacement.

Perhaps the best candidate for an example of hypoglycemia caused by epinephrine deficiency in childhood is the occasional episode of severe hypoglycemia, usually fasting, occurring in a young child taking propranolol or another beta-blocker (either therapeutically or accidentally).[321]

Impaired glucagon secretion also increases the vulnerability to hypoglycemia of people with type 1 diabetes, but no well-documented case of childhood hypoglycemia due to isolated glucagon deficiency has been reported. Insulin within islets is a major regulator of glucagon secretion, such that most forms of hyperinsulinism are accompanied by reversible suppression of glucagon. [125] The case most often cited has since been recognized as an instance of familial SCHAD hyperinsulinism.[126,127]

Genetic Disorders of Gluconeogenesis

In the early postabsorptive hours, glucose is maintained by both glycogenolysis and gluconeogenesis. As fasting continues more than 12 to 16 hours and liver glycogen is depleted, gluconeogenesis contributes an increasing proportion of circulating glucose. Alanine from muscle is the principal early substrate for gluconeogenesis, but lactic acid, oxidized to pyruvic acid, also contributes to the maintenance of normoglycemia via the Cori cycle, especially in larger children and adults. While substrate shortage can result in ketotic hypoglycemia during fasting, genetic defects of the four major enzymes of gluconeogenesis (pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase, and glucose 6-phosphatase) can do so as well. Deficiency of glucose 6-phosphatase produces the severe hypoglycemia of type 1 GSD, described previously. Lactic acidosis is a common feature of these defects of the last steps of gluconeogenesis.

Pyruvate Carboxylase Deficiency

Pyruvate carboxylase is a biotin-containing protein of four subunits that binds pyruvate, ATP, HCO_3, and acetyl CoA, and produces oxaloacetate. It is a major regulatory enzyme

at the beginning of the gluconeogenic pathway. Activation is dependent on acetyl CoA, and occurs principally during mobilization of fatty acids during fasting. Several forms of deficiency have been described, typically presenting as severe lactic acidosis and encephalopathy in early infancy, usually with metabolic decompensation during illness.[322] Hepatomegaly is common. Hypoglycemia has been reported in several forms of pyruvate carboxylase deficiency but is not invariably present.[323,324] Urine contains large amounts of α-ketoglutarate. The diagnosis can be confirmed by direct sequencing of the *PC* gene on 11q13. Management consists of frequent carbohydrate meals and intravenous dextrose support during illness.

Phosphoenolpyruvate Carboxykinase Deficiency

Phosphoenolpyruvate carboxykinase (PEPCK) enables conversion of oxaloacetate to phosphoenolpyruvate. Cytosolic and mitochondrial isoforms are encoded by *PEPCK1* and *PEPCK2*, respectively. Autosomal recessive deficiencies of both have been described with hypoglycemia but are rare.[322] Deficiency of the cytosolic form was described in 1976 in a patient with severe hypoglycemia and fatty infiltration of the liver.[325,326] The hypoglycemia was found in the second day of life and had features of hyperinsulinism, but did not respond to diazoxide. Deficiency of the mitochondrial form in several children has caused lactic acidosis, hypoglycemia, and liver failure in the first year of life.[327]

Fructose 1,6-Diphosphatase Deficiency

Fructose 1,6-diphosphatase (FDPase, or fructose 1,6-bisphosphatase) catalyzes the irreversible splitting of fructose 1,6-diphosphate to fructose-6-phosphate. It is encoded by *FBP1* on chromosome 9q22.32. This is a key enzyme of gluconeogenesis; deficiency impairs the formation of glucose from lactate, glycerol, and gluconeogenic amino acids such as alanine. Some of the clinical features of deficiency of FDPase resemble those of deficiency of glucose 6-phosphatase (GSD 1), another major enzyme of gluconeogenesis, though glycogenolysis is not affected in FDPase deficiency.

Hypoglycemia due to FDPase deficiency was first described in 1970 by Baker and Winegrad.[328] FDPase deficiency presents typically in the first days of life, but up to 50% of cases present later than this. Typical manifestations include hyperventilation from lactic acidosis and ketoacidosis, seizures and coma from hypoglycemia, and hepatomegaly. Attacks are precipitated in infancy and childhood by prolonged fasting and intercurrent illness rather than by fructose ingestion. Unlike in hereditary fructose intolerance, liver dysfunction and renal tubular dysfunction are rare. Uric acid is elevated. The diagnosis is suspected when fasting leads to hypoglycemia and lactic acidosis, with poor glycemic response to glucagon. The diagnosis may be confirmed by enzyme studies from liver biopsy material or by gene test. It may be differentiated from GSD type 1 by the fed glucagon stimulation test. Acute treatment is by intravenous glucose and bicarbonate infusion. Chronic treatment is avoidance of prolonged fasting and a reduction but not total elimination of fructose from the diet.[329]

Hereditary Fructose Intolerance

Hereditary fructose intolerance (HFI) was first described as an idiosyncrasy to fructose[330] and was later determined to be caused by mutation of the gene for aldolase B (*ALDOB* at 9q31.1).[331,332] Aldolase splits fructose 1-phosphate to dihydroxy-acetone phosphate and glyceraldehyde, substrates for gluconeogenesis via fructose 1,6-diphosphate. Fructose 1-phosphate accumulates in liver and kidney cells causing phosphate depletion and gradual long-term organ damage. Phosphate depletion further contributes to hypoglycemia by impairing glycogenolysis.[333] Although discussed here with FDPase deficiency, the hypoglycemia of HFI occurs in the hours after fructose ingestion and in timing is more a reactive than a fasting hypoglycemia.

HFI presents in infancy, after fructose is introduced to the diet in fruit or sucrose. Fructose ingestion induces vomiting, abdominal pain, diarrhea, and hypoglycemia.[334] With substantial ingestion, lactic and uric acids and magnesium rise, and phosphorus, potassium, and bicarbonate fall. Severity of symptoms is somewhat proportional to the amount of fructose and in early infancy large amounts may produce shock, acute liver failure, and death. Acute treatment of hypoglycemia by intravenous glucose rapidly reverses symptoms, and the patient remains healthy if fructose exposure is discontinued. Chronic fructose ingestion causes failure to thrive and increasing liver dysfunction. The earliest renal effect is proximal tubular damage with glucosuria and phosphaturia, but prolonged exposure to fructose can lead to kidney failure.

Fructose challenge is potentially dangerous and not necessary to make the diagnosis as the gene test is commercially available if clinical evidence suggests HFI.[329] Chronic treatment is the avoidance of all fructose-containing food (such as sucrose and high-fructose corn syrup); as sorbitol is metabolized to fructose, it needs to be excluded as well.[329]

Defects of Fatty Acid Oxidation

Fatty acid oxidation (FAO) provides a significant proportion of energy required to sustain normal glucose levels during fasting. Glycerol serves as a direct substrate for gluconeogenesis, and mitochondrial oxidation of acyl-CoA derived from free fatty acid metabolism produces ketones (β-hydroxybutyrate [BOHB] and acetoacetate [AcAc]) and reduces glucose consumption by muscle and other peripheral tissues. The key steps in carnitine uptake, mitochondrial transport, and oxidation of fatty acids are outlined in Chapter 6. The spectrum of genetic disorders of mitochondrial fatty acid transport and oxidation is remarkably wide, with specific disorders affecting every organ system at any age to produce many different presenting problems. Newborn screening and improved methods of investigation are demonstrating that disorders of fatty acid metabolism and mitochondrial function are relatively common.[335]

Hypoglycemia can be severe and persistent, manifest in the first days of life (Chapter 6), or may appear at several months of age as feeding intervals lengthen. Defects of FAO are especially likely to become clinically apparent

during the accelerated starvation of catabolic illness associated with vomiting. Because FAO and ketone body generation is impaired, hypoglycemia associated with FAO disorders is usually hypoketotic. The hypoglycemia may be accompanied by an encephalopathy that has mimicked Reye syndrome and does not always immediately respond to glucose.[336,337] Unrecognized, such episodes may be fatal and may resemble sudden infant death syndrome.[338] Diagnosis is often suggested an abnormal plasma acylcarnitine profile or abnormal urine organic acids (especially dicarboxylic aciduria). Confirmation can be made by metabolic studies of liver tissue or cultured fibroblasts and increasingly by genotyping.

Cardiac and skeletal muscle weakness is a prominent feature with certain defects, principally long-chain acyl CoA dehydrogenase, carnitine transport deficiency, and carnitine transferase deficiency. In patients with carnitine transport deficiency, supplementation with carnitine may improve the clinical features—including improvement in the cardiomyopathy over a period of weeks to months.[339]

The primary treatment of these disorders of FAO is avoidance of fasting. In older children, fasting of as long as 10 to 12 hours may be possible without the manifestations. Once an index case has been identified, acute illness with fasting should be treated by rapid institution of intravenous glucose to reverse an evolving episode or to prevent its occurrence.

Several drugs or chemicals that interfere with FAO may mimic the features of inherited defects in FAO, including hypoglycemia. Principal among these is the use of valproic acid, which has been associated with unusual episodes of hypoglycemia with a Reye-like syndrome and hypoketosis. Elevation of ammonia levels and secondary carnitine deficiency may make this side effect appear similar to an FAO defect, leading some to recommend carnitine as a supplement for infants receiving valproic acid.[99] Urine organic acids and an acyl carnitine profile will easily distinguish these conditions.

Plant toxins can occasionally produce hypoglycemia by impairing fatty acid oxidation as well. Hypoglycine, a component of unripe ackee fruit found in the Caribbean and Africa, causes hypoglycemia by interfering with FAO.[100] Atractyloside, found in certain Mediterranean plants, uncouples oxidative phosphorylation in the mitochondria.[101]

Defects of Organic or Amino Acid Metabolism

Hypoglycemia may occur with several inborn errors of organic acid and amino acid metabolism. Many of these are diagnosed in the newborn period by extended newborn screening tests based on mass spectrometry. In some patients with defects of organic or amino acid metabolism, hypoglycemia is a common manifestation presenting in early infancy; in most of the others it is atypical, infrequent, or mild. [340]

Hypoglycemia usually occurs during episodes of encephalopathic metabolic decompensation, sometimes spontaneous and sometimes triggered by a stressful illness or starvation. Vomiting and sometimes tachypnea may be prominent symptoms. Many of the conditions result in impairment of physical growth or neuromuscular development even when severe episodes are infrequent. The major clue to diagnosis is usually a pattern of characteristically abnormal organic acids in the urine. In most cases, management (or at least hypoglycemia prevention) consists of providing intravenous glucose at the onset of illness or at the earliest signs of decompensation.

Hypoglycemia also occurs in association with advanced liver disease in neonatal hemochromatosis, tyrosinosis type 1, S-adenosylhomocysteine hydrolase deficiency, type 3 glycogenosis, and some of the disorders of mitochondrial function. Though galactosemia results in a depletion of hepatic phosphate that is similar to the aldolase deficiency of hereditary fructose intolerance, hypoglycemia is uncommon until liver damage is advanced.

Hypoglycemia accompanied by ketosis and lactic acidosis has also been reported in late-onset maple syrup urine disease and glycerol kinase deficiency. In disorders of ketolysis (deficiencies of succinyl CoA transferase and 3-ketothiolase), ketone levels may be quite high but lactic acidosis may not be present.

Hypoglycemia in Fasting, Starvation, Illness, and Stress

Under controlled conditions, well-nourished children can maintain glucose above 70 mg/dL (3.9 mM) for increasing durations between meals. As fasting is prolonged and ketosis supplies a greater proportion of fuel, plasma glucose levels will fall below 50 mg/dL (2.8 mM) in some healthy children and young women without autonomic or neuroglycopenic symptoms or apparent ill effects.[17,22,341] This is occasionally found with preoperative fasting.[342,343] In uncontrolled, real-life illnesses, alterations of carbohydrate metabolism can occur that are less predictable and benign. Most operate to enhance peripheral glucose uptake and utilization, to increase glucose production, and to depress glycogen formation and insulin sensitivity, resulting in hyperglycemia or hypoglycemia, depending on the patient's nutritional state, severity and duration of illness, and other factors.[344] Rewarming after hypothermia and salt-water near-drowning are examples of severe stresses in which hypoglycemia has been repeatedly observed.[345,346]

Hypoglycemia During Starvation and Malnutrition

The ability to maintain euglycemia is compromised by malnutrition, and hypoglycemia in malnourished infants and adults can be both dangerous and difficult to reverse, even with intravenous glucose.[347,348] In African children with kwashiorkor, hypoglycemia has been reported to be both common and a predictor of death.[349] In the developed world, anorexia nervosa is one of the most common causes of severe malnutrition and hypoglycemia is common and occasionally severe.[350-353]

Hypoglycemia with Prolonged Exercise

Glucose is utilized at a higher rate by muscle during prolonged exercise. Insulin sensitivity is acutely enhanced, at least partly by myocytes secreting a peptide (irisin) that stimulates energy expenditure by adipocytes.[354,355] Hypoglycemia is normally prevented by increased secretion of

catecholamines.[356-358] However, hypoglycemia has long been recognized as an occasional effect of intense exercise even in presumably healthy adults and can be prevented by complex carbohydrates but not extra sugar.[359] Cases of hypoglycemia severe enough to cause seizures after a marathon or comparable exercise have been reported.[360-362] Demonstration that exercise can acutely reverse the compensatory insulin resistance of starvation provides a likely explanation for many. [363] With the discovery of exercise-induced hyperinsulinism (described previously), it may be that at least some of these instances reflect unsuspected disorders of metabolism.[137]

Diarrheal Illness

Hypoglycemia may occur in infants and children with severe diarrhea.[270] It is uncommon in previously healthy children with acute viral gastroenteritis (e.g., rotavirus) unless there has been a period of starvation (as occurs after prolonged use of water or sugar-free fluids).[364] Hypoglycemia in this setting is usually ketotic and has a good outcome. However, it is important that the history of prolonged starvation be confirmed and disorders of fatty acid oxidation be ruled out, as hypoglycemia is uncommon in healthy children. In one survey of sequential emergency department patients with hypoglycemia, 28% had a significant unsuspected metabolic or hormonal disorder.[281]

When diarrhea develops in malnourished children, such as during cholera or shigella outbreaks in the Third World, hypoglycemia is a dangerous problem.[365,366] During severe malnutrition, gluconeogenic substrates such as alanine and lactate are significantly reduced, the capacity to generate glucose by gluconeogenesis is markedly diminished, and alternative fuels such as ketones and lactate are also reduced. In this situation, almost half of patients with hypoglycemia plus diarrhea died with an encephalopathic picture—indicating that hypoglycemia in the malnourished child is a poor prognostic sign.[367]

Sepsis

Severe hypoglycemia is a potential presentation of sepsis, especially meningococcemia.[368,369] Animal research suggests that septicemia may produce early hyperglycemia followed by a drop of blood sugar attributed to increased glucose uptake by multiple organs, and that cytokines like tumor necrosis factor may amplify glucose uptake.[370] Limited measurements on humans indicate appropriate suppression of insulin and elevation of counter-regulatory hormones as well as cytokines.[371]

Specific Infections

Hypoglycemia has been described as occurring in approximately one third of children with severe malaria.[372] As in those with diarrhea and hypoglycemia, there is increased mortality in children with malaria associated with hypoglycemia. Insulin levels in such children were reported to be appropriately low, whereas ketones, lactate, and alanine levels were high—suggesting impaired gluconeogenesis.[373] Provision of glucose is therefore indicated for such patients. In addition, the therapy for malaria (in particular, quinine) may aggravate hypoglycemia because of its ability to stimulate insulin release.[374]

Hypoglycemia has been repeatedly reported in pertussis infection, with some animal evidence suggesting a hyperinsulinemic hypoglycemia rather than simply a fasting effect.[375] Though infant insulin levels after pertussis immunization have been reported slightly higher, no instances of hypoglycemia have been attributed to pertussis immunization.[171]

Hypoglycemia in Organ System Disease

Hypoglycemia is common in critically ill patients of all ages[376,377] and can occur in failure or severe disease of nearly every major organ system.

The liver is the principal source of glucose during postabsorptive periods, and under experimental conditions, hypoglycemia occurs after a loss of 80% of the liver. Hypoglycemia in human liver disease is less predictable. In cirrhosis and progressive liver failure, glucose levels usually remain normal even into hepatic coma. However, fasting hypoglycemia can occur sporadically in many forms of liver disease, with little dependence on the severity of liver impairment by other measures.

Hypoglycemia has been reported in adults and children with drug injury, poisoning,[378] infectious hepatitis,[379,380] suppurative cholangitis,[381] and poor hepatic perfusion.[382,383] Hypoglycemia can occur as an occasional complication of various rare genetic diseases that affect the liver. For example, citrin deficiency has an intermediate, childhood-onset form characterized by growth retardation, episodic neurologic and behavioral abnormalities with hyperammonemia, and sometimes hypoglycemia.[384]

Renal gluconeogenesis also normally contributes to maintenance of blood glucose during fasting, and hypoglycemia may occur in patients with end-stage chronic renal failure.[382,385-387] Because autonomic responses to hypoglycemia are often impaired in chronic renal failure, neuroglycopenic manifestations typically predominate. In many cases, hypoglycemia occurs as a complication of dialysis or other circumstances and the existence of a specific "uremic hypoglycemia" has been challenged.[388]

Many cases of fasting hypoglycemia associated with both acute and chronic pancreatitis have been reported in adults and children.[364,389-391] Both hypoglycemia and hyperglycemia were recognized complications of mumps pancreatitis in children.[390,392]

The causes of hypoglycemia associated with severe heart disease, both cyanotic congenital heart disease in young children[393] and congestive heart failure in older children,[394-396] are complex, with evidence of both increased glucose uptake and impaired gluconeogenesis. In infants, hypoglycemia can cause heart failure that improves with restoration of normal glucose levels.[397]

Skeletal muscle contributes substrates to gluconeogenesis during fasting, and fasting hypoglycemia occurs with several forms of muscular dystrophy and spinal muscle atrophy characterized by reduced muscle mass.[398-401] A novel mechanism for hypoglycemia due to intracranial disease would be alteration of the afferent or efferent limbs of hypothalamic glucose sensing; this can be demonstrated in rodents and has been invoked in

rare cases of hypoglycemia accompanying brain tumors and trauma.[402,403]

Hypoglycemia accompanied by lactic acidosis has been reported as both uncommon presentations and end-stage events in patients with acute and chronic leukemias and lymphomas.[404] However, children on prolonged chemotherapy regimens have been found to have a high rate of fasting hypoglycemia due to depleted glycogen and gluconeogenic precursors and to direct effects of oral purine analogs.[405-407]

Hypoglycemia in the Intensive Care Unit

New, symptomatic hypoglycemia in a previously well child is uncommon and usually attributable to a single, identifiable diagnosis. In contrast, hypoglycemia occurs in up to 10% of critically ill children, is often asymptomatic, and is associated with poorer outcomes.[376,408] In addition to processes specific to the underlying diseases, multiple contributing factors may include substrate depletion, accelerated glucose consumption, undernutrition, impaired gluconeogenesis, cytokine effects, and adrenal insufficiency. Iatrogenic factors such as misplaced infusion lines, changes of intravenous dextrose, or drug effects need to be considered.[409] Factors affecting measurement reliability (e.g., altered hematocrit, oxygenation, line draws, drugs) are more common in the intensive care unit (ICU).[410] Finally, the treating physician should not forget the possibility that an acute illness is unmasking a previously compensated disorder of glucose metabolism, especially in a young child.[281]

Insulin use is the most common cause of hypoglycemia in the ICU. With evidence suggesting that outcomes from stroke, myocardial infarction, surgery, and infection are better with suppression of hyperglycemia, intensive care physicians have striven for tighter glycemic control, and the frequency and risks of hypoglycemia are being scrutinized.[411] It is not clear that iatrogenic hypoglycemia during intensive glycemic control has the same association with adverse outcome reported with spontaneous ICU hypoglycemia.[412] One study detected no neurocognitive outcome differences in children a few years after surgical management with or without aggressive glycemic control.[413] A variety of methods and algorithms have been devised to reduce the hypoglycemia risk,[414] and it seems likely that these will be further improved in the near future with continuous glucose sensors and limited closed-loop systems.[376]

Hypoglycemia Induced by Exogenous Agents

Insulin and various diabetes medications that stimulate insulin release are the most common exogenous causes of hypoglycemia at all ages, as discussed earlier in this chapter. Although the literature of the 20th century contains reports of hypoglycemia associated with hundreds of other agents, the evidence of a causal relationship is weak for most and represented by very few cases.[171,173,415,416] In children, hypoglycemia due to exogenous agents usually represents accidental ingestions. Ethanol, salicylates, quinine, and beta-blockers such as propranolol are the most frequent single agents implicated in childhood hypoglycemia. Rare cases of hypoglycemia have also been reported with sulfonamide and cotrimoxazole use in children, as well as in hepatic failure due to acetaminophen overdose. Pentamidine is one of the most common nondiabetes medications reported to cause hypoglycemia in adults in therapeutic doses. Hypoglycemia is also an uncommon effect of recreational drugs such as ecstasy and paramethoxyamphetamine.[376]

Alcohol-Induced Hypoglycemia

Ethanol is a notorious cause of severe fasting hypoglycemia in young children and malnourished alcoholic adults, and occasionally in healthy adolescents who are intoxicated.[417] The liver consumes nicotinamide adenine dinucleotide (NAD+) and produces NADH as it oxidizes ethanol. The depletion of NAD+ slows oxidation of lactate to pyruvate, thus impairing gluconeogenesis and the production of glucose during later stages of fasting.[418] In young children who have been fasting overnight, consumption of even small quantities of alcohol can acutely precipitate hypoglycemia.[419] Cases of severe hypoglycemia have occurred from ingestion of mouthwash or skin absorption of rubbing alcohol.[420,421] Numerous home products—including cologne, deodorants, and hand sanitizers—contain substantial amounts of ethanol.[422]

Salicylate Intoxication

In high doses, salicylates have enough hypoglycemic potency that they have been used experimentally to treat diabetes, although the exact mechanism of action remains uncertain.[423] Suggested mechanisms include stimulation of insulin release, inhibition of gluconeogenesis, increased peripheral glucose uptake, and suppression of lipolysis.[424] Infants appear to be more susceptible than older children to salicylate-induced hypoglycemia, and prior to their therapeutic abandonment for young children in the 1980s because of concerns about a role for salicylates in causing Reye syndrome, numerous cases were reported.[425]

Reactive Hypoglycemia and "Spells"

Reactive hypoglycemia refers to symptomatic hypoglycemia occurring 1 to 4 hours after a meal. The pathophysiologic concept is simple and familiar: if insulin secretion after a carbohydrate meal is excessive or prolonged in relation to incoming glucose during digestion, plasma glucose can fall to hypoglycemic levels. Potential causes of a mismatch of insulin secretion and carbohydrate digestion include accelerated carbohydrate digestion, disordered gastrointestinal-beta cell signaling, delayed insulin secretion, abnormal prolongation of insulin action, and abnormal insulin release.

Symptomatic reactive hypoglycemia characterized by a measurably low glucose accompanying neuroglycopenic symptoms (i.e., Whipple's triad) is uncommon, and a specific diagnosis should be sought among the forms of hyperinsulinism discussed earlier in this chapter. Alimentary hyperinsulinism (discussed earlier in the chapter) after Nissen fundoplication or gastric bypass surgery

likely involves both acceleration of carbohydrate digestion and excessive intestinal secretin signaling. Delaying gastric emptying is the primary treatment. Case reports of spontaneous acceleration of gastric emptying have been less convincing.[426,427] The most common form of delayed insulin secretion occurs during the gradual development of type 2 diabetes, when first-phase insulin response is diminishing and postprandial glucose peaks are higher. A similar pattern can occur with more severe forms of insulin resistance, but in these conditions the plasma glucose rarely falls to neuroglycopenic levels and may be difficult to distinguish from the purely autonomic symptoms of idiopathic postprandial syndrome (discussed later). Insulin effects may be delayed and prolonged by antibody binding, as occurs with autoimmune hypoglycemia. In up to 25% of patients with insulinoma, the worst hypoglycemic episodes are reactive rather than fasting. In some forms of genetic hyperinsulinism detected after infancy, postprandial hypoglycemia may be more apparent than fasting hypoglycemia, but in these cases hypoglycemia is triggered by ingestion of protein, not carbohydrates. The initial evaluation should be directed toward obtaining a critical sample during documented hypoglycemia, during admission for a diagnostic fast if necessary.

History of the Reactive Hypoglycemia Controversy

In contrast to the theories presented earlier in this chapter, the popular conception of reactive hypoglycemia as a likely explanation for any postprandial symptoms has persisted since the 1930s. Patients suspicious that they (or their children) have such a condition regularly present to the endocrine clinic. Within a decade of the first successful diagnosis and surgical cure of an insulinoma in 1929,[428] it became clear that not all patients with symptoms of hypoglycemia had demonstrable insulinomas curable by surgery. Seale Harris postulated that in some people an exaggerated insulin response to carbohydrate meals could cause transient hypoglycemia 2 to 4 hours later.[219] In these patients, symptoms could often be alleviated or prevented by more frequent meals containing more protein and less sugar and starch. Some of the patients who demonstrated this pattern seemed to be in the early stages of developing diabetes. Very few had insulinomas, and Allen Whipple is credited with the criteria for identifying patients whose hypoglycemia was potentially dangerous and might be curable by surgery; referred to as Whipple's triad, the criteria include (1) measurably low glucose accompanying (2) symptoms characteristic of hypoglycemia that are (3) relieved by raising the glucose.[197] Nonetheless, diagnoses of reactive hypoglycemia, based solely on symptoms or on OGTT patterns, became epidemic. Various treatment regimens, ranging from benign (frequent snacks, avoiding sugar) to pernicious (oral diabetes drugs, elaborate dietary restrictions, vagotomy, expensive supplements, or "adrenal extracts" of uncontrolled composition), have been offered.

However, further research shed doubt on the nature, and even the existence, of this common form of reactive hypoglycemia.[429] The OGTT pattern often considered characteristic of reactive hypoglycemia, with a nadir below the fasting glucose, occurs in most people.[223,230] A plasma glucose level below 50 mg/dL in the third, fourth, or fifth hour occurs without symptoms in 10% of adults.[430,431] Few of the self-diagnosed patients' symptoms coincided with low glucose levels, and low glucose levels were often not accompanied by symptoms.[432,433] Some showed the same symptoms after a placebo.[431] Symptoms could not be correlated with objective evidence of neuroglycopenia (e.g., electroencephalographic changes). Attempts to identify pathognomonic counter-regulatory hormone responses have been uncontrolled, unconfirmed, or demonstrated patterns indistinguishable from asymptomatic people.[434-438] Few patients with self-diagnosed reactive hypoglycemia show any abnormalities of glucose metabolism as determined by more rigorous tests for hyperinsulinism.[439] A "diabetic pattern" of early hyperglycemia with an exaggerated decline can occur in prediabetic adults but can also be produced by low carbohydrate intake prior to the OGTT.[440] The reproducibility of patterns and symptoms within the normal range is limited.[441] Symptoms provoked by a mixed meal tolerance test differed from those resulting after an OGTT.[442,443] The diagnostic test with a stronger demonstrated association with reactive hypoglycemic symptoms than any measure of glucose metabolism was the Minnesota Multiphasic Personality Test.[433,444,445] These problems led some endocrinologists to reject the entire concept of reactive or "functional hypoglycemia" as illusory or at least unrelated to glucose metabolism, preferring the term *idiopathic postprandial syndrome* as a more accurate descriptor.[443] The American Diabetes Association and the Endocrine Society formulated a position statement in 1973 supporting the need to demonstrate low glucose values at the time of symptoms, as well as improvement after glucose ingestion, in order to validate a diagnosis of hypoglycemia.[446] The value of the Whipple's triad criteria for suspected reactive hypoglycemia was reiterated in the 2009 hypoglycemia guidelines of the Endocrine Society.[71]

Approach to the Patient with Episodic "Hypoglycemic" Symptoms

Pediatric endocrinologists are often asked to evaluate older children and adolescents who have had episodic autonomic or dysphoric symptoms attributed to hypoglycemia. A demonstration of low glucose at the time of neuroglycopenic symptoms warrants a diagnostic fast, as potentially dangerous forms of hypoglycemia are nearly always disorders of fasting.[447] The differential diagnosis list for symptoms suggestive of hypoglycemia is lengthy and includes seizures, hyperventilation, and anxiety attacks[448-451]; syncope with exercise warrants immediate cardiac evaluation.[452] Most adolescents with "spells" referred for hypoglycemia evaluation have simple orthostatic hypotension or vasovagal syncope[453] and, in a few, the more disabling postural orthostatic hypotension syndrome (POTS).[454,455]

If recurrent symptoms are frequent and sufficiently suggestive of hypoglycemia, an attempt should be made to measure plasma glucose concentration during symptoms. Although self-monitoring of plasma glucose by

meter may be useful, results may not be reliable. Because glucose meters often yield erratic patterns, especially in the hands of nondiabetic patients, multiple glucose tests at similar times of the day are needed for comparison. It is important to prepare the parent for interpreting the overall pattern, rather than focusing on one or two unusual glucose values, because healthy people can have occasional high or low glucoses.[456] Normal meter glucoses do not exclude the possibility that symptoms may improve with the standard dietary recommendations for mild reactive hypoglycemia, which are that affected individuals (1) eat breakfast, (2) reduce sugar and starch intake in favor of low-glycemic index carbohydrates but do not restrict carbohydrates, (3) include fat and protein with meals and snacks, and (4) try a morning or afternoon snack.[457] An oral glucose tolerance test is of no value for excluding serious disorders of fasting hypoglycemia and is also not useful for testing the possible benefit of the standard dietary modifications.[458]

Behavior Problems as Effects of Hypoglycemia or Dietary Sugar

Another category of problem often brought to the endocrine clinic as possible hypoglycemia is distressing or aggressive behavior, mood, or school problems that, to the parent, seem either caused by or relieved by sugar. Decades of psychologic,[459,460] social science,[461-463] criminological,[50,464] childrearing,[465] and alternative medicine literature[466] perpetuate the putative connection between problem behavior and hypoglycemia or sugar ingestion. Supportive evidence tends to be poorly controlled or speculative; better-designed studies provide little support for such an effect.[467-470] Although there is evidence that autonomic activity, sleep, mood, and behavior can be affected by dietary carbohydrates in humans and rodents,[471,472] these behavioral effects are not evidence of hypoglycemia and no tests of carbohydrate metabolism will provide guidance for diet or management. Even the link between hyperactive behavior and sugar ingestion evaporates when tested with careful controls.[473,474]

Artifactual Hypoglycemia

When a low glucose level is unexpectedly reported in a "well" child who has an outpatient chemistry profile drawn for reasons unrelated to carbohydrate metabolism, it is most often a spurious result. The most common cause of postphlebotomy artifactual hypoglycemia is glucose consumption by red blood cells during a long delay before measurement.[475,476] This can be reduced but not eliminated by chilling the specimen and by newer collection tubes designed to sequester cells from serum.[477] Fluoride tubes reduce but do not eliminate glucose consumption, and glucose levels may decline by 20% or more when processing is delayed for even a few hours.[478] Unfortunately, postphlebotomy glycolysis consumes a higher proportion of glucose when the specimen contains a low level when drawn. Spurious hypoglycemia is more common when either the red cell or the white cell counts are elevated.[479,480]

Blood glucose meters have an inherent imprecision of at least 15% even under optimal conditions.[79,481] Continuous glucose sensors are being increasingly used to track patterns of glucose, but absolute nadirs are often inaccurate and their usefulness in evaluating hypoglycemia is doubtful.[482-485] Nevertheless, it is important to stress that unexpected low glucose levels should not be ignored because of the possibility that a "fluke" lab test may detect an unrecognized chronic hypoglycemic condition.

FASTING SYSTEM APPROACH TO DIAGNOSIS

The pathophysiology of fasting and the intricate balance of hormonal changes and their effects on intermediary metabolites is the key to discovering the cause of hypoglycemia. Normal infants, children, and adults may develop hypoglycemia if fasted long enough. At the time of hypoglycemia, a blood and urine sample (the critical sample) should demonstrate the normal adaptive changes that occur with time. In an infant or child who presents with unexplained hypoglycemia, the testing of this hypoglycemic sample may be pivotal in making the diagnosis and may avoid prolonged, dangerous, or expensive testing.

History

For a previously well child, the timing of hypoglycemia in relation to the child's last meal may provide a useful clue. Hypoglycemia occurring in the immediate postprandial period—reactive hypoglycemia—suggests excess insulin secretion. A previous fundoplication or other gastric surgery suggests alimentary hyperinsulinism. Hypoglycemia occurring in the early postabsorptive hours may be hyperinsulinism but can also occur with the more severe glycogen storage diseases. Hypoglycemia after several hours of fasting suggests disorders of FAO, of gluconeogenesis, or of the hormones that control these processes. The presence of sulfonylureas, insulin, or alcohol in the household suggests ingestion or injection.

Clinical Examination

Physical findings are only occasionally useful, typically in infants. Hepatomegaly suggests a GSD or an FAO disorder. Short stature or failure to thrive often occurs in a GSD, Fanconi-Bickel syndrome, and hypopituitarism. Neuromuscular signs may occur in association with FAO disorders and adrenoleukodystrophy. Midline defects such as central cleft lip and palate, single central incisor, optic nerve hypoplasia, or micropenis are often associated with pituitary hormonal deficiencies.

Critical Sample

Finally, the third stage revolves around the critical sample (see Figure 21-5). The key feature to assess is the presence or absence of acidosis. This can be determined from basic studies before the results of the intermediary metabolites. A chemistry panel with measurement of bicarbonate levels and a urine dip for ketones will be immediately available in most centers. In addition, a lactate level should be easily available.

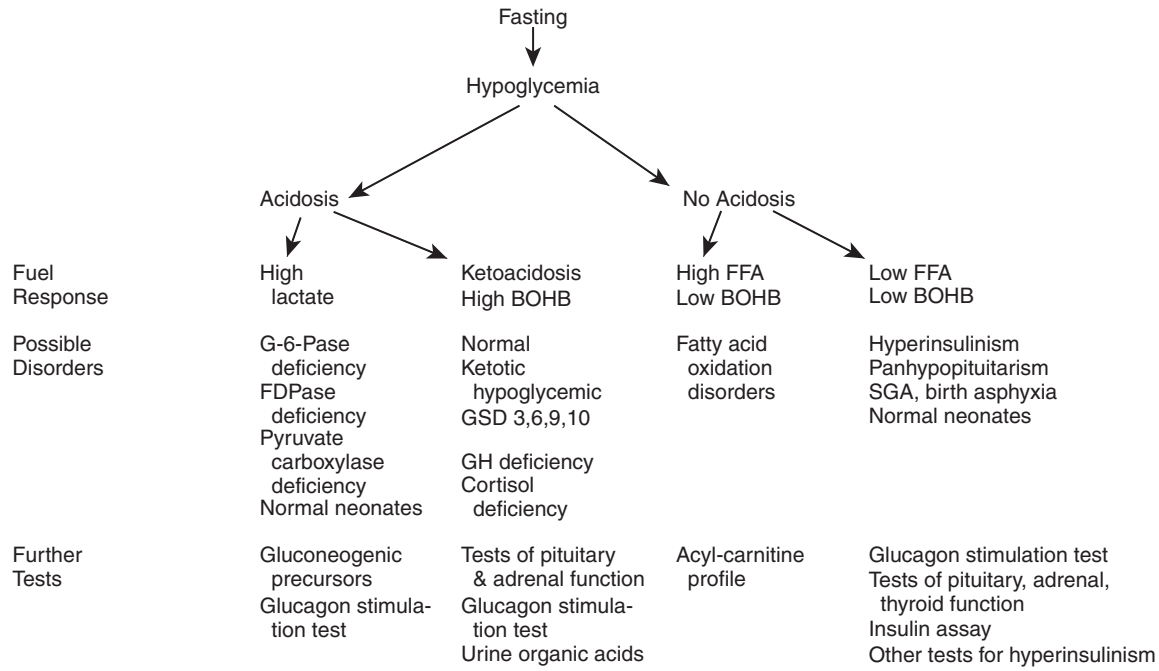


FIGURE 21-5 ■ An algorithmic approach to hypoglycemia. *FDPase,* fructose 1,6-diphosphatase; *FFA,* free fatty acids; *G-6-Pase,* glucose 6-phosphatase; *GH,* growth hormone; *GSD,* glycogen storage disorder; and *SGA,* small for gestational age.

Acidosis with elevated lactate and absent ketones suggests disorders of glycogenolysis or gluconeogenesis. GSD type 1 and a fructose 1,6-diphosphatase (FDPase) deficiency may be difficult to differentiate, and a fed glucagon stimulation test may help. There will be a rise in lactate and no rise in glucose in GSD type 1, with a rise in glucose in FDPase deficiency. An intravenous fructose tolerance test may be required. In the older child, lactic acidosis and hypoglycemia may suggest alcohol ingestion.

Acidosis with ketones occurs in normal fasting children, idiopathic ketotic hypoglycemia, and GSD types 3, 6, 9, and glycogen synthetase deficiency. Ketones also may be present in deficiencies of GH and cortisol. Disorders of FAO may sometimes present as small to moderate ketones in the urine, particularly when the patient is dehydrated. Some forms (such as the short-chain length disorders, the disorders of electron transport, and HMG-CoA lyase deficiency) may also have mild ketosis.

No acidosis or ketones in the urine is strongly suggestive of hyperinsulinism caused by genetic defects, insulinoma, accidentally administered excess insulin, deliberately administered insulin with intent to harm, or oral hypoglycemic agents. FAO defects will often be hypoketotic, but patients with some forms may have trace or small ketones in the urine. Those with hyperinsulinism will have neither elevated free fatty acids nor elevated ketones, whereas those with FAO will have elevated free fatty acids and low ketones. FAO may be diagnosed by acyl-carnitine profile or by urine organic acid measurement. Hyperinsulinism owing to genetic defects and insulinoma can be differentiated from insulin administration by the presence of C peptide in the plasma at the time of hypoglycemia. Oral hypoglycemic agents will have elevated insulin and C peptide, and the diagnosis will be missed unless suspected and unless a urine toxicology with a request for the specific drug to be sought is performed.

EMERGENCY TREATMENT OF HYPOGLYCEMIA

Once the critical sample has been obtained, a minibolus of 0.2 g/kg of dextrose should be administered by intravenous infusion over 1 minute (2 mL/kg of 10% dextrose; see Table 21-4). This should be followed by a continuous intravenous infusion of 8 mg/kg/min using dextrose 10% solution. This rate of glucose administration may be rapidly and conveniently calculated by using the simple formula that 5 mL/kg/hr of a 10% dextrose solution provides approximately 8 mg/kg/min of glucose. Glucose levels should be determined 15 minutes after the bolus has been given and while the maintenance glucose infusion is running. If hypoglycemia recurs, a bolus of 0.5 g/kg may be given (5 mL/kg of dextrose 10%) and the glucose infusion increased by 25% to 50%. For details of specific treatments of individual disorders, see the sections on each condition.

REFERENCES

1. Gregory, J. W., & Aynsley-Green, A. (1993). The definition of hypoglycaemia. *Baillieres Clin Endocrinol Metab*, *7*, 587–590.
2. Field, J. B. (1989). Hypoglycemia: definition, clinical presentations, classification, and laboratory tests. *Endocrinol Metab Clin North Am*, *18*, 27–43.
3. Bolli, G. B., & Fanelli, C. G. (1999). Physiology of glucose counterregulation to hypoglycemia. *Endocrinol Metab Clin North Am*, *28*, 467–493, v.
4. Cryer, P. E. (2011). Hypoglycemia. In S. Melmed, K. S. Polonsky, P. Reed Larson, & H. M. Kronenberg, (Eds.), *Williams textbook of endocrinology* (pp. 1552–1577). Philadelphia: Saunders.
5. Fanelli, C., Pampanelli, S., Epifano, L., et al. (1994). Relative roles of insulin and hypoglycaemia on induction of neuroendocrine responses to, symptoms of, and deterioration of cognitive function in hypoglycaemia in male and female humans. *Diabetologia*, *37*, 797–807.
6. Jackson, L., Williams, F. L., Burchell, A., et al. (2004). Plasma catecholamines and the counterregulatory responses to hypoglycemia in infants: a critical role for epinephrine and cortisol. *J Clin Endocrinol Metab*, *89*, 6251–6256.
7. Cryer, P. E. (2007). Hypoglycemia, functional brain failure, and brain death. *J Clin Invest*, *117*, 868–870.
8. Bendtson, I. (1993). Neurophysiologic changes of hypoglycaemia. In B. M. Frier, & B. M. Fisher (Eds.), *Hypoglycaemia and diabetes: clinical and physiological aspects* (pp. 72–79). London, Edward Arnold.
9. Holliday, M. A. (1971). Metabolic rate and organ size during growth from infancy to maturity and during late gestation and early infancy. *Pediatrics*, *47*(Suppl. 2), 169–179.
10. Bier, D. M., Leake, R. D., Haymond, M. W., et al. (1977). Measurement of "true" glucose production rates in infancy and childhood with 6,6-dideuteroglucose. *Diabetes*, *26*, 1016–1023.
11. Haymond, M. W., Karl, I. E., Clarke, W. L., et al. (1982). Differences in circulating gluconeogenic substrates during short-term fasting in men, women, and children. *Metabolism*, *31*, 33–42.
12. Stanley, C. A., & Baker, L. (1976). Hyperinsulinism in infancy: diagnosis by demonstration of abnormal response to fasting hypoglycemia. *Pediatrics*, *57*, 702–711.
13. Kaye, R., Davidson, M. H., Williams, M. L., et al. (1961). The response of blood glucose, ketones, and plasma nonesterified fatty acids to fasting and epinephrine injection in infants and children. *J Pediatr*, *59*, 836–847.
14. Chaussain, J. L. (1973). Glycemic response to 24 hour fast in normal children and children with ketotic hypoglycemia. *J Pediatr*, *82*, 438–443.
15. Chaussain, J. L., Georges, P., Olive, G., & Job, J. C. (1974). Glycemic response to 24-hour fast in normal children and children with ketotic hypoglycemia: II. Hormonal and metabolic changes. *J Pediatr*, *85*, 776–781.
16. Chaussain, J. L., Georges, P., Calzada, L., & Job, J. C. (1977). Glycemic response to 24-hour fast in normal children: III. Influence of age. *J Pediatr*, *91*, 711–714.
17. Hojlund, K., Wildner-Christensen, M., Eshøj, O., et al. (2001). Reference intervals for glucose, beta-cell polypeptides, and counterregulatory factors during prolonged fasting. *Am J Physiol Endocrinol Metab*, *280*, E50–E58.
18. Bonnefont, J. P., Specola, N. B., Vassault, A., et al. (1990). The fasting test in paediatrics: application to the diagnosis of pathological hypo- and hyperketotic states. *Eur J Pediatr*, *150*, 80–85.
19. Cryer, P. E. (1997). *Hypoglycemia: pathophysiology, diagnosis, and treatment.* New York: Oxford University Press.
20. Schwartz, N. S., Clutter, W. E., Shah, S. D., & Cryer, P. E. (1987). Glycemic thresholds for activation of glucose counterregulatory systems are higher than the threshold for symptoms. *J Clin Invest*, *79*, 777–781.
21. Pagliara, A. S., Kari, I. E., De Vivo, D. C., et al. (1972). Hypoalaninemia: a concomitant of ketotic hypoglycemia. *J Clin Invest*, *51*, 1440–1449.
22. Lamers, K. J., Doesburg, W. H., Gabre®ls, F. J., et al. (1985). Reference values of blood components related to fuel metabolism in children after an overnight fast. *Clinica Chimica Acta*, *145*, 17–26.
23. DeRosa, M. A., & Cryer, P. E. (2004). Hypoglycemia and the sympathoadrenal system: neurogenic symptoms are largely the result of sympathetic neural, rather than adrenomedullary, activation. *Am J Physiol Endocrinol Metab*, *287*, E32–E41.
24. Cooperberg, B. A., & Cryer, P. E. (2010). Insulin reciprocally regulates glucagon secretion in humans. *Diabetes*, *59*, 2936–2940.
25. Gosmanov, N. R., Szoke, E., Israelian, Z., et al. (2005). Role of the decrement in intraislet insulin for the glucagon response to hypoglycemia in humans. *Diabetes Care*, *28*, 1124–1131.
26. Hevener, A. L., Bergman, R. N., & Donovan, C. M. (2001). Hypoglycemic detection does not occur in the hepatic artery or liver: findings consistent with a portal vein glucosensor locus. *Diabetes*, *50*, 399–403.
27. McCrimmon, R. (2008). The mechanisms that underlie glucose sensing during hypoglycaemia in diabetes. *Diabet Med*, *25*, 513–522.
28. Towler, D. A., Havlin, C. E., Craft, S., & Cryer, P. (1993). Mechanism of awareness of hypoglycemia: perception of neurogenic (predominantly cholinergic) rather than neuroglycopenic symptoms. *Diabetes*, *42*, 1791–1798.
29. Freinkel, N., Metzger, B. E., Harris, E., et al. (1972). The hypothermia of hypoglycemia: studies with 2-deoxy-D-glucose in normal human subjects and mice. *N Engl J Med*, *287*, 841–845.
30. Macdonald, I. A., & Maggs, D. G. (1993). Cutaneous blood flow, sweating, tremor and temperature regulation in hypoglycaemia. In B. M. Frier, & B. M. Fisher (Eds.), *Hypoglycaemia and diabetes: clinical and physiological aspects* (pp. 132–143). London: Edward Arnold, 132–143.
31. Amiel, S. A., et al. (1987). Rate of glucose fall does not affect counterregulatory hormone responses to hypoglycemia in normal and diabetic humans. *Diabetes*, *36*, 518–522.
32. Davis, S. N., Shavers, C., Costa, F. (2000). Differential gender responses to hypoglycemia are due to alterations in CNS drive and not glycemic thresholds. *Am J Physiol Endocrinol Metab*, *279*, E1054–E1063.
33. Jones, T. W., Boulware, S. D., Kraemer, D. T., et al. (1991). Independent effects of youth and poor diabetes control on responses to hypoglycemia in children. *Diabetes*, *40*, 358–363.
34. Galassetti, P., Neill, A. R., Tate, D., et al. (2001). Sexual dimorphism in counterregulatory responses to hypoglycemia after antecedent exercise. *Clin Endocrinol Metab*, *86*, 3516–3524.
35. Jones, T. W., Porter, P., Sherwin, R. S., et al. (1998). Decreased epinephrine responses to hypoglycemia during sleep. *N Engl J Med*, *338*, 1657–1662.
36. Fujii, S., Tamai, H., Kumai, M., et al. (1989). Impaired glucagon secretion to insulin-induced hypoglycemia in anorexia nervosa. *Acta Endocrinol* (Copenh), *120*, 610–615.
37. Kerr, D., Sherwin, R. S., Pavalkis, F., et al. (1993). Effect of caffeine on the recognition of and responses to hypoglycemia in humans. *Ann Intern Med*, *119*, 799.
38. Robinson, A. M., Parkin, H. M., Macdonald, I. A., & Tattersall, R. B. (1995). Antecedent hypoglycaemia in non-diabetic subjects reduces the adrenaline response for 6 days but does not affect the catecholamine response to other stimuli. *Clin Sci* (Lond), *89*, 359–366.
39. Heller, S. R., & Cryer, P. E. (1991). Reduced neuroendocrine and symptomatic responses to subsequent hypoglycemia after 1 episode of hypoglycemia in nondiabetic humans. *Diabetes*, *40*, 223–226.
40. Hussain, K., Bryan, J., Christesen, H. T., et al. (1996). Serum glucagon counterregulatory hormonal response to hypoglycemia is blunted in congenital hyperinsulinism. *Diabetes*, *54*, 2946–2951.
41. Cryer, P. E. (2006). Mechanisms of sympathoadrenal failure and hypoglycemia in diabetes. *J Clin Invest*, *116*, 1470–1473.
42. Boyle, P. J., Schwartz, N. S., Shah, S. D., et al. (1988). Plasma glucose concentrations at the onset of hypoglycemic symptoms in patients with poorly controlled diabetes and in nondiabetics. *N Engl J Med*, *318*, 1487–1492.
43. Amiel, S. A., Sherwin, R. S., Simonson, D. C., & Tamborlane, W. V. (1988). Effect of intensive insulin therapy on glycemic thresholds for counterregulatory hormone release. *Diabetes*, *37*, 901–907.
44. De Feo, P., Gallai, V., Mazzotta, G., et al. (1988). Modest decrements in plasma glucose concentration cause early impairment in cognitive function and later activation of glucose counterregulation in the absence of hypoglycemic symptoms in normal man. *J Clin Invest*, *82*, 436–444.
45. Bjørgaas, M., Sand, T., Vik, T., & Jorde, R. (1998). Quantitative EEG during controlled hypoglycaemia in diabetic and non-diabetic children. *Diabet Med*, *15*, 30–37.
46. Amiel, S. A. (1998). Cognitive function testing in studies of acute hypoglycaemia: rights and wrongs? *Diabetologia*, *41*, 713–719.

47. Graveling, A. J., Deary, I. J., & Frier, B. M. (2013). Acute hypoglycemia impairs executive cognitive function in adults with and without type 1 diabetes. *Diabetes Care*. Advance online publication. doi:10.2337/dc13-0194
48. Cornblath, M., & Schwartz, R. (1991). *Disorders of carbohydrate metabolism in infancy* (3rd ed.). Boston, MA/St. Louis, MO: Blackwell Scientific.
49. Budner, L. J. (1997). Behavioral effects of hypoglycemia. *J Am Acad Child Adolesc Psychiatry*, *36*, 1651–1652.
50. Maher, G., & Frier, B. M. (1993). Hypoglycaemia and criminal responsibility. In B. M. Frier, & B. M. Fisher (Eds.), *Hypoglycaemia and diabetes: clinical and physiological aspects.* London: Edward Arnold, 380–386.
51. Ding, Y., Wang, S., Liu, J., et al. (2010). Neuropsychiatric profiles of patients with insulinomas. *Eur Neurol*, *63*, 48–51.
52. Rankins, D., Wellard, R. M., Cameron, F., et al. (2005). The impact of acute hypoglycemia on neuropsychological and neurometabolite profiles in children with type 1 diabetes. *Diabetes Care*, *28*, 2771–2773.
53. Pettigrew, L. C., Arieff, A. I., & McCandless, D. W. (1992). Central nervous system manifestations of hypoglycemia. In A. I. Arief, & R. C. Griggs (Eds.), *Metabolic brain dysfunction in systemic disorders* (pp. 129–138). Boston: Little, Brown and Company.
54. Suh, S. W., Hamby, A. M., & Swanson, R. A. (2007). Hypoglycemia, brain energetics, and hypoglycemic neuronal death. *Glia*, *55*, 1280–1286.
55. Auer, R. N. (2004). Hypoglycemic brain damage. *Forensic Sci Int*, *146*, 105–110.
56. Kinnala, A., Rikalainen, H., Lapinleimu, H., et al. (1999). Cerebral magnetic resonance imaging and ultrasonography findings after neonatal hypoglycemia. *Pediatrics*, *103*, 724–729.
57. Sharma, P., Eesa, M., & Scott, J. N. (2009). Toxic and acquired metabolic encephalopathies: MRI appearance. *Am J Roentgenol*, *193*, 879–886.
58. Boluyt, N., van Kempen, A., & Offringa, M. (2006). Neurodevelopment after neonatal hypoglycemia: a systematic review and design of an optimal future study. *Pediatrics*, *117*, 2231–2243.
59. Langan, S. J., Deary, I. J., Hepburn, D. A., & Frier, B. M. (1991). Cumulative cognitive impairment following recurrent severe hypoglycaemia in adult patients with insulin-treated diabetes mellitus. *Diabetologia*, *34*, 337–344.
60. Steinkrauss, L., Lipman, T. H., Hendell, C. D., et al. (2005). Effects of hypoglycemia on developmental outcome in children with congenital hyperinsulinism. *J Pediatr Nurs*, *20*, 109–118.
61. Hershey, T., Perantie, D. C., Warren, S. L., et al. (2005). Frequency and timing of severe hypoglycemia affects spatial memory in children with type 1 diabetes. *Diabetes Care*, *28*, 2372–2377.
62. Menni, F., de Lonlay, P., Sevin, C., et al. (2001). Neurologic outcomes of 90 neonates and infants with persistent hyperinsulinemic hypoglycemia. *Pediatrics*, *107*, 476–479.
63. Segel, S. A., Fanelli, C. G., Dence, C. S., et al. (2001). Blood-to-brain glucose transport, cerebral glucose metabolism, and cerebral blood flow are not increased after hypoglycemia. *Diabetes*, *50*, 1911–1917.
64. Amiel, S. A. (1995). Organ fuel selection: brain. *Proc Nutr Soc*, *54*, 151–155.
65. Smith, D., Pernet, A., Hallett, W. A., et al. (2003). Lactate: a preferred fuel for human brain metabolism in vivo. *J Cereb Blood Flow Metab*, *23*, 658–664.
66. Wahren, J., Ekberg, K., Fernqvist-Forbes, E., & Nair, S. (1999). Brain substrate utilisation during acute hypoglycaemia. *Diabetologia*, *42*, 812–818.
67. Evans, M. L., Hopkins, D., Macdonald, I. A., & Amiel, S. A. (2004). Alanine infusion during hypoglycaemia partly supports cognitive performance in healthy human subjects. *Diabetic Medicine*, *21*, 440–446.
68. Brown, A. M., & Ransom, B. R. (2007). Astrocyte glycogen and brain energy metabolism. *Glia*, *55*, 1263–1271.
69. Oz, G., Kumar, A., Rao, J. P., et al. (2009). Human brain glycogen metabolism during and after hypoglycemia. *Diabetes*, *58*, 1978–1985.
70. Luber, S., Meldon, S., & Brady, W. (1998). Hypoglycemia presenting as acute respiratory failure in an infant. *Am J Emerg Med*, *16*, 281–284.
71. Cryer, P. E., Axelrod, L., Grossman, A. B., et al. (2009). Evaluation and management of adult hypoglycemic disorders: an Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab, 94, 709–728.
72. Cornblath, M., Hawdon, J. M., Williams, A. F., et al. (2000). Controversies regarding definition of neonatal hypoglycemia: suggested operational thresholds. *Pediatrics*, *105*, 1141–1145.
73. Cornblath, M., Schwartz, R., Aynsley-Green, A., & Lloyd, J. K. (1990). Hypoglycemia in infancy: the need for a rational definition. A Ciba Foundation discussion meeting. *Pediatrics*, *85*, 834–837.
74. Koh, T. H., Aynsley-Green, A., Tarbit, M., & Eyre, J. A. (1988). Neural dysfunction during hypoglycaemia. *Arch Dis Child*, *63*, 1353–1358.
75. Koh, T. H., Eyre, J. A., & Aynsley-Green, A. (1988). Neonatal hypoglycaemia: the controversy regarding definition. *Arch Dis Child*, *63*, 1386–1388.
76. Tustison, W. A., Bowen, A. J., & Crampton, J. H. (1966). Clinical interpretation of plasma glucose values. *Diabetes*, *15*, 775–777.
77. Howanitz, P. J., & Howanitz, J. H. (1979). Carbohydrates. In J. B. Henry (Ed.), *Clinical diagnosis and management by laboratory methods* (pp. 153–188). Philadelphia: W. B. Saunders.
78. Caraway, W. T. (1976). Carbohydrates. In N. W. Tietz (Ed.), *Fundamentals of clinical chemistry* (pp. 234–263). Philadelphia: Saunders.
79. Gama, R., Anderson, N. R., & Marks, V. (2000). 'Glucose meter hypoglycaemia': often a non-disease. *Ann Clin Biochem*, *37*(Pt. 5), 731–732.
80. Clarke, W., Jones, T., & Rewers, A. (2009). Assessment and management of hypoglycemia in children and adolescents with diabetes. *Pediatr Diabetes*, *10*(Suppl. 12), 134–145.
81. De Leon, D. D., & Stanley, C. A. (2007). Mechanisms of disease: advances in diagnosis and treatment of hyperinsulinism in neonates. *Nat Clin Pract Endocrinol Metab*, *3*, 57–68.
82. Dekelbab, B. H., & Sperling, M. A. (2006). Recent advances in hyperinsulinemic hypoglycemia of infancy. *Acta Paediatr*, *95*, 1157–1164.
83. Stanley, C. A., & De Leon, D. D. (2012). *Monogenic hyperinsulinemic hypoglycemia disorders: frontiers in diabetes.* Basel: Karger.
84. Meissner, T., Rabl, W., Mohnike, K., et al. (2001). Hyperinsulinism in syndromal disorders. *Acta Paediatr*, *90*, 856–859.
85. Kukuvitis, A., Deal, C., Arbour, L., & Polychronakos C. (1997). An autosomal dominant form of familial persistent hyperinsulinemic hypoglycemia of infancy, not linked to the sulfonylurea receptor locus. *J Clin Endocrinol Metab*, *82*, 1192–1194.
86. Sayed, S., Langdon, D. R., Odili, S., et al. (2009). Extremes of clinical and enzymatic phenotypes in children with hyperinsulinism caused by glucokinase activating mutations. *Diabetes*, *58*, 1419–1427.
87. Thornton, P. S., Satin-Smith, M. S., Herold, K., et al. (1998). Familial hyperinsulinism with apparent autosomal dominant inheritance: clinical and genetic differences from the autosomal recessive variant. *J Pediatr*, *132*, 9–14.
88. Glaser, B., Kesavan, P., Heyman, M., et al. (1998). Familial hyperinsulinism caused by an activating glucokinase mutation. *N Engl J Med*, *338*, 226–230.
89. Ryan, F., Devaney, D., Joyce, C., et al. (1998). Hyperinsulinism: molecular aetiology of focal disease. *Arch Dis Child*, *79*, 445–447.
90. Stanley, C. A., Lieu, Y. K., Hsu, B. Y., et al. (1998). Hyperinsulinism and hyperammonemia in infants with regulatory mutations of the glutamate dehydrogenase gene. *N Engl J Med*, *338*, 1352–1357.
91. Palladino, A. A., Bennett, M. J., & Stanley, C. A. (2008). Hyperinsulinism in infancy and childhood: when an insulin level is not always enough. *Clin Chem*, *54*, 256–263.
92. Chevenne, D., Trivin, F., & Porquet, D. (1999). Insulin assays and reference values. *Diabetes Metab*, *25*, 459–476.
93. Levitt Katz, L. E., Satin-Smith, M. S., Collett-Solberg, P., et al. (1997). Insulin-like growth factor binding protein-1 levels in the diagnosis of hypoglycemia caused by hyperinsulinism. *J Pediatr*, *131*, 193–199.
94. Hardy, O. T., Hernandez-Pampaloni, M., Saffer, J. R., et al. (2007). Diagnosis and localization of focal congenital hyperinsulinism by 18F-fluorodopa PET scan. *J Pediatr*, *150*, 140–145.
95. de Lonlay-Debeney, P., Poggi-Travert, F., Fournet, J. C., et al. (1999). Clinical features of 52 neonates with hyperinsulinism. *N Engl J Med*, *340*, 1169–1175.

96. Thomas, P. M., Cote, G. J., Wohllk, N., et al. (1995). Mutations in the sulfonylurea receptor gene in familial persistent hyperinsulinemic hypoglycemia of infancy. *Science*, *268*, 426–429.
97. Pinney, S. E., MacMullen, C., Becker, S., et al. (2008). Clinical characteristics and biochemical mechanisms of congenital hyperinsulinism associated with dominant KATP channel mutations. *J Clin Invest*, *118*, 2877–2886.
98. Macmullen, C. M., Zhou, Q., Snider, K. E., et al. (2011). Diazoxide-unresponsive congenital hyperinsulinism in children with dominant mutations of the beta-cell sulfonylurea receptor SUR1. *Diabetes*, *60*, 1797–1804.
99. Thornton, P. S., Alter, C. A., Katz, L. E., et al. (1993). Short- and long-term use of octreotide in the treatment of congenital hyperinsulinism. *J Pediatr*, *123*, 637–643.
100. Aynsley-Green, A., Hussain, K., Hall, J., et al. (2000). Practical management of hyperinsulinism in infancy. *Arch Dis Child Fetal Neonatal Ed*, *82*, F98–F107.
101. Rahier, J., Sempoux, C., Fournet, J. C., et al. (1998). Partial or near-total pancreatectomy for persistent neonatal hyperinsulinaemic hypoglycaemia: the pathologist's role. *Histopathology*, *32*, 15–19.
102. Smith, V. V., Malone, M., & Risdon, R. A. (2001). Focal or diffuse lesions in persistent hyperinsulinemic hypoglycemia of infancy: concerns about interpretation of intraoperative frozen sections. *Pediatr Dev Pathol*, *4*, 138–143.
103. Suchi, M. T., Stanley C. A., Ruchelli, E. D. et al (2001). Hyperinsulinism: are there only two histologic forms? *Mod Pathol*, *14*, 5P.
104. Weinzimer, S. A., Stanley, C. A., Berry, G. T., et al. (1997). A syndrome of congenital hyperinsulinism and hyperammonemia. *J Pediatr*, *130*, 661–664.
105. Zammarchi, E., Filippi, L., Novembre, E., & Donati MA. (1996). Biochemical evaluation of a patient with a familial form of leucine-sensitive hypoglycemia and concomitant hyperammonemia. *Metabolism*, *45*, 957–960.
106. Treberg, J. R., Brosnan, M. E., Watford, M., & Brosnan, J. T. (2010). On the reversibility of glutamate dehydrogenase and the source of hyperammonemia in the hyperinsulinism/hyperammonemia syndrome. *Adv Enzyme Regul*, *50*, 34–43.
107. Hsu, B. Y., Kelly, A., Thornton, P. S., et al. (2001). Protein-sensitive and fasting hypoglycemia in children with the hyperinsulinism/hyperammonemia syndrome. *J Pediatr*, *138*, 383–389.
108. Huijmans, J. G., Duran, M., de Klerk, J. B., et al. (2000). Functional hyperactivity of hepatic glutamate dehydrogenase as a cause of the hyperinsulinism/hyperammonemia syndrome: effect of treatment. *Pediatrics*, *106*, 596–600.
109. Stanley, C. A. (2004). Hyperinsulinism/hyperammonemia syndrome: insights into the regulatory role of glutamate dehydrogenase in ammonia metabolism. *Mol Genet Metab*, *81*(Suppl. 1), S45–S51.
110. Matschinsky, F. M., Glaser, B., & Magnuson, M. A. (1998). Pancreatic beta-cell glucokinase: closing the gap between theoretical concepts and experimental realities. *Diabetes*, *47*, 307–315.
111. Gloyn, A. L. (2003). Glucokinase (GCK) mutations in hyper- and hypoglycemia: maturity-onset diabetes of the young, permanent neonatal diabetes, and hyperinsulinemia of infancy. *Hum Mutat*, *22*, 353–362.
112. Sayed, S., Matschinsky, F. M., & Stanley, C. A. (2012). Hyperinsulinism due to activating mutations of glucokinase. In C. A. Stanley, & D. D. De Leon (Eds.), *Monogenic hyperinsulinemic hypoglycemic disorders* (pp. 146–157). Basel: Karger.
113. Christesen, H. B. T., Herold, K. C., Noordam, K., et al. (2004). Glucokinase-linked hypoglycemia: clinical aspects of activating glucokinase mutations. In F. M. Matschinsky, & M. A. Magnuson (Eds.), *Glucokinase and glycemic disease: from basics to novel therapeutics.* Basel: Karger.
114. Sayed, S., Langdon, D. R., Odili, S., et al. (2009). Extremes of clinical and enzymatic phenotypes in children with hyperinsulinism due to glucokinase activating mutations. *Diabetes*, *58*, 1419–1427.
115. Christesen, H. B., Brusgaard, K., Beck Nielsen, H., & Brock Jacobsen, B. (2008). Non-insulinoma persistent hyperinsulinaemic hypoglycaemia caused by an activating glucokinase mutation: hypoglycaemia unawareness and attacks. *Clin Endocrinol* (Oxf), *68*, 747–755.
116. Cuesta-Muñoz, A. L., Huopio, H., Otonkoski, T., et al. (2004). Severe persistent hyperinsulinemic hypoglycemia due to a de novo glucokinase mutation. *Diabetes*, *53*, 2164–2168.
117. Narayan, S. B., Master, S. R., Sireci, A. N., et al. (2012). Short-chain 3-hydroxyacyl-coenzyme A dehydrogenase associates with a protein super-complex integrating multiple metabolic pathways. *PLoS One*, *7*, e35048.
118. Li, C., Chen, P., Palladino, A., Narayan, S., et al. (2010). Mechanism of hyperinsulinism in short-chain 3-hydroxyacyl-CoA dehydrogenase deficiency involves activation of glutamate dehydrogenase. *J Biol Chem*, *285*, 31806–31818.
119. Kapoor, R. R., Heslegrave, A., & Hussain, K. (2010). Congenital hyperinsulinism due to mutations in HNF4A and HADH. *Rev Endocr Metab Disord*, *11*, 185–191.
120. Molven, A., Helgeland, G., Sandal, T., et al. (2012). The molecular genetics and pathophysiology of congenital hyperinsulinism caused by short-chain 3-hydroxyacyl-CoA dehydrogenase deficiency. In C. A. Stanley, & D. D. De Leon (Eds.), *Monogenic hyperinsulinemic hypoglycemic disorders* (pp. 137–145). Basel: Karger.
121. Molven, A., Matre, G. E., Duran, M., et al. (2004). Familial hyperinsulinemic hypoglycemia caused by a defect in the SCHAD enzyme of mitochondrial fatty acid oxidation. *Diabetes*, *53*, 221–227.
122. Kapoor, R. R., James, C., Flanagan, S. E., et al. (2009). 3-Hydroxyacyl-coenzyme A dehydrogenase deficiency and hyperinsulinemic hypoglycemia: characterization of a novel mutation and severe dietary protein sensitivity. *J Clin Endocrinol Metab*, *94*, 2221–2225.
123. Clayton, P. T., Eaton, S., Aynsley-Green, A., et al. (2001). Hyperinsulinism in short-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency reveals the importance of β-oxidation in insulin secretion. *J Clin Invest*, *108*, 457–465.
124. Eaton, S., Chatziandreou, I., Krywawych, S., et al. (2003). Short-chain 3-hydroxyacyl-CoA dehydrogenase deficiency associated with hyperinsulinism: a novel glucose-fatty acid cycle? *Biochem Soc Trans*, *31*(Pt. 6), 1137–1139.
125. Finegold, D. N., Stanley, C. A., & Baker, L. (1980). Glycemic response to glucagon during fasting hypoglycemia: an aid in the diagnosis of hyperinsulinism. *J Pediatr*, *96*, 257–259.
126. Vidnes, J., & Oyasaeter, S. (1977). Glucagon deficiency causing severe neonatal hypoglycemia in a patient with normal insulin secretion. *Pediatr Res*, *11*(9, Pt. 1), 943–949.
127. Molven, A., Rishaug, U., Matre, G. E., et al. (2002). Hunting for a hypoglycemia gene: severe neonatal hypoglycemia in a consanguineous family. *Am J Med Genet*, *113*, 40–46.
128. Yang, S. Y., He, X. Y., & Schulz, H. (2005). 3-Hydroxyacyl-CoA dehydrogenase and short chain 3-hydroxyacyl-CoA dehydrogenase in human health and disease. *FEBS J*, *272*, 4874–4883.
129. Kapoor, R. R., Locke, J., Colclough, K., et al. (2008). Persistent hyperinsulinemic hypoglycemia and maturity-onset diabetes of the young due to heterozygous HNF4A mutations. *Diabetes*, *57*, 1659–1663.
130. Pearson, E. R., Boj, S. F., Steele, A. M., et al. (2007). Macrosomia and hyperinsulinaemic hypoglycaemia in patients with heterozygous mutations in the HNF4A gene. *PLoS Med*, *4*, e118.
131. Pingul, M. M., Hughes, N., Wu, A., et al. (2011). Hepatocyte nuclear factor 4alpha gene mutation associated with familial neonatal hyperinsulinism and maturity-onset diabetes of the young. *J Pediatr*, *158*, 852–854.
132. Stanescu, D. E., Hughes, N., Kaplan, B., et al. (2012). Novel presentations of congenital hyperinsulinism due to mutations in the MODY genes: HNF1A and HNF4A. *J Clin Endocrinol Metab*, *97*, E2026–E2030.
133. Meissner, T., Otonkoski, T., Feneberg, R., et al. (2001). Exercise induced hypoglycaemic hyperinsulinism. *Arch Dis Child*, *84*, 254–257.
134. Otonkoski, T., & Meissner, T. (2012). Exercise-induced hyperinsulinism: a failure of monocarboxylate transporter 1 expression silencing. In C. A. Stanley, & D. D. De Leon (Eds.), *Monogenic hyperinsulinemic hypoglycemic disorders* (pp. 172–181). Basel: Karger.
135. Meissner, T., Friedmann, B., Okun, J. G., et al. (2005). Massive insulin secretion in response to anaerobic exercise in exercise-induced hyperinsulinism. *Horm Metab Res*, *37*, 690–694.
136. Otonkoski, T., Jiao, H., Kaminen-Ahola, N., et al. (2007). Physical exercise-induced hypoglycemia caused by failed silencing of monocarboxylate transporter 1 in pancreatic beta cells. *Am J Hum Genet*, *81*, 467–474.

137. Marquard, J., Welters, A., Buschmann, T., et al. (2006). Association of exercise-induced hyperinsulinaemic hypoglycaemia with MCT1-expressing insulinoma. *Diabetologia*, *56*, 31–35.
138. Freeze, H. H. (2006). Genetic defects in the human glycome. *Nat Rev Genet*, *7*, 537–551.
139. Timal, S., Hoischen, A., Lehle, L., et al. (2012). Gene identification in the congenital disorders of glycosylation type I by whole-exome sequencing. *Hum Mol Genet*, *21*, 4151–4161.
140. Jaeken, J., Hennet, T., Matthijs, G., & Freeze, H. H. (2009). CDG nomenclature: time for a change! *Biochim Biophys Acta*, *1792*, 825–826.
141. Böhles, H., Sewell, A. A., Gebhardt, B., et al. (2001). Hyperinsulinaemic hypoglycaemia—leading symptom in a patient with congenital disorder of glycosylation Ia (phosphomannomutase deficiency). *J Inherit Metab Dis*, *24*, 858–862.
142. de Lonlay, P., Cuer, M., Vuillaumier-Barrot, S., et al. (1999). Hyperinsulinemic hypoglycemia as a presenting sign in phosphomannose isomerase deficiency: a new manifestation of carbohydrate-deficient glycoprotein syndrome treatable with mannose. *J Pediatr*, *135*, 379–383.
143. Sun, L., Eklund, E. A., Chung, W. K., et al. (2005). Congenital disorder of glycosylation id presenting with hyperinsulinemic hypoglycemia and islet cell hyperplasia. *J Clin Endocrinol Metab*, *90*, 4371–4375.
144. de Zegher, F., & Jaeken, J. (1995). Endocrinology of the carbohydrate-deficient glycoprotein syndrome type 1 from birth through adolescence. *Pediatr Res*, *37*(4, Pt. 1), 395–401.
145. Enns, G. M., Steiner, R. D., Buist, N., et al. (2002). Clinical and molecular features of congenital disorder of glycosylation in patients with type 1 sialotransferrin pattern and diverse ethnic origins. *J Pediatr*, *141*, 695–700.
146. Babovic-Vuksanovic, D., Patterson, M. C., Schwenk, W. F., et al. (1999). Severe hypoglycemia as a presenting symptom of carbohydrate-deficient glycoprotein syndrome. *J Pediatr*, *135*, 775–781.
147. de Lonlay, P., & Seta, N. (2009). The clinical spectrum of phosphomannose isomerase deficiency, with an evaluation of mannose treatment for CDG-Ib. *Biochim Biophys Acta*, *1792*, 841–843.
148. Harms, H. K., Zimmer, K. P., Kurnik, K., et al. (2002). Oral mannose therapy persistently corrects the severe clinical symptoms and biochemical abnormalities of phosphomannose isomerase deficiency. *Acta Paediatrica*, *91*, 1065–1072.
149. González-Barroso, M. M., Giurgea, I., Bouillaud, F., et al. (2008). Mutations in UCP2 in congenital hyperinsulinism reveal a role for regulation of insulin secretion. *PLoS One*, *3*, 3850.
150. Gonzalez-Barrosa MDM, de Lonlay, P., & Ricquier, D. (2012). Hyperinsulinism due to mutations of uncoupling protein 2. In C. A. Stanley, & D. D. De Leon (Eds.), *Monogenic hyperinsulinemic hypoglycemic disorders* (pp. 157–171) Basel: Karger.
151. Mitchell, G. A., Lambert, M., & Tanguay, R. M. (1995). Hypertyrosinemia. In C. R. Scriver, A. L. Beaudet, W. S. Sly, & D. Valle (Eds.), *The metabolic and molecular bases of inherited disease* (p. 1093). New York: McGraw-Hill.
152. Baumann, U., Preece, M. A., Green, A., et al. (2005). Hyperinsulinism in tyrosinaemia type I. J Inherit Metab Dis, 28, 131–135.
153. Hussain, K., Challis, B., Rocha, N., et al. (2011). An activating mutation of AKT2 and human hypoglycemia. *Science*, *334*(6055), 474.
154. Semple, R. K., Savage, D. B., Cochran, E. K., et al. (2011). Genetic syndromes of severe insulin resistance. *Endocr Rev*, *32*, 498–514.
155. Bier, D. M., Schedewie, H., Larner, J., et al. (1980). Glucose kinetics in leprechaunism: accelerated fasting due to insulin resistance. *J Clin Endocrinol Metab*, *51*, 988–994.
156. al-Gazali, L. I., Khalil, M., & Devadas, K. (1993). A syndrome of insulin resistance resembling leprechaunism in five sibs of consanguineous parents. *J Med Genet*, *30*, 470–475.
157. Rosenberg, A. M., Haworth, J. C., Degroot, G. W., et al. (1980). A case of leprechaunism with severe hyperinsulinemia. *Am J Dis Child*, *134*, 170–175.
158. Musso, C., Cochran, E., Moran, S. A., et al. (2004). Clinical course of genetic diseases of the insulin receptor (type A and Rabson-Mendenhall syndromes): a 30-year prospective. *Medicine* (Baltimore), *83*, 209–222.
159. Højlund, K., Hansen, T., Lajer, M., et al. (2004). A novel syndrome of autosomal-dominant hyperinsulinemic hypoglycemia linked to a mutation in the human insulin receptor gene. *Diabetes*, *53*, 1592–1598.
160. Agostini, M., Schoenmakers, E., Mitchell, C., et al. (2006). Non-DNA binding, dominant-negative, human PPARγ mutations cause lipodystrophic insulin resistance. *Cell Metab*, *4*, 303–311.
161. Marks, V., & Teale, J. D. (1999). Hypoglycemia: factitious and felonious. *Endocrinol Metab Clin North Am*, *28*, 579–601.
162. Meadow and Munchausen [Editorial]. (1983). *Lancet*, *1*, 456.
163. Price, W. A., Zimmer, B., Conway, R., & Szekely, B. (1986). Insulin-induced factitious hypoglycemic coma. *Gen Hosp Psychiatry*, *8*, 291–293.
164. Giurgea, I., Ulinski, T., Touati, G., et al. (2005). Factitious hyperinsulinism leading to pancreatectomy: severe forms of Munchausen syndrome by proxy. *Pediatrics*, *116*, e145–e148.
165. Aranibar, H., & Cerda, M. (2005). Hypoglycemic seizure in Munchausen-by-proxy syndrome. *Pediatr Emerg Care*, *21*, 378–379.
166. Marks, V. (1999). Murder by insulin. *Med Leg J*, *67*(Pt. 4), 147–163.
167. Moriyama, M., Hayashi, N., Ohyabu, C., et al. (2006). Performance evaluation and cross-reactivity from insulin analogs with the ARCHITECT insulin assay. *Clin Chem*, *52*, 1423–1426.
168. Owen, W. E., & Roberts, W. L. (2004). Cross-reactivity of three recombinant insulin analogs with five commercial insulin immunoassays. *Clin Chem*, *50*, 257–259.
169. Sperling, M. A. (1980). Insulin biosynthesis and C-peptide: practical applications from basic research. *Am J Dis Child*, *134*, 1119–1121.
170. Vezzosi, D., Bennet, A., Fauvel, J., & Caron, P. (2007). Insulin, C-peptide and proinsulin for the biochemical diagnosis of hypoglycaemia related to endogenous hyperinsulinism. *Eur J Endocrinol*, *157*, 75–83.
171. Marks, V., & Teale, J. D. (1999). Drug-induced hypoglycemia. *Endocrinol Metab Clin North Am*, *28*, 555–577.
172. Pavone, L., Mollica, F., Musumeci, S., et al. (1980). Accidental glibenclamide ingestion in an infant: clinical and electroencephalographic aspects. *Dev Med Child Neurol*, *22*, 366–371.
173. Seltzer, H. S. (1989). Drug-induced hypoglycemia: a review of 1418 cases. *Endocrinol Metab Clin North Am*, *18*, 163–183.
174. Spiller, H. A., Villalobos, D., Krenzelok, E. P., et al. (1997). Prospective multicenter study of sulfonylurea ingestion in children. *J Pediatr*, *131*, 141–146.
175. Quadrani, D. A., Spiller, H. A., & Widder, P. (1996). Five year retrospective evaluation of sulfonylurea ingestion in children. *J Toxicol Clin Toxicol*, *34*, 267–270.
176. Uchigata, Y., Eguchi, Y., Takayama-Hasumi, S., & Omori, Y. (1994). Insulin autoimmune syndrome (Hirata disease): clinical features and epidemiology in Japan. *Diabetes Res Clin Pract*, *22*, 89–94.
177. Redmon, J. B., & Nuttall, F. Q. (1999). Autoimmune hypoglycemia. *Endocrinol Metab Clin North Am*, *28*, 603–618, vii.
178. Goldman, J., Baldwin, D., Rubenstein, A. H., et al. (1979). Characterization of circulating insulin and proinsulin-binding antibodies in autoimmune hypoglycemia. *J Clin Invest*, *63*, 1050–1059.
179. Meschi, F., Dozio, N., Bognetti, E., et al. (1992). An unusual case of recurrent hypoglycaemia: 10-year follow up of a child with insulin auto-immunity. *Eur J Pediatr*, *151*, 32–34.
180. Rovira, A., Valverde, I., Escorihuela, R., & Lopez-Linares M. (1982). Autoimmunity to insulin in a child with hypoglycemia. *Acta Paediatr Scand*, *71*, 343–345.
181. Elias, D., Cohen, I. R., Schechter, Y., et al. (1987). Antibodies to insulin receptor followed by anti-idiotype: antibodies to insulin in child with hypoglycemia. *Diabetes*, *36*, 348–354.
182. Burch, H. B., Clement, S., Sokol, M. S., & Landry, F. (1992). Reactive hypoglycemic coma due to insulin autoimmune syndrome: case report and literature review. *Am J Med*, *92*, 681–685.
183. Uchigata, Y., Hirata, Y., & Iwamoto, Y. (2009). Drug-induced insulin autoimmune syndrome. *Diabetes Res Clin Pract*, *83*, e19–e20.
184. Bortolotti, D., Mothe-Satney, I., Ferrari, P., et al. (2006). Spontaneous hypoglycaemia in the presence of both anti-insulin antibody and anti-insulin receptor antibody. *Diabetes Metab*, *32*, 598–603.
185. Wilkin, T. J., Hammonds, P., Mirza, I., et al. (1988). Graves' disease of the beta cell: glucose dysregulation due to islet-cell stimulating antibodies. *Lancet*, *2*, 1155–1158.

186. Karachaliou, F., Vlachopapadopoulou, E., Kaldrymidis, P., et al. (2006). Malignant insulinoma in childhood. *J Pediatr Endocrinol Metab*, *19*, 757–760.
187. Jaksic, T., Yaman, M., Thorner, P., et al. (1992). A 20-year review of pediatric pancreatic tumors. *J Pediatr Surg*, *27*, 1315–1317.
188. Abood, G. J., Go, A., Malhotra, D., & Shoup, M. (2009). The surgical and systemic management of neuroendocrine tumors of the pancreas. *Surg Clin North Am*, *89*, 249–266, x.
189. Janem, W., Sultan, I., Ajlouni, F., et al. (2010). Malignant insulinoma in a child. *Pediatr Blood Cancer*, *55*, 1423–1426.
190. Goode, P. N., Farndon, J. R., Anderson, J., et al. (1986). Diazoxide in the management of patients with insulinoma. *World J Surg*, *10*, 586–592.
191. Grant, C. S. (2005). Insulinoma. *Best Pract Res Clin Gastroenterol*, *19*, 783–798.
192. Dizon, A. M., Kowalyk, S., & Hoogwerf, B. J. (1999). Neuroglycopenic and other symptoms in patients with insulinomas. *Am J Med*, *106*, 307–310.
193. Placzkowski, K. A., Vella, A., Thompson, G. B., et al. (2009). Secular trends in the presentation and management of functioning insulinoma at the Mayo Clinic, 1987–2007. *J Clin Endocrinol Metab*, *94*, 1069–1073.
194. Mitrakou, A., Fanelli, C., Veneman, T., et al. (1993). Reversibility of unawareness of hypoglycemia in patients with insulinomas. *N Engl J Med*, *329*, 834–839.
195. Nikfarjam, M., Warshaw, A. L., Axelrod, L., et al. (2008). Improved contemporary surgical management of insulinomas: a 25-year experience at the Massachusetts General Hospital. *Ann Surg*, *247*, 165–172.
196. Saddig, C., Goretzki, P. E., & Starke, A. A. (2008). Differentiation of insulin secretion patterns in insulinoma. *World J Surg*, *32*, 918–929.
197. Whipple, A. O., & Frantz, V. K. (1935). Adenoma of islet cells with hyperinsulinism: a review. *Ann Surg*, *101*, 1299–1335.
198. Hirshberg, B., Livi, A., Bartlett, D. L., et al. (2000). Forty-eight-hour fast: the diagnostic test for insulinoma. *J Clin Endocrinol Metab*, *85*, 3222–3226.
199. Service, F. J. (1996). Hypoglycemic disorders. *N Engl J Med*, *332*, 1144–1152.
200. Schiffman, A. B., & Langdon, D. R. (2009). Insulinomas in childhood: twenty year experience at the Children's Hospital of Philadelphia. *Horm Res*, *72*(Suppl. 3), 301.
201. Druce, M. R., Muthuppalaniappan, V. M., O'Leary, B., et al. (2010). Diagnosis and localisation of insulinoma: the value of modern magnetic resonance imaging in conjunction with calcium stimulation catheterisation. *Eur J Endocrinol*, *162*, 971–978.
202. Rostambeigi, N., & Thompson, G. B. (2009). What should be done in an operating room when an insulinoma cannot be found? *Clin Endocrinol*, *70*, 512–515.
203. McLean, A. M., & Fairclough, P. D. (2005). Endoscopic ultrasound in the localisation of pancreatic islet cell tumours. *Best Pract Res Clin Endocrinol Metab*, *19*, 177–193.
204. Eriksson, B., Orlefors, H., Oberg, K., et al. (2005). Developments in PET for the detection of endocrine tumours. *Best Pract Res Clin Endocrinol Metab*, *19*, 311–324.
205. Grossman, A. B., & Reznek, R. H. (2005). Commentary: imaging of islet-cell tumours. *Best Pract Res Clin Endocrinol Metab*, *19*, 241–243.
206. Guettier, J. M., Kam, A., Chang, R., et al. (2009). Localization of insulinomas to regions of the pancreas by intraarterial calcium stimulation: The NIH experience. *J Clin Endocrinol Metab*, *94*, 1074–1080.
207. Huppmann, A., et al. (2012). Spectrum of histopathologic and molecular features of pediatric insulinomas in American Diabetes Association 72nd Scientific Sessions. Philadelphia: American Diabetes Association.
208. Falchetti, A., Marini, F., Luzi, E., et al., (2009). Multiple endocrine neoplasia type 1 (MEN1): not only inherited endocrine tumors. *Genet Med*, *11*, 825–835.
209. Brandi, M. L., Gagel, R. F., Angeli, A., et al., (2001). CONSENSUS: guidelines for diagnosis and therapy of men type 1 and type 2. *J Clin Endocrinol Metab*, *86*, 5658–5671.
210. Tack, J., Arts, J., Caenepeel, P., et al., (2009). Pathophysiology, diagnosis and management of postoperative dumping syndrome. *Nat Rev Gastroenterol Hepatol*, *6*, 583–590.
211. De Leon, D. D. (2012). Role of incretin hormones in hyperinsulinemic hypoglycemia. In C. A. Stanley, & D. D. De Leon (Eds.), *Monogenic hyperinsulinemic hypoglycemic disorders* (pp. 79–86). Basel: Karger.
212. Samuk, I., Afriat, R., Horne, T., et al. (1996). Dumping syndrome following Nissen fundoplication, diagnosis, and treatment. *J Pediatr Gastroenterol Nutr*, *23*, 235–240.
213. Palladino, A. A., Sayed, S., Levitt Katz, L. E., et al. (2009). Increased glucagon-like peptide-1 secretion and postprandial hypoglycemia in children after Nissen fundoplication. *J Clin Endocrinol Metab*, *94*, 39–44.
214. Zung, A., & Zadik, Z. (2003). Acarbose treatment of infant dumping syndrome: extensive study of glucose dynamics and long-term follow-up. *J Pediatr Endocrinol Metab*, *16*, 907–915.
215. Ng, D. D., Ferry, R. J., Jr., Kelly, A., et al. (2002). Acarbose treatment of postprandial hypoglycemia in children after Nissen fundoplication. *J Pediatr*, *139*, 877–879.
216. De Cunto, A., Barbi, E., Minen, F., & Ventura, A. (2011). Safety and efficacy of high-dose acarbose treatment for dumping syndrome. *J Pediatr Gastroenterol Nutr*, *53*, 113–114.
217. McLaughlin, T., Peck, M., Holst, J., & Deacon, C. (2010). Reversible hyperinsulinemic hypoglycemia after gastric bypass: a consequence of altered nutrient delivery. *J Clin Endocrinol Metab*, *95*, 1851–1855.
218. Service, G. J., Thompson, G. B., Service, F. J., et al. (2005). Hyperinsulinemic hypoglycemia with nesidioblastosis after gastric-bypass surgery. *N Engl J Med*, *353*, 249–254.
219. Harris, S. (1932). Hyperinsulinism and dysinsulinism (insulinogenic hypoglycemia) with a chronologic review of cases reported in the United States and Canada. *Internat Clin*, *1*, 9–29.
220. Horton, E. S., Sheikholislam, B. M., & Bressler, R. (1968). Combined sulfonylurea-phenformin therapy of the dysinsulinism of early diabetes mellitus. *Ann N Y Acad Sci*, *148*, 778–786.
221. Danowski, T. S. (1978). *The hypoglycemia syndromes* (p. 164). Pittsburgh: Harper Printing Services.
222. Conn, J. W., Fajans, S. S., & Seltzer, H. S. (1956). Spontaneous hypoglycemia as an early manifestation of diabetes mellitus. *Diabetes*, *5*, 437–442.
223. Freinkel, N., & Metzger, B. E. (1969). Oral glucose tolerance curve and hypoglycemias in the fed state. *N Engl J Med*, *280*, 820–828.
224. Sussman, K. E., Stimmler, L., & Birenboim, H. (1966). Plasma insulin levels during reactive hypoglycemia. *Diabetes*, *15*, 1–4.
225. Chutorian, A. M., Nicholson, J. F., & Killian, P. (1973). Reactive hypoglycemia in children. *Trans Am Neurol Assoc*, *98*, 188–192.
226. Chow, C., & Chow, J. (2007). *Hypoglycemia for dummies* (2nd ed.) (p. 274). Hoboken, NJ: Wiley.
227. Traisman, H. S., Boehm, J. J., & Newcomb, A. L. (1959). Diabetes mellitus at ages one to five; findings at onset. *Diabetes*, *8*, 289–293.
228. Sperling, M., & Drash, A. (1971). Evolution of diabetes mellitus from hypoglycemia. *Am J Dis Child*, *121*, 5–9.
229. Poon, M., & Hussain, K. (2007). Postprandial hyperinsulinaemic hypoglycaemia and type 1 diabetes mellitus. *Arch Dis Child*, *92*, 714–715.
230. Lloyd, J.K. (1964) Diabetes mellitus presenting as spontaneous hypoglycaemia in childhood. *Proc R Soc Med*, 1964. *57*: p. 1061-4.
231. Cristaldi, C., & Becker, D. (2013). Reactive hypoglycemia unmasked by weight loss in early T1D. Washington, DC: Pediatric Academic Societies.
232. Battezzati, A., et al. (2007) Spontaneous hypoglycemia in patients with cystic fibrosis. *Eur J Endocrinol. 156*(3): p. 369-76.
233. Radike, K., et al. (2011) Prognostic relevance of hypoglycemia following an oral glucose challenge for cystic fibrosis-related diabetes. *Diabetes Care. 34*(4): p. e43.
234. Daughaday, W. H. (1989). Hypoglycemia in patients with non-islet cell tumors. *Endocrinol Metab Clin North Am*, *18*, 91–101.
235. Schutt, R. C., Gordon, T. A., Bhabhra, R., et al. (2013). Doege-Potter syndrome presenting with hypoinsulinemic hypoglycemia in a patient with a malignant extrapleural solitary fibrous tumor: a case report. *J Med Case Rep*, *7*, 11.
236. de Groot, J. W., Rikhof, B., van Doorn, J., et al. (2007). Non-islet cell tumour-induced hypoglycaemia: a review of the literature including two new cases. *Endocr Relat Cancer*, *14*, 979–993.

237. Agus, M. S., Katz, L. E., Satin-Smith, M., et al. (1995). Non-islet-cell tumor associated with hypoglycemia in a child: successful long-term therapy with growth hormone. *J Pediatr*, *127*, 403–407.
238. Chung, J., & Henry, R. R. (1996). Mechanisms of tumor-induced hypoglycemia with intraabdominal hemangiopericytoma. *J Clin Endocrinol Metab*, *81*, 919–925.
239. Le Roith, D. (1999). Tumor-induced hypoglycemia. *N Engl J Med*, *341*, 757–758.
240. Dynkevich, Y., Rother, K. I., Whitford, I., et al. (2013). Tumors, IGF-2 and hypoglycemia: insights from the clinic, the laboratory and the historical archive. Endocri Rev Advance online publication. doi:10.1210/er.2012-1033
241. Glasheen, J., & Sorensen, M. (2005). Burkitt's lymphoma presenting with lactic acidosis and hypoglycemia: a case presentation. *Leuk Lymphoma*, *46*, 281–283.
242. Elhomsy, G. C., Eranki, V., Albert, S. G., et al. (2012). "Hyperwarburgism," a cause of asymptomatic hypoglycemia with lactic acidosis in a patient with non-Hodgkin's lymphoma. *J Clin Endocrinol Metab*, *97*, 4311–4316.
243. Kishnani, P. S., Koeberl, D., & Chen Y.-T. (2009). Glycogen storage diseases. In D. Valle, A. L. Beaudet, B. Vogelstein, et al. (Eds.), *The online metabolic & molecular bases of inherited disease*. New York: McGraw-Hill.
244. Froissart, R., Piraud, M., Boudjemline, A. M., et al. (2011). Glucose-6-phosphatase deficiency. *Orphanet J Rare Dis*, *6*, 27.
245. Bandsma, R. H., Smit, G. P., & Kuipers, F. (2002). Disturbed lipid metabolism in glycogen storage disease type 1. *Eur J Pediatr*, *161*(Suppl. 1), S65–S69.
246. Lee, P. J., Dalton, R. N., Shah, V., et al. (1995). Glomerular and tubular function in glycogen storage disease. *Pediatr Nephrol*, *9*, 705–710.
247. Chen, Y. T., Coleman, R. A., Scheinman, J. I., et al. (1988). Renal disease in type I glycogen storage disease. *N Engl J Med*, *318*, 7–11.
248. Wang, D. Q., Fiske, L. M., Carreras, C. T., & Weinstein, D. A. (2011). Natural history of hepatocellular adenoma formation in glycogen storage disease type I. *J Pediatr*, *159*, 442–446.
249. Melis, D., Fulceri, R., Parenti, G., et al. (2005). Genotype/phenotype correlation in glycogen storage disease type 1b: a multicentre study and review of the literature. *Eur J Pediatr*, *164*, 501–508.
250. Matern, D., Seydewitz, H. H., Bali, D., et al. (2002). Glycogen storage disease type I: diagnosis and phenotype/genotype correlation. *Eur J Pediatr*, *161*(Suppl. 1), S10–S19.
251. Rake, J. P., Visser, G., Labrune, P., et al. (2002). Guidelines for management of glycogen storage disease type I: European Study on Glycogen Storage Disease Type I (ESGSD I). *Eur J Pediatr*, *161*(Suppl. 1), S112–S119.
252. Visser, G., Rake, J. P., Labrune, P., et al. (2002). Consensus guidelines for management of glycogen storage disease type 1b: European Study on Glycogen Storage Disease Type 1. *Eur J Pediatr*, *161*(Suppl. 1), S120–S123.
253. Wolfsdorf, J. I., Keller, R. J., Landy, H., & Crigler, J. F., Jr. (1990). Glucose therapy for glycogenosis type 1 in infants: comparison of intermittent uncooked cornstarch and continuous overnight glucose feedings. *J Pediatr*, *117*, 384–391.
254. Weinstein, D. A., & Wolfsdorf, J. I. (2002). Effect of continuous glucose therapy with uncooked cornstarch on the long-term clinical course of type 1a glycogen storage disease. *Eur J Pediatr*, *161*(Suppl. 1), S35–S39.
255. Wolfsdorf, J. I., & Crigler, J. F., Jr. (1997). Cornstarch regimens for nocturnal treatment of young adults with type I glycogen storage disease. *Am J Clin Nutr*, *65*, 1507–1511.
256. Chen, Y. T., Scheinman, J. I., Park, H. K., et al. (1990). Amelioration of proximal renal tubular dysfunction in type I glycogen storage disease with dietary therapy. *N Engl J Med*, *323*, 590–593.
257. Daublin, G., Schwahn, B., & Wendel, U. (2002). Type I glycogen storage disease: favourable outcome on a strict management regimen avoiding increased lactate production during childhood and adolescence. *Eur J Pediatr*, *161*(Suppl. 1), S40–S45.
258. Chou, J. Y., Jun, H. S., & Mansfield, B. C. (2010). Glycogen storage disease type I and G6Pase-[beta] deficiency: etiology and therapy. *Nat Rev Endocrinol*, *6*, 676–688.
259. Kishnani, P. S., Austin, S. L., Arn, P., et al. (2010). Glycogen storage disease type III diagnosis and management guidelines. *Genet Med*, *12*, 446–463.
260. McCallion, N. I., Naughten, E., & Thornton, P. S. (1998). Uncooked corn flour compared to high protein diet in the treatment of GSD type III. *J Inherit Metab Dis*, *21*(Suppl. 2), 93.
261. Dagli, A. I., & Weinstein, D. A. (2011). Glycogen storage disease type VI. In R. A. Pagon, M. P. Adam, T. D. Bird, et al. (Eds.), *GeneReviews*. Seattle: University of Washington.
262. Beauchamp, N. J., Dalton, A., Ramaswami, U., et al. (2007). Glycogen storage disease type IX: high variability in clinical phenotype. *Mol Genet Metab*, *92*, 88–99.
263. Goldstein, J., Austin, S., Kishnani, P., et al. (2011). Phosphorylase kinase deficiency. In R. A. Pagon, M. P. Adam, T. D. Bird, et al. (Eds.), *GeneReviews*. Seattle: University of Washington.
264. Kagalwalla, A. F., Kagalwalla, Y. A., al Ajaji, S., et al. (1995). Phosphorylase b kinase deficiency glycogenosis with cirrhosis of the liver. *J Pediatr*, *127*, 602–605.
265. Orho, M., Bosshard, N. U., Buist, N. R., et al. (1998). Mutations in the liver glycogen synthase gene in children with hypoglycemia due to glycogen storage disease type 0. *J Clin Invest*, *102*, 507–515.
266. Weinstein, D. A., Correia, C. E., Saunders, A. C., & Wolfsdorf, J. I. (2006). Hepatic glycogen synthase deficiency: an infrequently recognized cause of ketotic hypoglycemia. *Mol Genet Metab*, *87*, 284–288.
267. Aynsley-Green, A., Williamson, D. H., & Gitzelmann, R. (1977). Hepatic glycogen synthetase deficiency: definition of syndrome from metabolic and enzyme studies on a 9-year-old girl. *Arch Dis Child*, *52*, 573–579.
268. Santer, R., Steinmann, B., & Schaub, J. (2002). Fanconi-Bickel syndrome—a congenital defect of facilitative glucose transport. *Curr Mol Med*, *2*, 213–227.
269. Wolfsdorf, J. I., Sadeghi-Nejad, A., & Senior, B. (1984). Ketonuria does not exclude hyperinsulinemic hypoglycemia. *Am J Dis Child*, *138*, 168–171.
270. Reid, S. R., & Losek, J. D. (2005). Hypoglycemia complicating dehydration in children with acute gastroenteritis. *J Emerg Med*, *29*, 141–145.
271. Pershad, J., Monroe, K., & Atchison, J. (1998). Childhood hypoglycemia in an urban emergency department: epidemiology and a diagnostic approach to the problem. *Pediatr Emerg Care*, *14*, 268–271.
272. Ross, S. G., & Josephs, H. W. (1924). Observations on the metabolism of recurrent vomiting. *Am J Dis Child*, *28*, 447–457.
273. Haymond, M. W. (1989). Hypoglycemia in infants and children. *Endocrinol Metab Clin North Am*, *18*, 211–252.
274. Pagliara, A. S., Karl, I. E., Haymond, M., et al. (1973). Hypoglycemia in infancy and childhood. II. *J Pediatr*, *82*, 558–577.
275. Pagliara, A. S., Karl, I. E., Haymond, M., et al. (1973). Hypoglycemia in infancy and childhood. I. *J Pediatr*, *82*, 365–379.
276. Stanley, C. A. (2006). Parsing ketotic hypoglycaemia. *Arch Dis Child*, *91*, 460–461.
277. Senior, B. (1973). Ketotic hypoglycemia: a tale (tail) of Gauss? *J Pediatr*, *82*, 555–556.
278. Bodamer, O. A., Hussein, K., Morris, A. A., et al. (2006). Glucose and leucine kinetics in idiopathic ketotic hypoglycaemia. *Arch Dis Child*, *91*, 483–486.
279. Wolfsdorf, J. I., Sadeghi-Nejad, A., & Senior, B. (1982). Hypoalaninemia and ketotic hypoglycemia: cause or consequence? *Eur J Pediatr*, *138*, 28–31.
280. Huidekoper, H. H., Duran, M., Turkenburg, M., et al. (2008). Fasting adaptation in idiopathic ketotic hypoglycemia: a mismatch between glucose production and demand. *Eur J Pediatr*, *167*, 859–865.
281. Weinstein, D. A., et al. (2001). High incidence of unrecognized metabolic and endocrinologic disorders in acutely ill children with previously unrecognized hypoglycemia [Abstract 578]. Baltimore: Pediatric Academic Societies.
282. Correia, C. E., et al. (2005). High incidence of glycogen storage diseases in children with ketotic hypoglycemia [Abstract 789]. Washington, DC: Pediatric Academic Societies.
283. De Feo, P., Perriello, G., Torlone, E., et al. (1989). Demonstration of a role for growth hormone in glucose counterregulation. *Am J Physiol*, *256*, E835–E843.

284. Cryer, P. E. (1996). Role of growth hormone in glucose counterregulation. *Horm Res*, *46*, 192–194.
285. Møller, N., & Jørgensen, J. O L. (2009). Effects of growth hormone on glucose, lipid, and protein metabolism in human subjects. *Endocr Rev*, *30*, 152–177.
286. Steiner, M. M., & Lawrence, G. (1953). Rare dwarfism with chronic hypoglycemia and convulsions. *J Clin Endocrinol Metab*, *13*, 283–299.
287. Brasel, J. A., Wright, J. C., Wilkins, L., & Blizzard, R. M. (1965). An evaluation of seventy-five patients with hypopituitarism beginning in childhood. *Am J Med*, *38*, 484–498.
288. Goodman, H. G., Grumbach, M. M., & Kaplan, S. L. (1968). Growth and growth hormone. II. A comparison of isolated growth-hormone deficiency and multiple pituitary-hormone deficiencies in 35 patients with idiopathic hypopituitary dwarfism. *N Engl J Med*, *278*, 57–68.
289. Hopwood, N. J., Forsman, P. J., Kenny, F. M., & Drash, A. L. (1975). Hypoglycemia in hypopituitary children. *Am J Dis Child*, *129*, 918–926.
290. Johnson, J. D., Hansen, R. C., Albritton, W. L., et al. (1973). Hypoplasia of the anterior pituitary and neonatal hypoglycemia. *J Pediatr*, *82*, 634–641.
291. Thornton, P. S. (2005). Hypoglycemia. In J. T. Moshang (Ed.), *Pediatric endocrinology: the requisites in pediatrics*, St. Louis: Elsevier Mosby.
292. Spray, C. H., Mckiernan, P., Waldron, K. E., et al. (2000). Investigation and outcome of neonatal hepatitis in infants with hypopituitarism. *Acta Paediatr*, *89*, 951–954.
293. Kelly, A., Tang, R., Becker, S., & Stanley, C. A. (2008). Poor specificity of low growth hormone and cortisol levels during fasting hypoglycemia for the diagnoses of growth hormone deficiency and adrenal insufficiency. *Pediatrics*, *122*, e522–e528.
294. Mehta, A., & Dattani, M. T. (2008). Developmental disorders of the hypothalamus and pituitary gland associated with congenital hypopituitarism. *Best Pract Res Clin Endocrinol Metab*, *22*, 191–206.
295. Roe, T. F., & Kogut, M. D. (1971). Hypopituitarism and ketotic hypoglycemia. *Am J Dis Child*, *121*, 296–299.
296. Lorentz, W. B. (1979). Ketotic hypoglycemia and hypopituitarism. *Pediatrics*, *63*, 414–415.
297. Wolfsdorf, J. I., Sadeghi-Nejad, A., & Senior, B. (1983). Hypoketonemia and age-related fasting hypoglycemia in growth hormone deficiency. *Metabolism*, *32*, 457–462.
298. Hussain, K., Hindmarsh, P., & Aynsley-Green, A. (2003). Spontaneous hypoglycemia in childhood is accompanied by paradoxically low serum growth hormone and appropriate cortisol counterregulatory hormonal responses. *J Clin Endocrinol Metab*, *88*, 3715–3723.
299. Perkins, R. F., & Rynearson, E. H. (1952). Practical aspects of insufficiency of the anterior pituitary gland in the adult. *J Clin Endocrinol Metab*, *12*, 574–603.
300. Rosenfeld, R. G., Rosenbloom, A. L., & Guevara-Aguirre, J. (1994). Growth hormone (GH) insensitivity due to primary GH receptor deficiency. *Endocr Rev*, *15*, 369–390.
301. Laron, Z. (2004). Laron syndrome (primary growth hormone resistance or insensitivity): the personal experience 1958–2003. *J Clin Endocrinol Metab*, *89*, 1031–1044.
302. De Feo, P., Perriello, G., Torlone, E., et al. (1989). Contribution of cortisol to glucose counterregulation in humans. *Am J Physiol*, *257*, E35–E42.
303. Zuckerman-Levin, N., Tiosano, D., Eisenhofer, G., et al. (2001). The importance of adrenocortical glucocorticoids for adrenomedullary and physiological response to stress: a study in isolated glucocorticoid deficiency. *J Clin Endocrinol Metab*, *86*, 5920–5924.
304. Cryer, P. E., Binder, C., Bolli, G. B., et al. (1989). Hypoglycemia in IDDM. *Diabetes*, *38*, 1193–1199.
305. Artavia-Loria, E., Chaussain, J. L., Bougnères, P. F., & Job, J. C. (1986). Frequency of hypoglycemia in children with adrenal insufficiency. *Acta Endocrinol Suppl* (Copenh), *279*, 275–278.
306. Christiansen, J. J., Djurhuus, C. B., Gravholt, C. H., et al. (2007). Effects of cortisol on carbohydrate, lipid, and protein metabolism: studies of acute cortisol withdrawal in adrenocortical failure. *J Clin Endocrinol Metab*, *92*, 3553–3559.
307. Hinde, F. R., & Johnston, D. I. (1984). Hypoglycaemia during illness in children with congenital adrenal hyperplasia. *Br Med J* (Clin Res Ed), 289, 1603–1604.
308. Böttner, A., Keller, E., Kratzsch, J., et al. (2004). PROP1 mutations cause progressive deterioration of anterior pituitary function including adrenal insufficiency: a longitudinal analysis. *J Clin Endocrinol Metab*, *89*, 5256–5265.
309. Pernasetti, F., Toledo, S. P., Vasilyev, V. V., et al. (2000). Impaired adrenocorticotropin-adrenal axis in combined pituitary hormone deficiency caused by a two-base pair deletion (301–302delAG) in the prophet of Pit-1 gene. *J Clin Endocrinol Metab*, *85*, 390–397.
310. Krude, H., Biebermann, H., Luck, W., et al. (1998). Severe early-onset obesity, adrenal insufficiency and red hair pigmentation caused by POMC mutations in humans. *Nat Genet*, *19*, 155–157.
311. Nussey, S. S., Soo, S. C., Gibson, S., et al. (1993). Isolated congenital ACTH deficiency: a cleavage enzyme defect? *Clin Endocrinol* (Oxf), *39*, 381–385.
312. Andrioli, M., Giraldi, F., & Cavagnini, F. (2006). Isolated corticotrophin deficiency. *Pituitary*, *9*, 289–295.
313. Jensen, M. D., Handwerger, B. S., Scheithauer, B. W., et al. (1986). Lymphocytic hypophysitis with isolated corticotropin deficiency. *Ann Intern Med*, *105*, 200–203.
314. Clark, A. J., & Weber, A. (1994). Molecular insights into inherited ACTH resistance syndromes. *Trends Endocrinol Metab*, *5*, 209.
315. Pinney, S., et al. (2007). Hypoglycemia and growth failure due to inhaled corticosteroids. Toronto: The Endocrine Society 89th Annual Meeting.
316. Verma, S., Green-Golan, L., VanRyzin, C., et al. (2010). Adrenomedullary function in patients with nonclassic congenital adrenal hyperplasia. *Horm Metab Res*, *42*, 607–6012.
317. Keil, M. F., Bosmans, C., Van Ryzin, C., & Merke, D. P. (2010). Hypoglycemia during acute illness in children with classic congenital adrenal hyperplasia. *J Pediatr Nurs*, *25*, 18–24.
318. Merke, D. P., Chrousos, G. P., Eisenhofer, G., et al. (2000). Adrenomedullary dysplasia and hypofunction in patients with classic 21-hydroxylase deficiency. *N Engl J Med*, *343*, 1362–1368.
319. Christensen, N. J. (1974). Hypoadrenalinemia during insulin hypoglycemia in children with ketotic hypoglycemia. *J Clin Endocrinol Metab*, *38*, 107–112.
320. Brunjes, S., Hodgman, J., Nowack, J., & Johns, V. J., Jr. (1963). Adrenal medullary function in idiopathic spontaneous hypoglycemia of infancy and childhood. *Am J Med*, *34*, 168–176.
321. McBride, J. T., McBride, M. C., & Viles, P. H. (1973). Hypoglycemia associated with propranolol. *Pediatrics*, *51*, 1085–1087.
322. Robinson, B. H. (2009). Lactic acidemia: disorders of pyruvate carboxylase and pyruvate dehydrogenase. In D. Valle, A. L. Beaudet, B. Vogelstein, et al. (Eds.), *The online metabolic & molecular bases of inherited disease*. New York: McGraw-Hill.
323. Robinson, B. H., Oei, J., Saudubray, J. M., et al. (1987). The French and North American phenotypes of pyruvate carboxylase deficiency, correlation with biotin containing protein by 3H-biotin incorporation, 35S-streptavidin labeling, and Northern blotting with a cloned cDNA probe. *Am J Hum Genet*, *40*, 50–59.
324. García-Cazorla, A., Rabier, D., Touati, G., et al. (2006). Pyruvate carboxylase deficiency: Metabolic characteristics and new neurological aspects. *Ann Neurol*, *59*, 121–127.
325. Vidnes, J., & Sovik, O. (1976). Gluconeogenesis in infancy and childhood. II. Studies on the glucose production from alanine in three cases of persistent neonatal hypoglycaemia. *Acta Paediatr Scand*, *65*, 297–305.
326. Vidnes, J., & Sovik, O. (1976). Gluconeogenesis in infancy and childhood. III. Deficiency of the extramitochondrial form of hepatic phosphoenolpyruvate carboxykinase in a case of persistent neonatal hypoglycaemia. *Acta Paediatr Scand*, *65*, 307–312.
327. Hommes, F. A., Bendien, K., Elema, J. D., et al. (1976). Two cases of phosphoenolpyruvate carboxykinase deficiency. *Acta Paediatr Scand*, *65*, 233–240.

328. Baker, L., & Winegrad, A. I. (1970). Fasting hypoglycaemia and metabolic acidosis associated with deficiency of hepatic fructose-1,6-diphosphatase activity. *Lancet*, *2*, 13–16.
329. Steinmann, B., Gitzelmann, R., & Van den Bergh, G. (2009). Disorders of fructose metabolism. In D. Valle, A. L. Beaudet, B. Vogelstein, et al. (Eds.), *The online metabolic & molecular bases of inherited disease.* New York: McGraw-Hill.
330. Chambers, R. A., & Pratt, R. T. (1956). Idiosyncrasy to fructose. *Lancet*, *271*, 340.
331. Hers, H. G., Joassin, G. (1961). [Anomaly of hepatic aldolase in intolerance to fructose.]. *Enzymol Biol Clin* (Basel) *1*, 4–14.
332. Bouteldja, N., & Timson, D. J. (2010). The biochemical basis of hereditary fructose intolerance. *J Inherit Metab Dis*, *33*, 105–112.
333. Kaufmann, U., & Froesch, E. R. (1973). Inhibition of phosphorylase-a by fructose-1-phosphate, alpha-glycerophosphate and fructose-1,6-diphosphate: explanation for fructose-induced hypoglycaemia in hereditary fructose intolerance and fructose-1, 6-diphosphatase deficiency. *Eur J Clin Invest*, *3*, 407–413.
334. Ali, M., Rellos, P., & Cox, T. M. (1998). Hereditary fructose intolerance. *J Med Genet*, *35*, *353*–365.
335. Ziadeh, R., Hoffman, E. P., Finegold, D. N., et al. (1995). Medium chain acyl-CoA dehydrogenase deficiency in Pennsylvania: neonatal screening shows high incidence and unexpected mutation frequencies. *Pediatr Res*, *37*, 675–678.
336. Hale, D. E., & Bennett, M. J. (1992). Fatty acid oxidation disorders: a new class of metabolic diseases. *J Pediatr*, *121*, 1–11.
337. Roe, C. R., & Ding, J. (2009). Mitochondrial fatty acid oxidation disorders. In D. Valle, A. L. Beaudet, B. Vogelstein, et al. (Eds.), *The online metabolic & molecular bases of inherited disease.* New York: McGraw-Hill.
338. Treacy, E. P., Lambert, D. M., Barnes, et al. (2000). Short-chain hydroxyacyl-coenzyme A dehydrogenase deficiency presenting as unexpected infant death: a family study. *J Pediatr*, *137*, 257–259.
339. Treem, W. R., Stanley, C. A., Finegold, D. N., et al. (1988). Primary carnitine deficiency due to a failure of carnitine transport in kidney, muscle, and fibroblasts. *N Engl J Med*, *319*, 1331–1336.
340. Saudubray J.-M., & Charpentier, C. (2009). Diagnostic approaches. In D. Valle, A. L. Beaudet, B. Vogelstein, et al. (Eds.), *The online metabolic & molecular bases of inherited disease.* New York: McGraw-Hill.
341. Merimee, T. J., & Tyson, J. E. (1974). Stabilization of plasma glucose during fasting; Normal variations in two separate studies. *N Engl J Med*, *291*, 1275–1278.
342. Morrice, J. J., Taylor, K. M., Blair, J. I., & Young, D. G. (1974). Preoperative plasma glucose level. *Arch Dis Child*, *49*, 898–900.
343. O'Flynn, P. E., & Milford, C. A. (1989). Fasting in children for day case surgery. *Ann R Coll Surg Engl*, *71*, 218–219.
344. Mizock, B. A. (1995). Alterations in carbohydrate metabolism during stress: a review of the literature. *Am J Med*, *98*, 75–84.
345. Strapazzon, G., Nardin, M., Zanon, P., et al. (2012). Respiratory failure and spontaneous hypoglycemia during noninvasive rewarming from 24.7°C (76.5°F) core body temperature after prolonged avalanche burial. *Ann Emerg Med*, *60*, 193–196.
346. Boles, J. M., Mabille, S., Scheydecker, J. L., et al. (1988). Hypoglycaemia in salt water near-drowning victims. *Intensive Care Med*, *14*, 80.
347. Elias, A. N., & Gwinup, G. (1982). Glucose-resistant hypoglycemia in inanition. *Arch Intern Med*, *142*, 743–746.
348. Kerpel-Fronius, E., & Kaiser, E. (1967). Hypoglycaemia in infantile malnutrition. *Acta Paediatr Scand*, *56*(Suppl. 173), 119–127.
349. Wharton, B. (1970). Hypoglycaemia in children with kwashiorkor. *Lancet*, *1*, 171–173.
350. Ratcliffe, P. J., & Bevan, S. J. (1995). Severe hypoglycaemia and sudden death in anorexia nervosa. *Psychol Med*, *15*, 679–681.
351. Yamada, Y., Fushimi, H., Inoue, T., et al. (1996). Anorexia nervosa with recurrent hypoglycemic coma and cerebral hemorrhage. *Intern Med*, *35*, 560–563.
352. Rich, L. M., Caine, M. R., Findling, J. W., & Shaker, J. L. (1990). Hypoglycemic coma in anorexia nervosa: case report and review of the literature. *Arch Intern Med*, *150*, 894–895.
353. Sakurai-Chin, C., Ito, N., Taguchi, M., et al. (2010). Hypoglycemic coma in a patient with anorexia nervosa coincident with acute exacerbation of liver injury induced by oral intake of nutrients. *Intern Med*, *49*, 1553–1556.
354. Boström, P., Wu, J., Jedrychowski, M. P., et al. (2012). A PGC1-alpha-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature*, *481*, 463–468.
355. Pedersen, B. K. (2012). A muscular twist on the fate of fat. *N Engl J Med*, *366*, 1544–1545.
356. Hirsch, I. B., Marker, J. C., Smith, L. J., et al. (1991). Insulin and glucagon in prevention of hypoglycemia during exercise in humans. *Am J Physiol*, *260*, E695–E704.
357. Hoelzer, D. R., Dalsky, G. P., Clutter, W. E., et al. (1986). Glucoregulation during exercise: hypoglycemia is prevented by redundant glucoregulatory systems, sympathochromaffin activation, and changes in islet hormone secretion. *J Clin Invest*, *77*, 212–221.
358. Marker, J. C., Hirsch, I. B., Smith, L. J., et al. (1991). Catecholamines in prevention of hypoglycemia during exercise in humans. *Am J Physiol*, *260*(5, Pt. 1), E705–E712.
359. Brun, J. F., Dumortier M., Fedou, C., & Mercier, J. (2001). Exercise hypoglycemia in nondiabetic subjects. *Diabete Metab* (Paris) *27*, 102–106.
360. Field, J. B. (1989). Exercise and deficient carbohydrate storage and intake as causes of hypoglycemia. *Endocrinol Metab Clin North Am*, *18*, 155–161.
361. French, J. K., & Frengley, P. A. (1983). Hypoglycemia induced seizures following a marathon. *N Z Med J*, *96*, 407.
362. Levine, S. A., Gordon, B., & Derick, C. L. (1924). Some changes in the chemical constituents of the blood following a marathon race with special reference to the development of hypoglycemia. *JAMA*, *82*, 1778–1779.
363. Frank, P., Katz, A., Andersson, E., & Sahlin, K. (2013). Acute exercise reverses starvation-mediated insulin resistance in humans. *Am J Physiol*, *304*, E436–E443.
364. Nigro, G. (1991). Pancreatitis with hypoglycemia-associated convulsions following rotavirus gastroenteritis. *J Pediatr Gastroenterol Nutr*, *12*, 280–282.
365. Haymond, M. W. (1990). Diarrhea, malnutrition, euglycemia, and fuel for thought. *N Engl J Med*, *322*, 1390–1391.
366. Khan, W. A., Dhar, U., Salam, M. A., et al. (1999). Central nervous system manifestations of childhood shigellosis: prevalence, risk factors, and outcome. *Pediatrics*, *103*, e18.
367. Bennish, M. L., Azad, A. K., Rahman, O., & Phillips, R. E. (1990). Hypoglycemia during diarrhea in childhood: prevalence, pathophysiology, and outcome. *N Engl J Med*, *322*, 1357–1363.
368. Miller, S. I., Wallace, R. J., Jr., Musher, D. M., et al. (1980). Hypoglycemia as a manifestation of sepsis. *Am J Med*, *68*, 649–654.
369. Preyra, I., & Worster, A. (2003). Hypoglycemia in bacterial septicemia. *CJEM*, *5*, 268–270.
370. Maitra, S. R., Wojnar, M. M., & Lang, C. H. (2000). Alterations in tissue glucose uptake during the hyperglycemic and hypoglycemic phases of sepsis. *Shock*, *13*, 379–385.
371. Romijn, J. A., Godfried, M. H., Wortel, C., & Sauerwein, H. P. (1990). Hypoglycemia, hormones and cytokines in fatal meningococcal septicemia. *J Endocrinol Invest*, *13*, 743–747.
372. White, N. J., Miller, K. D., Marsh, K., et al. (1987). Hypoglycaemia in African children with severe malaria. *Lancet*, *1*, 708–711.
373. Krishna, S., Waller, D. W., ter Kuile, F., et al. (1994). Lactic acidosis and hypoglycaemia in children with severe malaria: pathophysiological and prognostic significance. *Trans R Soc Trop Med Hyg*, *88*, 67–73.
374. Phillips, R. E., Looareesuwan, S., White, N. J., et al. (1986). Hypoglycaemia and antimalarial drugs: quinidine and release of insulin. *Br Med J* (Clin Res Ed), *292*, 1319–1321.
375. Olson, L. C. (1975). Pertussis. *Medicine* (Baltimore) *54*, 427–469.
376. Faustino, E. V., Hirshberg, E. L., & Bogue, C. W. (2012). Hypoglycemia in critically ill children. *J Diabetes Sci Technol*, *6*, 48–57.
377. Fischer, K. F., Lees, J. A., & Newman, J. H. (1986). Hypoglycemia in hospitalized patients. Causes and outcomes. *N Engl J Med*, *315*, 1245–1250.
378. deCastro, F. J., Jaeger, R., & Gleason, W. A., Jr. (1977). Liver damage and hypoglycemia in acute iron poisoning. *Clin Toxicol*, *10*, 287–289.

379. Felig, P., Brown, W. V., Levine, R. A., & Klatskin, G. (1970). Glucose homeostasis in viral hepatitis. *N Engl J Med*, *283*, 1436–1440.
380. Senadhi, V. (2011). Recurrent hypothermia and hypoglycemia as the initial presentation of hepatitis C. *Clinics and Practice*, *1*, 30.
381. Conn, J. W. (1947). The diagnosis and management of spontaneous hypoglycemia. *JAMA*, *134*, 130–138.
382. Marks, V. (1981). Hepatogenous and nephrogenic hypoglycaemia. In V. Marks, & F. C. Rose (Eds.), *Hypoglycaemia* (pp. 216–226). Oxford: Blackwell Scientific.
383. Arky, R. A. (1989). Hypoglycemia associated with liver disease and ethanol. *Endocrinol Metab Clin North Am*, *18*, 75–90.
384. Kobayashi, K., Saheki, T., & Song, Y. Z. (2012). Citrin deficiency. In R. A. Pagon, M. P. Adam, T. D. Bird, et al. (Eds.), *GeneReviews*. Seattle: University of Washington.
385. Wells, T. G., Ulstrom, R. A., & Nevins, T. E. (1988). Hypoglycemia in pediatric renal allograft recipients. *J Pediatr*, *113*, 1002–1007.
386. Peitzman, S. J., & Agarwal, B. N. (1977). Spontaneous hypoglycemia in end-stage renal failure. *Nephron*, *19*, 131–139.
387. Arem, R. (1989). Hypoglycemia associated with renal failure. *Endocrinol Metab Clin North Am*, *18*, 103–121.
388. Toth, E. L., & Lee, D. W. (1991). 'Spontaneous'/uremic hypoglycemia is not a distinct entity: substantiation from a literature review. *Nephron*, *58*, 325–329.
389. Friedlander, E. O. (1960). Hyperinsulinism secondary to disease of the pancreas and organs adjacent to the pancreas: a review of the world literature. *Ann Intern Med*, *52*, 838–848.
390. Marks, V. (1981). Pancreatic hypoglycaemia—other causes. In V. Marks, & F. C. Rose (Eds.), *Hypoglycaemia* (pp. 158–178). Oxford: Blackwell Scientific.
391. Laraya-Cuasay, L. R., Wolfe, R., & Hughes, W. T. (1968). Acute pancreatitis and hypoglycemia. *Clin Pediatr* (Phila), *7*, 525–528.
392. Teng, R. J., Wu, T. J., & Ho, M. M. (1997). Mumps infection complicated by transient hyperinsulinemic hypoglycemia. *Pediatr Infect Dis J*, *16*, 416–417.
393. Haymond, M. W., Strauss, A. W., Arnold, K. J., & Bier, D. M. (1979). Glucose homeostasis in children with severe cyanotic congenital heart disease. *J Pediatr*, *95*, 210–213.
394. Benzing, G., 3rd, Schubert, W., Sug, G., & Kaplan S. (1969). Simultaneous hypoglycemia and acute congestive heart failure. *Circulation*, *40*, 209–216.
395. Hedayati, H. A., & Beheshti, M. (1977). Profound spontaneous hypoglycaemia in congestive heart failure. *Curr Med Res Opin*, *4*, 501–504.
396. Mellinkoff, S. M., & Tumulty, P. A. (1952). Hepatic hypoglycemia: its occurrence in congestive heart failure. *N Engl J Med*, *247*, 745–750.
397. Amatayakul, O., Cumming, G. R., & Haworth, J. C. (1970). Association of hypoglycaemia with cardiac enlargement and heart failure in newborn infants. *Arch Dis Child*, *45*, 717–720.
398. Shu, S., Cruse, R. P., & Redmond, G. P. (1989). Hypoglycemia in a child with congenital muscular dystrophy. *Brain Dev*, *11*, 62–65.
399. Bruce, A. K., Jacobsen, E., Dossing, H., & Kondrup, J. (1995). Hypoglycaemia in spinal muscular atrophy. *Lancet*, *346*, 609–610.
400. Aboumousa, A., Hoogendijk, J., Charlton, R., et al. (2008). Caveolinopathy—new mutations and additional symptoms. *Neuromuscul Disord*, *18*, 572–578.
401. Ørngreen, M. C., Zacho, M., & Hebert, A. (2003). Patients with severe muscle wasting are prone to develop hypoglycemia during fasting. *Neurology*, *61*, 997–1000.
402. Hadfield, M. G., Vennart, G. P., & Rosenblum, W. I. (1972). Hypoglycemia: invasion of the hypothalamus by lymphosarcoma: metastasis to blood glucose regulating centers. *Arch Pathol*, *94*, 317–321.
403. Deloof, T., Berre, J., Genette, F., et al. (1979). Disturbances of the carbohydrate metabolism in acute head trauma. *Acta Neurochir Suppl* (Wien), *28*, 113–114.
404. Eadington, D. W. (1988). Hypoglycaemia and metabolic acidosis in a patient with an acute leukaemia. *Scott Med J*, *33*, 309–310.
405. Halonen, P., Salo, M. K., & Mäkipernaa, A. (2001). Fasting hypoglycemia is common during maintenance therapy for childhood acute lymphoblastic leukemia. *J Pediatr*, *138*, 428–431.
406. Halonen, P., Salo, M. K., Schmiegelow, K., & Mäkipernaa A. (2003). Investigation of the mechanisms of therapy-related hypoglycaemia in children with acute lymphoblastic leukaemia. *Acta Pædiatrica*, *92*, 37–42.
407. Ziino, O., Russo, D., Orlando, M. A., et al. (2002). Symptomatic hypoglycemia in children receiving oral purine analogues for treatment of childhood acute lymphoblastic leukemia. *Med Pediatr Oncol*, *39*, 32–34.
408. Wintergerst, K. A., Buckingham, B., Gandrud, L., et al. (2006). Association of hypoglycemia, hyperglycemia, and glucose variability with morbidity and death in the pediatric intensive care unit. *Pediatrics*, *118*, 173–179.
409. Stout, S. M., & Cober, M. P. (2010). Metabolic effects of cyclic parenteral nutrition infusion in adults and children. *Nutr Clin Pract*, *25*, 277–281.
410. Dungan, K., Chapman, J., Braithwaite, S. S., & Buse, J. (2007). Glucose measurement: confounding issues in setting targets for inpatient management. *Diabetes Care*, *30*, 403–409.
411. Vriesendorp, T. M., DeVries, J. H., & Hoekstra, J. B. (2008). Hypoglycemia and strict glycemic control in critically ill patients. *Curr Opin Crit Care*, *14*, 397–402.
412. Kandil, S. B., Miksa, M., & Faustino, E. V. (2013). Control of serum glucose concentration in critical illness. *Curr Opin Pediatr*, *25*, 297–303.
413. Mesotten, D., Gielen, M., Sterken, C., et al. (2012). Neurocognitive development of children 4 years after critical illness and treatment with tight glucose control: a randomized controlled trial. *JAMA*, *308*, 1641–1650.
414. Meijering, S., Corstjens, A. M., Tulleken, J. E., et al. (2006). Towards a feasible algorithm for tight glycaemic control in critically ill patients: a systematic review of the literature. *Crit Care*, *10*, R19.
415. Murad, M. H., Coto-Yglesias, F., Wang, A. T., et al. (2009). Drug-induced hypoglycemia: a systematic review. *J Clin Endocrinol Metab*, *94*, 741–745.
416. Ben Salem, C., Fathallah, N., Hmouda, H., & Bouraoui, K. (2011). Drug-induced hypoglycaemia. *Drug Saf*, *34*, 21–45.
417. Ernst, A. A., Jones, K., Nick, T. G., & Sanchez, J. (1996). Ethanol ingestion and related hypoglycemia in a pediatric and adolescent emergency department population. *Acad Emerg Med*, *3*, 46–49.
418. Freinkel, N., Arky, R. A., Singer, D. L., et al. (1965). Alcohol hypoglycemia: IV: current concepts of its pathogenesis. *Diabetes*, *14*, 350–361.
419. Bradford, D. (1981). Ethanol poisoning in children. *Br J Alcohol Alcoholism*, *16*, 27.
420. Moss, M. H. (1970). Alcohol-induced hypoglycemia and coma caused by alcohol sponging. *Pediatrics*, *46*, 445–447.
421. Massey, C. C., & Shulman, J. D. (2006). Acute ethanol toxicity from ingesting mouthwash in children younger than age 6, 1989–2003. *Pediatr Dent*, *28*, 405–409.
422. Rayar, P., & Ratnapalan, S. (2013). Pediatric ingestions of house hold products containing ethanol: a review. *Clin Pediatr* (Phila), *52*, 203–209.
423. Baron, S. H. (1982). Salicylates as hypoglycemic agents. *Diabetes Care*, *5*, 64–71.
424. Fang, V., Foye, W. O., Robinson, S. M., Jenkins, H. J. (1968). Hypoglycemic activity and chemical structure of the salicylates. *J Pharm Sci*, *57*, 2111–2116.
425. Limbeck, G. A., Ruvalcaba, R. H., Samols, E., & Kelley, V. C. (1965). Salicylates and hypoglycemia. *Am J Dis Child*, *109*, 165–167.
426. Permutt, M. A., Kelly, J., Berstein, R., et al. (1973). Alimentary hypoglycemia in the absence of gastrointestinal surgery. *N Engl J Med*, *288*, 1206–1210.
427. Middleton, S. J., & Balan, K. (2012). Idiopathic accelerated gastric emptying presenting in adults with post-prandial diarrhea and reactive hypoglycemia: a case series. *J Med Case Rep*, *6*, 132.
428. Howland, G., et al. (1929). Dysinsulinism: convulsions and coma due to islet cell tumor of the pancreas, with operation and cure. *JAMA*, *93*, 674–679.

429. Service, F. J. (1999). Classification of hypoglycemic disorders. *Endocrinol Metab Clin North Am*, *28*, 501–517, vi.
430. Luyckx, A. S., & Lefebvre, P. J. (1971). Plasma insulin in reactive hypoglycemia. *Diabetes*, *20*, 435–442.
431. Lev-Ran, A., & Anderson, R. W. (1981). The diagnosis of postprandial hypoglycemia. *Diabetes*, *30*, 996–999.
432. Snorgaard, O., & Binder, C. (1990). Monitoring of blood glucose concentration in subjects with hypoglycaemic symptoms during everyday life. *BMJ*, *300*, 16–18.
433. Johnson, D. D., Dorr, K. E., Swenson, W. M., & Service, F. J. (1980). Reactive hypoglycemia. *JAMA*, *243*, 1151–1155.
434. Ahmadpour, S., & Kabadi, U. M. (1997). Pancreatic α-cell function in idiopathic reactive hypoglycemia. *Metabolism*, *46*, 639–643.
435. Berlin, I., Grimaldi, A., Landault, C., et al. (1994). Suspected postprandial hypoglycemia is associated with beta-adrenergic hypersensitivity and emotional distress. *J Clin Endocrinol Metab*, *79*, 1428–1433.
436. Chalew, S. A., McLaughlin, J. V., Mersey, J. H., et al. (1984). The use of the plasma epinephrine response in the diagnosis of idiopathic postprandial syndrome. *JAMA*, *251*, 612–615.
437. Charles, M. A., & Waldeck, N. (1981). Excessive circulating large molecular weight immunoreactive glucagon components in subjects with the idiopathic postprandial syndrome. *J Clin Endocrinol Metab*, *53*, 366–371.
438. Lefebvre, P. J., Luyckx, A. S., & Lecomte, M. J. (1976). Studies on the pathogenesis of reactive hypoglycemia: role of insulin and glucagon. *Horm Metab Res*, *8*, (Suppl. 6), 91–98.
439. Alken, J., Petriczko, E., & Marcus, C. (2008). Effect of fasting on young adults who have symptoms of hypoglycemia in the absence of frequent meals. *Eur J Clin Nutr*, *62*, 721–726.
440. Anderson, J. W., & Herman, R. H. (1975). Effects of carbohydrate restriction on glucose tolerance of normal men and reactive hypoglycemic patients. *Am J Clin Nutr*, *28*, 748–755.
441. Chalew, S. A., Koetter, H., Hoffman, S., et al. (1986). Diagnosis of reactive hypoglycemia: pitfalls in the use of the oral glucose tolerance test. *South Med J*, *79*, 285–287.
442. Brun, J. F., Fédou, C., Bouix, O., et al. (1995). Evaluation of a standardized hyperglucidic breakfast test in postprandial reactive hypoglycaemia. *Diabetologia*, *38*, 494–501.
443. Charles, M. A., Hofeldt, F., Shackelford, A., et al. (1981). Comparison of oral glucose tolerance tests and mixed meals in patients with apparent idiopathic postabsorptive hypoglycemia: absence of hypoglycemia after meals. *Diabetes*, *30*, 465–470.
444. Hofeldt, F. D. (1987). Psychological profile in reactive hypoglycemia. In D. Andreani, V. Marks, P. J. Lefebvre (Eds.), *Hypoglycemia* (pp. 229–236). New York: Raven.
445. Ford, C. V., Bray, G. A., & Swerdloff, R. S. (1976). A psychiatric study of patients referred with a diagnosis of hypoglycemia. *Am J Psychiatry*, *133*, 290–294.
446. Anonymous (1973). Special report from the American Diabetes Association, The Endocrine Society, and the American Medical Association: Statement on hypoglycemia. *Diabetes*, *22*, 137.
447. Blasetti, A., Di Pietro, L., di Corcia, G., et al. (2007). Can insulinoma cause generalised epilepsy? *J Pediatr Endocrinol Metab*, *20*, 837–840.
448. Kapoor, W. N. (2000). *Syncope. N Engl J Med*, *343*, 1856–1862.
449. Wieling, W., Thijs, R. D., van Dijk, N., et al. (2009). Symptoms and signs of syncope: a review of the link between physiology and clinical clues. *Brain*, *132*, 2630–2642.
450. Griffith, L. S., McGill, J. B., & Lustman, P. J. (1995). Panic disorder misperceived as hypoglycemia. Atlanta: American Diabetes Association 55th Annual Meeting & Scientific Sessions.
451. Moodley, M. (2013). Clinical approach to syncope in children. *Semin Pediatr Neurol*, *20*, 12–17.
452. Johnsrude, C. L. (2000). Current approach to pediatric syncope. *Pediatr Cardiol*, *21*, 522–531.
453. Stewart, J. M. (2013). Common syndromes of orthostatic intolerance. *Pediatrics*, *131*, 968–980.
454. Grubb, B. P., Kanjwal, Y., & Kosinski, D. J. (2006). The postural tachycardia syndrome: a concise guide to diagnosis and management. *J Cardiovasc Electrophysiol*, *17*, 108–112.
455. Stewart, J. M. (2012). Update on the theory and management of orthostatic intolerance and related syndromes in adolescents and children. *Expert Rev Cardiovasc Ther*, *10*, 1387–1399.
456. Marks, V. (1987). Glycemic stability in healthy subjects: fluctuations in blood glucose concentration through the day. In D. Andreani, V. Marks, & P. J. Lefebvre (Eds.), *Hypoglycemia* (pp. 19–24). New York: Raven.
457. Barry, J. A., Bouloux, P., & Hardiman, P. J. (2011). The impact of eating behavior on psychological symptoms typical of reactive hypoglycemia: a pilot study comparing women with polycystic ovary syndrome to controls. *Appetite*, *57*, 73–76.
458. Palardy, J., Havrankova, J., Lepage, R., et al. (1989). Blood glucose measurements during symptomatic episodes in patients with suspected postprandial hypoglycemia. *N Engl J Med*, *321*, 1421–1425.
459. Virkkunen, M. (1983). Insulin secretion during the glucose tolerance test in antisocial personality. *Br J Psychiatry*, *142*, 598–604.
460. Gailliot, M. T., & Baumeister, R. F. (2007). The physiology of willpower: linking blood glucose to self-control. *Pers Soc Psychol Rev*, *11*, 303–327.
461. Bolton, R. (1973). Aggression and hypoglycemia among the Qolla: a study in psychobiological anthropology. Ethnology, 12, 227–257.
462. Bolton, R. (1981). Susto, hostility, and hypoglycemia. Ethnology, 20, 261–276.
463. Bolton, R., et al. (1984). The hypoglycemia-aggression hypothesis: debate versus research [and comments and reply]. *Curr Anthropol*, *25*, 1–53.
464. Roy, A., Virkkunen, M., Guthrie, S., & Linnoila, M. (1986). Indices of serotonin and glucose metabolism in violent offenders, arsonists, and alcoholics. *Ann N Y Acad Sci*, *487*, 202–220.
465. Smith, L. (1977). *Improving your child's behavior chemistry* (p. 270). New York: Pocket Books.
466. Crook, W. G., & Stevens L. J. (1987). *Solving the puzzle of your hard-to-raise child* (p. 369). New York: Random House.
467. Benton, D. (1988). Hypoglycemia and aggression: a review. *Int J Neurosci*, *41*, 163–168.
468. Bachorowski, J. A., Newman, J. P., Nichols, S. L., et al. (1990). Sucrose and delinquency: behavioral assessment. *Pediatrics*, *86*, 244–253.
469. Wender, E. H., & Solanto, M. V. (1991). Effects of sugar on aggressive and inattentive behavior in children with attention deficit disorder with hyperactivity and normal children. *Pediatrics*, *88*, 960–966.
470. Gans, D. A., Harper, A. E., Bachorowski, J. A., et al. (1990). Sucrose and delinquency: oral sucrose tolerance test and nutritional assessment. *Pediatrics*, *86*, 254–262.
471. Lowden, A., Holmbäck, U., Akerstedt, T., et al. (2004). Performance and sleepiness during a 24 h wake in constant conditions are affected by diet. *Biol Psychol*, *65*, 251–263.
472. Jones, T. W., Borg, W. P., Boulware, S. D., et al. (1995). Enhanced adrenomedullary response and increased susceptibility to neuroglycopenia: mechanisms underlying the adverse effects of sugar ingestion in healthy children. *J Pediatr*, *126*, 171–177.
473. Wolraich, M. L., Wilson, D. B., & White, J. W. (1995). The effect of sugar on behavior or cognition in children: a meta-analysis. *JAMA*, *274*, 1617–1621.
474. Wolraich, M. L., Lindgren, S. D., Stumbo, P. J., et al. (1994). Effects of diets high in sucrose or aspartame on the behavior and cognitive performance of children. *N Engl J Med*, *330*, 301–307.
475. Horwitz, D. L. (1989). Factitious and artifactual hypoglycemia. *Endocrinol Metab Clin North Am*, *18*, 203–210.
476. Bergmeyer, H. U., et al. (1974). Handling of biochemical reagents and samples. In H. U. Bergmeyer (Ed.), *Methods of enzymatic analysis* (p. 165). Weinheim New York: Verlag Chemie, Academic Press.
477. Giampietro, O., Navalesi, R., Buzzigoli, G., et al. (1980). Decrease in plasma glucose concentration during storage at (20 degrees C. *Clin Chem*, *26*, 1710–1712.
478. de Pasqua, A., Mattock, M. B., Phillips, R., & Keen, H. (1984). Errors in blood glucose determination. *Lancet*, *2*, 1165.
479. Billington, C. J., Casciato, D. A., Choquette, D. L., & Morley, J. E. (1983). Artifactual hypoglycemia associated with polycythemia vera. *JAMA*, *249*, 774–775.

480. Macaron, C. I., Kadri, A., & Macaron, Z. (1981). Nucleated red blood cells and artifactual hypoglycemia. *Diabetes Care*, *4*, 113–115.
481. Clarke, W. L., Cox, D., Gonder-Frederick, L. A., et al. (1987). Evaluating clinical accuracy of systems for self-monitoring of blood glucose. *Diabetes Care*, *10*, 622–628.
482. Mauras, N., Beck, R. W., Ruedy, K. J., et al. (2004). Lack of accuracy of continuous glucose sensors in healthy, nondiabetic children: results of the Diabetes Research in Children Network (DirecNet) accuracy study. *J Pediatr*, *144*, 770–775.
483. Munir, A., Choudhary, P., Harrison, B., et al. (2008). Continuous glucose monitoring in patients with insulinoma. *Clin Endocrinol* (Oxf), *68*, 912–918.
484. Hay, W. W., Jr., & Rozance, P. J. (2010). Continuous glucose monitoring for diagnosis and treatment of neonatal hypoglycemia. *J Pediatr*, *157*, 180–182.
485. Harris, D. L., Battin, M. R., Weston, P. J., & Harding, J. E. (2010). Continuous glucose monitoring in newborn babies at risk of hypoglycemia. *J Pediatr*, *157*, 198–202, e1.
486. Mitrakou, A., Ryan, C., Veneman, T., et al. (1991). Hierarchy of glycemic thresholds for counterregulatory hormone secretion, symptoms, and cerebral dysfunction. *Am J Physiol*, *260*, E67–E74.
487. Buchwald, P. (2011). A local glucose-and oxygen concentration-based insulin secretion model for pancreatic islets. *Theor Biol Med Model*, *8*, 20.
488. Quinkler, M., Strelow, F., Pirlich, M., et al. (2007). Assessment of suspected insulinoma by 48-hour fasting test: a retrospective monocentric study of 23 cases. *Horm Metab Res*, *39*, 507–510.

QUESTIONS

A 30-month-old boy was too lethargic to be fed after his mother tried to awaken him in the morning. He was rushed to the local emergency department where he was still obtunded and lab glucose was 37 mg/dL. He improved with intravenous dextrose. An analysis of bagged urine shows a large amount of ketones. No other diagnostic tests have been done when you are called for advice 2 hours later, around noon.

1. Which history may be most relevant to the cause of his hypoglycemia?
   a. His grandmother, who lives with him, takes glyburide,
   b. His mother had gestational diabetes requiring insulin,
   c. His length is small compared to family heights,
   d. He has been taking both inhaled and nasal fluticasone for asthma and rhinitis,
   e. Both c and d

Answer: e. The ketonuria makes it unlikely that his hypoglycemia is due to injected insulin or sulfonylurea ingestion. However, both growth hormone deficiency and adrenal insufficiency due to suppression can cause morning hypoglycemia with ketosis.

2. Tests that will be of most value if obtained in the next hour include which of the following?
   a. Basic chemistry panel
   b. Insulin and C-peptide
   c. Cortisol and growth hormone
   d. Acylcarnitine profile and urine organic acids
   e. Both a and d

Answer: e. It is important to see if he is acidotic, and both the acylcarnitine profile and the urine organic acid will remain abnormal even after immediate correction of hypoglycemia for most organic acidoses and some of the disorders of fatty acid oxidation. Insulin and c-peptide will have already risen from the dextrose, and single GH and cortisol levels at noon would be of limited value for excluding adrenal or pituitary deficiency.

A 15-year-old girl complained to her family doctor of "almost passing out" on three occasions late in the morning at school. Her doctor ordered a fasting chemistry panel that was unremarkable except for a glucose level of 62. Her doctor then ordered a 5-hour oral glucose tolerance test that produced the following glucose levels without symptoms: 88, 123, 78, 61, 69, and 75. Which of the following statements are true?

3. A low fasting glucose in a routinely ordered chemistry panel drawn when this patient was asymptomatic is more likely to be an artifact of delayed specimen processing than hypoglycemia. True or false?

Answer: True. Routine chemistry panels are often subject to delayed separation of serum.

4. The OGTT is abnormal, as less than 10% of healthy young women reach glucose levels below 60 mg/dL in the latter half of an extended oral glucose tolerance test, and the glucose level at 2 to 3 hours should not fall below the baseline fasting value. True or false?

Answer: False. More than 10% of healthy young women will fall below 50 mg/dL during an extended OGTT. Later glucose levels during an OGTT are often lower than the baseline in healthy people.

5. An insulinoma is excluded by these test results. True or false?

Answer: False. Insulinomas are uncommon, but neither a random fasting glucose nor an OGTT changes the small probability that the patient has one.

6. An insulinoma is excluded because her symptoms did not occur while fasting. True or false?

Answer: False. Hypoglycemia from insulinomas may occur at any time in relation to meals.

7. The next step to exclude an insulinoma would be to obtain a glucose reading during symptoms, either by comparing a meter glucose obtained during symptoms with some glucoses without symptoms, or by conducting an extended diagnostic fast. True or false?

Answer: True. An insulinoma is a rare cause of such spells in adolescents, but this is a possible presentation and the next step is to try to demonstrate hypoglycemia during symptoms.

CHAPTER 22

Obesity, Metabolic Syndrome, and Disorders of Energy Balance

Ram Weiss, MD, PhD • Robert H. Lustig, MD, MSL

CHAPTER OUTLINE

INTRODUCTION

Energy balance is the "final frontier" of endocrinology. Prior to 1994, with the discovery of leptin, disorders of energy balance were not even considered to be endocrine diseases. Today, obesity can account for up to 25% of pediatric endocrine practice referrals, and type 2 diabetes accounts for up to 33% of the new referrals for pediatric diabetes, the majority of whom are also obese. Since the discovery of leptin, the negative feedback pathway of energy balance has been elucidated, and endocrinologists have embraced disorders of energy balance as part of their management portfolio. Thus, the study of energy balance has become a matter of continuing education for pediatric endocrinologists. The whole field is a work in progress, which is problematic because our diagnostic armamentarium and our treatment options for the most part do not target the multiple hormonal feedback loops that govern energy balance. This chapter conveys a clear and up-to-date basic understanding of the energy balance pathway and provides a clinical rationale and formulation for evaluating and treating these patients.

NEUROENDOCRINE REGULATION OF ENERGY BALANCE

The negative feedback axis of energy balance and its function during homeostasis has been largely delineated through studies in animal models; human data are presented where available. The axis is composed of three arms (Figure 22-1). The first is the afferent arm, which conveys peripheral information on hunger and peripheral metabolism, in the form of hormonal and neural inputs, to the hypothalamus. The second is a central processing unit, consisting of various areas within the hypothalamus. The ventromedial hypothalamus (VMH; consisting of the ventromedial [VMN] and arcuate [ARC] nuclei), integrates the afferent peripheral signals, along with other central stimuli; the paraventricular nuclei (PVN) and lateral hypothalamic area (LHA) serve as a gated neurotransmitter system to alter neural signals for changes in feeding and energy expenditure. The third component is the efferent arm and consists of a complex network of autonomic effectors, which regulate energy intake, and energy expenditure versus storage.[1,2] Anatomic disruptions or genetic or metabolic alterations of the afferent, central processing, or efferent arms can alter energy intake or expenditure in stereotyped ways, which can lead to either obesity or cachexia.

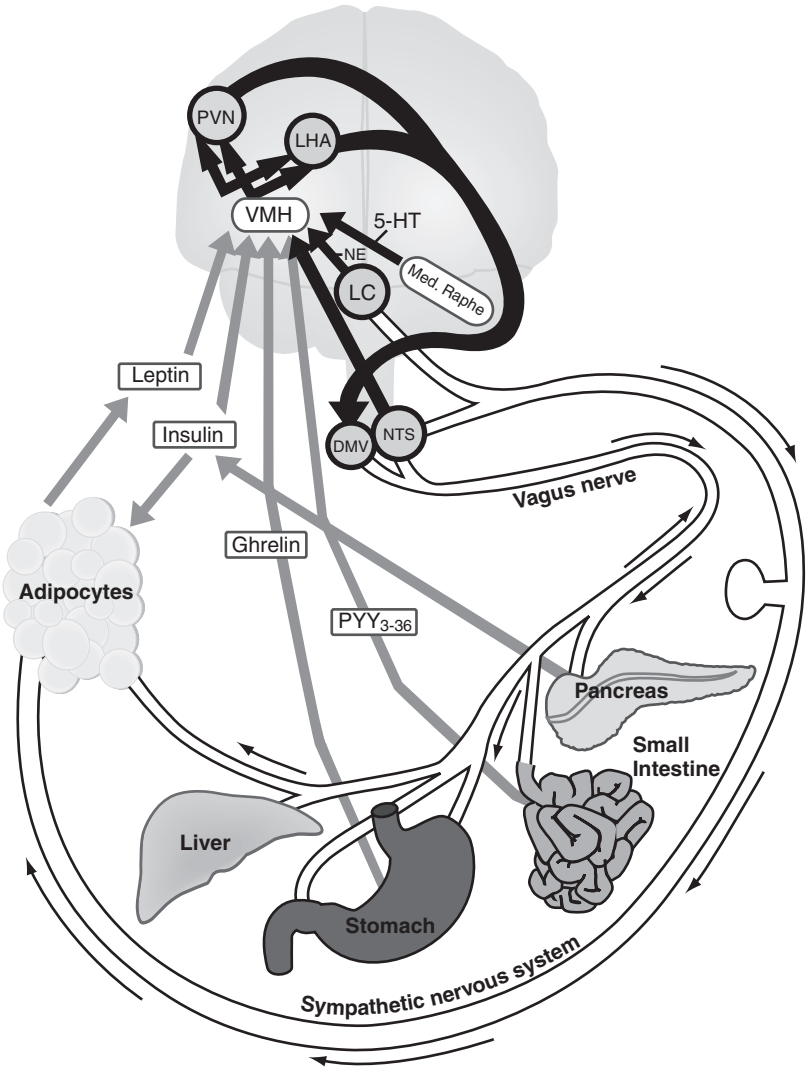


FIGURE 22-1 ■ The homeostatic pathway of energy balance. Afferent (gray), central (black), and efferent (white) pathways are delineated. The hormones insulin, leptin, ghrelin, and peptide $YY_{(3\text{-}36)}$ ($PYY_{3\text{–}36}$) provide afferent information to the ventromedial hypothalamus regarding short-term energy metabolism and energy sufficiency. From there, the ventromedial hypothalamus elicits anorexigenic (α-melanocyte stimulating hormone, cocaine-amphetamine regulated transcript) and orexigenic (neuropeptide Y, agouti-related protein) signals to the melanocortin-4 receptor in the paraventricular nucleus and lateral hypothalamic area. These lead to efferent output via the locus coeruleus, via the nucleus tractus solitarius, which activates the sympathetic nervous system, causing the adipocyte to undergo lipolysis, or via the dorsal motor nucleus of the vagus, which activates the vagus nerve to store energy, both by increasing pancreatic insulin secretion, and (in rodents) by increasing adipose tissue sensitivity to insulin. 5-HT, serotonin (5-hydroxytryptamine); DMV, dorsal motor nucleus of the vagus; LC, locus coeruleus; LHA, lateral hypothalamic area; NE, norepinephrine; NTS, nucleus tractus solitarius; PVN, paraventricular nucleus; VMH, ventromedial hypothalamus. (From Lustig, R. H. (2006). Childhood obesity: behavioral aberration or biochemical drive? Reinterpreting the First Law of Thermodynamics. *Nature Clin Pract Endo Metab, 2*, 447–458. Courtesy of Nature Publishing Group, with permission.)

The Afferent System

Alimentary Afferents

Hunger

The Afferent Vagus. The vagus nerve is the primary neural connection between the brain and the gut. The afferent vagus nerve conveys sensory information regarding the mechanical stretch of the stomach and duodenum and sensations of gastric fullness to the nucleus tractus solitarius (NTS).[3] Of note is that each of the following alimentary neuropeptide effects on hunger and satiety is obviated by concomitant vagotomy, implicating the afferent vagus as the primary mediator of alimentary energy balance signals.[4-6]

Ghrelin. Ghrelin, an octanoylated 28-amino-acid peptide, was discovered serendipitously while looking for the endogenous ligand of the growth hormone secretagogue receptor (GHS-R).[7] Ghrelin induces the release of growth hormone (GH) through stimulation of the pituitary GHS-R. The endogenous secretion of ghrelin from the fasting stomach is high, but the administration of nutrients decreases it; volumetric stretching of the stomach wall has no effect. However, ghrelin also binds to the GHS-R in the VMH, which increases hunger, food intake, and fat deposition.[8,9] Ghrelin also increases the respiratory quotient (RQ) in rats, suggesting a reduction of fat oxidation and promotion of fat storage. Ghrelin appears to tie the lipolytic effect of GH with the hunger signal and is probably important in the acute response to fasting. In humans, ghrelin levels rise with increasing subjective hunger and peak at the time of voluntary food consumption,[10] suggesting that ghrelin acts on the VMH to trigger meal initiation. Ghrelin infusion increases food intake in humans.[11] However, plasma ghrelin levels are low in obese individuals and increase with fasting,[12] suggesting that ghrelin is a response to, rather than a cause of, obesity.

Satiety

Peptide YY $_{3\text{-}36}$ ($PYY_{3\text{-}36}$). A hormonal signal to control meal volume is $PYY_{3\text{-}36}$.[13] This peptide fragment is secreted by intestinal L cells in response to the exposure to nutrient, crosses the blood-brain barrier, and binds to the Y_2 receptor in the VMH. Activation of this receptor causes a decrease in neuropeptide Y (NPY) mRNA in neurons of the orexigenic arm of the central processing system (discussed later). In nonobese humans, infusion of $PYY_{3\text{-}36}$ during a 12-hour period decreased the total amount of food ingested from 2200 to 1500 kcal, but without an effect on food ingested during the next 12-hour interval.[13] Although the pharmacology of this peptide is being elucidated, its specific role in obesity is not yet known.

Glucagon-Like Peptide-1 (GLP-1). Those same intestinal L cells produce GLP-1 through the posttranslational processing of preproglucagon. Two equipotent forms of GLP-1 are generated: a glycine-extended form GLP-1 (7–37) and the amidated peptide GLP-1(7–36)amide.[14] GLP-1 acts on the stomach to inhibit gastric emptying; this prolongs the time it takes to absorb a meal. GLP-1 also activates its receptor on pancreatic β cells to stimulate cAMP production, protein kinase A activation, and insulin secretion (Figure 22-2), thereby improving glucose tolerance, a mechanism of the "incretin" effect. GLP-1 also acts on rodent β cells to stimulate neogenesis, thereby increasing β-cell mass.[15] Lastly, GLP-1 also exerts potent effects on reduction of appetite, both through reduction in gastric emptying and through direct decreases of corticotropin-releasing hormone (CRH) signaling in the PVN and increasing leptin signaling in the VMH.[16]

Cholecystokinin (CCK). CCK is an 8-amino-acid gut peptide released in response to a caloric load. It circulates and binds to CCK_A receptors in the pylorus, vagus nerve, NTS, and area postrema to promote satiety.[3]

Metabolic Afferents

Leptin. Energy intake versus expenditure is normally regulated very tightly (within 0.15% per year) by the hormone leptin. Leptin is a 167-amino-acid hormone, produced by adipocytes, which transmits the primary long-term signal of energy depletion/repletion to the VMH.[17,18] Leptin's primary neuroendocrine role is to mediate information about the size of peripheral adipocyte energy stores to the VMH. Leptin is a prerequisite signal to the VMH for the initiation of high-energy processes, such as puberty and pregnancy.[19,20] Leptin reduces food intake and increases the activity of the sympathetic nervous system (SNS).[21] Conversely, low circulating levels of leptin infer diminished energy stores, which signal via the VMH to reduce energy expenditure, inhibit metabolic processes, and increase appetite. Serum leptin concentrations drop precipitously and in excess of body fat loss during periods of short-term fasting,[22,23] and it seems likely that leptin functions primarily as a peripheral signal to the hypothalamus of inadequate caloric intake rather than specifically as a satiety signal.[24]

In the fed state, circulating levels of leptin correlate with the percentage of body fat.[25,26] Leptin production by adipocytes is stimulated by insulin and glucocorticoids[27,28] and is inhibited by β-adrenergic stimulation.[24] Programming of relative leptin concentrations by early caloric intake may be one mechanism that links early overnutrition with later obesity.[29]

Leptin binds to its receptor (a member of the cytokine receptor superfamily) on target VMH neurons. Four receptor isoforms are formed by differential mRNA splicing: ObRa, an isoform with a shortened intracellular domain, which may function as a transporter; ObRb, the intact full-length receptor; ObRc, also with a short intracellular domain; and ObRe, without an intracellular domain, but which may function as a soluble receptor.[30] As leptin binds to its VMH receptor, three neuronal signals are transduced. The first is the opening of an adenosine triphosphate (ATP)–sensitive potassium channel, which hyperpolarizes the neuron and decreases its firing rate.[31] The second is the activation of a cytoplasmic janus kinase 2 (JAK2), which phosphorylates a tyrosine moiety on proteins of a family called signal transducers and activators of transcription (STAT-3).[32] The phosphorylated STAT-3 translocates to the nucleus, where it promotes leptin-dependent gene transcription.[33] However, leptin also activates the insulin receptor substrate 2/phosphatidyl inositol-3-kinase (IRS2/PI3K) second messenger system in VMH neurons, which increases neurotransmission of the central anorexigenic signaling pathway.[34]

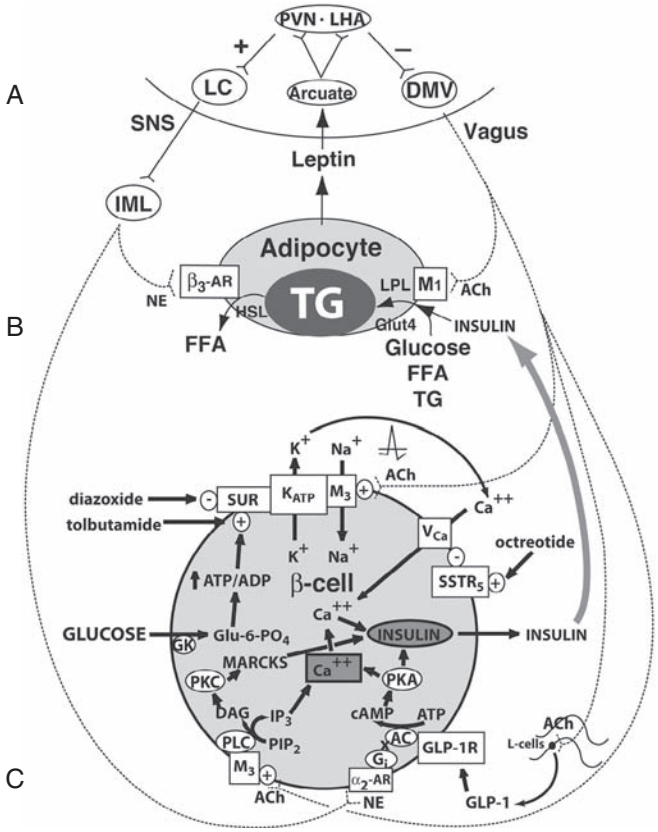


FIGURE 22-2 ■ Central regulation of leptin signaling, autonomic innervation of the adipocyte and β cell, and the starvation response. **A,** The arcuate nucleus transduces the peripheral leptin signal as one of sufficiency or deficiency. In leptin sufficiency, efferents from the hypothalamus synapse in the locus coeruleus, which stimulates the sympathetic nervous system. In leptin deficiency or resistance, efferents from the hypothalamus stimulate the dorsal motor nucleus of the vagus. **B,** Autonomic innervation and hormonal stimulation of white adipose tissue. In leptin sufficiency, norepinephrine binds to the β_3-adrenergic receptor, which stimulates hormone-sensitive lipase, promoting lipolysis of stored triglyceride into free fatty acids. In leptin deficiency or resistance, vagal acetylcholine increases adipose tissue insulin sensitivity (documented only in rats to date), promotes uptake of glucose and free fatty acids for lipogenesis, and promotes triglyceride uptake through activation of lipoprotein lipase. **C,** Autonomic innervation and hormonal stimulation of the β cell. Glucose entering the cell is converted to glucose-6-phosphate by the enzyme glucokinase, generating ATP, which closes an ATP-dependent potassium channel, resulting in cell depolarization. A voltage-gated calcium channel opens, allowing for intracellular calcium influx, which activates neurosecretory mechanisms leading to insulin vesicular exocytosis. In leptin sufficiency, norepinephrine binds to α_2-adrenoceptors on the β-cell membrane to stimulate inhibitory G proteins, decrease adenyl cyclase and its product cAMP, and thereby reduce protein kinase A levels and insulin release. In leptin deficiency or resistance, the vagus stimulates insulin secretion through three mechanisms.[99] First, acetylcholine binds to a M_3 muscarinic receptor, opening a sodium channel, which augments the ATP-dependent cell depolarization, increasing the calcium influx, and insulin exocytosis. Second, acetylcholine activates a pathway that increases protein kinase C, which also promotes insulin secretion. Third, the vagus innervates L cells of the small intestine, which secrete glucagon-like peptide-1, which activates protein kinase A, contributing to insulin exocytosis. Octreotide binds to a somatostatin receptor on the β cell, which is coupled to the voltage-gated calcium channel, limiting calcium influx and the amount of insulin released in response to glucose (reprinted with kind permission of Springer Science and Business media). α_2-AR, α_2-adrenergic receptor; β_3-AR, β_3-adrenergic receptor; AC, adenyl cyclase; ACh, acetylcholine; DAG, diacylglycerol; DMV, dorsal motor nucleus of the vagus; FFA, free fatty acids; G_i, inhibitory G protein; GK, glucokinase; GLP-1, glucagon-like peptide-1; GLP-1R, GLP-1 receptor; Glu-6-PO_4, glucose-6-phosphate; Glut4, glucose transporter-4; HSL, hormone-sensitive lipase; IML, intermediolateral cell column; IP_3, inositol triphosphate; LC, locus coeruleus; LHA, lateral hypothalamic area; LPL, lipoprotein lipase; MARCKS, myristoylated alanine-rich protein kinase C substrate; NE, norepinephrine; PIP_2, phosphatidylinositol pyrophosphate; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; PVN, paraventricular nucleus; $SSTR_5$, somatostatin-5 receptor; TG, triglyceride; V_{Ca}, voltage-gated calcium channel; VMH, ventromedial hypothalamus; SUR, sulfonylurea receptor. (From Lustig, R. H. (2006). Childhood obesity: behavioral aberration or biochemical drive? Reinterpreting the first law of thermodynamics. *Nature Clin Pract Endo Metab, 2*, 447–458. Courtesy of Nature Publishing Group, with permission.)

Insulin. Insulin plays an extremely important role in energy balance,[35] as it is part of both the afferent and efferent systems. On the afferent side, there is a significant insulin receptor density in a subpopulation of VMH neurons,[36] and there is coordinated transport of insulin across the blood-brain barrier,[37] suggesting a central role for this hormone. In animals, acute and chronic intracerebroventricular (ICV) infusions of insulin decrease feeding behavior and induce satiety.[38-40] The data on acute and chronic peripheral insulin infusions are less clear. Studies of overinsulinized diabetic rats demonstrate increased caloric intake (to prevent subacute hypoglycemia) and the development of peripheral insulin resistance.[41,42] Chronic experimental peripheral insulin infusions decrease hepatic and skeletal muscle glucose uptake by decreasing Glut4 expression, but they do not alter

adipose tissue glucose uptake.[43,44] One study on humans showed that injecting short-term insulin peripherally during meals did not have an effect on satiety.[45]

Insulin normally activates the insulin receptor substrate 2/phosphatidyl inositol-3-kinase (IRS2/PI3K) second messenger system in VMH neurons,[46] which increases neurotransmission of the central anorexigenic signaling pathway (discussed later). The importance of central nervous system (CNS) insulin action was underscored by the development of a brain/neuron–specific insulin receptor knockout (NIRKO) mouse, which cannot transduce a CNS insulin signal.[47] Such mice become hyperphagic, obese, and infertile, with high peripheral insulin levels. These data suggest that peripheral insulin mediates a satiety signal in the VMH to help control energy balance.[48] Various knockouts of the insulin signal transduction pathway that reduce insulin signaling lead to an obese phenotype,[49,50] whereas those that improve insulin signaling lead to a lean phenotype.[51,52]

Central Processing

The peripheral afferent signals outlined earlier reach neurons in the VMH, where they are integrated by a gated neural circuit, designed to promote or diminish both energy intake and expenditure (see Figure 22-2). This circuit consists of two arms: the anorexigenic arm, which contains neurons expressing the colocalized peptides proopiomelanocortin (POMC) and cocaine/amphetamine-regulated transcript (CART); and the orexigenic arm, which contains neurons with the colocalized peptides neuropeptide Y (NPY) and agouti-related protein (AgRP). Ghrelin receptor-immunoreactivity colocalizes with NPY and AgRP neurons, whereas insulin and leptin receptors are located on both POMC/CART and NPY/AgRP neurons in the VMH,[53] suggesting divergent regulation of each arm. These two arms compete for occupancy of melanocortin receptors (MCRs; either MC_3R or MC_4R) in the PVN and LHA.

Anorexigenesis, POMC/α-MSH, and CART

POMC is differentially cleaved in different tissues and neurons. The ligand α-melanocyte stimulating hormone (α-MSH) is the primary product involved in anorexigenesis. Both overfeeding and peripheral leptin infusion induce the synthesis of POMC and α-MSH within the ARC.[54] α-MSH induces anorexia by binding to melanocortin receptors within the PVN or LHA. CART is a hypothalamic neuropeptide induced by leptin and reduced by fasting. Intrahypothalamic infusion blocks appetite, whereas antagonism of endogenous CART increases caloric intake.[55]

Orexigenesis, NPY, and AgRP

NPY and AgRP colocalize to a different set of neurons within the ARC, immediately adjacent to those expressing POMC/CART.[56] NPY has numerous functions within the hypothalamus, including initiation of feeding, puberty, regulation of gonadotropin secretion, and adrenal responsiveness.[57,58] NPY is the primary orexigenic peptide. ICV infusion of NPY in rats rapidly leads to hyperphagia, energy storage, and obesity,[59,60] mediated through Y_1 and Y_5 receptors. Fasting and weight loss increase NPY expression in the ARC, accounting for increased hunger, whereas $PYY_{3\text{-}36}$ (through Y_2 receptors) and leptin decrease NPY mRNA[13,61] AgRP is the human homolog of the protein agouti, which is present in abundance in the yellow (A^y-a) mouse.[62] This protein is an endogenous competitive antagonist of all melanocortin receptors (MCRs), accounting for the yellow color in these mice. In the presence of large amounts of AgRP at the synaptic cleft in the PVN, α-MSH cannot bind to the MC_4R to induce satiety.[63]

Other Neuroendocrine Modulators of Energy Balance

Norepinephrine (NE). NE neurons in the locus coeruleus synapse on VMH neurons to regulate food intake.[64] The actions of NE on food intake seem paradoxical, as intrahypothalamic NE infusions stimulate food intake through effects on central α_2- and β-adrenergic receptors,[65] whereas central infusion of α_1-agonists markedly reduces food intake.[66]

Serotonin (5-HT). 5-HT has been implicated in the perception of satiety based on many lines of evidence: (1) injection of 5-HT into the hypothalamus increases satiety, particularly with respect to carbohydrate[67]; (2) central administration of 5-HT_{2c} receptor agonists increase satiety, whereas antagonists induce feeding[68]; (3) administration of selective 5-HT reuptake inhibitors induce early satiety[69]; (4) leptin increases 5-HT turnover[70]; and (5) the 5-HT_{2c}R-KO mouse exhibits increased food intake and body weight.[71] The role of 5-HT in the transduction of the satiety signal may have both central and peripheral components, as intestinal 5-HT is secreted into the bloodstream during a meal, where it may have an impact on gastrointestinal (GI) neuronal function and muscle tone, and may bind to 5-HT receptors in the NTS (discussed previously) to promote satiety.[72]

Melanin-Concentrating Hormone (MCH). MCH is a 17-amino-acid peptide expressed in the zona incerta and LHA. MCH neurons synapse on neurons in the forebrain and the locus coeruleus. MCH appears to be important in conditions such as anxiety and aggression around food.[73] Expression of this peptide is up-regulated in *ob/ob* mice. MCH-knockout mice are hypophagic and lean,[74] whereas transgenic MCH-overexpressing mice develop obesity and insulin resistance.[75] ICV administration of MCH stimulates food intake, similar to that seen with NPY administration.[76]

Orexins A and B. These 33- and 28-amino-acid peptides, respectively, have been implicated in both energy balance and autonomic function in mice.[77] Orexin knockout mice demonstrate narcolepsy, hypophagia, and obesity,[78] suggesting that orexins bridge the gap between the afferent and efferent energy balance systems.[79] Orexins in the LHA stimulate neuropeptide

Y (NPY) release, which may account for their effects on orexigenesis, and also stimulate the corticotropin-releasing factor (CRF) and sympathetic nervous system (SNS) output to increase wakefulness and energy expenditure, learning and memory, and the hedonic reward system (discussed later).[80] Conversely, orexin neurons in the perifornical and dorsomedial hypothalamus regulate arousal and response to stress.

Endocannabinoids (EC). It has long been known that tetrahydrocannabinol stimulates food intake. This observation led to the identification of endogenous EC and their receptor, termed CB_1.[81] The CB_1 receptor is expressed in corticotropin-releasing factor (CRH) neurons in the PVN, in CART neurons in the VMN, and in MCH- and orexin-positive neurons in the LHA and perifornical region. Fasting and feeding are associated with high and low levels of ECs in the hypothalamus, respectively. For example, CB_1 receptor-knockout mice have increased CRH and reduced CART expression. In the *ob/ob* mouse, hypothalamic EC levels are increased, whereas leptin infused intravenously reduces these levels, indicating that a direct negative control is exerted by leptin on the EC system. Glucocorticoids increase food intake by stimulating EC synthesis and secretion, whereas leptin blocks this effect.[82] Finally, the presence of CB_1 receptors on afferent vagal neurons suggest that endocannabinoids may be involved in mediating satiety signals originating in the gut.

Melanocortin Receptors (MCR) and Central Neural Integration

The human MC_4R localizes to chromosome 2, and is a 7-transmembrane G-coupled receptor, encoded by an intron-less 1 kB gene. The binding of hypothalamic α-MSH to the MC_4R in the PVN and LHA results in a state of satiety, whereas ICV administration of MC_4R antagonists stimulates feeding, suggesting that MC_4R transduces satiety information on caloric sufficiency. A different phenotype is observed in the MC_3R knockout mouse. These animals are obese, but they are instead hypophagic and have increased body fat for their lean mass. They gain weight on either low- or high-fat chow and do not change substrate oxidation in response to changes in dietary fat content, suggesting a defect in energy expenditure.[83] Thus, these two hypothalamic MCRs appear to modulate different aspects of energy metabolism. One hypothesis is that the MC_4R modulates energy intake, whereas the MC_3R modulates energy expenditure.[84]

The Efferent System

The MCRs in the PVN and LHA transduce the anorexigenic and orexigenic information coming from the VMH, in order to modulate activity of the sympathetic nervous system (SNS), which promotes energy expenditure, and the efferent vagus, which promotes energy storage (see Figure 22-2). In this way, peripheral energy balance can be modulated acutely to provide requisite energy for metabolic needs, and store the rest.

The Sympathetic Nervous System (SNS) and Energy Expenditure

Anorexigenic pressure increases energy expenditure through activation of the SNS.[85] For instance, leptin administration to *ob/ob* mice promotes increased brown adipose tissue lipolysis, thermogenesis, renovascular activity, and increased movement, all associated with increased energy expenditure, which assists in weight loss.[86] Similarly, insulin administration acutely increases SNS activity in normal rats and in humans.[87,88] The magnitude of energy expenditure also has a salutary effect on quality of life; factors that reduce resting energy expenditure (REE) (e.g., hypothyroidism) reduce quality of life, whereas factors that increase REE (e.g., caffeine) increase quality of life (at least acutely).

The SNS increases energy expenditure in four ways: (1) by innervating the hypothalamus and appetite centers in the medulla to reduce appetite; (2) by increasing thyroid-stimulating hormone (TSH) secretion to increase thyroid hormone release and energy expenditure; (3) by innervating skeletal muscles to increase energy expenditure; and (4) by innervating β_3-adrenergic receptors in white adipose tissue to promote lipolysis.

Activation of the SNS increases energy expenditure by the skeletal muscle, by activating β_2-adrenergic receptors,[89] which in turn increase the expression of numerous genes in skeletal muscle,[90] especially those involved in carbohydrate metabolism. SNS activation stimulates glycogenolysis, incites myocardial energy expenditure, increases in glucose and fatty acid oxidation, and increases protein synthesis.[91]

Activation of the SNS in rodents stimulates the β_3-adrenergic receptor of brown adipose tissue to promote lipolysis.[92] In humans, activation of the β_3-adrenergic receptor increases cAMP, which activates protein kinase A. PKA acts in two separate molecular pathways to increase energy expenditure. First, PKA phosphorylates the cyclic AMP response element binding protein (CREB), which induces expression of PPARγ-coactivator-1α (PGC-1α). PGC-1α then binds to enhancer elements on the uncoupling protein-1 (UCP1) gene, which increases the expression and activity of uncoupling proteins (UCPs) 1 and 2.[93,94] Uncoupling proteins reduce the proton gradient across the inner membranes of mitochondria, thereby diverting protons from storage in the form of ATP to heat production. Originally, uncoupling proteins were discovered in brown adipose tissue and were found to be responsible for thermogenesis. UCP1 is an inner membrane mitochondrial protein that uncouples proton entry from ATP synthesis[95]; therefore, UCP1 expression dissipates energy as heat, thus reducing the energy efficiency of the adipose tissue. However, UCP2 has been found in most tissues and UCP3 in skeletal muscle. Second, PKA activation activates the enzyme hormone sensitive lipase (HSL), which is responsible for lipolysis of intracellular triglyceride to its component free fatty acids (FFAs). The FFAs also induce UCP1, further increasing energy expenditure. The FFAs released from the adipocyte also travel to the liver where they are utilized for energy by metabolizing into two-carbon fragments. Lipolysis reduces leptin

expression; thus, a negative feedback loop is achieved between leptin and the SNS (see Figure 22-2).

The Efferent Vagus and Energy Storage

In response to declining levels of leptin or persistent orexigenic pressure, the LHA and PVN send efferent projections residing in the medial longitudinal fasciculus to the dorsal motor nucleus of the vagus nerve (DMV), activating the efferent vagus.[96] The efferent vagus opposes the SNS by promoting energy storage in four ways: (1) by slowing the heart rate, myocardial oxygen consumption is reduced; (2) the vagus nerve promotes alimentary peristalsis, pyloric opening, and energy substrate absorption; (3) through direct effects on the adipocyte, the vagus nerve promotes insulin sensitivity to increase the clearance of energy substrate into adipose tissue; and (4) through effects on the β cells, the vagus increases postprandial insulin secretion, which promotes energy deposition into adipose tissue.[97-100]

Retrograde tracing of white adipose tissue reveals a wealth of efferents originating at the DMV.[100] These efferents synapse on the M_1 muscarinic receptor on the adipocyte, which increases insulin sensitivity of the adipocyte. Denervation of white adipose tissue results in a reduction of glucose and FFA uptake, and an induction of HSL, which promotes lipolysis—both of which reduce the efficiency of insulin-induced energy storage. Thus, vagal modulation of the adipocyte augments storage of both glucose and FFAs by improving adipose insulin sensitivity [101] (see Figure 22-2).

The DMV also sends efferent projections to the β cells of the pancreas.[102] This pathway is responsible for the "cephalic" or preabsorptive phase of insulin secretion, which is glucose independent and can be blocked by atropine.[103] Overactive vagal neurotransmission increases insulin secretion from β cells in response to an oral glucose load through three distinct but overlapping mechanisms[104] (see Figure 22-2):

1. Vagal firing increases acetylcholine availability and binding to the M_3 muscarinic receptor on the β cell, which is coupled to a sodium channel within the pancreatic β-cell membrane.[105] As glucose enters the β cell after ingestion of a meal, the enzyme glucokinase phosphorylates glucose to form glucose-6-phosphate, increasing intracellular ATP, which induces closure of the ATP-dependent potassium channel. Upon channel closure, the β cell experiences an ATP concentration-dependent β cell depolarization[106,107] and the opening of a separate voltage-gated calcium channel within the membrane. Intracellular calcium influx increases acutely, which results in rapid insulin vesicular exocytosis. Concomitant opening of the sodium channel by vagally mediated acetylcholine augments β-cell depolarization, which in turn augments the intracellular calcium influx and results in insulin hypersecretion.[108-110]
2. Vagally mediated acetylcholine increases phospholipases A_2, C, and D within the β cell, which hydrolyze intracellular phosphatidylinositol to diacylglycerol (DAG) and inositol triphosphate (IP_3).[104] DAG is a potent stimulator of protein kinase C (PKC),[111] which phosphorylates myristoylated alanine-rich protein kinase C substrate (MARCKS), which then binds actin and calcium-calmodulin and induces insulin vesicular exocytosis.[112] IP_3 potentiates the release of calcium within β cells from intracellular stores, which also promotes insulin secretion.[113]
3. The vagus also stimulates the release of GLP-1 from intestinal L cells, which circulates and binds to a GLP-1 receptor within the β-cell membrane. Activation of this receptor induces a calcium-calmodulin-sensitive adenyl cyclase, with conversion of intracellular ATP to cAMP, which then activates protein kinase A. PKA causes both the release of intracellular calcium stores and the phosphorylation of vesicular proteins, each contributing to an increase in insulin exocytosis.[14,114]

In the efferent pathway, insulin is responsible for shunting blood-borne nutrients into adipose for storage. Indeed, the primary hormonal signal for adipogenesis is insulin.[115] Within the adipocyte, insulin increases (1) glut4 expression, (2) acetyl-CoA carboxylase, (3) fatty acid synthase, and (4) lipoprotein lipase.[116] Thus, the net effect of insulin on the adipocyte is the rapid clearance and storage of circulating glucose and lipid. Thus, insulin promotes energy storage.

CNS MODULATION OF FOOD INTAKE

The Hypothalamus and the Starvation Response

The regulation of the various components of the energy balance system is manifest during the starvation response. Everyone has a "personal leptin threshold," probably genetically set, above which the brain interprets a state of energy sufficiency.[117] Thus, the leptin-replete state is characterized by increased physical activity, decreased appetite, and increased feelings of well-being. However, in response to caloric restriction, leptin levels decline even before weight loss is manifest,[22,23] which the VMH interprets as starvation. Gastric secretion of ghrelin increases, which increases pituitary GH release, in order to stimulate lipolysis to provide energy substrate for catabolism. Ghrelin stimulates NPY/AgRP to antagonize α-MSH/CART. Decline of leptin reduces α-MSH/CART as well. This leads to decreased MC_4R occupancy so that the reduced anorexigenic pressure on the MC_4R increases feeding behavior and energy efficiency (with reduced fat oxidation) in order to store energy as fat. In response, the efferent pathway of energy balance coordinates efforts at improving energy efficiency and increasing energy storage. Total and resting energy expenditures decline in an attempt to conserve energy.[118] Specifically, UCP1 levels within adipose tissue decline[119] as a result of decreased SNS activity in response to starvation.[120] In spite of decreased SNS tone at the adipocyte, there is clearly an obligate lipolysis (due to insulin suppression and up-regulation of hormone-sensitive lipase), which is necessary to maintain energy delivery to the musculature and brain in the form of liver-derived ketone bodies. Additionally, in the starved state, vagal tone is increased in order to slow the heart rate and

myocardial oxygen consumption, increase β-cell insulin secretion in response to glucose, and increase adipose insulin sensitivity—all directed to increase energy storage.[120] These revert back to baseline once caloric sufficiency is reestablished, and leptin levels rise.

The Nucleus Accumbens and the Hedonic Pathway of Food Reward

The negative feedback pathway delineated beforehand is not the only site of central regulation of food intake. Complementary to insulin and leptin's ability to alter energy balance, these hormones also modify the "hedonic pathway," or the pleasurable and motivating responses to food. This is the same pathway that responds to drugs of abuse, such as nicotine and morphine. The hedonic pathway comprises the ventral tegmental area (VTA) and the nucleus accumbens (NA), with inputs from various components of the limbic system, including the striatum, amygdala, hypothalamus, and hippocampus. Food intake is a readout of the hedonic pathway; administration of morphine to the NA increases food intake in a dose-dependent fashion.[121] When functional, the hedonic pathway helps curtail food intake in situations where energy stores are replete; however, when dysfunctional, this pathway can increase food intake leading to obesity.

The VTA appears to mediate feeding on the basis of palatability rather than energy need. The dopaminergic projection from the VTA to the NA mediates the motivating, rewarding, and reinforcing properties of various stimuli, such as food and addictive drugs. Leptin and insulin receptors are expressed in the VTA, and both hormones have been implicated in modulating rewarding responses to food and other pleasurable stimuli.[122] For instance, fasting and food restriction (where insulin and leptin levels are low) increase the addictive properties of drugs of abuse, whereas ICV leptin can reverse these effects.[123] In rodent models of addiction, increased addictive behavior (and pleasurable response from a food reward), as measured by dopamine release and dopamine receptor signaling, is greater after food deprivation.[124] In humans with leptin deficiency, alterations in activity in the nucleus accumbens can be seen using functional MRI scanning, and these changes subside with administration of exogenous leptin.[125] Acutely, insulin increases expression and activity of the dopamine transporter, which clears and removes dopamine from the synapse; thus, acute insulin exposure blunts the reward of food.[126] Furthermore, insulin appears to inhibit the ability of VTA agonists (e.g., opioids) to increase intake of sucrose.[127] Finally, insulin blocks the ability of rats to form a conditioned place preference association to a palatable food.[128] However, insulin resistance of this pathway may lead to increased reward of food.

One question that has garnered increasing interest is whether any macronutrient has addictive properties. In animal studies, sugar has been shown to induce the four criteria for addiction: (1) bingeing, (2) withdrawal, (3) craving, and (4) cross-sensitization with other drugs of abuse.[129] Within fast food, sugar and caffeine satisfy the criteria presented in the fifth edition of the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-V) for dependence in humans.[130] However, the question of whether food addiction exists, and whether it can explain patients with obesity, remains hotly contested.[131]

The Amygdala and the Stress Response

The VMH and VTA-NA mediate satiety when energy stores are replete, but they appear to be easily overridden by amygdala activation and resultant stress, a state of physiologic insulin resistance. Numerous lines of evidence suggest that the stress glucocorticoid corticosterone (in the rat) or cortisol (in the human) is essential for the full expression of obesity, which helps to explain the disruptive role of stress in weight regulation.[132]

Stress and glucocorticoids are integral in promoting adiposity and the metabolic syndrome. Adrenalectomized rats maintained pharmacologically with high levels of corticosterone demonstrate that exogenous fat intake is directly proportional to circulating corticosterone concentrations,[133] whereas amygdala activation by stress is dampened by the ingestion of energy-dense food.[134] In intact rats, corticosterone stimulates eating, particularly of high-fat food, and in humans, cortisol administration increases food intake.[135] Human research shows increased caloric intake of "comfort foods" (i.e., those with high energy density) after acute stress,[136] and that the stress response contributes to leptin resistance (discussed later).[137] Several studies in children have observed relationships between stress and unhealthy dietary practices, including increased snacking, and an elevated risk for problems with weight during adolescence and adulthood.[138] In a controlled study of 9-year-olds, children who scored high on dietary restraint and who felt more stressed by lab challenges tended to eat more comfort food.[139]

Leptin Resistance

Most obese children have high leptin levels but do not have receptor mutations, manifesting what is commonly referred to as "functional leptin resistance." Leptin resistance prevents exogenous leptin administration from promoting weight loss.[140] The response to most weight-loss regimens plateaus rapidly due to the rapid fall of peripheral leptin levels below a personal "leptin threshold,"[141] which is likely genetically determined. Leptin decline causes the VMH to sense a reduction in peripheral energy stores, which modulates a decrease in REE to conserve energy, analogous to the starvation response (discussed earlier),[118] but occurring at elevated leptin levels.

The cause of leptin resistance is unknown, but it may have several etiologies. Leptin crosses the blood-brain barrier via a saturable transporter, which limits the amount of leptin reaching its receptor in the VMH [142,143]; this transporter operates more efficiently at lower levels of leptin, while preventing increased signaling at higher levels.[144] Activation of the leptin receptor induces the intraneuronal expression of suppressor of cytokine signaling-3 (SOCS-3), which limits leptin signal transduction in an autoregulatory fashion.[51]

The standard method for producing insulin resistance and obesity in rodents is a high-fat diet. Dietary

fat promotes leptin resistance through its effects on hypertriglyceridemia,[145] which limits access of peripheral leptin to the VMH, and also by interfering with leptin signal transduction upstream of STAT-3, its primary second messenger.[146] One likely modulator of this pathway is the enzyme phosphatidyl inositol-3-kinase (PI3K), which is the downstream effector of insulin action in POMC neurons[147] and which appears to account for the effects of dietary fat on leptin resistance and obesity.[148]

Two clinical paradigms have been shown to improve leptin sensitivity. After weight loss through caloric restriction, exogenous administration of leptin can then increase REE back to baseline and permit further weight loss,[149,150] suggesting that the weight loss itself improves leptin sensitivity. Second, suppression of insulin correlates with improvement in leptin sensitivity and promotes weight loss,[151] suggesting that hyperinsulinemia promotes leptin resistance by interfering with leptin signal transduction in the VMH and VTA.[152] Indeed, insulin reduction strategies can effectively promote weight loss in children with hyperinsulinemia by improving leptin sensitivity.[153] These clinical substantiations of the PI3K data in animals has led to the hypothesis that chronic hyperinsulinemia blocks leptin signal transduction at the VMH and VTA, which turns a negative feedback cycle into a vicious feed-forward cycle.

Based on the concept that hyperinsulinemia promotes weight gain at the adipocyte, yet blocks leptin signaling in the hypothalamus and nucleus accumbens, the role of starvation, reward, and stress on weight gain and adiposity via their effects on insulin are a reasonable hypothesis, termed the "limbic triangle" (Figure 22-3).[154] However, this hypothesis remains to be proven.

ENERGY EXCESS—OBESITY

The rise in the prevalence of obesity in children and adolescents is one of the most alarming public health issues facing the world today. Although the rise in the prevalence of obesity in children and adolescents seems to have leveled in some parts of the world,[155] many others, especially developing countries, are still experiencing a steady increase. Obesity is associated with significant health problems in children, is an early risk factor for a great

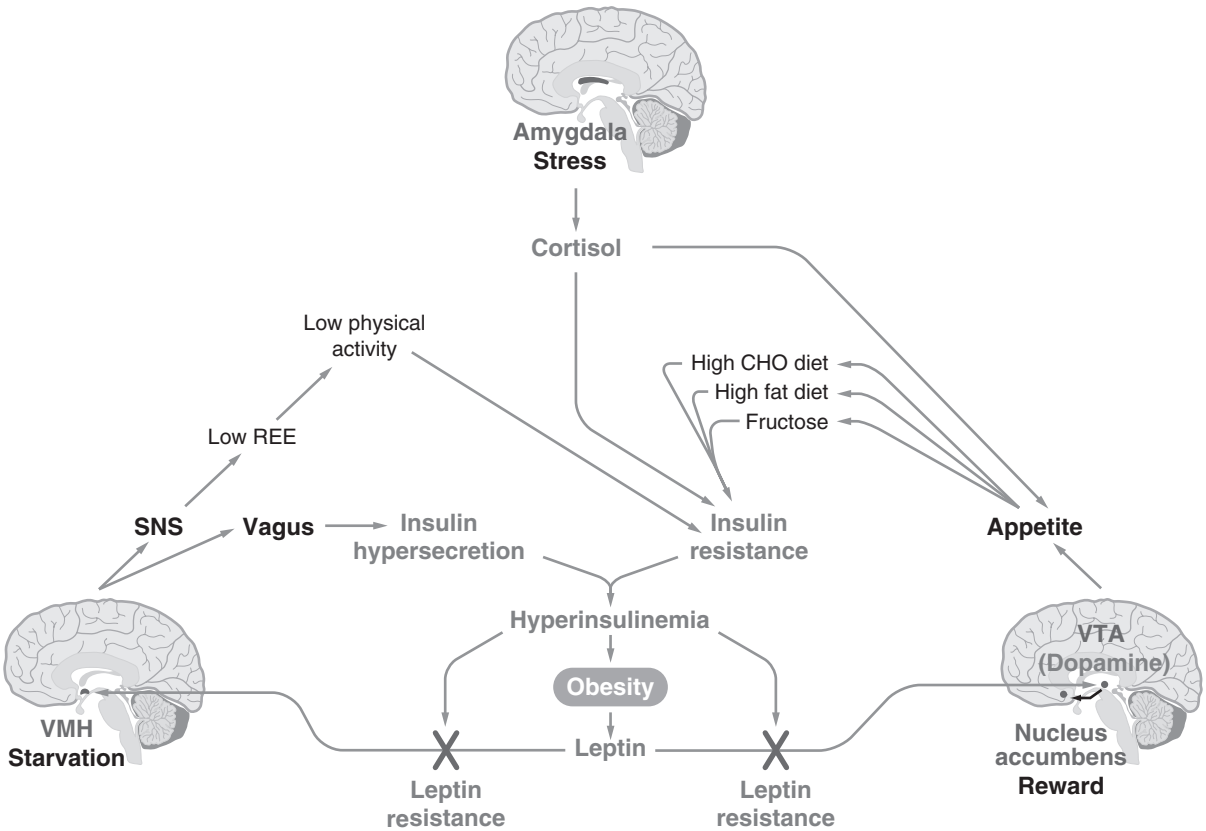


FIGURE 22-3 ■ The "limbic triangle." Three areas of the CNS conspire to drive food intake and reduce physical activity, resulting in persistent weight gain. The ventromedial hypothalamus (VMH) transduces the leptin signal from adipocytes to reduce energy intake and increase energy expenditure; however, hyperinsulinemia prevents leptin signaling, promoting the "starvation response." The ventral tegmental area (VTA) transduces the leptin signal to reduce dopamine neurotransmission to the nucleus accumbens (NA), reducing food intake; however, hyperinsulinemia prevents leptin signaling here as well, increasing dopamine and promoting the "reward" of food. The amygdala transduces fear and stress, which results in increased cortisol release from the adrenal cortex. The elevated cortisol also drives energy-rich food intake and promotes insulin resistance, further interfering with leptin signaling at the other two CNS sites. Thus, activation of any aspect of the limbic triangle turns on a positive feedback loop, promoting continued weight gain and obesity. (From Mietus-Snyder, M. L., & Lustig, R. H. (2008). Childhood obesity: adrift in the "limbic triangle." *Ann Rev Med, 59*, 119–134.)

many cases of adult morbidity and mortality,[156] and is an important factor in increasing health care expenditures.[157] Childhood obesity tends to track to adulthood,[158] and those who remain obese as adults have a significant risk for the development of type 2 diabetes, atherosclerosis, and dyslipidemia. In contrast, obese children who lost weight and became nonobese adults do not have an increased risk for such morbidity[159]; thus, early and effective intervention against obesity during childhood is crucial.

Definition

The theoretical definition of obesity is a degree of somatic overweight that affords detrimental health consequences.[160] Based on morbidity and mortality statistics, and with a desire to prevent future risk of morbidity, we practically define obesity as a statistical magnitude of overweight for a population, keeping in mind that morbidity and mortality vary with degree of overweight in different racial, ethnic, and socioeconomic groups,[161] and indeed for individuals as well. The World Health Organization[162] categorizes adult overweight into four subgroups based on body mass index (BMI; weight [kg] ÷ height [m]^2): BMI 25 to 30 (overweight); BMI 30 to 35, grade 1 (moderately obese); BMI 35 to 40, grade 2 (severely obese); and BMI > 40, grade 3 (morbidly obese). Some make a further delineation at BMI > 60, denoting this as "superobesity," as even surgical therapies are less effective in this range.

Most obesity in adulthood has its origins in childhood,[163,164] making obesity a pediatric concern and the prevention and treatment of obesity a pediatric goal. BMI is also the accepted marker in children.[165] In childhood, a comparison of BMI to normal curves for age[166] allows for categorization of BMI above the 85th percentile as overweight and that above the 95th percentile as obese (Figure 22-4A and B).

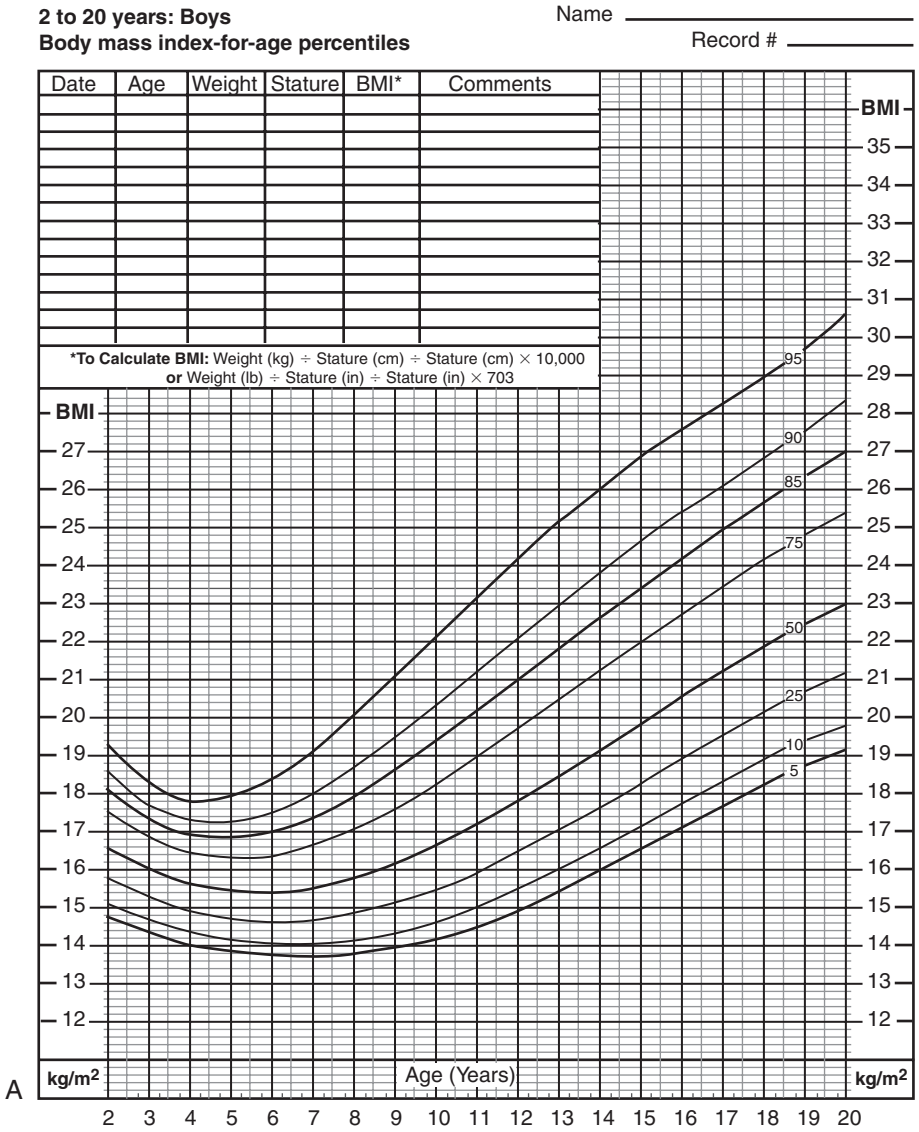


FIGURE 22-4 ■ Body mass index (BMI)-for-age percentiles for U.S. boys (**A**) and U.S. girls (**B**), 2 to 20 years of age. Note that the 95th percentile signifies obesity. The adiposity rebound occurs at approximately 5 years of age in both sexes; the earlier the adiposity rebound occurs, the more likely an organic etiology for the weight gain can be inferred. (From www.cdc.gov/growthcharts [National Center of Health Statistics, 2000].)

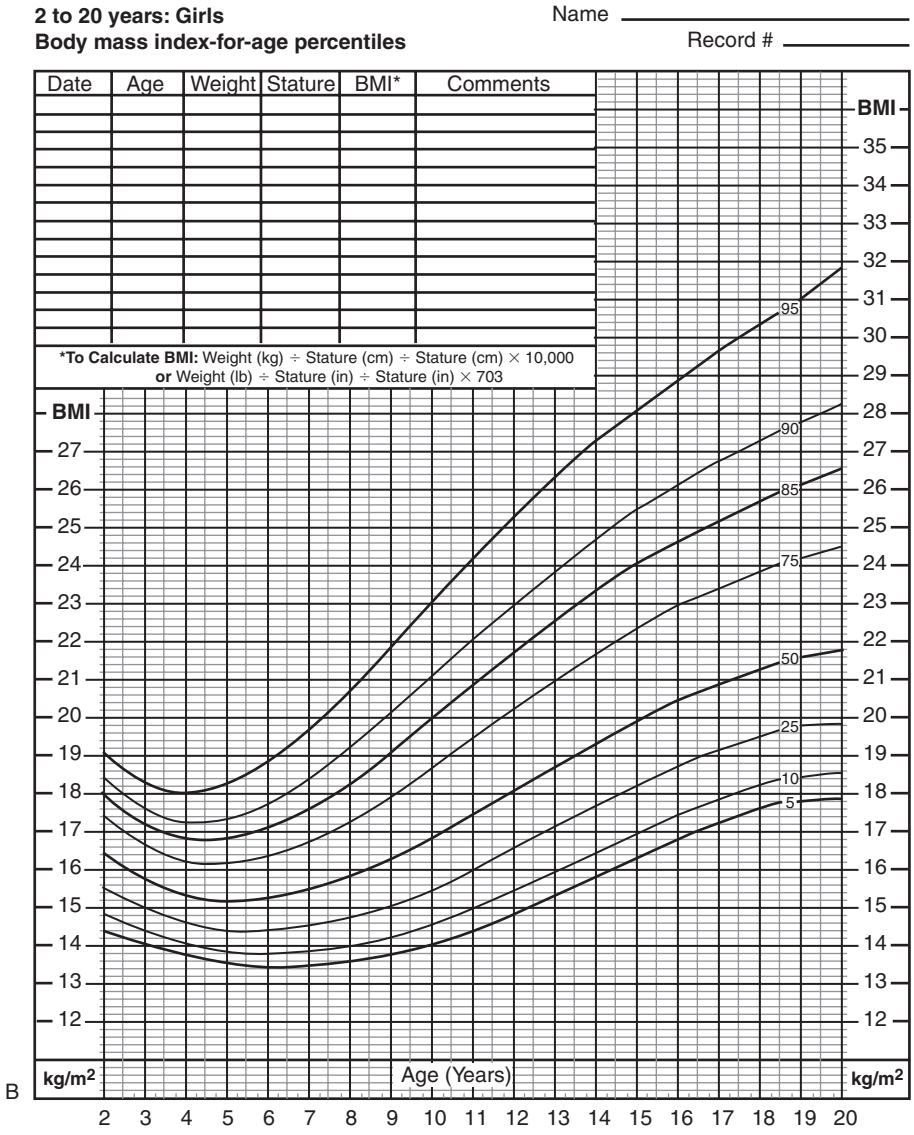


FIGURE 22-4, cont'd

Although BMI is the standard for obesity for statistical purposes and within populations, it should be noted that BMI takes into account muscle and bone, as well as both subcutaneous and visceral fat. Furthermore, BMI in children is age and puberty dependent, thus the BMI z-score is a more accurate assessment of childhood adiposity. Lastly, waist circumference (an indirect measure of visceral fat) has emerged as a more accurate indicator of metabolic disturbance in children.[167]

Prevalence and Epidemiology

The prevalence of childhood obesity in the United States has increased dramatically since the 1980s[168,169] and continues to do so, although the comparison of longitudinal and cross-sectional data is difficult due to different definitions and measurement parameters between epidemiologic studies. Estimates of obesity prevalence in the United States based on data from the 2009-2010 National Health and Nutrition Examination Survey (NHANES IV)[155] demonstrate that the epidemic of childhood obesity is occurring at earlier ages: In 2009 to 2010, 9.7% (95% CI, 7.6% to 12.3%) of infants and toddlers had a high weight-for-recumbent length and 16.9% (95% CI, 15.4% to 18.4%) of children and adolescents from 2 through 19 years of age were obese. Trend analyses over a 12-year period indicated a significant increase in obesity prevalence between 1999-2000 and 2009-2010 in males ages 2 through 19 years (odds ratio, 1.05; 95% CI, 1.01 to 1.10) but not in females (odds ratio, 1.02; 95% CI, 0.98 to 1.07) per 2-year survey cycle. There was a significant increase in BMI among adolescent males ages 12 through 19 years ($P = .04$) but not among any other age group or among females. Thus, in 2009-2010, the prevalence of obesity in children and adolescents was 16.9%; this was not changed compared with 2007-2008. Importantly, whereas the prevalence of obesity in Caucasian children declined slightly, the prevalence of African-American and Latino obesity

continued to climb. Furthermore, the prevalence of severe obesity (BMI > the 97th percentile) is still on the rise.[170] Lastly, projections argue that by 2030, 42% of American adults will be obese.[157]

Global Prevalence

Obesity has overtaken AIDS and malnutrition as the number one public health problem in the world.[171] The global prevalence of childhood obesity has been increasing worldwide at an alarming rate since the 1990s. Rates have increased 2.7-fold to 3.8-fold over 29 years in the United States,[169] 2-fold to 2.8-fold over 10 years in England, 3.4-fold to 4.6-fold over 10 years in Australia, and 3.4-fold to 3.6-fold over 23 years in Brazil. In Asia, the prevalence has increased 1.1-fold to 1.4-fold over 6 years in China and 2.3-fold to 2.5-fold over 26 years in Japan. In Africa, the prevalence has increased 3.9-fold over 18 years in Egypt, 3.8-fold over 6 years in Ghana, and 2.5-fold over 5 years in Morocco.[172] Based on data from 2008, nearly 20% of Indian boys and 18% of Indian girls from 2 to 17 years of age are considered overweight. Even higher prevalence rates are reported from New Zealand and Taiwan, making the Far East and Oceania regions the current hub of the obesity epidemic.[170]

In developed countries, the urban poor are more susceptible for developing obesity, presumably due to poor dietary practices and limited opportunity for physical activity.[173,174] In contrast, obesity is more frequent in upper socioeconomic classes of developing countries, probably due to transition to a more Western lifestyle with a more energy-dense diet consisting of higher fats and sugar, which tend to be more palatable at a lower cost.[175,176] This may be due to specific properties of processed food, which promote leptin resistance.[152]

Racial and Ethnic Considerations

The NHANES surveys only list prevalence among Caucasians, African Americans, and Hispanics, despite the fact that Native Americans, Pacific Islanders, Asians, and other racial/ethnic groups are experiencing rapid increases in prevalence as well. Across racial groups, there is a marked dichotomy in the prevalence and in the rate of increase of childhood obesity.[170,177] For instance, the prevalence among African-American (24.4%), Hispanic (21.7%), and Mexican-American adolescents (22.2%) is significantly higher than that among white adolescents (15.6%). Importantly, the prevalence of severe obesity (BMI > the 97th percentile) among African-American (18.5%), Hispanic (15.2%), and Mexican-American adolescents (15.2%) by far exceeds that of non-Hispanic white adolescents (10.5%). The rate of increase in the prevalence of obesity among African-American and Hispanic adolescents almost doubled between 1988-1994 and 1999-2000, from 13.4% to 23.6% in African Americans and from 13.8% to 23.4% in Hispanics. In the National Heart, Lung and Blood Institute (NHLBI) Growth and Health Study,[178] the prevalence of obesity in 9-year-old African-American girls was 17.7%, in Caucasian girls 7.7%, and both of these prevalences doubled over the 10 years of study. These differences in prevalence hold true at younger ages as well. The 1994 Pediatric Nutrition Surveillance System (PedNSS) indicated that 12% of 2- to 4-year-old Native American children were overweight, which is similar to Hispanic children at the same age (12%) but much higher than white children (6%). The prevalence of overweight at 5 to 6 years in Native Americans is twice that in U.S. youth in general, and the prevalence of obesity is even three times higher.[179] Among infants and toddlers younger than 2 years of age, the prevalence of obesity is highest in African Americans (18.5%), as compared to 10.1% in Caucasians and 13.7% in Hispanics. It is possible that different dietary practices may account for some of these differences. For instance, a study of 2-year-old Latino children in California correlated obesity with early consumption of sugar-sweetened beverages.[180]

Within racial populations, ethnic variability in the prevalence of childhood obesity has also been noted. The United States National Longitudinal Study of Adolescent Health (Add Health) indicated that the BMI ≥ the 85th percentile in adolescent Hispanics was more common among Mexican Americans (32.1%) and Puerto Ricans (30.3%), as compared with Cuban Americans (27.1%) and Central South Americans (26.2%).[174] Only 25% of first-generation Hispanic adolescents were overweight based on a BMI ≥ the 85th percentile, as compared with 32% of second- and third-generation Hispanics. The prevalence of overweight in Asian-American adolescents in this study was 20.6%, with comparable prevalence among Filipinos (18.5%) and Chinese (15.3%). Again, only 12% of first-generation Asian Americans were overweight, compared with 27% and 28% of those from the second and third generations, respectively. In Native Americans, there is great variation in the prevalence of obesity from 12% to 77%, based on tribes, age groups, measurement tools, and cutoff values, among the studies performed between 1990 and 2000.[179] These studies indicate that obesity in Native Americans begins very early in childhood.

Predictive Factors

The higher the BMI during childhood, the more likely adult obesity will manifest. In general, children with a BMI ≥ the 95th percentile have a very high risk for adult obesity.[181] Obesity in adolescence is a primary risk factor for obesity in adulthood, with an increased odds ratio from 1.3 for obesity at 1 to 2 years of age to 17.5 for obesity at 15 to 17 years of age.[182] The change of BMI during and after adolescence is the most important predictive variable for adult obesity.[183] Children and adolescents with BMI ≥ the 95th percentile have a 62% to 98% chance of being obese at 35 years of age, with a 50% chance in males age ≥ 13 years and 66% chance in girls age ≥ 13 years.[184] Importantly, an elevated BMI in adolescence—one that is well within the range currently considered to be normal—constitutes a substantial risk factor for obesity-related disorders in midlife. Although the risk of diabetes is mainly associated with increased BMI close to the time of diagnosis, the risk of coronary heart disease is associated with an elevated BMI both in adolescence and in adulthood.[185]

The age of adiposity rebound, the point of the BMI nadir before the body fatness begins to rise (between 5 and 6 years of age) (see Figure 22-4A and B), is also an important predictor for adult obesity.[186] Girls tend to have a slightly earlier adiposity rebound than do boys. Children with an early adiposity rebound have a fivefold greater chance of becoming obese as adults, compared to those with a late adiposity rebound. At the age of adiposity rebound, children already overweight have a sixfold greater risk for adult obesity, as compared to lean children. Therefore, the earlier the onset of childhood obesity, the greater is the risk for adult obesity.

Infant overnutrition plays an extremely important role in the future development of obesity. Numerous studies have implicated bottle feeding as a specific risk factor.[187] The prevalence of obesity in children who were never breastfed was 4.5%, as compared with 2.8% in breastfed children, and a clear time-response effect was identified for the duration of breastfeeding on the decline in the prevalence of obesity as well.[188] Early overnutrition has been correlated with elevated leptin concentrations in later life.[29] Differences in both volume and composition of commercial formula versus breast milk have been proposed as etiologic factors.

Parental obesity is also an important predictor of childhood obesity. Children with at least one overweight parent at the age of adiposity rebound have a four- to fivefold greater chance of becoming obese adults. Lean children 5 years old or younger have a 13-fold risk of adult obesity if both parents are obese. Excessive BMI gains of parents during childhood and adulthood are also associated with a higher BMI and risk of obesity in the offspring.[189] Conversely, older obese children (10 to 14 years of age) have a 22.3-fold increased risk of adult obesity regardless of parental weight,[164] suggesting that parental obesity is important in early childhood weight gain.[190] Parental obesity is also related to early adiposity rebound, although it is unclear whether the relation between parental and childhood obesity is genetic, epigenetic, or environmental.

METABOLIC IMPACT OF CHILDHOOD OBESITY

Many of the metabolic and cardiovascular (CV) complications of obesity are already evident during childhood and are closely related to the development of insulin resistance causes of hyperinsulinemia, the most common biochemical abnormality seen in obesity.[191] The obesity-related comorbidities that emerge early in childhood are alterations in glucose metabolism, dyslipidemia, and hypertension. Although an accelerated atherogenic process is present in obese children, thrombotic CV events do not usually appear until adulthood. The clustering of these manifestations is termed the *metabolic syndrome* or *the insulin resistance syndrome*, suggesting that peripheral insulin resistance may be the driving force behind most cases of related morbidity.

Insulin Resistance

Insulin resistance is defined as the decreased tissue response to insulin-mediated cellular actions and is the inverse of insulin sensitivity. The term *insulin resistance*, as generally applied, refers to whole-body reduced glucose uptake in response to physiologic insulin levels and its consequent effects on glucose and insulin metabolism. However, it is now clear that not all tissues are equally insulin resistant. Generalized insulin resistance would result in global metabolic dysfunction, such as leprechaunism or Rabson-Mendenhall syndrome. Thus, the insulin resistance of obesity must, of necessity, affect different tissues quantitatively (see Chapter 19 on diabetes mellitus and insulin receptor mutations).

Hepatic Insulin Resistance

The liver plays a major role in substrate metabolism and is the primary target of insulin action. After insulin's release from the β cell following a glucose load, it travels directly to the liver via the portal vein, where it binds to the insulin receptor and elicits two key actions at the level of gene transcription. First, insulin stimulates the phosphorylation of FoxO1, which prevents it from entering the nucleus,[192,193] and thus diminishes the expression of genes required for gluconeogenesis, mainly phosphoenolpyruvate carboxykinase and glucose 6-phosphatase. The net effect is diminished hepatic glucose output. Second, insulin activates the transcription factor sterol regulatory element-binding protein (SREBP)-1c, which in turn increases the transcription of genes required for fatty acid and triglyceride (TG) biosynthesis, most notably ATP-citrate lyase, acetyl-coenzyme A carboxylase, and fatty acid synthase, which together constitute the process of *de novo* lipogenesis (DNL). TGs synthesized by DNL are then packaged with apoliprotein B (apoB) into very low-density lipoproteins (VLDL) for export to the periphery for storage or utilization by reciprocal activation of lipoprotein lipase (LPL) on the surfaces of endothelial cells in adipose or muscle tissues.[194]

For reasons that remain unclear, insulin-resistant subjects typically have "selective" or "dissociated" hepatic insulin resistance—that is, they have impaired glucose homeostasis (mediated by the FoxO1 pathway) but enhanced insulin-mediated hepatic DNL (mediated by the SREBP-1c pathway).[195] The increase in free fatty acid (FFA) flux within the liver, either by DNL or FFA delivery via the portal vein, impairs hepatic insulin action,[196] leading to increases in hepatic glucose output, the synthesis of proinflammatory cytokines, excess triglyceride secretion by the liver, low HDL cholesterol levels, and an increase of relatively cholesterol-depleted LDL particles.[197] Furthermore, the intrahepatic accumulation of FFA and lipid is also detrimental to liver insulin sensitivity, as this leads to the generation of toxic lipid-derived metabolites such as diacylglycerol (DAG), fatty acyl CoA, and ceramides. These in turn trigger the activation of protein kinase C-ε (PKCε) and serine/threonine phosphorylation of IRS-1, which attenuates hepatic insulin signal transduction.[198]

Adipose Tissue Insulin Resistance

The expanded adipose tissue mass that accompanies obesity often leads to increased lipolysis and FFA turnover.

Normally, insulin inhibits adipose tissue lipolysis; however, in the insulin-resistant state, the process is accelerated, leading to increased FFA release into the circulation. Moreover, visceral adipocytes are more sensitive to catecholamine-stimulated lipolysis than subcutaneous adipocytes, further increasing FFA flux.[199] Macrophages also infiltrate into adipose tissue and contribute to both adipocyte hypertrophy and cytokine release.[200-202] These circulating cytokines also affect insulin action in other tissues, such as liver and muscle.

Muscle Insulin Resistance

Downstream of an insulin-resistant liver, increased plasma FFA levels disrupt the glucose-fatty acid or "Randle" cycle and insulin-mediated glucose transport in skeletal muscle,[203,204] facilitating the development of hyperglycemia. The ectopic deposition in skeletal muscle of fat as intramyocellular lipid may also play a direct role in the pathogenesis of insulin resistance and metabolic syndrome via the lipid metabolite-induced activation of PKCϵ with the subsequent impairment of insulin signaling.[198]

Assessment of Insulin Resistance

The euglycemic hyperinsulinemic clamp is the gold standard for measuring insulin sensitivity; the frequently sampled intravenous (IV) glucose tolerance test (FSIVGTT) and steady-state plasma glucose (SSPG) methods are also valid measurements. Euglycemic hyperinsulinemic clamp studies have shown that insulin resistance is determined primarily by the response of skeletal muscle, with over 75% of infused glucose taken up by muscle and only 2% to 3% by adipose tissue.[205] All three methods are generally time consuming, require IV infusions and frequent blood sampling, are burdensome for participants, are costly, and require a research setting. In an attempt to simplify the measurement of insulin sensitivity, a number of methods using single simultaneously obtained samples of fasting insulin and glucose have been developed, such as the homeostatic model assessment of insulin resistance (HOMA-IR). Each of these methods uses a mathematical formula that adjusts for individual variability in insulin and glucose secretion and clearance. Although the goal for these methods was to improve the accuracy of fasting insulin alone by the addition of fasting glucose, it is now agreed that they yield similar results to fasting insulin. When correlated with gold standard methods in children, fasting insulin is a poor measure of whole body insulin sensitivity in an individual child. Although the primary interest has been in insulin resistance, the adverse effects related to insulin resistance are more likely mediated via compensatory hyperinsulinemia.

The two most important biologic conditions associated with insulin resistance in childhood are ethnicity and puberty. Studies show that African-American, Hispanic, Pima Indian, and Asian children are less insulin sensitive compared with Caucasian children.[206] The insulin resistance in minority ethnic groups is manifested as lower insulin-stimulated glucose uptake, concomitant with hyperinsulinemia, evidence of increased insulin secretion from the β cell, and decreased insulin clearance. During puberty there is ~25% to 50% decline in insulin sensitivity with recovery when pubertal development is complete. The compensatory increase in insulin secretion during puberty may be blunted in African-American and Hispanic youth, thus increasing their risk for T2DM around the time of puberty. The development of T2DM is covered in depth in Chapter 9, yet it is worth noting that impaired glucose tolerance (IGT), known as prediabetes, is a relatively common condition in obese children and adolescents. IGT in obese youth is typically characterized by obesity with an unfavorable pattern of lipid partitioning, with an increased deposition of fat in the visceral and intramyocellular lipid (IMCL) compartments.[207]

Lipid Partitioning

The term *lipid partitioning* refers to the distribution of body fat in various organs and compartments. The majority of excess fat is stored in its conventional subcutaneous depot, yet other potential storage sites exist as well, such as the intra-abdominal (visceral) fat compartment and insulin-responsive tissues such as muscle and liver. One hypothesis to explain the relation between obesity and insulin resistance is the "portal-visceral" paradigm.[208] This hypothesis claims that increased adiposity causes an accumulation of fat in the visceral depot, leading to an increased portal and systemic free fatty acid (FFA) flux[209] (Figure 22-5). Associations between visceral adiposity, insulin resistance, and comorbidities have been demonstrated across most age groups and ethnicities.[210] Of note, studies of *in vivo* free fatty acid fluxes from the visceral and subcutaneous truncal and abdominal depots have failed to demonstrate a substantial difference in net fluxes between these depots.

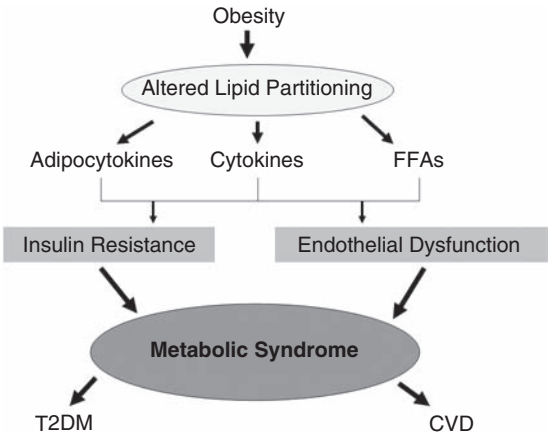


FIGURE 22-5 ■ A hypothesis on the relation between obesity and the metabolic syndrome. The metabolic impact of obesity is determined by the pattern of lipid partitioning. Lipid storage in insulin-sensitive tissues such as liver or muscle and in the visceral compartment is associated with a typical metabolic profile characterized by elevated free fatty acids and inflammatory cytokines alongside reduced levels of adiponectin. This combination can independently lead to peripheral insulin resistance and to endothelial dysfunction. The combination of insulin resistance and early atherogenesis (manifested as endothelial dysfunction) drives the development of altered glucose metabolism and of cardiovascular disease.

Subcutaneous fat, which does not drain into the portal system, is strongly related to insulin resistance in healthy obese and diabetic men.[211] Similarly, truncal subcutaneous fat mass has been demonstrated to independently predict insulin resistance in obese women. Visceral and subcutaneous fat differ in their biologic responses,[212] as visceral fat is more resistant to insulin and has increased sensitivity to catecholamines. These observations emphasize that both visceral and subcutaneous abdominal fat can contribute to insulin resistance, possibly by different mechanisms.[213]

Studies performed in obese adolescents highlight the fact that the ratio of visceral to subcutaneous fat may be the determinant of their metabolic impact rather than their absolute quantity. Indeed, obese adolescents with a high ratio, which are not necessarily more obese than others, demonstrate a markedly adverse metabolic phenotype of severe insulin resistance and alterations in glucose and lipid metabolism.[214] Moreover, intrahepatic fat, though strongly associated with high levels of visceral fat, is also associated with the insulin-resistant state in obese adolescents, independent of all other fat depots.[215]

An alternative theory to explain the relation between obesity and insulin resistance is the "ectopic lipid deposition" paradigm.[216] This theory is based on the observations that lipid content in liver or muscle is increased in obesity and in T2DM and is a strong predictor of insulin resistance.[217,218] Moreover, in conditions such as lipodystrophies, all fat is stored in liver and muscle due to lack of subcutaneous fat tissue, causing severe insulin resistance and diabetes.[219] In obese adults (BMI $>$ 30), muscle attenuation on computed tomography (CT) (representing lipid content) is a stronger predictor of insulin resistance than is visceral fat.[220] Studies performed *in vivo* using ^{1}H-NMR spectroscopy demonstrated increased IMCL content to be a strong determinant of insulin resistance in adults[221] and in obese adolescents.[222] Alternatively, lipid deposition in hepatocytes to produce intrahepatocellular lipid (IHCL) is highly predictive of insulin resistance, even more so than visceral fat.[223] Thus, obesity-driven morbidity may begin when the subcutaneous fat reaches its capacity to store excess fat and begins to shunt lipid to ectopic tissues, such as liver and muscle, leading to peripheral insulin resistance,[224] or possibly when liver or muscle accumulates lipid produced *de novo* in response to dietary manipulation (discussed later). Another postulated cause of IMCL and IHCL accumulation is a reduction of fat β oxidation,[225] related to low aerobic capacity, a reduced number or malfunction of mitochondria, or reduced SNS tone. The effect of IMCL or IHCL accumulation on peripheral sensitivity is postulated to be due to an alteration of the insulin signal transduction pathway in muscle, caused by derivates of fat such as long chain fatty acyl-CoA and diacylglycerol within the hepatocyte or myocyte. These derivates activate the serine/threonine kinase cascade and cause serine phosphorylation of IRS-1, which inhibits insulin signaling.[226] A comparable mechanism has been demonstrated in the liver, where the accumulation of lipids, in particular diacylglycerol, activates the inflammatory cascade by inducing *c-jun* N-terminal kinase (JNK-1), which causes serine rather than tyrosine phosphorylation of IRS-1, leading to an inhibition of hepatic insulin signaling.[227,228]

Vascular Changes

Early stages of the atherosclerotic process may be detected in obese children. It has become clear that endothelial dysfunction represents a key early step in the development of atherosclerosis.[229] The hallmark and cause of endothelial dysfunction is impairment in nitric oxide (NO)-mediated vasodilatation.[230] This is due to decreased NO production by endothelial nitric oxide synthase (eNOS), which has been postulated to result from high levels of free fatty acids and inflammatory cytokines (IL-6, TNF-α) in insulin-resistant obese individuals, increased reactive oxygen species, or increased uric acid, which inhibits eNOS activity.[231] Decreased NO bioavailability leads to an imbalance between vasodilating and vasoconstricting factors (such as endothelin), which leads to impaired vascular smooth muscle relaxation, increased adhesion of inflammatory cells to the endothelium, increased expression of plasminogen activator inhibitor–1 (PAI-1; a pro-thrombotic molecule), and increased vascular smooth muscle cell proliferation. Thus, decreased NO bioavailability is thought to create a proinflammatory, prothrombotic environment, which promotes atherosclerosis.[232] Endothelial function represents an integrated index of the overall CV risk burden in any given individual. Since the early 2000s, noninvasive techniques for the assessment of endothelial function, including high-resolution external vascular ultrasound to measure flow-mediated endothelium-dependent dilatation (FMD) of the brachial artery during hyperemia, have been developed.[233,234] Impaired FMD correlates with arterial wall stiffness, coronary dilatation, and endothelial dysfunction in obese children.[235] Similarly, anatomic changes in peripheral arterial vessels such as increased intimal medial thickness (IMT) have also been demonstrated in obese children and adolescents,[236] which mimics early coronary pathology and predicts adverse CV outcomes.

There are no studies that directly measure *in vivo* insulin sensitivity and its relationship to atherosclerotic abnormalities in children. Very limited observations suggest a relationship between HOMA-IR and arterial stiffness and fasting insulin levels in youth. However, a role for insulin resistance in the early abnormalities of vascular smooth muscles is proposed based on the observation that circulating biomarkers of endothelial dysfunction (intercellular adhesion molecule and E-selectin) are highest, whereas adiponectin, the anti-atherogenic adipocytokine, is lowest among the most insulin-resistant youth. The landmark Bogalusa heart study demonstrated that CV risk factors present in childhood are predictive of coronary artery disease in adulthood.[191,237] Among these risk factors, LDL-cholesterol and body mass index (BMI) measured in childhood were found to predict intima-media thickness (IMT) in young adults.[238] There is now substantial evidence that the insulin resistance of childhood obesity creates the metabolic platform for adult CV disease.[239-241] Moreover, the constellation of peripheral insulin resistance, an unfavorable adipocytokine profile, subacute inflammation, and

endothelial dysfunction work in parallel to promote the pathologic processes of aging.

Adipocytokines

Leptin

The discovery of leptin in 1994 has dramatically changed the view of adipose tissue in the regulation of energy balance.[17] Adipocytes secrete several proteins that act as regulators of glucose and lipid homeostasis.[242] These proteins have been collectively referred to as adipocytokines because of their structural similarity with cytokines. Circulating leptin levels correlate with the degree of obesity. As stated earlier, the primary role of leptin is to serve as an adiposity sensor to protect against starvation. Leptin probably has a permissive role in high-energy metabolic processes such as puberty, ovulation, and pregnancy, but its role in states of energy excess is less known. In obesity, the development of leptin resistance may result in a breakdown of the normal partitioning of surplus lipids in the adipocyte compartment.[243]

Adiponectin

The cytokine adiponectin is peculiar in obesity because, in contrast to the other adipocytokines, its level is reduced in obesity.[244] The adiponectin gene is expressed exclusively in adipose tissue and codes a protein with a carboxyl terminal globular head domain and an amino terminal collagen domain, which is structurally reminiscent of the complement factor 1q.[245] The gene is located on chromosome 3q27, a location previously linked to the development of type 2 diabetes and the metabolic syndrome. Several single nucleotide polymorphisms (SNPs) in the adiponectin gene have been reported to be associated with the development of T2DM in populations around the world, suggesting that adiponectin plays a major role in glucose metabolism.[246] Adiponectin circulates in plasma in three major forms: a low-molecular-weight trimer, a middle-molecular-weight hexamer, and a high-molecular-weight 12 to 18-mer.[247] Circulating plasma adiponectin concentrations demonstrate a sexual dimorphism (females have greater concentrations), suggesting a role for sex hormones in the regulation of adiponectin production or clearance. Dietary factors such as linoleic acid or fish oil versus a high-carbohydrate diet or increased oxidative stress have been shown to increase or decrease adiponectin concentrations, respectively. These observations suggest that the circulating levels of adiponectin are regulated by complex interactions between genetic and environmental factors.[248]

The receptors for adiponectin have been characterized in rodent models and cloned. Two receptors, named ADIPOR1 and ADIPOR2, have been characterized. ADIPOR1 is expressed in numerous tissues including muscle, whereas ADIPOR2 is mostly restricted to the liver. Both receptors are bound to the cell membrane yet are unique in comparison to other G protein–coupled receptors in the fact that the C terminal is external whereas the N terminal is intracellular.[249] Both ADIPOR1 and ADIPOR2 are receptors for the globular head of adiponectin and serve as initiators of signal transduction pathways that lead to increased PPARα and increased AMP kinase activities, which promote glucose uptake and increased fatty acid oxidation. Adiponectin has been shown to have potent anti-atherogenic functions, as it accumulates in the subendothelial space of injured vascular walls to reduce the expression of adhesion molecules and the recruitment of macrophages.[250]

Studies in obese children and adolescents have shown that adiponectin is inversely related with the degree of obesity, insulin sensitivity, visceral adiposity, IHCL, and IMCL, whereas weight loss increases adiponectin. A fall in adiponectin has been shown to coincide with the onset of insulin resistance[251] and the development of diabetes in monkeys.[252] All of these observations along with human clinical data support a pivotal role for adiponectin in the prevention of the comorbidities of the metabolic syndrome.

Family studies using parent-offspring regressions revealed that most adipocytokines show evidence for significant inheritance. A principal component (PC) analysis of standardized hormone levels demonstrates surprising heritability of the three most common axes of variation. The main axis, which explained 21% of the variation, was most strongly loaded on levels of leptin, TNFα, insulin, and PAI-1, and inversely with adiponectin. It was significantly associated with body mass index (BMI), phenotypically stronger in children, and showed a heritability of 50%, after adjustment for age, gender, and generational effects. Thus, adipocytokines are highly heritable and their pattern of covariation significantly influences BMI as early as the preteen years.[253]

Myokines and Natriuretic Peptides. Skeletal and heart muscle may serve as an endocrine organ as well. Some of the effects of exercise on skeletal muscle are mediated by the transcriptional coactivator PPAR-γ coactivator 1α (PGC-1α). In the mouse, PGC-1α expression in muscle stimulates an increase in expression of FNDC5, a membrane protein that is cleaved and secreted as a newly identified hormone, named irisin. Irisin acts on white adipose cells in culture and in vivo to stimulate UCP1 expression and a broad program of brown fatlike development. Irisin is induced with exercise in mice and humans, and mildly increased irisin levels in the blood cause an increase in energy expenditure in mice with no changes in movement or food intake. This results in improvements in obesity and glucose homeostasis. This novel myokine is actually the first hormonal link between exercise and the adipose tissue changes it may induce.[254] Atrial natriuretic peptides (ANPs) also have been implicated in fat metabolism. These natriuretic peptides are produced with exercise, cardiac wall stress, weight loss, and cold exposure, and they are inhibited by obesity and insulin resistance. The ANP binds to its natriuretic receptor, facilitating the formation of cGMP from GTP. In turn, cGMP phosphorylates cGMP-dependent protein kinase, which activates lipolysis and phosphorylates p38 mitogen-activated protein kinase to enhance mitochondrial biogenesis with increased energy expenditure and increased heat generation, as part of the brown-fat thermogenic program. Thus, both skeletal

and cardiac muscle may respond to exercise by inducing changes in fat metabolism to enhance caloric expenditure and limit obesity. These new findings are likely to open new avenues of clinical research to limit the consequences of the "obesity epidemic."[255,256]

Inflammatory Cytokines. Accumulating evidence indicates that obesity is associated with subclinical chronic inflammation.[257] The adipose tissue serves not merely a simple reservoir of energy stored as triglycerides, but serves as an active secretory organ releasing many peptides, including inflammatory cytokines, into the circulation. In obesity, the balance between these numerous peptides is altered, such that larger adipocytes and macrophages embedded within them produce more inflammatory cytokines (i.e., TNF-α, IL-6) and fewer anti-inflammatory peptides such as adiponectin.[258] One theory posits that as energy accumulates in adipocytes, the perilipin border of the fat vacuole breaks down, causing the adipocyte to die.[259] Cell death recruits macrophages in the adipose tissue, especially the visceral compartment, which in the process of clearing debris also elaborate inflammatory cytokines, initiating a pro-inflammatory milieu that predates and possibly drives the development of systemic insulin resistance, diabetes, and endothelial dysfunction.[260,261] Systemic concentrations of C-reactive protein (CRP) and IL-6, two major markers and participants of the inflammatory process, are increased in obese children and adolescents. CRP levels within the "high-normal" range have been shown to predict CV disease[262] and development of T2DM[263] in adults. Elevated levels of CRP correlate with other components of the metabolic syndrome in obese children.[264,265] Thus, inflammation may be one of the links between obesity and insulin resistance and may also promote endothelial dysfunction and early atherogenesis.

Reactive Oxygen Species (ROS). The "free radical theory" holds that an imbalance between ROS generation and antioxidant defenses is a major factor in the determination of lipid peroxidation and protein misfolding, with resultant DNA and cellular damage.[266] Excessive intracellular ROS formation occurs via three pathways: (1) inflammatory cytokines derived from visceral fat accumulation,[267] (2) dysfunctional mitochondrial energetics,[268] and (3) glycation (discussed later). Excessive nutrient processing by mitochondria can result in uncoupling of oxidative phosphorylation and increased generation of ROS; this, in turn, leads to altered mitochondrial function and further ROS generation.[269] ROS accumulation can also impair endoplasmic reticulum (ER) function, causing ER stress and the compensatory unfolded protein response (UPR). The UPR can itself be overwhelmed by persistent excessive nutrient processing and ROS generation, leading to cellular shutdown, defective insulin secretion, and T2DM[270 271] (Figure 22-6).

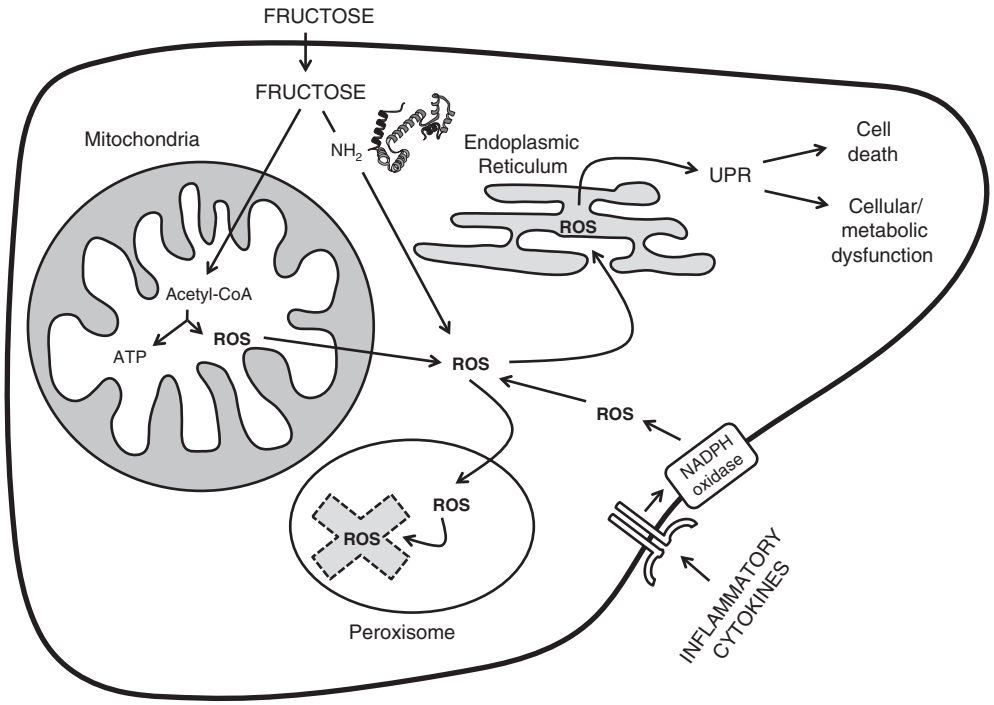


FIGURE 22-6 ■ Mechanisms of subcellular metabolic dysfunction, using fructose as an example. The formation of acetyl-CoA leads to lipid deposition and activation of inflammatory pathways, which serine phosphorylate IRS-1, leading to insulin resistance. Furthermore, metabolic processing in the mitochondria, the glycation of protein ϵ-amino groups via the Maillard reaction, and circulating inflammatory cytokines, due to their receptor-mediated activation of NADPH oxidase, all increase intracellular levels of reactive oxygen species (ROS). In the absence of sufficient peroxisomal quenching and degradation, the ROS moieties lead to endoplasmic reticulum (ER) stress, promoting the unfolded response (UPR), and cause either cell death (apoptosis) or cellular/metabolic dysfunction. ATP, adenosine triphosphate; CoA, coenzyme A; JNK-1, *c-jun* N-terminal kinase 1; NADPH, nicotinamide adenine dinucleotide phosphate; PKCϵ, protein kinase C−ϵ; pSer-IRS-1, serine phosphorylated IRS-1; ROS, reactive oxygen species; UPR, uncoupled protein response. (From Bremer, A. A., Mietus-Snyder, M. L., & Lustig, R. H. (2012). Toward a unifying hypothesis of metabolic syndrome. *Pediatrics*, *129*, 557–570.)

As ROSs are inherent by-products of cellular metabolism, endogenous cellular antioxidants (e.g., catalase and glutathione) quench the ROS before they have a chance to promote peroxidation. These antioxidants are found primarily in peroxisomes, which abut the mitochondria and act as "support staff" for ROS processing. Reduction in peroxisomal activity results in mitochondrial dysfunction and endoplasmic reticulum stress. Furthermore, cytokines such as TNF-α can reduce peroxisomal number and function, rendering cells even more vulnerable.[272,273]

Others. Several other novel adipocytokines have been identified, but their clinical significance in humans is still unclear. For instance, resistin, a 12.5-kDa polypeptide hormone, is produced by adipocytes in rodents and by immunocompetent cells in humans. In rodents, resistin appears to have an important role in the development of hepatic insulin resistance, yet its role in humans is less clear but may be related to involvement in the regulation of inflammatory processes, rather than tissue-specific insulin sensitivity.

Comorbidities Related to Insulin Resistance

The Metabolic Syndrome

The association and clustering of T2DM, hypertension, dyslipidemia, and CV disease in adults has led to the hypothesis that they may arise from a common antecedent. The World Health Organization (WHO) argues that this antecedent is insulin resistance and defines this association as the "metabolic syndrome."[274-277] A consensus definition of the metabolic syndrome for the pediatric age group has been published[278] and declares that children younger than 10 years of age should not be defined as having this condition. For children older than 10 years of age, the obesity component of the definition is waist circumference and not BMI, indicating the clinical importance of intra-abdominal fat. The metabolic syndrome affects approximately 25% of the U.S. adult population.[279] Because of its wide prevalence, the metabolic syndrome is of enormous clinical and public health importance, even at its earliest stages. Although still debated, one scheme of the pathophysiology of the metabolic syndrome is shown in Figure 22-5. According to this paradigm, the impact of obesity is determined by the pattern of lipid partitioning (i.e., the specific depots in which excess fat is stored). This pattern of lipid storage determines the adipocytokine secretion profile, on circulating concentrations of inflammatory cytokines and on the flux of FFA. The combined effect of these factors determines the sensitivity of insulin target organs (such as muscle and liver) to insulin and impacts the vascular system by affecting endothelial function. Peripheral insulin resistance and endothelial dysfunction are the early promoters of overt pathology, culminating in T2DM and cardiovascular disease. Regardless of the definition of *metabolic syndrome* used, insulin resistance and high insulin levels are associated with the clustering of cardiometabolic risks associated with metabolic syndrome in a variety of ethnic groups.

Nonalcoholic Fatty Liver Disease (NAFLD)

NAFLD represents fatty infiltration of the liver in the absence of alcohol consumption.[280] The spectrum of NAFLD ranges from pure fatty infiltration (steatosis) to inflammation (nonalcoholic steatohepatitis, or NASH), to fibrosis and even cirrhosis.[281] NAFLD was found in the NHANES III survey to be more prevalent in obese African-American and Hispanic males, with T2DM, hypertension, and hyperlipidemia.[282] These associations have led to the hypothesis that NAFLD may precede the onset of T2DM in some individuals. NAFLD is now the most common liver disease among children in North America.[283,284] NAFLD in children is associated with increased visceral fat deposition[285] and may progress to cirrhosis and related complications.[286] The association between abdominal obesity and fatty liver may be partially explained by sustained exposure of the liver to an increased flux of FFA from the visceral depot.[213] NAFLD may represent an early manifestation of ectopic lipid deposition in the liver and represents a challenge to the clinician due to the contrast of its minimal early manifestations and its potential serious outcomes. Studies using the clamp methodology demonstrate that NAFLD is associated with hepatic and peripheral insulin resistance. The relation between insulin sensitivity and NAFLD seems to be, in part, driven by abdominal fat content.

Insulin plays a key role in regulating transcription factors such as sterol response element binding protein-1c (SREBP-1c), which are abundantly expressed in the liver.[287] SREBP-1c is pivotal in the control of hepatic lipogenesis and is increased in proportion to circulating insulin levels.[288] These data raise the possibility that fasting hyperinsulinemia may contribute to hepatic steatosis, rather than vice versa. Alternatively, inflammatory cytokines released by visceral fat or by the hepatic immunoreactive cells may contribute to altered hepatic lipid metabolism.[280] The majority of patients probably experience NAFLD without progressing on to NASH. It is likely that subsequent inflammation from increased ROS formation without appropriate quenching is necessary to promote progression to NASH (the so-called second hit theory).[286,289] As hepatic imaging modalities improve, noninvasive quantification of hepatic lipid deposition may enable us to use it as a target for intervention. For the time being, NAFLD can be surmised by an elevated ALT. However, ALT does not have to be very elevated; the 95th percentile for ALT in children is 25.8 U/mL for boys and 22.1 for girls.[290]

Polycystic Ovarian Syndrome (PCOS)

The association of hyperandrogenism and oligomenorrhea or amenorrhea in females, termed PCOS, is a frequent comorbidity of obesity, which can extend down to childhood. This disorder is covered in detail in Chapter 15. A 2003 consensus statement[291] defined the diagnostic criteria for PCOS as two out of the following three (after exclusion of other hyperandrogenic disorders): oligo/anovulation, clinical or biochemical manifestations of hyperandrogenism, and polycystic ovaries by ultrasound. PCOS is the most common cause of infertility due to anovulation and a

major risk factor for development of the metabolic syndrome and altered glucose metabolism in females. The antecedents of PCOS have been identified in prepubertal girls, suggesting a developmental lesion.[292]

Adolescent girls with PCOS can have moderate to severe insulin resistance with increased risk for altered glucose metabolism, and the impairment in insulin sensitivity is more pronounced in obese rather than lean PCOS girls. In some ethnic groups, girls with premature pubarche, a potential antecedent of PCOS, have relatively increased insulin levels, thus a causal link between hyperinsulinemia and androgen hypersecretion (of adrenal or ovarian origin) has been hypothesized. Population studies of normal girls have shown that rapid weight gain is associated with higher adrenal androgens and body fatness, and that hyperinsulinemia is related to early menarche. Thus, the association of higher insulin levels with premature pubarche and subsequent PCOS may be driven, at least in part, by obesity. Obesity characterizes about 50% of women with classic PCOS,[293] although it is even more common among adolescents. Increased peripheral insulin resistance occurs in approximately 50% of patients with PCOS and almost certainly plays a role in the pathogenesis of this condition. On the other hand, almost all forms of severe insulin resistance, such as T2DM or rare lipodystrophy syndromes, are also associated with PCOS. Of note, insulin resistance has not been included as a diagnostic criterion for PCOS mainly because of the difficulty of its measurement. Fasting hyperinsulinemia and an increased insulin secretory response to an oral glucose load have been demonstrated in girls with PCOS.[294] Indeed, obese adolescent girls with PCOS have been shown to be 50% more insulin resistant than weight-matched controls without PCOS.[295] The constellation of metabolic abnormalities typically seen in insulin-resistant individuals is commonly encountered in obese adolescents with PCOS, including NAFLD[296] and T2DM.[297] The increased prevalence of the metabolic syndrome may be related to the hyperandrogenism independent of obesity-related insulin resistance.[298] Early markers of accelerated atherogenesis are already present in young females with PCOS,[299] indicating that early intervention aimed at reducing CV risk may be beneficial.

Metabolic examination of patients with PCOS demonstrates hepatic and muscle resistance, but not ovarian insulin resistance, possibly accounting for insulin stimulation of theca cell androgen production.[300] The correlation between insulin resistance and hyperandrogenism begs a unifying hypothesis as to their pathogenesis, which is proffered by the "serine phosphorylation hypothesis," which suggests that both P450c17 and the insulin receptor are aberrantly serine phosphorylated; in the case of P450c17, this leads to excess activity and increased androgen production,[301] and in the case of the insulin receptor, this leads to tissue-specific insulin resistance.[302] However, this hypothesis remains to be proven.

Other Endocrine Comorbidities

Obesity causes changes in other hormonal systems, some of which confer specific morbidities. The age of pubertal initiation has been creeping earlier, particularly in African Americans. This advancement is explained in part by the increasing overnutrition and BMI seen in this population.[303] Infertility in older adolescents and adult women may occur either as a manifestation of PCOS due to excessive ovarian androgen production in females or due to excessive aromatization of androgen to estrogen by peripheral adipose tissue with suppression of the hypothalamic-pituitary gonadal axis in both sexes.[304] The hyperestrogenemia may also promote gynecomastia in males.[305] In addition, the hypercapnia associated with obstructive sleep apnea can suppress hypothalamic GnRH function, leading to a syndrome of delayed puberty.[306]

Obesity is associated with decreased growth hormone (GH) secretion, and indeed most obese subjects, despite normal or excessive statural growth, fail GH stimulation testing. However, caloric restriction for 24 hours can restore normal GH responsivity.[307] Despite the functional GH inadequacy, statural growth is accelerated, bone age is advanced, and peripheral total and free IGF-1 levels are normal or elevated in obesity, suggesting normal or accentuated GH sensitivity,[308] or possibly due to the suppression of IGFBP-1 and the effects of hyperinsulinemia on activation of the growth plate IGF-1 receptor.[309] Free thyroxine levels tend to be lower and TSH higher in obese children, although mostly within the normal range along with some TSH levels within the subclinical hypothyroidism category; the mechanism is unknown. The elevated TSH levels in obesity seem a consequence rather than a cause of obesity. Therefore, treatment of hyperthyrotropinemia with thyroxine is unnecessary in obese children. Lastly, obesity can be associated with increased cortisol exposure, possibly due to conversion of circulating cortisone to cortisol by the enzyme 11β-hydroxysteroid dehydrogenase-1 (11β−HSD1) located within adipocytes.[310]

Other Nonendocrine Comorbidities

Childhood obesity is associated with numerous other comorbidities. Pseudotumor cerebri[311] is a rare and poorly understood condition leading to intracranial hypertension, whose manifestations include papilledema and headache. Treatment includes serial lumbar puncture, acetazolamide to reduce CSF production; in severe cases there is a need for a ventriculoperitoneal shunt, and occasionally optic nerve sheath fenestration is necessary to save eyesight. Obstructive sleep apnea occurs frequently in morbidly obese children; presumably due to the large amount of retropharyngeal fat that compresses the upper airway during sleep.[312] Affected patients snore, often stop breathing for more than 20 seconds during sleep, and wake up during the night with headache. Treatment includes nocturnal positive airway pressure and, when appropriate, tonsilloadenoidectomy; however, symptoms often recur. Obese children manifest numerous orthopedic difficulties, including fractures, knee pain, anatomic lower limb malalignment, and impairment in mobility.[313] Cholelithiasis occurs in approximately 2.5% of obese adolescents, especially in females, but is not usually seen in prepubertal obese children.[314] Lastly, psychological distress, including clinical depression, is clearly manifested in obese children[315] and specifically in

the severely obese. These various comorbidities all appear to be associated with a BMI z-score in a curvilinear fashion[316]; thus, the more obese a patient is, the more likely he or she will manifest comorbidity.

FACTORS ASSOCIATED WITH THE CURRENT EPIDEMIC OF OBESITY

Genetics

The association between obesity and genetics owes to two separate lines of investigation: (1) the discoveries of monogenic disorders of the energy balance pathway (discussed later) and (2) studies of specific racial and ethnic groups, in which obesity seems to segregate, such as the Pimas and Hispanics in the Southwest United States.[317,318] These observations are combined with an attractive theory on the natural selection of individuals in response to drastic environmental/ecologic pressure (i.e., famine), termed the *thrifty gene hypothesis*,[319] to yield a very strong driving force for the elucidation of specific genetic loci in the pathogenesis of obesity.[320] However, the rapid timescale of the increased prevalence of childhood obesity cannot possibly reflect a population genetic change. Therefore, the current model is that obesity is a result of gene-environment interactions; an ancient genetic selection to deposit fat efficiently may have provided a survival advantage in old times but is maladaptive with our current food overabundance. In the common forms of obesity, relating single nucleotide polymorphisms with associated risks for obesity is difficult, as the effects are uncertain and the results not always confirmed. Several single nucleotide polymorphisms in specific genes have been identified, such as the fat mass and obesity associated (FTO) gene. Variation in the FTO gene has provided the most robust associations with common obesity to date, yet the FTO variant that confers a predisposition to obesity does not appear to be involved in the regulation of energy expenditure but may have a role in the control of food intake and food choice, suggesting a link to a hyperphagic phenotype or a preference for energy-dense foods.[321] The large genome wide association studies (GWAS) have yielded to date 34 loci that may be relevant to the development of adult obesity, yet they explain less than 5% of the phenotype.[322] However, an analysis done specifically in children reveals two more loci that may provide more genetic susceptibility.[323]

Epigenetics and Developmental Programming

Follow-up studies of newborns who were small for their gestational age (SGA), large for gestational age (LGA), and premature have noted markedly increased risks for obesity and the metabolic syndrome. The "fetal origins hypothesis"[324] states that some aspect of the *in utero* environment contributes to the development of obesity and chronic disease in later life. The specific developmental aberration that promotes obesity remains unknown. However, each of these three antenatal conditions is associated with insulin resistance. The "developmental model" of chronic diseases postulates that early-life events affect individual differences in vulnerability to lifestyle and environment. This concept is supported by studies of *in utero* or at-birth conditions on future diseases by the fact that differences in adult lifestyles only partially explain the development of diseases. One possible mechanism for such developmental programming includes epigenetic changes, which may contribute to alterations in gene expression. For instance, the pattern of DNA methylation noted in cord blood predicted the degree of adiposity at age 9 years.[325]

Documentation of the relationship of SGA with adult obesity and CV disease started with studies of the Dutch famine during World War II and its aftermath.[326] Several studies of SGA newborns demonstrate that they are hyperinsulinemic and insulin resistant at birth, exhibit rapid catch-up growth in the early postnatal period, and develop obesity in childhood, which remains and promotes persistent insulin resistance and the metabolic syndrome. An analysis of Indian newborns born in India versus the United Kingdom.[327] demonstrate that even though those born in India weigh 700 g less at birth, their glucose and insulin levels are markedly elevated. After adjustment for birth weight, the India-born babies demonstrate increased adiposity, four times higher insulin levels, and two times higher leptin levels than babies born in the U.K. Thus, these babies are insulin resistant even at birth, which translates into increased adiposity. Following such babies into childhood, numerous studies document insulin resistance during early childhood.[328-330]

Babies born LGA are hyperinsulinemic at birth.[331] Although most LGA babies are due to gestational diabetes mellitus (GDM) and exposure to hyperglycemia throughout the pregnancy, this is not always the cause. A follow-up of LGA babies without GDM demonstrates a doubling of prevalence of insulin resistance and metabolic syndrome, whereas LGA babies resulting from GDM manifest a threefold increase.[332,333] Indeed, the "vertical" transmission of maternal diabetes to the offspring in the form of later obesity and diabetes has been documented in studies of Pima Indians.[334,335] Lastly, weight gain during pregnancy increases birthweight, the risk for LGA, and obesity in the offspring[336,337] (Figure 22-7).[661]

Although there are no studies documenting hyperinsulinemia at birth in premature infants due to technical reasons, follow-up of these babies into early childhood also demonstrates increased weight gain and insulin resistance and compensatory insulin secretion that is inappropriately high for their degree of weight gain.[338]

The protective effect of breastfeeding against the development of future obesity has long been known,[188] and there appears to be a dose response; the longer the breastfeeding, the more protective.[339] However, this may be complicated by confounding factors, such as socioeconomic status, maternal smoking during pregnancy, and maternal BMI.[340] The mechanism of breastfeeding's antiobesity effect is also unclear. Some think infant feeding self-regulation is most relevant, whereas one study suggests that leptin in breast milk may contribute to this protection,[341] and finally the concern about the fructose/sucrose content in infant formula has also received attention.

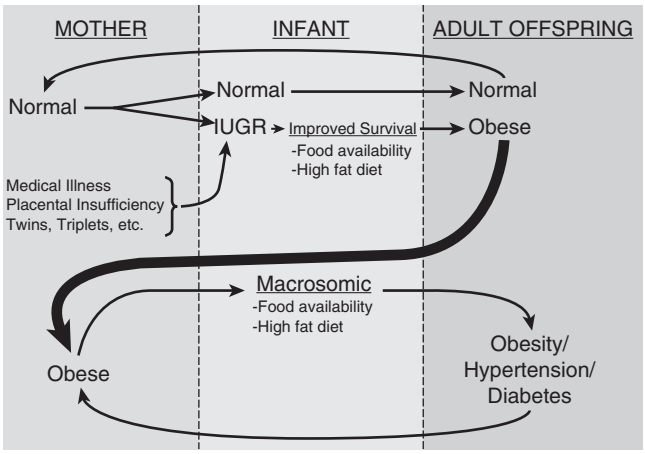


FIGURE 22-7 ■ Postulated mechanisms by which developmental programming (either intrauterine growth retardation or neonatal macrosomia) can lead to adult obesity in the offspring. (From Ross, M. G., Huber, I., & Desai, M. (2010). Intrauterine growth restriction, small for gestational age, and experimental obesity. In R. H. Lustig (Ed.), *Obesity before birth: maternal and prenatal effects on the offspring* (pp. 215–239) New York: Springer.)

Environmental Factors

Numerous environmental factors have also been associated with the obesity epidemic, particularly in children. However, most of these associations are derived from cross-sectional rather than longitudinal studies, and in many instances, the mechanism remains lacking.[342] Several longitudinal studies in adults have clearly demonstrated that specific dietary and other lifestyle behaviors are independently associated with long-term weight gain, with a substantial aggregate effect. For instance, on the basis of increased daily servings of individual dietary components, 4-year weight change was most strongly associated with the intake of potato chips (0.767 kg), potatoes (0.58 kg), sugar-sweetened beverages (0.45 kg), unprocessed red meats (0.43 kg), and processed meats (0.42 kg) and was inversely associated with the intake of vegetables (−0.1 kg), whole grains (−0.168 kg), fruits (−0.224 kg), nuts (−0.25 kg), and yogurt (−0.37 kg) ($P \leq .005$ for each comparison).[343] Similarly, the sociodemographic environment has been shown to affect the chance of being obese in adults. Indeed, the opportunity to move from a neighborhood with a high prevalence of poverty to one with lesser poverty was associated with modest but potentially important reductions in the prevalence of extreme obesity and diabetes.[344] Similar relationships are likely, but not proven, for children and adolescents.

Stress and Cortisol

In humans, elevated cortisol or markers of HPA axis dysregulation correlate with abdominal fat distribution and the metabolic syndrome.[345] Although circulating cortisol is clearly important in determining visceral adiposity, the identification of a reduction of circulating cortisone to cortisol within visceral fat tissue by the enzyme 11β-hydroxysteroid dehydrogenase-1 (11β−HSD1) has also been linked to the metabolic syndrome.[310,346] These data suggest that cortisol is important for both increasing visceral adiposity and promoting the metabolic syndrome.

In adults, job stress and depression stress with increased cortisol secretion[347] lead to insulin resistance and the metabolic syndrome. Psychosocial stresses correlate with the risk of myocardial infarction in adults[348]; it is assumed that such patients exhibit increased HPA axis activation.[349] Even exogenous glucocorticoid administration is a risk factor for CV events.[350] Evidence of associations between elevated cortisol and psychological distress with abdominal fat distribution in adults is compelling. For instance, urinary glucocorticoid excretion is linked to aspects of the metabolic syndrome, including blood pressure, fasting glucose, insulin, and waist circumference.[345] Some individuals seem to be "high-responders" to a stress stimulus and demonstrate higher cortisol secretion. These individuals seem more prone for an alteration in satiety recognition and consume larger amounts of calories following the stress exposure (see Figure 22-3). However, the role of stress and cortisol in childhood obesity is currently undefined.

Sleep Deprivation

Americans get significantly less sleep than they did in the 1980s. Adults in the United State currently average less than 7 hours of sleep per night—almost 2 hours less than in 1980—and about one third of them get less than 6 hours per night.[352] Analyses of data from the first National Health and Nutrition Examination Survey (NHANES I) revealed that adults (ages 32 to 49 years old) who got less than 7 hours of sleep were more likely to be obese 5 to 8 years later than those who got 7 or more hours of sleep.[353] Similarly, a 13-year prospective cohort study in which participants were interviewed at ages 27, 29, 34, and 40 years of age found that sleep duration correlated negatively with obesity.[354] The link between short sleep duration and obesity has also been observed among children.[355] Like adults, increasing numbers of children are chronically sleep deprived. This is especially true of obese children, who have been found to get less sleep than children of normal weight. In addition to its other effects, sleep is one of the most powerful cross-sectional[356] and longitudinal[357] predictors of childhood obesity in prepubertal children. Although relatively little is known about the mechanism for the sleep-obesity relationship, especially among children, there are reasons to assume increased stress (discussed earlier) and altered activity of various hormones, such as leptin, ghrelin, and cortisol.

Television Viewing and "Screen Time"

Television watching is considered one of the most modifiable causes of childhood obesity.[358] There are four possible mechanisms linking television watching and obesity. First, television watching may increase stress levels and cortisol (discussed earlier), thereby increasing food intake and promoting obesity.[359] Second, television watching displaces physical activity. Most, but not all studies find inverse correlations between television watching and

physical activity and fitness.[360,361] Third, television watching increases calorie consumption because people eat while viewing or are affected by food advertising. Television viewing is also associated with increased high-fat food intake, decreased fruit and vegetable consumption, and increased soft drink intake.[362] "Junk food" is the most frequently advertised product category on children's television. Lastly, REE and nonexercise-associated thermogenesis (NEAT) seem to decrease during television watching.[363] According to NHANES III (1988-1994), the prevalence of childhood obesity is lowest among children who watch television ≤ 1 hour/day and highest among those who watch ≥ 4 hours/day.[364] The relationship between television watching and obesity has been examined in a large number of cross-sectional epidemiologic studies but in few longitudinal studies.[358] Several experimental studies have been conducted on the impact of reduced television watching, and their results support the suggestion that reduced television watching may help to reduce the obesity risk or promote weight loss in obese children.[365] These studies represent the strongest direct evidence that altering television watching alone is a promising strategy for preventing childhood obesity. Other forms of "screen time," such as playing videogames and using cell phones, are also implicated in the obesity pathogenesis.

Dietary Factors

Although the primary focus in terms of obesity has been on total calories ingested, an emerging body of evidence suggests that the quality of those calories play an important role in the pathogenesis of metabolic syndrome, by increasing hepatic insulin resistance or increasing ROS formation.

Dietary Fat versus Carbohydrate

Fat is generally considered more obesogenic than other macronutrients, because it is more energy dense, highly palatable, and more effectively converted to body fat.[366] A high-fat meal induces less thermogenesis and a higher positive fat balance than an isocaloric and isoprotein-containing low-fat meal.[367] Excessive fat intake is believed to cause weight gain,[368] but the relationships between dietary fat intake and childhood adiposity remain controversial.[369]

The prevalence of overweight in the United States has increased despite a decreased percentage of dietary energy derived from fat. A meta-analysis of 12 studies in overweight or obese adults who were given dietary advice on low-fat diet and followed for 6 to 18 months suggested that low-fat diets are no better than calorie-restricted diets for long-term weight loss.[370] Similarly, in children, total fat consumption expressed as a percentage of energy intake has decreased.[371] This decrease in fat consumption is largely due to increased total energy intake in the form of carbohydrates. Much of this imbalance is attributed to changing beverage consumption patterns, characterized by declining milk intakes and substantial increases in soft-drink consumption,[372] which may have its own etiopathogenesis (discussed later). Most interventions with a low-fat, "heart-healthy" diet have not been successful in childhood overweight prevention.[373]

The reduction in carbohydrate intake is taken to the extreme in the Atkins diet, which restricts adult subjects to less than 25 g/day of ingested carbohydrate. Adult evaluations of the diet have been disappointing long term,[374,375] and the popular diet has been abandoned. There are currently no data on the effects of this diet on children or adolescents. However, it should be noted that the ketogenic diet used to control seizures is similar in composition to the Atkins diet. A 2-year study of the ketogenic diet demonstrated persistent decreases in weight z-scores in children who were above average when the diet was initiated, without a significant compromise in general nutrition or height.[376]

Trans-unsaturated Fatty Acids (Trans Fats)

Trans-unsaturated fats in processed foods have been a staple in the Western diet since the early 20th century. This is because the trans-isomerization of the double bond prevents fatty acid breakdown by bacteria, prolonging the shelf life of foods. Like its bacterial predecessors, human mitochondria cannot subject trans fats to β oxidation in the liver,[377] contributing to ectopic intrahepatic lipid accumulation. Fortunately, due to the recognized association between trans fat consumption and cardiovascular disease in the mid-1980s and more stringent labeling requirements since 2006, the percentage of calories from trans fats consumed in the "Western diet" has gradually declined.[378] Trans fats have no health benefit and cause hepatic steatosis and insulin resistance[379]; however, their current consumption trends are temporally disparate with the current increasing prevalence of metabolic syndrome, suggesting that other factors are involved.

Glycemic Index and Fiber

Not all sugars exert the same insulinogenic response. Complex carbohydrates can be taken in two forms: either a combination of α1-4 linkages and α1-6 linkages, which gives the starch a globular structure called amylopectin, as seen in bread, rice, pasta, potatoes, and glycogen; or a linear polymer of α1-4 linkages called amylose, as seen in beans, lentils, and other legumes. Digestion and absorption of the former in the intestine is rapid due to the simultaneous actions of both α1-4 and α1-6 glucosidases, whereas that of the latter is much slower, because the α1-4 glucosidase can only cleave single glucose moieties on either side of the polymer. This phenomenon constitutes the basis of the glycemic index (GI),[380] which refers to the glucose area under the curve after consumption. High-GI foods lead to an accentuated insulin response, which can shunt energy substrate to adipose tissue.[381] Controlled studies on children with a high-GI diet demonstrate that energy intake is 53% higher than it is for children on a low-GI diet.[382] One study on adolescents demonstrated that an *ad libitum* low-GI diet was more effective in promoting weight loss than an energy-restricted low-fat diet.[383] Therefore, the GI may be a simple concept to

institute, although the "toxic environment" of American foodstuffs may make it difficult to maintain.

Dietary fiber consists of the nonstarch, polysaccharide portion of plant foods, including cellulose, hemicellulose, pectins, β-glucans, fructans, gums, mucilages, and algal polysaccharides. Major sources of dietary fiber include whole grains, fruits, vegetables, legumes, and nuts. Fiber content accounts for 50% of the variability in glycemic load (GL; GI × volume) between foods. Cohort studies of adults demonstrate that fiber intake is inversely associated with weight gain, fasting insulin levels, and risk of T2DM.[384,385] Fiber may influence the regulation of body weight by several mechanisms involving intrinsic, hormonal, and colonic effects, which eventually decrease food intake by promoting satiation (lower meal energy content), satiety (longer duration between meals), or by increasing fat oxidation and decreasing fat storage.[386] A fiber-rich meal is processed more slowly, has less caloric density, and is lower in fat and added sugars. Fiber-containing foods engender slower glucose absorption, which lessens the postprandial insulin surge and decreases lipogenesis.[387] In addition, high-fiber meals allow undigested triglyceride to be delivered to the colon, where fermentation to short-chain fatty acids and their absorption improve lipids and insulin sensitivity.[388] Archeologists surmise that our ancestors consumed approximately 100 grams of fiber/day.[389] However, the dietary fiber intake throughout childhood and adolescence currently averages approximately 12 g/day and has not changed since the 1980s.[390] Therefore, parents and school food service personnel should strive to offer fiber-rich foods to children so that their acceptance and consumption of these foods will increase.[391]

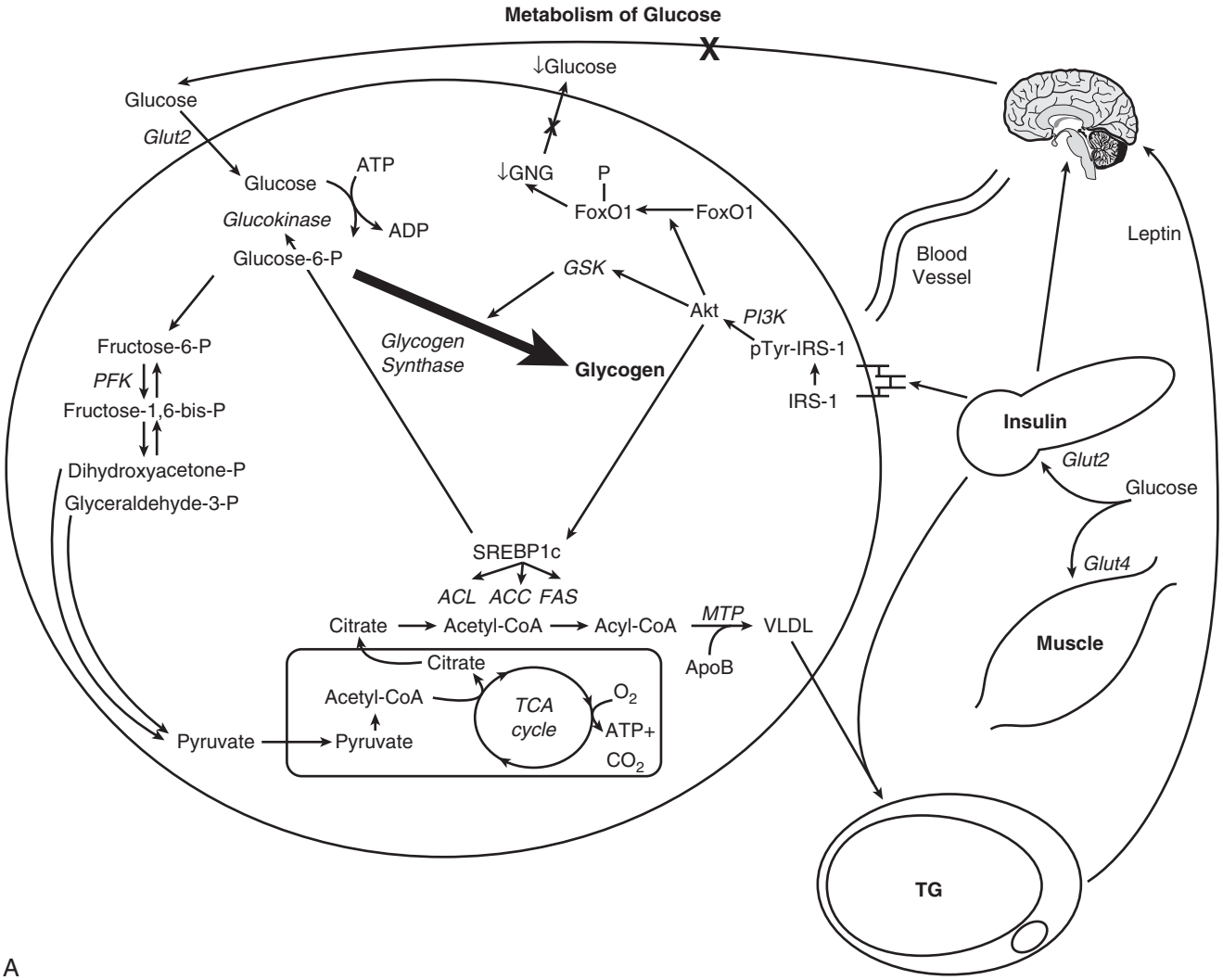
Fructose

The most commonly used sweetener in the U.S. diet is the disaccharide sucrose (e.g., table sugar), which contains 50% fructose and 50% glucose. However, in North America and many other countries, nondiet soft drinks are sweetened with high-fructose corn syrup (HFCS), which contains up to 55% of the monosaccharide fructose. Thanks to its abundance, sweetness, and low price, HFCS has become the most common sweetener used in processed foods. The issue is not that HFCS is biologically more ominous than sucrose, it is that its low cost has made it available to everyone, especially low socioeconomic groups. HFCS is found in processed foods ranging from soft drinks and candy bars to crackers, hot dog buns, and ketchup. Average daily fructose consumption has increased by over 25% since the 1980s. The growing dependence on fructose in the Western diet may be fueling the obesity and T2DM epidemics.[392] The highest fructose loads are soda (1.7 g/30 mL) and juice (1.8 g/30 mL). Although soda has received most of the attention,[180,393] high fruit juice intake is also associated with childhood obesity, especially among lower-income families.[394] The American Academy of Pediatrics has altered its recommendations, suggesting that fruit juice consumption be limited to 120 to 150 mL/day for 1- to 6-year-old children and 240 to 360 mL/day for 7- to 18-year-old children.[395]

Animal models demonstrate that high-fructose diets lead to increased energy intake, decreased resting energy expenditure, excess fat deposition, and insulin resistance[396]; these outcomes suggest that fructose consumption is playing a role in the epidemics of insulin resistance and obesity and T2DM in humans.[397,398]

Fructose in the gut is transported into the enterocyte via the fructose transporter Glut5, independent of ATP hydrolysis and sodium absorption. Once inside in the enterocyte, a small portion of the fructose load is converted to lactic acid and released in the portal circulation; another small portion may also be converted to glucose. However, the majority of ingested fructose is secreted into the portal circulation and delivered to the liver. There, fructose is rapidly metabolized to fructose-1-phosphate (F1P) via fructokinase, an insulin-independent process that also bypasses the negative feedback regulation of phosphofructokinase in the glycolytic pathway. Thus, fructose metabolism generates lipogenic substrates (e.g., glyceraldehyde-3-phosphate and acetyl-CoA) in an unregulated fashion, which are delivered straight to the mitochondria. This excessive mitochondrial substrate then drives hepatic DNL, which can then overwhelm apoB and the lipid export machinery, leading to intrahepatic lipid deposition and steatosis.[289] Hepatic DNL also limits further fatty acid oxidation in the liver via excess production of malonyl-CoA, which reduces the entry of fatty acids into the mitochondria by inhibiting carnitine palmitoyl transferase-1 (CPT-1). F1P also stimulates sterol regulatory binding protein-1c (SREBP-1c) via peroxisome proliferator-activated receptor gamma coactivator (PGC)-1β[399] independently of insulin, which activates the genes involved in DNL; moreover, fructose has been shown to induce activation of carbohydrate-response element binding protein (ChREBP), which also increases the expression of all the enzymes of DNL. Furthermore, F1P activates dual-specificity mitogen-activated protein kinase 7 (MKK7), which subsequently stimulates Janus kinase-1 (JNK-1), a hepatic enzyme considered to act as a bridge between hepatic metabolism and inflammation.[400] In addition, the lipogenic intermediate diacyl glycerol (DAG) (formed during fructose metabolism in the liver) activates protein kinase C epsilon (PKCε), which phosphorylates serine residues on insulin receptor substrate-1(IRS-1), inactivating it, and leading to hepatic insulin resistance.[289] This impairs insulin-mediated phosphorylation of FoxO1, leading to increased expression of the genes required for gluconeogenesis and promoting increased hepatic glucose output, also contributing to hyperglycemia and the development of T2DM. The excess TGs secreted from the liver into the circulation as fat-laden VLDL particles following the ingestion of fructose, coupled with a fructose-induced reduction in LPL activity, cause sustained postprandial dyslipidemia, thereby augmenting the risk for cardiovascular disease[401,402] (Figure 22-8).[662]

Fructose also does not suppress secretion of the so-called hunger hormone ghrelin, levels of which correlate with perceived hunger. In sum, fructose consumption has metabolic and hormonal consequences that facilitate the development of obesity and the metabolic syndrome.[398]



A

FIGURE 22-8 ■ Hepatic (**A**) glucose and (**B**) fructose metabolism. Of an ingested glucose load, 20% is metabolized by the liver; thus, in a 120-calorie glucose load (two slices of white bread), 24 calories are hepatically metabolized. Under the action of insulin, glycogen synthase is increased, and the majority of the glucose load is stored as glycogen. While insulin activation of sterol response element binding protein-1c (SREBP-1c) activates the lipogenic pathway, there is little citrate formed to act as substrate for lipogenesis. In addition, insulin action on the liver phosphorylates forkhead protein-O1 (FoxO1), excluding it from the nucleus, and suppressing the enzymes involved in gluconeogenesis (GNG). In comparison, virtually 100% of a fructose load is hepatically metabolized; thus, in a 120-calorie sucrose load (an 8-ounce glass of orange juice), a bolus of 72 calories reach the liver. In contrast to glucose, fructose induces (1) substrate-dependent hepatocellular phosphate depletion, which increases uric acid and contributes to hypertension through inhibition of endothelial nitric oxide synthase and reduction of nitric oxide (NO); (2) stimulation of *de novo* lipogenesis and excess production of VLDL and serum triglyceride, promoting dyslipidemia; (3) accumulation of intrahepatic lipid droplets, promoting hepatic steatosis; (4) production of FFA, which promotes muscle insulin resistance; (5) *c-jun* N-terminal kinase (JNK-1) activation, which serine phosphorylates and the hepatic insulin receptor, rendering it inactive, and contributing to hepatic insulin resistance, which promotes hyperinsulinemia and influences substrate deposition into fat; and (6) CNS hyperinsulinemia, which antagonizes leptin signaling (see Figure 22-3) and promotes continued energy intake. Glut2, glucose transporter 2; Glut4, glucose transporter 4; Glut 5, glucose transporter 5; SREBP-1c, sterol regulatory element binding protein-1c; PGC-1β, peroxisome proliferator-activated receptor-γ coactivator-1β; MKK7, MAP kinase kinase 7; PKCε, protein kinase C-ε; IRS-1, insulin receptor substrate-1; ChREBP, carbohydrate response element binding protein; PI3K, phosphatidyl inositol-3-kinase; CPT-1, carnitine palmitoyl transferase-1; GSK, glycogen synthase kinase; PFK, phosphofructokinase; PP2A, protein phosphatase 2a; ACL, ATP citrate lyase; ACC, acetyl CoA carboxylase; FAS, fatty acid synthase; ApoB, apolipoprotein B; MTP, microsomal transfer protein; VLDL, very low density lipoprotein; FFA, free fatty acids; LPL, lipoprotein lipase; IR, insulin resistance; ACSS2, acyl-CoA synthetase short-chain family member 2. (From Lustig, R. H. (2010). Fructose: metabolic, hedonic, and societal parallels with ethanol. *J Am Diet Assoc, 110*, 1307–1321.)

Continued

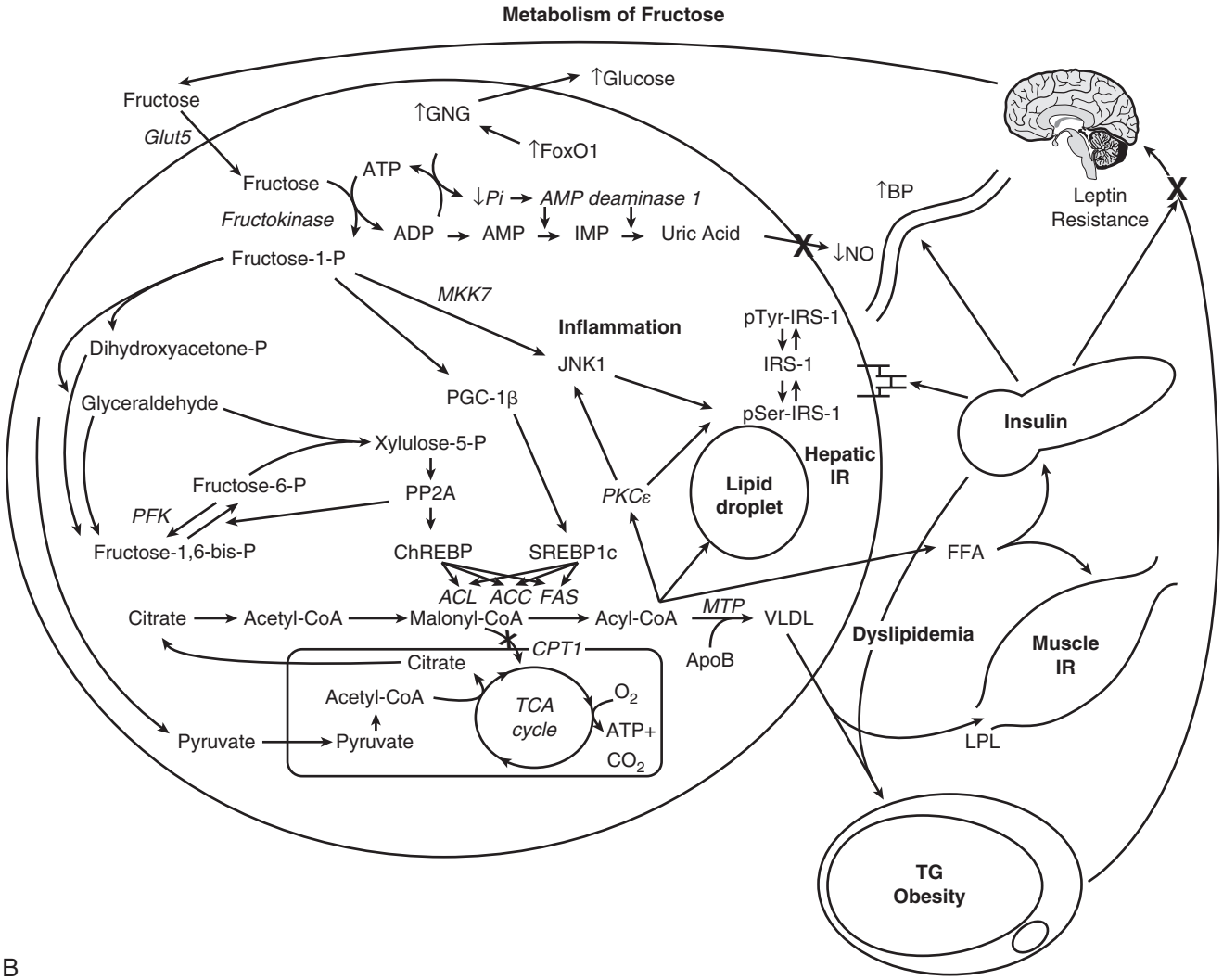


FIGURE 22-8, cont'd

Due to its unique stereochemistry, the ring form of fructose (a five-membered furan with axial hydroxymethyl groups) is under a great deal of ionic strain, which favors the linear form of the molecule, exposing the reactive 2-keto group, which can readily engage in the nonenzymatic fructosylation of exposed amino moieties of proteins via the Maillard reaction, in the same way that the 1-aldehyde position of glucose is reactive.[289] Each Maillard reaction generates one ROS, which must be quenched by an antioxidant or risk cellular damage. In an *in vitro* study, incubation of hepatocytes with fructose yielded no direct damage; however, when these hepatocytes were preincubated with sublethal doses of hydrogen peroxide to reduce their peroxisomal ROS-quenching ability, fructose then became as hepatotoxic as other organic aldehydes.[403] Furthermore, an *in vivo* study in antioxidant-deficient mice demonstrated that intrahepatic lipid toxicity and hepatocellular death occurred following sucrose administration.[404] These data thus suggest that excessive ROS, in combination with micronutrient insufficiencies that impair antioxidant reserves, can lead to cellular damage and promote the metabolic syndrome.

Branched-Chain Amino Acids

Branched-chain amino acids (BCAAs: valine, leucine, and isoleucine) are essential amino acids that account for > 20% of the amino acids in the typical "Western diet."[405] Although normally utilized for protein biosynthesis and cell growth, when provided in excess they are diverted away from protein synthesis and toward energy utilization.[406]

In the liver, BCAAs increase the transcription of carbohydrate regulatory element-binding protein (ChREBP) and SREBP-1c,[407] facilitating DNL. Furthermore, BCAAs limit insulin-induced PI3-K signaling and stimulate the activation of the mammalian target of rapamycin (mTOR), promoting the serine phosphorylation of IRS-1 and impairment of insulin signaling. In addition, just as there are obesity-related changes in adipokines and cardiovascular risk markers, there also appear to be obesity-associated changes in BCAA metabolism and subsequent serum levels. In particular, valine and leucine/isoleucine

levels have been reported to be 20% and 14% higher, respectively, in obese compared to lean subjects.[406] Mechanistically, this appears to be accounted for by a high rate of flux through the BCAA catabolic pathway, resulting in the increased production of alanine. Since alanine is a highly gluconeogenic amino acid, increased BCAA catabolism may thus contribute to increased hepatic glucose output.[408] Furthermore, the increased α-ketoacids generated by increased flux of the BCAAs through their catabolic pathways also potentially suppress mitochondrial β-oxidation.

Furthermore, chronic BCAA elevation impairs the transport of aromatic amino acids into the brain; the reduced production of serotonin (derived from tryptophan) and catecholamines (derived from phenylalanine and tyrosine) may drive hunger.[406] The "BCAA overload" hypothesis suggests that in the context of a dietary pattern that includes high fat consumption, BCAAs may make an independent contribution to the development of insulin resistance, a hypothesis supported by metabolomics studies demonstrating high BCAA levels in normoglycemic individuals who subsequently develop insulin resistance and diabetes.[409,410]

Ethanol

Although adult epidemiologic studies associate light to moderate ethanol consumption with improved insulin sensitivity and wine consumption with reduced cardiovascular risk, other cross-sectional and prospective studies implicate a dose-dependent effect of alcohol in the metabolic syndrome and suggest that chronic consumption of large amounts of ethanol worsen insulin sensitivity. Ethanol bypasses glycolysis by being converted by alcohol dehydrogenase-1B to form acetaldehyde, which promotes ROS formation and must also be quenched by hepatic antioxidants such as glutathione or ascorbic acid. Acetaldehyde is then metabolized by the enzyme aldehyde dehydrogenase-2 to acetic acid, which in turn is metabolized by the enzyme acyl-CoA synthetase short-chain family member 2 to form acetyl-CoA. The acetyl-CoA can then enter the mitochondria; or, in the presence of other caloric substrates, it is preferentially used for the synthesis of fatty acids through DNL. The excess malonyl-CoA produced from ethanol metabolism inhibits CPT-1, limiting mitochondrial fatty acid β-oxidation. Ethanol also blocks fatty acid β-oxidation by inhibiting both peroxisome proliferator-activated receptor (PPAR)-α, which suppresses microsomal triglyceride transfer protein, thereby altering the liver's lipid export machinery.[411-413] Buildup of intrahepatic lipid metabolites leads to subsequent activation of the enzyme *c-jun* N-terminal kinase-1 (JNK-1) and serine-phosphorylation of IRS 1, driving further hepatic insulin resistance. Thus, ethanol metabolism results in intrahepatic lipid accumulation and liver injury,[414,415] driving hepatic insulin resistance and promoting the metabolic syndrome.[416] However, although clearly a concern in adults, it is unlikely that ethanol contributes significantly to metabolic syndrome in children.

Calcium and Dairy

There have been several reports of an inverse relationship between dietary calcium and obesity indices.[417,418] Dietary calcium plays an important role in regulating energy metabolism. Increased calcitriol (1,25-dihydroxyvitamin D) in response to low calcium diets stimulates Ca^{2+} influx in human adipocytes and may lead to the stimulation of lipogenic gene expression and lipogenesis, as well as the inhibition of lipolysis.[418] This may result in an expansion of adipocyte triglyceride stores, which can promote adiposity. Increased dietary calcium reduces calcitriol levels and leads to a reduction of fat mass without caloric restriction in mice,[419] and this antiobesity effect of dietary calcium is supported by human clinical and epidemiologic studies.[420] Vitamin D deficiency correlates with increasing BMI, especially in African Americans[421]; however, it is not known if this is due to the substitution of soft drinks for dairy, lactose intolerance, or other factors. One adult study[422] revealed a consistent effect of higher calcium intake on lower body weight and body fat; however, pediatric studies are lacking.

Trace Minerals

Chromium and vanadium appear to be involved in the insulin signaling process. In diabetic animals, chromium or vanadium supplementation results in improvement in insulin sensitivity and glycemic control,[423,424] and there is a suggestion of decreased weight. It is not yet known whether the pathogenesis of insulin resistance in humans involves a deficiency of a trace mineral; however, inadequate dietary intakes of vitamins and minerals are widespread, most likely due to the excessive consumption of energy-rich, micronutrient-poor, refined food. Inadequate intake of micronutrients may result in chronic metabolic disruption, mitochondrial decay, DNA damage, oxidant leakage, and cellular aging associated with late onset diseases such as obesity and cancer.[425]

Infectious Causes

The pattern of increase in prevalence during the current obesity epidemic is reminiscent of an infectious transmission. Studies in animals implicate adenovirus-36 in the conversion from lean to obese.[426] Studies in adults thus far demonstrate a correlation between BMI and antibodies to this virus.[427] Adenovirus-36 appears to differentiate fat cells. Ad-36 antibody levels correlate with BMI in certain populations, particularly in children. In one study, 15% of obese children were Ad-36 positive, in contrast to 7% of normal-weight children. But *within* the obese population, those who were Ad-36 positive weighed, on average, ~16 kg more than those who were not. Presently, all human data on Ad-36 and obesity are based on correlations. Bacteria also are implicated in obesity. The predominance of certain human intestinal flora species (Firmicutes versus bacteroides) may predispose both animals and humans to obesity,[428] possibly by increasing efficiency of energy absorption[429]; however, factors that determine their predominance are unknown.

Medications

Numerous medications promote excessive weight gain in children. The most commonly prescribed are pharmacologic doses of glucocorticoids (e.g., prednisone, methylprednisolone, dexamethasone), used for their anti-inflammatory and antineoplastic activities. Patients so treated frequently become obese[430,431] and develop many of the features of Cushing syndrome (e.g., visceral adiposity, hyperlipidemia, hypertension, glucose intolerance), which typify the metabolic syndrome.[350] Sex hormone administration also promotes excessive weight gain, presumably by inducing insulin resistance.[432] In patients with type 1 diabetes, strict glycemic control is usually accompanied by slight overinsulinization of the patient, leading to a greater occurrence of mild hypoglycemic episodes requiring nonhunger-driven calorie consumption and potentially to excessive weight gain.[433] Lastly, more and more children are being placed on the atypical antipsychotics risperidone, olanzapine, quetiapine, clozapine, aripiprazole, and ziprasidone to affect mood and behavior.[434] These medications induce insulin resistance, which foments persistent hyperphagia and weight gain and increases the risk for the metabolic syndrome specifically during the first months following their initiation.[435]

DISORDERS OF OBESITY

The concept that obesity is a phenotype of numerous pathologies is evident from the examination of specific endocrine disorders leading to obesity in early childhood (Box 22-1). Some involve neural mechanisms, others involve classic hormonal mechanisms, whereas others involve dysregulation of increased energy intake, decreased energy expenditure, or increased energy storage at the adipocyte. It is important to remember that even in referral centers for obese children, children with an "organic" cause of their obesity represent less than 2% of the population; the majority represents the classic genetic-environmental complex interaction.

BOX 22-1 Classification of Childhood Obesity Disorders

Monogenetic Disorders of the Energy Balance Pathway

- Leptin deficiency
- Leptin receptor deficiency
- POMC mutation ("red" hair, adrenal insufficiency)
- Prohormone convertase-1 deficiency
- MC_3R mutation
- MC_4R mutation
- SIM-1 mutation
- Brain-derived neurotrophic factor (BDNF) deficiency

Syndromic Disorders (Mental Retardation Prominent)

- Prader-Willi syndrome
  - Short stature
  - Hypogonadism
  - Hypotonia
  - Ghrelin overproduction
- Bardet-Biedl syndrome
  - Retinitis pigmentosa
  - Polydactyly
  - Hypogonadism
- *Trk*B mutation
  - Hypotonia
  - Impaired short-term memory
  - Decreased nociception
- Borjeson-Forssman-Lehmann syndrome
  - Microcephaly
  - Large ears
  - Hypogonadism
- Carpenter syndrome
  - Variable craniosynostosis
  - Brachydactyly, polydactyly, syndactyly
  - Congenital heart disease
  - Hypogonadism
- Cohen syndrome
  - Persistent hypotonia
  - Microcephaly
  - Maxillary hypoplasia
  - Prominent incisors
- Alstrom syndrome
  - Hypogonadism
  - Short stature
  - Neurosensory deficits

"Classic" Endocrine Disorders (Short Stature/Growth Failure Prominent)

- Hypothyroidism
  - Primary
  - Central
- Cushing syndrome (adrenal hypercorticism)
  - Adrenal adenoma/carcinoma
  - Adrenal micronodular hyperplasia
  - Pituitary ACTH-secreting tumor
  - Ectopic ACTH-secreting tumor
  - Exogenous glucocorticoid administration
- Growth hormone deficiency
- Pseudohypoparathyroidism 1a
  - Maternal transmission (AHO + multihormone resistance)
  - Paternal transmission (pseudopseudohypoparathyroidism, AHO only)

Insulin Dynamic Disorders

- Hypothalamic obesity (insulin hypersecretion)
- Insulin resistance
- Leptin resistance

Other Disorders

- Rapid-onset obesity with hypoventilation, hypothalamic, autonomic dysregulation, often with neural crest tumor syndrome (ROHHAD)

"Classic" Endocrine Disorders with an Obesity Phenotype

In children, linear or statural growth accounts for up to 20% of ingested calories. Endocrine states that allow for normal energy intake for age, but inhibit linear growth, will of necessity lead to excessive energy storage. This is the case for the four "classic" endocrine disorders associated with obesity. These can be distinguished from other causes of pediatric obesity on the basis of their suboptimal growth rate, as opposed to overnutrition, which tends to increase the rate of both growth and skeletal maturation, probably due to excess insulin cross-reacting with the IGF-1 receptor.[309]

Hypothyroidism results in a lower REE due to insufficient circulating T_3, along with decreased voluntary energy expenditure (VEE) due to fatigue. The decrease in total energy expenditure, despite a relatively low caloric intake, promotes persistent energy storage and increases adiposity. Thyroid hormone replacement is sufficient to increase growth, REE, and VEE to resolve the obesity over time.

Cushing syndrome arrests growth and results in cortisol-induced hyperphagia,[135] along with a decrease in REE and VEE due to muscle wasting. A reduction of circulating glucocorticoid through medical or surgical means usually reverses the obesity to some degree. Exogenous glucocorticoid therapy can result in the same obesity phenotype. Hypercortisolism may be more related to obesity than previously realized, due to the transgenic model of 11βHSD1 overexpression in visceral adipose tissue, which converts inactive circulating cortisone to cortisol.[310] However, its role in human obesity is not clear, as correlations between 11βHSD1 polymorphisms and BMI or waist:hip ratio were weak at best,[436] and enzyme activity was not elevated in one human study.[437]

GH deficiency prevents lipolysis and promotes visceral adiposity, although obesity is usually not severe. The disorder should be diagnosed long before the obesity phenotype develops. GH deficiency is also associated with fatigue and decreased VEE. GH deficiency is often accompanied by other pituitary hormone deficiencies (e.g., central hypothyroidism), which can also decrease REE. GH therapy is able to reverse these defects, increase muscle mass, and promote weight loss.

Pseudohypoparathyroidism type 1a (PHP1a) is an autosomal dominant mutation of *GNAS1*, which codes for the $G_{s\alpha}$ subunit, necessary for peptide hormone signal transduction. Maternal transmission leads to PHP1a (multihormone resistance) along with Albright hereditary osteodystrophy (AHO), of which a cardinal feature is obesity, probably due to the inability to stimulate cAMP in response to β-adrenergic stimulation within adipocytes, due to defective G-protein signal transduction.[438] Paternal transmission leads to AHO without multihormone resistance, also known as pseudopseudohypoparathyroidism. This form of obesity is not responsive to current medications.

Monogenetic Disorders of the Negative Feedback Pathway

The elucidation of the regulation of the energy balance pathway is exemplified by the discovery of specific defects within that pathway leading to early-onset obesity. Numerous obesity syndromes within the pathway have been described and are reviewed in detail elsewhere.[439,440]

Leptin Deficiency

Mutations of the leptin gene in humans recapitulate the phenotype of the *ob/ob* leptin-deficient mouse.[441] Only a few such patients have been described, primarily of Pakistani and Turkish descent, and for the most part they were born to consanguineous parents. These patients manifest hyperphagia from birth, with obesity documented as early as 6 months of age. The lack of leptin induces the starvation response in the form of reduced thyroid hormone concentrations, lack of sympathetic tone, lack of pubertal progression, and defective immunity.[442] Despite the modest hypothyroidism, the concomitant hyperinsulinemia allows excess insulin to cross-react with the IGF-1 receptor in order to maintain growth rate and bone age until the usual time of puberty. However, because of the important role of leptin in initiating and maintaining puberty, untreated patients with leptin deficiency are short, due to the lack of a pubertal growth spurt. The diagnosis is made by demonstrating extremely low or unmeasurable serum concentrations of leptin. However, treatment with recombinant leptin effectively restores leptin signaling, thereby reducing hyperphagia, resolving obesity, inducing puberty, and regulating immunity.[442] Heterozygotes for leptin deficiency assume an intermediate phenotype.[443]

Leptin Receptor (LEPR) Deficiency

Three family members in France of Algerian ancestry were originally found to have a mutation truncating the leptin receptor prior to its insertion in the membrane.[444] This family had symptoms and signs similar to those with leptin deficiency; they also had growth retardation, low thyroid, and low IGF-1 and IGFBP-3 concentrations. The reason for this dichotomy is not known. When testing a large cohort of subjects with severe, early-onset obesity, the prevalence of pathogenic LEPR mutations was 3%. Affected subjects were characterized by hyperphagia, severe obesity, alterations in immune function, and delayed puberty due to hypogonadotropic hypogonadism. Serum leptin levels were within the range predicted by the elevated fat mass in these subjects (thus, serum leptin cannot be used as a marker for leptin-receptor deficiency). Their clinical features were less severe than those of subjects with congenital leptin deficiency.[445]

POMC Splicing Mutation

The inability to synthesize POMC due to missense or truncation mutations results in the inability to splice out α-MSH in the brain, leading to defective anorexigenesis at the MC_4R and early-onset obesity; in the periphery, this defect leads to "red" hair resulting from a lack of α-MSH action at the skin MC_1R; and in the pituitary the defect results in an inability to splice out ACTH, leading to secondary adrenal insufficiency.[446] A Turkish patient with this mutation was reported to have dark hair, indicating that

pigmentation of the hair is not entirely explained by the mutation's effect on MC_1R.[447] The diagnosis is easily established due to the unusual phenotype, ACTH levels, and hypocortisolemia. There is currently no treatment for the obesity.

Prohormone Convertase-1 (PC-1) Deficiency

Defects in this enzyme lead to the inability to process various preprohormones to their active ligands, such as POMC to ACTH and α-MSH, proinsulin to insulin, and various gut propeptides to active hormones.[448] Affected patients manifest severe early-onset obesity, ACTH deficiency, hypogonadism, hyperproinsulinemia, and small intestinal dysfunction due to inability to cleave intestinal propeptides to their mature form. The diagnosis can be made by finding extremely high levels of proinsulin, and molecular diagnostics may be required to confirm the gene defect. Treatment currently does not exist for the obesity.

Melanocortin-3 Receptor (MC_3R) Mutation

Two family members in Singapore with mutations of the MC_3R manifested with early-onset obesity.[449] This receptor appears to have a slightly different function from the MC_4R, as it seems to be involved in regulating energy expenditure as opposed to energy intake.[84] Diagnosis can only be made by gene sequencing. No treatment currently exists.

Melanocortin-4 Receptor (MC_4R) Mutation

Mutations in the MC_4R appear to account for up to 5% of morbid obesity cases, especially those beginning in childhood.[450,451] This mutation is transmitted as a codominant inheritance. Mutation carriers have severe obesity, increased lean mass, increased linear growth, hyperphagia, and severe hyperinsulinemia; homozygotes are more severely affected than heterozygotes. Subjects with mutations retaining residual signaling capacity have a less severe phenotype. Thus, mutations in MC4R result in a distinct obesity syndrome that is inherited in a codominant manner.[451] As the melanocortin system has been shown to directly control lipid metabolism as well as systemic blood pressure, mutation carriers usually manifest the typical elements of the insulin-resistance syndrome.[452] The diagnosis is made by gene sequencing. There is currently no treatment for this disorder.

SIM-1 Mutation

SIM-1 stands for *single minded*, a Drosophila gene involved in neurogenesis, particularly the PVN, which expresses the MC_4R. SIM-1 appears to act as a signal transduction mechanism integrating information downstream from MC_4R activation.[453] Heterozygous null mice for SIM-1 are obese. The human homolog is on chromosome 6q. A girl with hyperphagia, obesity, and developmental delay with a balanced translocation between 1p22.1 and 6q16.2 was found to have a mutation of SIM-1.[454] Only a few polymorphisms of SIM-1 in obesity have been reported, and their significance is not yet established.[455]

Chromosome 16p11.2

Large deletions of chromosome 16p11.2 have been shown to account for 0.7% of a large cohort of morbidly obese patients.[456]

Brain-Derived Neurotrophic Factor Deletion

The brain-derived neurotrophic factor (BDNF) inhibits food intake, and rodent models of BDNF disruption all exhibit increased food intake and obesity, as well as hyperactivity. Serum concentration of BDNF protein was significantly reduced by 50% in obese patients with the WAGR syndrome compared to those who were not obese and compared with age- and BMI-matched subjects. The WAGR syndrome (*W*ilms tumor, *a*niridia, *g*enital abnormality, *m*ental *r*etardation) is caused by heterozygous gene deletions on chromosome 11p13, which result in the haploinsufficiency of WT1 and PAX6 genes that are in close relation to the BDNF gene at 11p14.1. Haploinsufficiency for BDNF was associated with increased ad libitum food intake, severe early-onset obesity, hyperactivity, and cognitive impairment.[457]

Pleiotropic Obesity/Mental Retardation Disorders

Approximately 30 obesity syndromes have been described, and most if not all are associated with mental retardation.[458] Each has some other distinguishing phenotype, which makes the diagnosis obvious on clinical grounds. Various genetic linkages have been noted, but the etiologies for the obesity in these disorders remain obscure.

Prader-Willi Syndrome (PWS)

PWS is a defect in the paternal allele of chromosome 15q11-13,[459] either from deletion, mutation, defective imprinting, or uniparental disomy of the paternal chromosome 15. Subjects affected with PWS present with hypotonia and failure to thrive in the neonatal period, and they develop the classic findings of hypogonadism, short stature, mental retardation, and severe obesity as they get older. PWS patients are classically described as voracious eaters, although they can be easily dissuaded from food if they are removed from behavioral signals for food intake. REE is approximately 60% of normal in PWS, promoting adiposity.[460] In addition, ghrelin levels are massively elevated in PWS,[461] which may be one etiology for their persistent hyperphagia. Others have suggested that the obesity in PWS is a function of growth hormone (GH) deficiency, with defective lipolysis.[462]

Bardet-Biedl Syndrome

This syndrome is characterized by obesity, mild mental retardation, dysmorphic extremities (including polydactyly), retinitis pigmentosa, hypogonadism, and renal malformations. The inheritance of Bardet-Biedl is complex, but it is

usually described as autosomal recessive. Eight different genetic loci have been implicated,[463] although their functions continue to remain elusive. The cause of the obesity remains unknown, but it is postulated to be due to the failed development of ciliated hypothalamic neurons.[464,465] Cilia are conserved sensory organelles that protrude from the apical membrane of cells and play vital developmental and physiologic roles in all vertebrates. Moreover, mouse genetic models and *in vitro* experiments suggest a positive role for cilia in leptin signaling in the hypothalamus and a negative role for cilia in the formation of adipocytes in peripheral tissues—offering two potential explanations on how ciliary lesions lead to obesity. Diagnosis usually rests on clinical grounds. Although no specific treatment is available, anecdotal instances of the efficacy of metformin exist.

TrkB Mutation

The NTRK2 gene codes for the ligand-specific subunit for the receptor *Trk*B, which has brain-derived growth factor (BDNF) as its ligand. *Trk*B is important for neural development. An 8-year-old boy with early hypotonia, developmental delay, impaired short-term memory, decreased responsiveness to nociception, and severe hyperphagia starting at 6 months culminating in morbid obesity was reported.[466] Examination of the NTRK2 gene demonstrated an A to G transition in codon 722, substituting cysteine for tyrosine, which inhibited the phosphorylation of *Trk*B. A second patient with obesity, impaired cognitive function, and hyperactivity was found to have an isocentric inversion at the BDNF locus.[467] The etiology of the hyperphagia and obesity remain unclear.

Carpenter Syndrome

This syndrome consists of obesity plus variable craniosynostosis, brachydactyly, polydactyly, syndactyly, congenital heart disease, and hypogonadism. Its etiology remains obscure.

Cohen Syndrome

This disorder presents with persistent hypotonia, microcephaly, maxillary hypoplasia, and prominent incisors. The gene for Cohen syndrome has been mapped to chromosome 8q,[468] and it codes for a transmembrane protein of unknown function.

Alstrom Syndrome

This syndrome presents with neurosensory deficits, such as deafness, with various other endocrinopathies, which cause early-onset type 2 diabetes. The ALMS1 gene has been identified[469]; it appears to be important in cilia function[465] and may account for abnormal neuronal migration or leptin signaling.

Börjeson-Forssman-Lehmann Syndrome

This syndrome is characterized by microcephaly, seizures, large ears, and hypogonadism. The defective gene is a zinc-finger protein of unknown function.[470]

Rapid Onset Obesity, Hypoventilation, Hypothalamic, Autonomic and Thermal Dysregulation, with and without Neural Tumor (ROHHAD)

A recently identified syndrome includes hyperphagia and rapidly evolving obesity, combined with various forms of hypothalamic dysfunction (including GH deficiency, hypogonadotropic hypogonadism, central hypothyroidism, and ACTH abnormalities), central hypoventilation or altered respiratory control, thermal dysregulation, and risk for the development of tumors of neural crest origin.[471] The cause and treatment of this syndrome remain unknown.

Insulin Dynamic Disorders

Hypothalamic Obesity and Insulin Hypersecretion

It is well known that bilateral electrolytic lesions or deafferentation of the VMH in rats leads to intractable weight gain,[109,110,472-474] even upon food restriction.[475] Originally, the obesity was considered to be due to damage to a "satiety" center, which promoted hyperphagia and increased energy storage.[476] However, we now understand that a dysfunction of leptin signal transduction in the VMH due to hypothalamic damage— secondary to CNS tumor, surgery, radiation, or trauma—can alter both the afferent and efferent pathway of energy balance and lead to severe and intractable weight gain.[477,478] In this syndrome of "hypothalamic obesity," hypothalamic insult prevents the integration of peripheral afferent signals; the VMH cannot transduce these signals into a sense of energy sufficiency and a subjective state of satiety.[54,473] Patients with hypothalamic obesity exhibit weight gain, even in response to forced caloric restriction.[477] This seems paradoxical, as one would expect that if hyperphagia were the reason for the obesity, then caloric restriction would be effective. The reason for this paradox is similar to that for the *db/db* mouse, which is that these subjects exhibit "organic leptin resistance"—that is, the inability to respond to their own leptin due to the VMH damage.

Numerous assessments of weight gain following cancer therapy in children have been performed. Most of these evaluations have been retrospective and performed in the acute lymphoblastic leukemia (ALL) survivor population.[479] An extremely high frequency of hypothalamic obesity of 30% to 77% has been documented after craniopharyngioma treatment.[480] In each of these cancer types, hypothalamic damage is the primary risk factor for development of this syndrome.[481] However, the syndrome has also been reported in cases of pseudotumor cerebri, trauma, and infiltrative or inflammatory diseases of the hypothalamus.[477] Aside from the symptoms of tumor-induced increased intracranial pressure, patients with hypothalamic obesity classically exhibit signs of limbic system involvement, such as hypogonadism, somnolence, rage, and hyperphagia[482]; however, such classic presentations are actually rare.[474]

Hypothalamic obesity is the result of "organic" leptin resistance due to death of VMH neurons, leading to

defective autonomic neurotransmission.[99] This is akin to the starvation response, in that it manifests (1) defective activation of the SNS, which retards lipolysis and energy expenditure[483,484] (indeed, such patients demonstrate significantly reduced resting energy expenditure)[485] and (2) overactivation of the vagus,[486] which promotes an obligate insulin hypersecretion and energy storage.[487] In both animals and humans, vagal hyperreactivity can be prevented by pancreatic vagotomy.[110,488-490] Therapy for this disorder remains extremely problematic, as the brain seems to be "locked" in an orexigenic balance favoring energy consumption and storage. As the crucial period of weight gain is within the first year following the brain insult, it is crucial to focus on preventive measures during this narrow window of opportunity.

Primary Insulin Resistance

Insulin resistance is a primary entity in some pediatric populations. It is associated with the development of the metabolic syndrome, especially in certain racial and ethnic groups.[491] For example, the Pima Indians of Arizona exhibit a 50% incidence of obesity and type 2 diabetes.[492] In studies of adult twins, approximately half of the variance in insulin sensitivity and secretion can be attributed to genetic factors. Healthy children with a family history of T2DM are more insulin resistant, with an impaired balance between insulin sensitivity and secretion. Insulin resistance correlates with abdominal adiposity and CV morbidity.[493,494] The presence of acanthosis nigricans, a hyperpigmented and hypertrophic patch of skin at extensor surfaces such as the nape of the neck, is a clinical marker of hyperinsulinemia,[495] due to cross-reactivity between insulin and the epidermal growth factor receptor of the skin.[496] Fasting hyperinsulinemia, an indicator of inherent insulin resistance in children, is an important predictor of adult obesity.[497,498] This is further exacerbated by sex hormones (especially estrogen), contributing to the increased incidence of insulin resistance and obesity in teenage girls. The cause is unknown, but primary insulin resistance may be a manifestation of all three inciting factors: genetic, epigenetic, and environmental (discussed previously). There may be specific genetic predispositions,[317] which have been enriched by natural selection. In addition, the high incidence of gestational DM in Pima mothers promotes obesity and T2DM in the offspring.[335] Lastly, the consumption of low-quality processed food[152] made available by government subsidies promote continued obesity. It should be noted that in primary insulin resistance, dietary and exercise interventions have been notoriously ineffective at reducing the obesity.[499,500] The locus of the resistance to insulin is not defined; reduced numbers and function of insulin receptors may in part be secondary to the hyperinsulinemia itself, rather than due to a primary defect.

EVALUATION AND TREATMENT OF THE OBESE CHILD

Diagnostic Approach

The key to successful obesity therapy is accurate diagnosis. Our diagnostic armamentarium is not yet fully developed, so matching treatment to diagnosis is still uncertain. Specific points in the evaluation and their rationale are listed in Table 22-1. In eliciting the history, birth weight, parent's BMIs, gestational diabetes, prematurity, history of breastfeeding, and neonatal complications (especially CNS injury) are all relevant. The younger the patient's obesity is noted (i.e., early adiposity rebound), the more likely an organic reason will be identified. Neurodevelopmental abnormalities and signs of dysmorphism may signify the need for a genetic referral. The medication list must be reviewed, especially for atypical antipsychotics. Orthopedic pain, headache, and snoring must be assessed. Dietary history must include skipping breakfast, daily ingestion of sodas and juices, and frequency and type of snacking. The degree of perceived stress by the patient is also likely a major contributor to visceral adiposity. A corollary is the number of caretakers the child has, as a higher number increases stress, family chaos, and lack of child supervision.

On physical examination, linear growth is key, as a classic endocrine evaluation (e.g., hypothyroidism, Cushing's, GH deficiency, pseudohypoparathyroidism [PHP]) is not necessary if linear growth is not attenuated. However, hyperinsulinemia and insulin resistance may cause accelerated growth, due to cross-reactivity of insulin with the IGF-1 receptor.[309] Important physical features to assess include acanthosis nigricans and waist circumference (both of which are associated with insulin resistance and the metabolic syndrome), funduscopic examination to rule out pseudotumor cerebri, liver enlargement to suggest hepatic steatosis, hirsutism to suggest PCOS, and muscle tone to evaluate hypotonia and myopathy, which reduce energy expenditure.

Laboratory evaluation includes tests of obesity-related morbidity (e.g., AST, ALT, lipids, fasting glucose and HbA1c, knee and hip x-rays [in cases of suspected Blount deformity]). Specific diagnostic studies must be tailored to the individual patient. For instance, growth attenuation requires endocrine evaluation, including thyroid function tests, IGF-1 and IGFBP-3, 24-hour urinary cortisol or midnight serum cortisol, and possibly magnetic resonance imaging (MRI) of the hypothalamus and pituitary. Luteinizing hormone (LH), follicle stimulating hormone (FSH), and testosterone levels may be appropriate when evaluating for delayed puberty in males or PCOS in females. Patients with developmental delay will require karyotype and MRI. Severe obesity in a toddler may require a leptin level and genetic testing for a MC_4R mutation. Evaluating patients for insulin resistance remains controversial. A proper identification of risk factors by family history and a prudent physical examination is certainly appropriate. At this point in time, obtaining a fasting insulin level remains controversial. In the clinical setting, fasting insulin is an unreliable measure of insulin sensitivity. Testing of aliquots of a common sample assayed in different laboratories has shown disparate results; however, there is a current effort to standardize insulin assays nationwide in the United States. Even if a uniformly reliable insulin assay became available, separate standards would need to be developed by genders, ethnic groups, and pubertal stages. Also, the correlation between fasting insulin and euglycemic-hyperinsulinemic clamp-derived whole-body insulin sensitivity in children

TABLE 22-1 **Diagnostic Evaluation of Childhood Obesity and Its Comorbidities**

	History
Genetic etiology	Parent BMI, race, mental retardation
Epigenetic etiology	Birth weight, gestational difficulties, prematurity
CNS etiology	CNS insult, mental retardation or developmental delay
Endocrine etiology	Slowdown in linear growth, red hair
Medication etiology	Medication history, esp. atypical antipsychotics
Dietary etiology	Calorie recall, esp. sugar-containing liquids, breastfeeding Hx
Physical activity etiology	Exercise history, television, computer, and cell phone recall
Stress etiology	Socioeconomic status, number of caregivers, television recall, sleep status, atypical depression
Sleep apnea	History of snoring, headache, waking up with headache
PCOS	Hirsutism, oligomenorrhea, amenorrhea
Type 2 diabetes	Polyuria, polydipsia, nocturia, recent weight loss
Orthopedic morbidity	Knee or hip pain, limitation of motion
Depression	Affect, activity level, school performance
	Physical
Insulin resistance	Acanthosis nigricans, skin tags, waist circumference
Hypertension	SBP or DBP > the 90th percentile for age
Pseudotumor cerebri	Papilledema
Hepatic steatosis	Hepatomegaly
PCOS	Hirsutism
Precocious/delayed puberty	Gonadal and pubic hair status
Sleep apnea	Tonsillar hypertrophy
Myopathy	Decreased muscle tone, hyporeflexia
Syndromic obesity	Specific neurocutaneous stigmata (see Box 22-1), retardation
	Laboratory
Hepatic steatosis	ALT, hepatic ultrasound
Glucose intolerance	Fasting glucose > 100 or 2-hour glucose > 140
Type 2 diabetes mellitus	Fasting glucose > 125 or 2-hour glucose > 200; HbA1c > 6.5%
Dyslipidemia	Lipid profile with increased VLDL, TG:HDL > 2.5
Insulin resistance	Fasting insulin, glucose
Insulin hypersecretion	3-hour OGTT with insulin levels
CNS lesion	MRI, especially with hypothalamic coned-down views

is relatively poor, with an r value of 0.42,[501] and other surrogate measures of insulin resistance do not exhibit superior correlation. This may have to do with the fact that fasting insulin levels likely reflect hepatic rather than total body insulin resistance.[271] Lastly, there is currently no recommended pharmacologic treatment for isolated insulin resistance. The mere presence of obesity should call for intervention to lower weight and consequently improve insulin sensitivity. An OGTT may become necessary in an obese child, to evaluate for type 2 diabetes in cases where a suggestive history and familial risk factors are present.

Lifestyle Modification

Lifestyle modification is and remains the cornerstone of obesity therapy, especially in children. This approach is common sense and based on a handful of early studies demonstrating the efficacy of lifestyle in a select hand-picked group of children with intensive follow-up.[502] Indeed, the Diabetes Prevention Trial for T2DM demonstrated the efficacy of lifestyle on weight loss in adults; however, the cost per patient of administering such an intervention was prohibitive.[503] Data supporting the long-term efficacy of lifestyle modification in the "real world" is not particularly persuasive. An analysis of numerous methodologies concluded that although there are limited quality data to recommend one treatment program over another, combined behavioral lifestyle interventions compared to standard care or self-help can produce a significant and clinically meaningful reduction in overweight in children and adolescents.[504] Importantly follow-up studies of numerous large-scale interventions often find a rapidly diminishing effect once the study has been completed. Finally, a meta-analysis of 39 published intervention studies designed to prevent childhood obesity showed that 40% of the 33,852 participating children had a reduction in BMI, whereas the remaining 60% exhibited no effect.[505] Of note, it has been shown that a year after initial weight reduction, levels of the circulating mediators of appetite that encourage weight regain after diet-induced weight loss do not revert to the levels recorded before weight loss. This emphasizes the problem of maintaining long-term weight loss and the difficulties facing those who wish to lose weight and maintain it.[506] Optimistically, the effectiveness of interventions to prevent, rather than reverse, obesity in childhood (particularly for programs targeted to children ages 6 to 12 years) seems to be much more effective.[507]

Although weight loss is the conventional goal for intervention among adults, weight maintenance is recommended for the majority of children. The prevention of weight gain is easier, less expensive, and more effective than treating obesity itself,[508] and the prevention of overweight

among children prior to the presence of risk-related behaviors is crucial to stem the obesity epidemic. The primary goal of obesity prevention should be to promote physical activity and healthy diet with an emphasis on improving overall health, rather than weight loss.[509] There are at least four reasons to promote interventions to improve nutrition and physical activity in children.[510] First, the child may receive immediate benefits such as better fitness, energy, or micronutrient intake. Second, intervention at critical periods may improve adult health. Third, modifying chronic disease risks in childhood may lead to lower rates and risk factors in adults. Lastly, modifying children's behaviors may lead to improved behaviors in adulthood that would protect against chronic diseases.

Behavioral-cognitive therapy is designed to deal with both parent and patient, with behavior restructuring and reinforcement. Behavior changes include counseling sessions, teaching parenting skills, praise and contracts, self-monitoring tools, stimulus control within the home, role-modeling of behaviors by parents, and vigorous and long-term exercise programs. These programs have been successful in small studies with handpicked subjects by specific investigators,[511,512] but they have not yet been successful when attempted in clinic populations. One new clinical approach involves motivational interviewing,[513] a method for helping patients work through their ambivalence about behavior change. This method has been shown to be effective for substance abuse and in cases of adult diabetes; whether it will be successful in treating pediatric obesity remains to be seen.[514]

Dietary Intervention

Dietary intervention is essential in order to reduce not just caloric intake but the insulin response that promotes excessive energy deposition into adipose tissue. A myriad of studies demonstrate an association between the consumption of high-calorie, high-fat, high-carbohydrate, low-fiber foods and the development of pediatric obesity.[373] Specific maneuvers that have been successful in reducing the insulin response and promoting weight loss or stability in children include (1) the elimination of sugar-containing beverages (including both soda and juice)[515,516] and (2) a shift to a low-glycemic-load diet.[383] Not only does dietary modification to reduce the insulin response promote weight loss,[517] but it also promotes increased energy expenditure during the weight maintenance phase,[518] presumably by improving leptin sensitivity, which would help prevent weight regain. Other commonsense approaches in adults (although pediatric efficacy data are lacking) include (3) eating breakfast, (4) reducing portion sizes, (5) increasing fruit and vegetable consumption, (6) reducing between-meal snacking, and (7) reducing fast-food consumption.[519,520] However, dietary intervention alone is not a successful strategy for reducing pediatric overweight, unless the treatment is very intensive,[521] in which case the majority drop out. Furthermore, studies of nonsupervised dieting have demonstrated the opposite effect—that is, increased attempts in female adolescents predict a greater increase in weight and risk for obesity.[522]

Importantly, diets are a crucial part of weight maintenance and not only aimed at weight loss. Specifically, following a successful weight loss a weight maintenance diet is crucial to prevent weight regain due to the metabolic "starvation response" mentioned earlier in the text. Comparison of dietary approaches to weight maintenance following weight loss in children has shown that among overweight and obese young adults compared with pre–weight loss energy expenditure, isocaloric feeding following 10% to 15% weight loss resulted in decreases in REE and TEE that were greatest with a low-fat diet, intermediate with the low-glycemic index diet, and least with the very low-carbohydrate diet. This suggests that a low-carbohydrate diet may attenuate the "starvation response" induced by weight loss and help sustain the new weight achieved.[518]

Physical Activity Intervention

In adults, exercise is not an effective means of inducing weight loss unless combined with a reduction of caloric intake. The role of exercise may have a greater impact on weight maintenance rather than weight loss.[523] Similarly, there is question as to whether physical activity regimens for children can stabilize or reduce BMI.[524,525] Short-term studies show that vigorous exercise can result in short-term reductions in adiposity[526] and improved insulin sensitivity.[527] This amounts to a minimum of 30 minutes of vigorous exercise, 5 days per week, as proposed activity guidelines from the Institute of Medicine.[528] However, the effectiveness of these interventions eventually plateaus, and even short-term cessation rapidly reverses any accrued benefit.[529] To succeed, physical activity interventions must be long-term, sustained, and incorporated into behavioral modifications aimed at the individual, at the family, at the school, and within the community in general.[526,529] In other words, in order to make physical activity work, it must become a priority.

Lifestyle programs including supervised exercise can improve fasting insulin levels as quickly as 2 weeks before measurable weight loss occurs. Furthermore, lifestyle intervention has improved body composition without a change in body weight. Available studies suggest that fitness may play a more important role than body mass index reduction on an improvement in insulin sensitivity in obese adolescents. Appropriate interventions include (1) making school-based physical education mandatory for every child and school, (2) increasing access to after-school recreation, (3) increasing culturally appropriate activities, (4) reducing the competitive nature of sports so that more children will participate, (5) increasing the incorporation of physical activity into daily life (e.g., stairs, walking to school as appropriate), and (6) increasing the participation of parents in physical recreation, for their own weight management and as role models for their children. Lastly, the effects of reducing sedentary behavior by restricting television viewing has been efficacious in both small and large studies, and in diverse ethnic groups,[358] although fitness is not improved. Restriction of television also has the secondary effect of reducing caloric intake while watching television.

Results using either dietary or exercise intervention alone are not encouraging. Those studies that use behavioral, dietary, and exercise components together appear to be more successful,[373,530] although long-term efficacy is lacking.

School Intervention

Numerous school-based interventions have focused on reducing obesity rates, most of which have not been successful, in part because school cafeteria fare in most districts remains problematic. However, interventions that increased time spent in vigorous physical activity (20 minutes in elementary schools and 30 minutes in middle schools) were more successful.[531] One approach is to improve the self-esteem and self-efficacy of students, as opposed to simply educating them about eating disorders, as this increases physical activity.[532] Schools can model health-promoting environments and provide appropriate health education. Doing so may increase self-efficacy, reduce coping stress, and empower children to make healthy lifestyle choices.

Family Intervention

Invariably, the patient is not the only obese member of the family. There is frequently family chaos, which promotes obesity in other family members. Various caretakers (grandmothers, babysitters, etc.) will feed children and allow unrestricted television as a method of confining their activities indoors, particularly in dangerous neighborhoods. Some parents will not alter their shopping for "junk" food because they believe siblings should not be deprived; usually it is those same parents who will not be deprived. Divorced parents often use food as reward in order to buy the child's love and loyalty. Thus, the family itself must be the target of lifestyle intervention. Parental involvement is critical, and the concept of "food is not love" must be emphasized. Families need training to modify behavior and thereby make healthy dietary choices, increase activity, and reduce perceived stress. A meta-analysis of randomized trials of combined lifestyle interventions for treating pediatric obesity yielded a significant although underwhelming decrease in BMI of 1.5 kg/m^2 with targeted family intervention, and a nonsignificant decrease in BMI of 0.4 kg/m^2 in those that targeted the patient alone.[533]

Pharmacotherapy

Indications for Pharmacotherapy

Pharmacologic therapies in children must be considered adjuncts to standard lifestyle modification. The main problem is that presently only one drug has been approved for use in obese children, though a new medication (lorcaserin) and a combination of approved medications (phentermine-topiramate) are now approved for adults, and studies are starting in children. In addition, several limitations preclude physicians from the early implementation of drug therapies for the treatment of childhood obesity—for example, (1) the youngest age for which the U.S. Food and Drug Administration (FDA) has approved any obesity pharmacotherapy is 10 years; (2) the long-term use of pharmacologic intervention has not always proven to be more efficacious than behavior modification; (3) there exists a limited number of well-controlled studies of safe and effective pharmacologic intervention in obese children; (4) the relative risk for the development of adverse events in children must be weighed against the long-term potential for improvement of morbidity and mortality, which is difficult to estimate in children; and (5) targeting the pathology is still in its infancy. Also, we must not forget that many drugs used to treat adult obesity led to unforeseen complications, which resulted either in their restriction (thyroid hormone, amphetamine) or recall (e.g., sibutramine, rimonabant, dinitrophenol, fenfluramine, dexfenfluramine, phenylpropanolamine, ephedra).[534-539] Despite these concerns, the negative health impact of childhood obesity may justify long-term medication to control its progression.

In the current pharmacopoeia of childhood obesity (Table 22-2), only a nonspecific fat absorption reduction approach is available for obesity per se. Targeting obesity-related insulin resistance adds further agents, yet their effect in regard to weight reduction is disappointing.

Reduction of Energy Absorption: Orlistat

Orlistat is a modified bacterial drug that specifically inhibits intestinal lipase and can reduce fat and cholesterol absorption by approximately 30% in subjects eating a 30% fat diet.[540] Orlistat irreversibly binds to the active site of the lipase, preventing the intraluminal deacylation of triglycerides and resulting in a 16 gram/day increase in fecal fat excretion.[541] Orlistat does not inhibit other intestinal enzymes. It has minimal absorption and exerts no effect on systemic lipases.[542,543]

Although there have been several open-label trials of orlistat in adolescents, only two randomized control trials (RCTs) have been published.[544,545] The side effects with orlistat are predictable from its mechanism of action on intestinal lipase.[534] Orlistat appears to be well tolerated in adults, with the principal complaints being borborygmi, flatus, and abdominal cramps. The most troubling side effects are fecal incontinence, oily spotting, and flatus with discharge, which are highly aversive in the pediatric population. Orlistat does not affect the pharmacokinetic properties of most other pharmaceutical agents. Absorption of vitamins A and E and β-carotene may be slightly reduced, and this may require vitamin therapy in a small number of patients. In one study,[546] vitamin D supplementation was required in 18% of subjects despite the prescription of a daily multivitamin containing vitamin D, although in the company-sponsored study, effects on vitamin levels were minor.[545] Orlistat must be taken with each meal, which reduces its attractiveness for children, who are in school during lunchtime. Orlistat is currently approved for the treatment of children as young as 12 years. An over-the-counter lower-dose preparation has obtained FDA approval and should be available soon.

TABLE 22-2 **Medications for the Treatment of Pediatric Obesity**

Drug	Dosage	Efficacy	Side Effects	Monitoring and Contraindications
Orlistat Not FDA approved for < 12 years of age	120 mg PO tid	Open-label: Wt—5.4 kg, BMI—2 kg/m^2 at 6 months RCT: Wt—2.6 kg, BMI—0.85, WC—2.7 cm over placebo at 12 months	Borborygmi, flatus, abdominal cramps, fecal incontinence, oily spotting, vitamin malabsorption	Monitor 25OHD$_3$ levels. MVI supplementation is strongly recommended. A lower dose preparation has been approved for over-the-counter sale.
Metformin Not FDA approved for treatment of obesity. Approved for ≥ 10 years of age for type 2 diabetes mellitus	250-1000 mg PO bid	RCT: BMI Z score—0.35 SD versus placebo at 6 months RCT: Wt—2.7% versus placebo at 6 months Post-hoc analysis: efficacy dependent on degree of insulin resistance; BMI Z score—0.23 SD in first 4 months,—0.12 SD in next year	Nausea, flatulence, bloating, diarrhea; usually resolves; lactic acidosis not yet reported in children	Do not use in renal failure or with intravenous contrast. MVI supplementation is strongly recommended.
Octreotide Not FDA approved for treatment of obesity, otherwise ≥ 18 years of age	5-15 μg/kg/day SQ ÷ tid	Open-label: Wt—4.8 kg, BMI—2 kg/m^2 in 6 months RCT:—7.6 kg, BMI—2.5 kg/m^2 over placebo at 6 months Post-hoc analysis: BMI Z score—0.70 SD in 6 months, dependent on insulin secretion and sensitivity	Gallstones, diarrhea, edema, abdominal cramps, nausea, bloating, reduction in thyroxine concentrations	Monitor fasting glucose, FT$_4$, HbA$_{1c}$. *Useful only for hypothalamic obesity.* Ursodiol coadministration strongly recommended.
Leptin Not approved by FDA	Titration of dose to serum levels, SQ	Anecdotal: BMI—19 kg/m^2 over 4 years	Local reactions	Useful *only* in leptin deficiency.
Topiramate Not FDA approved for treatment of obesity	96-256 mg PO qd	RCT: Wt—8% over placebo at 6 months	Paresthesias, difficulty with concentration/attention, depression, difficulty with memory, language problems, nervousness, psychomotor slowing	No pediatric data
Growth hormone Not FDA approved for treatment of obesity	1-3 mg/m^2 SQ qd	Decreases in percentage body fat, with increases in absolute lean body mass	Edema, carpal tunnel syndrome, death in patients with preexisting obstructive sleep apnea	*Recommended only in Prader-Willi syndrome primarily to increase height velocity.* It also decreases fat mass but should only be used after screening to rule out obstructive sleep apnea. Must closely monitor pulmonary function, glucose, HbA$_{1c}$.

Should be considered only after an unsuccessful 6-month trial of lifestyle intervention. All drugs effective only when combined with appropriate lifestyle intervention.
RCT, randomized controlled trial, MVI, multivitamin.

Improvement of Insulin Resistance: Metformin

Metformin is a bisubstituted, short-chain hydrophilic guanidine derivative used to treat children and adults with type 2 diabetes mellitus (T2DM).[547-550] Metformin also decreases fasting hyperinsulinemia, prevents T2DM,[551] and promotes weight loss in some obese individuals[552,553] by improving hepatic and muscle insulin sensitivity. Metformin has little effect on energy expenditure.[548] Although some believe that metformin promotes weight loss though a primary anorectic effect (as initial side effects of nausea and gastrointestinal distress limit caloric intake acutely),[554] most believe that the decline in caloric intake observed with metformin is related to its enhancement of glucose clearance, through a reduction of hepatic glucose output, and reduction in fasting hyperinsulinemia.[555,556] Metformin improves hepatic insulin resistance by inducing hepatic AMP kinase,[557] which reduces hepatic gluconeogenesis; therefore, pancreatic insulin secretion and peripheral insulin levels fall. Metformin also restores PI3-kinase and MAP kinase activity in muscle cells, improving muscle insulin sensitivity.[558] Another possible mechanism of metformin action is through stimulation of glucagon-like peptide-1 (GLP-1),[14,559] which may inhibit food intake through central actions on the VMH.[15]

Thus far, results of two RCTs in children and adolescents have been reported.[560,561] Examination of the open-label responses to metformin in a multivariate analysis demonstrated two predictors for efficacy: race (Caucasian > African American) and the degree of insulin resistance prior to therapy.[153] Metformin has also been used "off-label" for the treatment of polycystic ovarian syndrome and nonalcoholic steatohepatitis, with varying degrees of success.[562-567] One particular use for metformin may be to combat the weight gain associated with atypical antipsychotics.[568] However, the cessation of metformin therapy leads to a rebound hyperinsulinemia and rapid weight gain, which may negate any beneficial effects seen during the medication window.

Side effects with metformin include nausea, flatulence, bloating, and diarrhea upon the initiation of therapy, which appears to be self-limited and resolves within 3 to 4 weeks of initiation. Approximately 5% of pediatric patients discontinue metformin therapy because of its severe side effects. The most feared complication of metformin in adults is lactic acidosis, which is estimated to occur at a rate of 3 per 100,000 patient-exposure years, primarily in patients with contraindications to the use of metformin; however, no documented cases in children have been reported. Metformin increases the urinary excretion of vitamins B_1 and B_6, which are important in the tricarboxylic acid cycle and which may hasten the lactic acidosis.[569] Vitamin B_{12} deficiency also has been reported in as many as 9% of adult subjects using metformin. Therefore, prophylactic multivitamin supplementation is recommended with metformin use. Contraindications to metformin use include renal insufficiency, congestive heart failure or pulmonary insufficiency, acute liver disease, and alcohol use sufficient to cause acute hepatic toxicity. Metformin also should be withheld when patients are hospitalized with any condition that may decrease systemic perfusion or when the use of contrast agents is anticipated.[555] It should be noted that metformin has been FDA-approved for the treatment of T2DM in children, but it is unlikely to be approved for childhood obesity or insulin resistance.

Suppression of Insulin Hypersecretion: Octreotide

It is well known that bilateral electrolytic lesions or deafferentation of the VMH in rats leads to intractable weight gain,[109,110,472-474] even upon food restriction.[475] In humans, hypothalamic damage due to CNS tumor, surgery, radiation or trauma can alter both the afferent and efferent pathways of energy balance and lead to severe and intractable weight gain.[477,478] In this syndrome of "hypothalamic obesity," hypothalamic insult confers an "organic leptin resistance" as the VMH senses starvation[54,473]; therefore, energy intake is high, and expenditure is low.[480] Children with hypothalamic obesity exhibit weight gain, even in response to forced caloric restriction,[570] secondary to (1) overactivation of the vagus, which promotes an obligate insulin hypersecretion and energy storage, and (2) defective activation of the SNS, which retards lipolysis and energy expenditure.[99,479] Insulin hypersecretion with normal insulin sensitivity is noted on oral glucose tolerance testing in these children.[487] This same phenomenon of insulin hypersecretion has also been documented in a subset of obese adults without CNS damage.[571]

The voltage-gated calcium channel of the β-cell is coupled to a somatostatin ($SSTR_5$) receptor.[572,573] Octreotide binds to this receptor, which limits the opening of this calcium channel, reduces the influx of calcium into the β cells, and in turn reduces calmodulin activation and vesicle exocytosis, thereby acutely decreasing the magnitude of insulin response to glucose[574] (see Figure 22-2), which results in weight loss or stabilization. Two RCTs and an observational prediction study using octreotide for obesity have been performed.[502,503] An examination of BMI responses to octreotide in pediatric hypothalamic obesity in a multivariate analysis demonstrated that insulin hypersecretion with a concomitant retention of insulin sensitivity prior to therapy augured for success.[153] A larger-scale study of patients following a brain insult showed limited efficacy, probably due to the timing of the intervention—most weight gain in cases of hypothalamic obesity occurs early following the insult. Pharmacotherapy that begins too late may fail to reverse this condition, whereas early an initiation of treatment may prevent excessive weight gain.

Octreotide is usually well tolerated. The most common side effects include diarrhea, abdominal cramps, nausea, and bloating, which are self-limited and usually resolve in 3 to 4 weeks.[575,576] Other adverse events include gallstones (which are preventable by coadministration of ursodiol), edema, development of sterile abscess at the injection sites, B_{12} deficiency, suppression of growth hormone and TSH secretion, and mild hyperglycemia, especially in those with severe insulin resistance.[577] At present octreotide offers a promising approach for the treatment of insulin hypersecretion as seen in

hypothalamic obesity, but it is not FDA approved. The use of octreotide in obese children with acute glucose-stimulated insulin hypersecretion without cranial pathology has not yet been evaluated.

Other Targeted Therapies

Leptin. Mutations of the leptin gene in humans recapitulate the phenotype of the *ob/ob* leptin-deficient mouse.[441] Approximately 11 such patients have been described; they manifest hyperphagia from birth, with obesity documentable as early as 6 months of age. Leptin deficiency induces the starvation response,[442] with increased energy intake and decreased REE. The diagnosis is made by extremely low or unmeasurable serum leptin levels. In children with leptin deficiency, leptin therapy results in an extraordinary loss of weight and fat mass,[578,579] along with reduced hyperphagia, a resolution of obesity, induced puberty, and improved immunity.[442] Although leptin administration in adults did not prove effective by itself due to leptin resistance,[140] leptin may serve as an adjunct in combination with other medications after leptin sensitivity is ameliorated through weight loss.[149,580]

Growth Hormone (GH). GH fosters anabolism and lipolysis. GH therapy has been shown to increase REE, promote linear growth, increase muscle mass, and decrease the percentage of body fat in subjects with Prader-Willi syndrome.[462,581] It has also been shown to decrease the percentage of body fat in children with GH deficiency[582] due to its effect on lipoprotein lipase.[583] However, it is not clear whether these reductions in the percentage of body fat are primary effects on the adipose tissue compartment or rather due to the increase in lean body mass. Obesity results in a state of functional GH insufficiency, which can be ameliorated through weight loss.[584] GH therapy also improves the lipid profile in GH-deficient adults.[585] Currently, the role of GH therapy in the treatment of nonsyndromic childhood obesity is unclear and not approved.

GLP-1 Analogs. Several long-acting GLP-1 analogs have been introduced, some with an antiobesity indication. GLP-1 is a gut-derived incretin that also serves a satiety signal, as described earlier. As its half-life in the circulation is brief (~2 minutes) due to degradation by the enzyme DPP-4, stable analogs have been designed aiming initially at β-cell enhancement but also showing some significant weight loss effects.[586] None of these agents have thus far been approved for use in children, yet studies are under way to test their efficacy in this population.

The Future of Pediatric Obesity Pharmacotherapy

In response to the relative lack of efficacy of lifestyle interventions, the ever-expanding knowledge of the physiology of energy balance, and particularly as a business decision of potential financial reward, many pharmaceutical companies have launched obesity research programs. The following agents are currently in human study; however, use of any of these new agents in children will depend on proof of safety and efficacy based on experience in adults. *Topiramate* is a novel anticonvulsant, which blocks voltage-dependent sodium channels, enhances the activity of the $GABA_A$ receptor, and antagonizes a glutamate receptor other than the N-methyl-D-aspartate (NMDA) receptor.[587] Topiramate promotes weight loss in a dose-dependent fashion.[588] An RCT in adults demonstrated a 9.1% weight loss in subjects taking topiramate 192 mg/day along with significant improvements in blood pressure, waist circumference, and fasting glucose and insulin.[589] However, almost 33% of the subjects dropped out due to adverse events, which included paresthesias, somnolence, anorexia, fatigue, nervousness, decreased concentration, difficulty with memory, and aggression. There are currently no studies of topiramate use to address childhood obesity. *Oxyntomodulin* is an analog of $PYY_{(3-36)}$, which has been shown in a 4-week RCT to reduce energy intake and weight in adults.[590] There are no studies in children. Two new formulations have been approved for adults, including combination phentermine-topiramate (QSymia) and Lorcaserin, a serotonin 2c-agonist (Belviq). There are no studies in children as of yet.

Bariatric Surgery

In comparison to the criteria used for adults, stricter and more conservative criteria must be applied to adolescents due to the fact that only 85% of obese adolescents will become obese adults, the slightly improved rate of lifestyle and pharmacotherapeutic efficacy versus adults, a longer time interval before comorbidities become life threatening, and their inability to give legal consent. For all these reasons, an expert panel with representation from the American Pediatric Surgical Association and the American Academy of Pediatrics has suggested that bariatric surgery for adolescents should be done only in institutions committed to long-term management of these patients[591] and is justified in situations when obesity-related comorbid conditions (like obstructive sleep apnea) threaten the child's health. The panel provided stringent recommendations that bariatric surgery be limited to adolescents with a BMI > 40 with the presence of severe comorbidity or those with a BMI > 50 with a less severe comorbidity. Very few long-term studies on obese adolescents who have undergone bariatric surgery have been published, and none has identified predictors of success. It is not clear what should be the procedure of choice and what fashion of preparation and follow-up will optimize long-term results.

Bariatric procedures in children are still in their infancy. Gastric bypass has only been performed in children and adolescents since the beginning of the 21st century. This procedure appears to be safe and effective when candidates are carefully selected and the bariatric surgeon has advanced laparoscopic skills. Thus far, we have limited data on long-term complications, including those that might be associated with pregnancy. As physicians, we are confident in saying that this procedure will add years to the lives of the children who are morbidly obese and receiving treatment. But the degree to which it should be used as a solution cannot be determined until we have further data.

Indications for Bariatric Surgery

Conventional treatment of childhood obesity has proven to be time consuming, difficult, frustrating, and expensive. Although numerous short-term successes have been noted, long-term weight reductions are modest, and recidivism is the rule. In adolescents with extreme and morbid obesity that may be life-threatening, surgical therapy may be indicated in extreme and defined circumstances.[592,593] However, in comparison to the criteria used for adults, stricter and more conservative criteria must be applied to adolescents for the following reasons:

1. Not all obese adolescents will become obese adults.[594]
2. The rate of lifestyle and pharmacotherapeutic efficacy has modestly improved.
3. The time interval before comorbidities become life threatening has lengthened.
4. Adolescents are not able to give legal consent.

Therefore, it is virtually impossible to perform RCT surgical studies on children. The efficacy of any given approach will continue to be suspect, and different procedures cannot be compared. For all these reasons, an expert panel with representation from the American Pediatric Surgical Association and the American Academy of Pediatrics[593] suggested that bariatric surgery in adolescents could be justified in situations when obesity-related comorbid conditions threaten the child's health. The panel provided stringent recommendations that bariatric surgery be limited to adolescents with a BMI > 40 kg/m^2 with presence of severe comorbidity or those with a BMI > 50 kg/m^2 with a less severe comorbidity. However, these stringent criteria are undergoing careful scrutiny in an attempt to liberalize them.[595]

Special consideration should be taken to avoid bariatric surgery at very late stages of obesity, when the presence of obesity-related comorbidities and the inaccessibility of imaging (most magnetic resonance imaging [MRI] scanners have a weight limit of 450 pounds) may affect surgical outcome. Indeed, a review of eight retrospective studies in adolescents found that bariatric surgery in adolescents can promote durable weight loss in most patients, but there appears to be a significant complication and mortality rate.[596] Therefore, guidance is needed to determine the ideal circumstances at which the balance of risk versus benefit favors health preservation and reversal of complications with the lowest risk of morbidity and mortality from the procedure.

Bariatric procedures for weight loss can be divided into purely restrictive, those that bypass a segment of the foregut, and combination procedures. Purely malabsorptive procedures aim to decrease the functional length or efficiency of the intestinal mucosa through anatomic rearrangement of the intestine. These procedures include the jejunoileal bypass and the biliopancreatic diversion with duodenal switch. Due to the high morbidity and mortality of these procedures, they cannot be recommended for children and will not be discussed further. The restrictive procedures reduce stomach volume to decrease the volume of food ingested. They include the bariatric intragastric balloon (no data are available concerning this treatment on children) and the laparoscopic adjustable gastric band (LAGB). The Roux-en-Y gastric bypass (RYGB) is a combination procedure.[597] The sleeve gastrectomy seems to be a restrictive procedure, yet it probably shares some of the hormonal effects of the RYGB and is gaining popularity.

Restrictive: Laparoscopic Adjustable Gastric Banding (LAGB)

LAGB utilizes a prosthetic band to encircle and compartmentalize the proximal stomach into a small pouch and a large remnant.[597] The theoretical advantage of this technique is a decreased risk of staple line dehiscence. The introduction of a new laparoscopic approach and the use of an adjustable band (allowing the stomach size to change) make this procedure more attractive. Finally, this procedure is reversible (at least theoretically, although some surgeons scoff at this notion) or can be modified into the RYGB at a later date. Results vary widely in adults. A single RCT comparing LAGB to conservative obesity management in obese children has shown superior weight loss that was maintained over 2 years. Among obese adolescent participants, the use of gastric banding compared with lifestyle intervention resulted in a greater percentage achieving a loss of 50% of excess weight, corrected for age. There were associated benefits to health and quality of life.[598]

Combination: Roux-en-Y Gastric Bypass (RYGB)

RYGB involves dividing the stomach to create a small (15 to 30 mL) stomach pouch into which a segment of jejunum approximately 15 to 60 cm inferior to the ligament of Treitz is inserted, while the proximal portion of the jejunum that drains the bypassed lower stomach and duodenum is reanastomosed 75 to 150 cm inferior to the gastrojejunostomy.[597] This procedure combines the restrictive nature of gastrectomy with the consequences of dumping physiology as a negative conditioning response when high-calorie liquid meals are ingested. In addition, RYGB is associated with a decline in the circulating level of ghrelin, which may be in part responsible for the decrease in hunger associated with this procedure.[12] The procedure not only leads to extraordinary weight loss, but it can reverse type 2 diabetes as well.[599]

RYGB appears to result in significant early weight reduction in adults[597,600]; however, long-term studies demonstrate weight regain in many patients.[601] Limited data are available regarding the efficacy of these surgical procedures to induce weight loss in severely obese children and adolescents, and most of these are case series from individual surgeons or institutions.[602] In a case review, 10 severely obese adolescents (BMI 52.5 ± 10 kg/m^2) who underwent RYGB were followed for a mean 69 months (but ranging from 8 to 144 months).[603] In this series, weight loss was significant in 9 of 10 adolescents and was maintained as long as 10 years. The average weight loss was 53.6 ± 25.6 kg, which represents approximately 59% excess weight lost. Weight loss was also associated with an improvement of associated comorbidities including sleep apnea and hypertension. Finally, a large retrospective series focused on 33 obese adolescents[604] age 16 ± 1 year with a BMI of

52 ± 11 (ranging from 38 to 91) and obesity comorbidities. Researchers followed these subjects for up to 14 years after bariatric surgery, mostly RYGB. Adolescents participating in a multicenter study reported by the Pediatric Bariatric Study Group experienced excellent weight loss after laparoscopic RYGB with mean BMI change from 58 kg/m^2 to 35.8 kg/m^2 at 1 year.[605] Gastrojejunostomy stenosis (21 patients) requiring endoscopic balloon dilation and internal hernia (14 patients) requiring either laparoscopic or open reduction were the most common complications. This procedure appears to be safe and effective when candidates are carefully selected and the bariatric surgeon has advanced laparoscopic skills.

RYGB seems to provide complete remission of T2DM in obese adolescents. Importantly, when performed in superobese adolescents, the nadir BMI achieved is typically ~37% from baseline. If one performs the procedure on a patient with a BMI in the low 40s, the result may be a BMI that is within an acceptable healthy range. In contrast, performing the procedure in patients with a BMI > 50 leaves them, even after an optimal result, in the obese BMI range without further reductions.[606]

The most commonly reported complications of RYGB include iron-deficiency anemia (50%), transient folate deficiency (30%), and events requiring surgical intervention (40%: cholecystectomy in 20%, small bowel obstruction in 10%, and incisional hernia in 10%).[597] Because most of the stomach and duodenum is bypassed in this procedure, there is an increased risk for deficiencies in vitamin B_{12}, iron, calcium, and thiamine. Although beriberi has been reported in teenagers after RYGB,[607] compliance with daily supplementation and regular monitoring of patients can prevent such nutritional deficiencies.

Laparoscopic Sleeve Gastrectomy (LSG)

This procedure is becoming more widely used. It reduces gastric capacity by cutting out the greater curvature of the stomach (in other words, turning the stomach into a tube). The death rate is halfway between that for LAGB and the RYGB, and the efficacy is halfway between the two as well.[608]

Who Should Perform Bariatric Surgery in Children?

Surgical outcomes in adults vary widely among surgeons and institutions.[609-611] Furthermore, there is a clear learning curve, as the morbidity of bariatric surgery varies inversely with the number of procedures performed.[612] Also, because RCTs in adolescents are unlikely, the only method to validate and refine the use of these procedures will come from following patients carefully and long term. Lastly, the increased risk of readmission after bariatric surgery in adults[613] argues for close and careful follow-up and monitoring in adolescents. Therefore, it is essential that bariatric surgery in adolescents be performed in regional pediatric academic centers with programs equipped to handle the data acquisition, long-term follow-up, and the multidisciplinary nature of these difficult patients.[593]

A multidisciplinary team with medical, surgical, nutritional, and psychological expertise should carefully select adolescents who are well informed and motivated to become potential candidates for LAGB or RYGB. Attention to the principles of growth, development, and compliance is essential to avoid adverse physical, cognitive, and psychosocial outcomes following bariatric surgery.[593] It must be clear to the subject and the parent that bariatric surgery is in fact an adjunct to a sincere commitment to lifestyle, rather than a magic bullet. Indeed, evidence of relapse in adults after RYGB is now commonplace.

Subjects and families must be well informed as to the risks and complications of such surgery. The medical team will require endocrine, GI, cardiology, pulmonary, and otolaryngologic support. Prophylactic tracheostomy is rarely required to maintain airway patency and to allow for the resolution of the hypercapnia prior to surgery.[614] Adolescents undergoing bariatric surgery require lifelong medical and nutritional surveillance postoperatively.[592] Extensive counseling, education, and support are required both before and after bariatric surgery; patients left to their own recognizance tend to regain weight over time. Indeed, studies in adults document an increased risk of hospitalization after RYGB, due to difficulties from the procedure.[613] Monitoring of long-term weight maintenance, improvements in CV morbidity, and longevity are all necessary to determine the cost-effectiveness of bariatric surgery in the pediatric population.

ENERGY INADEQUACY

Starvation versus Cachexia

Although both are weight loss syndromes, understanding the neuroendocrine mechanisms that distinguish starvation from cachexia is integral to both understanding and treating these disorders properly. In starvation, the negative feedback energy balance pathway is intact. The signal of leptin inadequacy from the weight loss is transduced by the VMH neuron into reduced sympathetic activity (to conserve energy) and increased vagal activity (to store energy). However, in cachexia, this pathway is short circuited by cytokine action on the hypothalamus. The VMH POMC neuron expresses receptors for various cytokines, including IL-1 and TNF-α.[615] In response to cytokine exposure, POMC neurons are activated, resulting in anorexigenesis, increased sympathetic activity, decreased vagal activity, and energy wastage.[616,617] Proinflammatory cytokines increase epinephrine, growth hormone, and cortisol, and they reduce insulin. These long-term hormonal changes accelerate muscle proteolysis (cortisol), increase resting energy expenditure (SNS), contribute to insulin resistance (both epinephrine and cortisol-glucagon), increase catabolism (cortisol), and suppress appetite and intestinal transit (vagus). This is clearly adaptive in the short term during times of infection (in order to generate body heat to eradicate the organism), but it is maladaptive in the long term, when chronic cytokine signaling can lead to cachexia. Thus, even in the situations of leptin decline or inadequacy, cytokine activation of POMC neurons promotes continued cachexia and weight loss through persistent SNS activation.

Failure to Thrive

Failure to thrive (FTT) is not a disease per se but rather a sign of multiple organic and nonorganic conditions and the interactions between them leading to compromised growth at a young age. FTT still represents a common pediatric medical problem mostly managed in the outpatient setting. Although FTT can rarely be a manifestation of critical illness, the majority of cases are the result of undernutrition due to the combination of biologic, environmental, and psychological factors. The diagnosis of FTT requires a thorough, prudent, and oriented history taking by the caregiver and is not always simple to establish. Moreover, this diagnosis may carry several legal implications that are not within the scope of this text.

Definition

There is no consensus on a single definition for FTT. The condition reflects inadequate physical growth recorded over time using standard growth charts. The commonly used definitions in clinical practice include length or weight below the fifth percentile for age and gender, a downward cross of two major percentile lines of the growth chart over time, or a weight per height below the 10th percentile of expected. The practicality of usually using weight and not length curves evolves from the fact that undernutrition and chronic disease tend to primarily affect weight gain while preserving linear growth. Ultimately, linear growth is also affected if these conditions persist. It is important to emphasize that single measurements without longitudinal follow-up growth points are inadequate to make the diagnosis of FTT, and a wrongful diagnosis may be established in infants who were born small for gestational age or prematurely, and in some healthy growing infants growing along the lower percentiles.

Classification and Etiology

The traditional classification of FTT is to segregate between organic and nonorganic causes. The nonorganic causes refer to environmental and psychological factors such as sensory deprivation, parental and emotional deprivation, and feeding difficulties of no organic source that occur in infancy. This traditional classification seems to lack the insight that the majority of cases suffer from a combination of the two, reflecting a mixed etiology.[618] A different approach is to classify the disorder based on its pathophysiology (i.e., inadequate caloric intake, inadequate absorption, excess metabolic requirements, defective utilization of intake, and reduced growth potential).[619] Common causes of FTT based on this classification scheme are shown in Box 22-2.

Of note, normal growth variation can confound the diagnosis of FTT, as some infants may be born large for gestational age as a result of intrauterine causes (such as gestational diabetes) and later experience a "catch down" pattern of growth during infancy toward the actual growth potential curves. Another cause of such negative crossing of percentiles can be constitutional growth delay. It is estimated that up to 25% of children can cross curves by more than 25 percentile lines (representing a cross of two major growth percentile lines), for the previously mentioned reasons.[620] These infants reach a new point from which they display a normal growth rate and weight gain pattern, yet they do not have FTT.

Endocrine causes of FTT are uncommon, as typical hormonal deficiencies such as growth hormone deficiency or hypothyroidism present as growth failure but with

BOX 22-2 Differential Diagnosis of Failure to Thrive

1. Inadequate caloric intake
   - Poverty and low food resources
   - Mechanical feeding difficulties (altered swallowing ability, congenital anomalies, central nervous system damage, severe gastroesophageal reflux)
   - Wrongful preparation of infant formula (too diluted, too concentrated)
   - Unsuitable feeding habits by parent
   - Behavioral problems affecting eating
   - Child neglect
   - Poor parent-child interaction
2. Inadequate absorption of caloric intake
   - Reduced absorption surface area (short bowel syndrome, s/p necrotizing enterocolitis)
   - Chronic liver disease, biliary atresia
   - Celiac disease
   - Cystic fibrosis
   - Cow's milk allergy
   - Chronic diarrhea
   - Vitamin or mineral deficiencies (acrodermatitis enteropathica)
   - Vomiting due to CNS abnormalities (tumor, raised ICP)
3. Increased metabolism
   - Hyperthyroidism
   - Chronic infection (due to immune deficiency)
   - Occult malignancy
   - Congenital heart defects or acquired heart disease (mainly right to left shunts and heart failure)
   - Chronic lung disease with hypoxemia (bronchopulmonary dysplasia)
   - Burns
4. Defective utilization of calories
   - Renal failure, renal tubular acidosis
   - Inborn errors of metabolism (storage diseases, amino acid disorders)
5. Reduced growth potential
   - Genetic disorders (trisomies, skeletal dysplasias, Russell-Silver syndrome)
   - Specific genetic syndromes
   - Primordial dwarfism

preserved or increased weight gain. Hyperthyroidism (representing a state of increased metabolic demands and characteristically manifested by increased linear growth) and disorders of salt metabolism, such as hypoaldosteronism and pseudohypoaldosteronism, may have FTT as part of their clinical manifestations.[621] Hypophosphatemic rickets may also present as FTT.

Diagnosis and Evaluation

The key to making the diagnosis of FTT is in plotting anthropometric data (weight and length) during a reasonable follow-up period. Although a prudent and focused history and physical examination are the keys to diagnosis, often the correct diagnosis is made in retrospect. The lack of an organic etiology to explain the findings is not enough to establish a diagnosis of nonorganic FTT. A response to an active intervention, manifested at least as a limited period of adequate growth while altering a behavioral element by the caregiver or child, can help establish a diagnosis of nonorganic FTT.

History should focus on the dietary and feeding history, past and present medical history, social environment, and family history. The dietary history is aimed at assessing, as accurately as possible, the actual caloric intake of the patient. An important tool for this assessment can be the use of food logs of several days. The important details concern the actual amounts of food, the way the food is prepared (specifically relevant for the dilution technique of infant formulas and to cereals added to the formula), and beverages consumed, with a specific emphasis on sweetened juices and formula. These details should allow the practitioner to estimate the caloric intake.

The important details regarding feeding begin with the location of the meals and their timing throughout the day. Who feeds the patient or supervises the feeding process is of major importance. The feeding technique should be appropriate for the child's developmental stage. The timing is relevant in regard to frequent snacking in between meals that may cause early satiety during mealtimes.

A standard pediatric medical history should be taken from all patients, yet it should be focused on details that may be relevant to the diagnosis of FTT. The pregnancy and birth history are important for differentiating infants who were born small for gestational age from those who suffer from FTT. The timing at which poor weight gain began, especially in relation to changes in feeding, is critical for the diagnosis. Chronic medical conditions such as congenital heart disease, asthma, multiple recurrent infections, and anemia can all be causes of organic FTT. Multiple hospitalizations and a history of injuries can raise the suspicion of parental neglect. Gastrointestinal manifestations of relevant medical conditions such as frequent vomiting (in cases of milk allergy or gastroesophageal reflux) and stool frequency and consistency (in order to rule out malabsorption, celiac, inflammatory bowel disease, or cystic fibrosis) should be elicited in detail.

The social history should focus on identifying the patient's primary caregivers and determining whether there are economical issues that may affect the ability to nourish the patient adequately. Potential external and intrafamilial stressors that may affect the supply of food to the child should be sought (any stressor or life event that can affect the functioning of the caregiver in a way that could compromise the well being of the child). The family history should focus on the body habitus of parents and siblings in order to obtain clues regarding the genetic potential for height and weight. Medical conditions in siblings and relatives can suggest a predisposition to genetic disorders. The caregivers should be asked about mental illnesses, such as depression, that may hamper their ability to provide adequate care for the child. A family history of previous children who suffered from FTT should be investigated as well. A call to the local Department of Family Services may be warranted.

The physical examination begins with plotting the child's' length, weight and head circumference on standard growth charts, along with previous measurements (if available). The severity of the FTT can be estimated by assessing the present weight in comparison to the expected weight for age. If the weight is less than 60% of expected by the 50th percentile for age and length, the condition is severe, whereas a weight between the 60th and 75th percentile of expected is considered moderate FTT. Microcephaly accompanied by neurologic signs may suggest a CNS lesion. It should be remembered that head circumference is the last parameter to change in FTT, and only in the severest cases. The detection of dysmorphism may suggest a genetic cause for impaired growth and development. Measures of nutritional status (such as thickness of skin folds and body fat distribution) can be examined. It is important to carefully observe the interaction of the caregiver, and the child during feeding. Impaired parental-child interactions can have a major impact on feeding habits, and their identification is critical to the design of effective behavioral interventions tailored for the patient and family.

The majority of children with FTT have no laboratory abnormalities and no hormonal alterations. Minimal literature is available about comprehensive laboratory workups in children with FTT, although a classic manuscript about the workup of more than 180 infants in an inpatient setting found laboratory abnormalities in less than 1.4% of tests taken.[622] The choice of tests that may be beneficial should be based on the history and physical examination and usually is focused on the assessment of malnutrition in severe cases. A minimal workup, although not cost effective, may include a blood count, chemistry panel (including liver and renal function tests, electrolytes, serum protein, and albumin concentrations as well as blood acid-base status), and a urinalysis with pH. Additional tests should be oriented at specific findings from the history and physical examination. No hormonal tests are warranted initially unless a clinical suspicion of a specific disorder arises. In children older than 6 months, screening for iron deficiency and lead poisoning is warranted. Hospitalization and inpatient workup does not add any yield to the workup,[623] unless the degree of FTT is severe, or if there are concerns about child safety and neglect.

Management

The management of FTT is based on identifying the underlying cause and correcting it. The majority of cases are handled by a combination of nutritional and behavioral intervention. Importantly, the intervention should begin before the workup is complete (i.e., less than from the first evaluation). All medical problems are treated independently of nutritional and behavioral interventions and should not delay or hamper them. The mainstay of treatment of all infants with FTT is a calorie-rich diet accompanied by frequent and close monitoring of weight response. An effective intervention will document a catch-up weight and height gain that is maintained over time.

Feeding and eating behaviors should be addressed by walking the fine line between encouragement and pressure to promote eating. Timing meals and snacks and eating as a family in a pleasant environment of low stress may be important for improving eating and feeding practices. The feeding intervention is dependent on the infant's age at presentation. For breastfeeding infants, it is beneficial to attempt to increase breast milk supply[624] by pumping milk, treatment with metoclopramide to induce oxytocin secretion,[625] improving maternal nutrition and fluid intake, and making adaptations at the home and workplace that can promote and simplify the breastfeeding process. Suckling problems in neurologically impaired infants can be solved by providing expressed human milk via bottle feeding. Bottle-fed and older infants allow more interventions to increase the caloric content of the diet. Infants with FTT should receive ~150% of the recommended daily caloric intake based on their expected weight (rather than their actual weight).[626] Formula may be enriched by adding cereals, and toddlers may benefit from the addition of palatable high energy density foods (such as cheese and peanut butter) to their diet. High-calorie milk-based drinks (such as PediaSure, which provides 30 calories per ounce, compared to whole milk, which provides 19 calories ounce) can be added along with vitamin supplements. Zinc supplements have been shown to increase IGF-1 levels without affecting IGFBP-3 in infants with nonorganic FTT, yet this effect did not actually promote growth.[627]

Prognosis

The majority of infants and children with FTT show improvement with intervention. Others may even show progress when they achieve a more independent stage of development where they can attain their own food. Those who require gastrostomy feeding due to neurologic dysfunction may require assisted enteral nutrition for life. The cognitive and intellectual function outcomes of those who suffered from FTT seem worse than their peers, although this association has only been well established in cases of iron deficiency anemia.[628] It seems conceivable that deficiencies of other elements critical for brain development during infancy may have a similar adverse impact on intellectual properties at later ages, although this has not been studied systematically. The effects of nonorganic factors (such as emotional deprivation) on intellectual development, often coexisting with organic factors, may also contribute to decreased cognitive ability at later ages.

Cancer Cachexia

Cancer activates a complex set of CNS metabolic pathways, which result in cachexia (Box 22-3).[629] Peripheral cytokines gain access to the CNS through the central circumventricular organs, which bypass the blood-brain barrier, or by stimulation and amplification of CNS microglial cytokine or eicosanoid production. For instance, TNF-α stimulates VMH POMC neurons, which stimulates the SNS, which increases resting energy expenditure, increases cortisol and glucagon levels, and contributes to insulin resistance. IL-1 decreases neuropeptide Y within the VMH and thus decreases appetite. IL-1 also increases CRF, which indirectly inhibits appetite. IL-6 bears a striking similarity to ciliary neurotrophic factor (CNTF), which has been shown to reduce weight by activating VMH POMC neurons through a leptin-independent mechanism.[630] Conversely, due to a reduction in vagal activity, gastrointestinal motility is impaired in cancer cachexia and is clinically manifest by early and inappropriate satiety, which occurs in 40% to 60% of cancer patients.

In addition, cytokines have adverse peripheral effects. Uncoupling proteins are up-regulated by cytokines and contribute to increased energy expenditure. Cancer cachexia leads to the overexpression of UCP1 in brown adipose tissue; UCP2 in brain, skeletal muscle, and liver; and UCP3 in skeletal muscle. Levels of UCP2 and UCP3 in the liver and muscle are regulated by prostaglandins,

BOX 22-3 Metabolic Changes in Cachexia

Expression of Cytokines

- Increased production of acute phase proteins (APP)
- Up-regulation of transcription factor NFκB and AP-1
- Increased interleukin-1, IL-6, and tumor necrosis factor-α and interferon-γ
- Increased expression of tumor-specific cachexins (proteolysis inducing factor, lipid mobilizing factor, and anemia inducing substance)
- Increased expression of ubiquitin, E_1, E_2, E_3, and proteasome components (cell death and removal)

Increased SNS Tone

- Increased expression of hormone sensitive lipase in adipose tissue
- Up-regulation of uncoupling proteins (UCP2 and UCP3) in muscle and adipose tissue
- Increased hepatic gluconeogenesis

Reduced Vagal Tone

- Reduced lipoprotein lipase expression in adipose tissue
- Reduced intestinal transit
- Reduced hunger

and UCP3 is also regulated by triglycerides, all of which are increased in cancer.[631]

Cytokines cause insulin resistance in skeletal muscle, liver, and adipose tissue. TNF-α decreases insulin receptor and PPAR activity. In addition, there is an inverse correlation between interleukin-6 levels and insulin sensitivity.[632] Adipose tissue insulin resistance increases fat oxidation and decreases lipoprotein lipase activity, resulting in continued lipolysis.[633] The insulin resistance of cancer is not related to defective insulin clearance and therefore differs from other forms of primary insulin resistance.[634]

Cancers are uniformly anaerobic and depend on glucose for survival; thus, the glucose manufactured from gluconeogenesis secondary to hepatic insulin resistance is essential for tumor growth. Cancers release large amounts of lactate, which is converted in the liver back to glucose. Such gluconeogenesis consumes ATP, which also increases energy expenditure.[635] Additional raw materials for gluconeogenesis are alanine derived from skeletal muscle proteolysis and glycerol from lipolysis.

Treatment is difficult. Many methods have been tried (SNS antagonists, prostaglandin inhibitors, omega-3 fatty acids, melatonin, thalidomide, interleukins, anticytokine monoclonal antibodies, IL-1 receptor antagonists, and chemotherapy), but all are lacking. One promising new avenue is that of melanocortin antagonists,[615] but this is still in preclinical evaluation.

Diencephalic Syndrome

Originally described by Russell in 1951,[636] this rare disorder presents in infants younger than 1 year old and is an indication of a hypothalamic lesion, usually an anterior hypothalamic glioma, or other neoplasm affecting hypothalamic function. Although the clinical spectrum is variable, emaciation with paucity of subcutaneous fat but with normal linear growth and head circumference is inviolate. Other frequent features include hyperalertness, hyperkinesis, nystagmus, and vomiting.[637,638]

Although numerous patients have been anecdotally reported and characterized, the cause of the emaciation remains unclear. Subjects with diencephalic syndrome have extremely elevated baseline GH levels but normal IGF-1 levels, suggesting a modicum of GH resistance.[637] It has been suggested that the high GH leads to lipolysis and accounts for the emaciation, but this finding is not consistent in all patients. Only one evaluation of energy balance has been performed, which demonstrated 30% to 50% greater REE in comparison to normal babies, and 13% greater energy expenditure compared to intake.[639]

The recommended treatment is surgical extirpation of the lesion whenever feasible. Radiation is usually contraindicated for the very young patient. Frequently, these patients postoperatively manifest hypopituitarism and ultimately develop hypothalamic obesity.[638]

Anorexia Nervosa (AN)

Definition

Anorexia nervosa (AN) is an eating disorder that typically begins during adolescence and consists of persistent dieting and intense physical activity, usually accompanied by compulsive behavioral traits and sometimes purging behavior and binge eating. Most subjects manifest a disturbed body image and a persistent fear of fatness, both of which promote further weight loss. The result of this behavior is a pathologic weight loss, with pathophysiologic consequences. The risk of developing AN among females in Western societies is estimated to be between 0.5% and 1%.[640] There are two subtypes of anorexia: the food restrictive type, characterized by very low caloric intake plus excessive exercise, and the purging type, characterized by varying levels of food purging, usually by way of self-induced vomiting and laxative abuse. Alongside the obvious mental elements, the definition in the DSM-IV includes anthropometric as well as metabolic components: a significantly low weight (defined as a sustained weight below the 85th percentile of the expected weight per height due to weight loss or failure to gain weight during growth and development) and secondary amenorrhea in pubertal girls and women (defined as no menses in 3 months).

Medical complications driven by the chronically reduced caloric intake, purging behavior, and excessive exercise may affect several organ systems. Typically, patients develop a marked loss of subcutaneous fat tissue, impaired menstrual function, bradycardia and orthostatic hypotension, hypothermia, and increased hair loss. Importantly, anorexia nervosa that develops during adolescence may create adverse clinical effects that persist into adulthood,[641] including osteopenia and osteoporosis,[642] higher rates of miscarriage, and reduced offspring birth weight.[643] Anorexia may also alter cognitive abilities as during extreme weight loss, and a reduction of both gray and white matter occurs; conversely, during weight restoration white matter returns to premorbid levels, but gray matter does not.[644] Anorexia carries an increased mortality risk, specifically for suicide yet also from medical causes such as starvation per se and purging-induced arrhythmias. Full recovery of body habitus and of growth and development occurs in 50% to 70% of treated adolescents, yet achieving a full physiologic and psychological recovery may require a comprehensive treatment intervention that may last as long as 5 to 7 years.[645] Greater weight loss, lower sustained weight, and the coexistence of other psychiatric disorders adversely affect the probability of recovery. The outcomes for adults with anorexia nervosa are poorer than they are for those who are diagnosed and treated during adolescence.

Endocrine Associations

Obesity and malnutrition usually result in opposing effects on normal physiology and are both associated with changes in the hormonal profile. The majority of these changes represent an adaptive response yet should be considered as part of the differential diagnosis of specific hormonal excess or deficiency disorders. Figure 22-9 demonstrates the typical hormonal profile of patients with AN, aimed at energy preservation and the cessation of energetically costly and nonvital processes.

Hypothalamic-Pituitary-Thyroid Axis. The starvation status of AN may resemble the sick euthyroid syndrome.

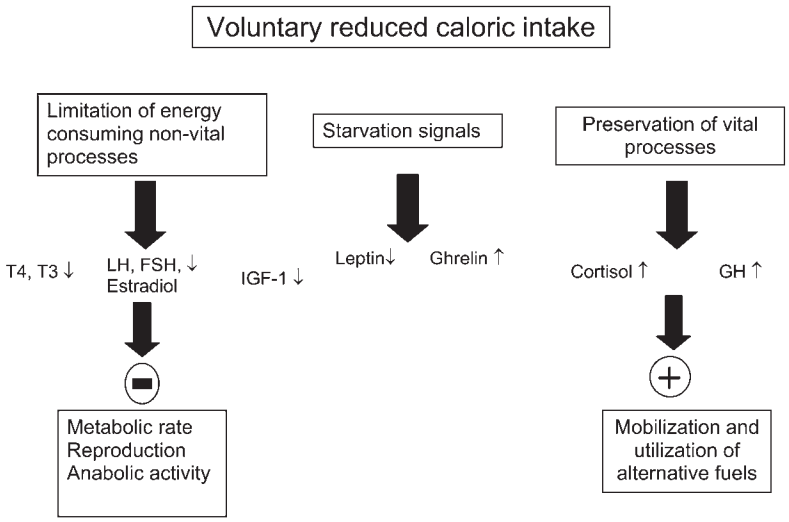


FIGURE 22-9 ■ Hormonal changes in anorexia nervosa. The hormonal profile of the patient with anorexia nervosa represents an adaptive response aimed at conserving energy. Processes that require significant energy such as reproduction and growth are limited by a complete suppression of the gonadotropic axis and by reduced IGF-1 levels, respectively. A seemingly chronic stress response characterized by increased growth hormone and cortisol is aimed at efficient utilization of the limited energetic sources present. Reduced leptin serves as a signal for down-regulating the hypothalamic-pituitary-gonadal axis. Reduced leptin also serves as a starvation signal, although this signal appears to be circumvented in this disorder.

In patients with AN, serum total and free T_4, total and free T_3, TSH, and TBG are significantly lower than normal, whereas rT_3 levels are significantly greater than they are in healthy controls.[646] Most AN patients have a hyporesponsiveness or delayed responsiveness of TSH to TRH stimulation. Of note, weight regain reverses the effects of AN on the hypothalamic-pituitary-thyroid axis back to normal; thus, the reduction in thyroxinemia may actually be a normal adaptive physiologic response to starvation. The differential diagnosis of hypothyroidism should be considered in patients with AN, as both disorders are characterized by low T_4 and T_3 levels. In primary hypothyroidism, TSH levels are greater than those observed in patients with AN, although in mild secondary hypothyroidism this distinction may be difficult. Obtaining a serum reverse T_3 (rT_3) level may be helpful in distinguishing a true thyroid disorder from the euthyroid sick syndrome associated with systemic illness.

Growth Hormone-IGF-1 Axis. Patients with AN typically have GH hypersecretion accompanied by low IGF-1 levels. Whether this profile is due to a primary hypothalamic dysfunction, a peripheral target organ resistance to GH, or an impaired negative IGF-1 central feedback mechanism is unclear. An increased GH response to GHRH has been demonstrated in AN, possibly reflecting an impairment of beta-adrenergic suppression of GH secretion.[647] The clinical presentation of weight loss, cessation of menses, and cold intolerance alongside low levels of pituitary-derived hormones may resemble panhypopituitarism, yet patients with AN present with GH hypersecretion.

Hypothalamic-Pituitary-Adrenal Axis. Patients with AN typically present with elevated cortisol levels in the presence of normal ACTH levels.[648] The elevated cortisol levels are apparently due to increased cortisol secretion alongside a reduction in cortisol clearance. Elevated CRH levels found in the cerebrospinal fluid of patients with AN suggest that AN represents an overall state of activation of the hypothalamic stress response, manifested peripherally by hypercortisolemia. An abnormal response in the dexamethasone suppression test, mainly of reduced suppression, suggests that an element of decreased feedback sensitivity occurs in this disorder.

Bone Metabolism. Adolescence represents a critical period for the accumulation of bone mineral, thus building bone strength and density for later years. The achievement of peak bone mass depends several hormonal effects characteristic of puberty (such as estradiol and IGF-1) as well as on adequate nutrition, all of which are compromised in patients with AN. Adolescents and adults with AN have a low bone mineral density. In adults this condition is due to increased bone resorption and reduced bone formation, but in adolescents it is characterized by an overall reduced bone turnover.[649] The reduced bone density is caused by the typical hormonal profile of AN (i.e., reduced estrogens and androgens, low IGF-1, and relative hypercortisolemia). Moreover, the reduced lean body mass and lower mechanical forces acting on long bones may also contribute to the overall reduced bone mineral density. Some publications also suggest that elevated levels of gut-derived hormones $PYY_{(3\text{-}36)}$ and ghrelin may also contribute to impaired bone metabolism in AN.[650] Importantly, resolving AN with weight gain often does not bring full recovery of the bone mineral density status. Osteopenia resulting from undernutrition and the typical hormonal dynamics of AN is one of several long-term complications of AN during adolescence, and thus is a major treatment target.

Hypothalamic-Pituitary-Gonadal Axis. Amenorrhea is one of the hallmarks of AN, yet it is not always

explained by the severe weight loss; thus, hypothalamic amenorrhea in AN often precedes weight loss and may persist after refeeding and achievement of normal weight. Gonadotropin levels are reduced in patients with AN, and GnRH stimulation testing in patients with AN demonstrates a blunted LH response with a preserved FSH response and very low levels of estradiol. LH pulsatility may revert to prepubertal patterns in adolescents who have previously achieved pubertal status. The amenorrhea of AN is also marked by strikingly reduced leptin levels. Leptin serves as a metabolic signal of energy status and nutritional reserve and thus may have a permissive role for the initiation of the complex hormonal dynamics necessary for normal reproductive function.[651] Teleologically, conserving energy for immediate and necessary metabolic demands while suppressing energetically expensive processes such as reproduction can serve as a protective measure in severely malnourished individuals, as seen in AN. The rise in leptin upon weight gain is associated with increases in gonadotropin secretion, suggesting that leptin serves a permissive role for the activation of the hypothalamic-pituitary-gonadal axis.

Fat-Derived Hormones. Adipocytes secrete a wide array of adipocytokines, the normal profile of which is altered in AN. Patients with AN typically have very low plasma leptin concentrations alongside a marked disturbance of the leptin diurnal secretion profile.[652] The concentration of leptin-binding protein (the soluble isoform of leptin receptor) has been reported to be increased in patients with AN, contributing to a further reduction in the concentration of free leptin.[653] Leptin has a major role in the neuroendocrine adaptations to the chronic starvation and undernutrition typical of AN, such as a reduction in gonadotropins, a reduction in thyroid hormone in order to conserve energy, a modified stress response manifested by hypercortisolemia, and elevated GH aimed at mobilizing and utilizing alternative energy sources.[654] Weight gain in AN patients can induce relative hyperleptinemia in comparison to controls matched for body mass index; circulating leptin concentrations in AN patients thus traverse from subnormal to supranormal levels within a few weeks.

Adiponectin is the only adipocytokine whose plasma concentrations are inversely related to fat mass. Conflicting results regarding adiponectin concentration in AN, ranging from hyperadiponectinemia[655] to hypoadiponectinemia,[656] have been reported. Interestingly, weight gain in AN is not necessarily associated with reciprocal changes in adiponectin concentration.[657]

Treatment

Indications for hospitalization and inpatient treatment of AN include dehydration, electrolyte disturbances (mainly hypokalemia), arrhythmias, CV instability (significant bradycardia, hypotension, and orthostatic changes), hypothermia, acute food refusal, acute complications of malnutrition (seizures, pancreatitis, cardiac failure), and psychiatric emergencies.

Along with psychological interventions that may consist of psychotherapy or pharmacotherapy (based on the spectrum of psychiatric pathology), an increase in caloric intake must be part of the treatment protocol. A protocol of starting with 1200 to 1500 kcal per day and increasing by 500 kcal is aimed at a gain of 0.5 to 1 kg per week.[658] There is no superior feeding regimen as long as adequate caloric intake is supplied. Extreme cases may be handled through hospitalization in dedicated inpatient units, and caloric intake in this setting may initially be provided by nasogastric and in extreme cases parenteral feeding.

One major treatment decision facing the caregiver of patients diagnosed with AN is whether to utilize estrogen replacement therapy to treat amenorrhea and protect the skeleton from osteopenia. There is a lack of a proven efficacious beneficial effect of estrogen replacement therapy in regard to improved bone mass in AN in comparison to placebo,[659] although this treatment modality is still commonly used.[660] This treatment approach may have several adverse effects on the treatment of adolescents, as it masks the beneficial effect of weight gain on the resumption of menses and may provide an erroneous sense of security in patients who are still at a critically low weight status. Increased calcium intake alongside 400 IU of vitamin D to accelerate absorption should be encouraged in all patients with AN as another potential protective measure of bone status.

CONCLUSIONS

The overwhelming majority of childhood obesity cases are not due to a documentable genetic or neuroanatomic lesion. Indeed, although classic endocrinopathies account for less than 2% of childhood obesity, every obese patient does manifest an endocrine disturbance (i.e., insulin resistance and/or leptin resistance). We must get past the obvious—that the child eats too much and exercises too little. The question that physicians must internally pose when seeing an obese patient is, "Where in the negative feedback energy balance pathway is this patient's dysfunction?" Only then can appropriate treatment be proffered. Similarly, in cachexia, we must go beyond the lack of appetite to understand the reasons for the wasting and illness. These are endocrine paradigms that may permit modulation, especially with endocrine therapies. Understanding the energy balance pathway, and where these various disorders impair its regulation, is the key to further research, and successful prevention and treatment.

REFERENCES

1. Druce, M. R., Small, C. J., & Bloom, S. R. (2004). Minireview: gut peptides regulating satiety. *Endocrinology*, *145*, 2660–2665.
2. Morton, G. J., Cummings, D. E., Baskin, D. G., et al. (2006). Central nervous system control of food intake and body weight. *Nature*, *443*, 289–295.
3. Hellstrom, P. M., Geliebter, A., Naslund, E., et al. (2004). Peripheral and central signals in the control of eating in normal, obese and binge-eating human subjects. *Br J Nutr*, *92*, S47–S57.
4. Date, Y., Murakami, N., Toshina, I. K., et al. (2002). The role of the gastric afferent vagal nerve in ghrelin-induced feeding and growth hormone secretion in rats. *Gastroenterology*, *123*, 1120–1128.
5. Bi, S. M., & Moran, T. H. (2002). Actions of CCK in the controld of food intake and body weight: lessons from the CCK-A receptor deficient OLETF rat. *Neuropeptides*, *36*, 171–181.

6. Abbott, C. R., Monteiro, M., Small, C. J., et al. (2005). The inhibitory effects of peripheral administration of peptide YY3–36 and glucagon-like peptide-1 on food intake are attenuated by ablation of the vagal–brainstem–hypothalamic pathway. *Brain Res*, *1044*, 127–131.
7. Kojima, M., Hosoda, H., Date, Y., et al. (1999). Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature*, *402*, 656–660.
8. Kamegai, J., Tamura, H., Shimizu, T., et al. (2000). Central effect of ghrelin, an endogenous growth hormone secretagogue, on hypothalamic peptide gene expression. *Endocrinology*, *141*, 4797–4800.
9. Tschöp, M., Smiley, D. L., & Heiman, M. L. (2000). Ghrelin induces adiposity in rodents. *Nature*, *407*, 908–913.
10. Cummings, D. E., Purnell, J. Q., Frayo, R. S., et al. (2001). A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes*, *50*, 1714–1719.
11. Druce, M. R., Neary, N. M., Small, C. J., et al. (2006). Subcutaneous administration of ghrelin stimulates energy intake in healthy lean human volunteers. *Int J Obes*, *30*, 293–296.
12. Cummings, D. E., Weigle, D. S., Frayo, R. S., et al. (2002). Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. *N Engl J Med*, *346*, 1623–1630.
13. Batterham, R. L., Cowley, M. A., Small, C. J., et al. (2002). Gut hormone PYY_{3-36} physiologically inhibits food intake. *Nature*, *418*, 650–654.
14. Kiefer, T. J., & Habener, J. F. (1999). The glucagon-like peptides. *Endocr Rev*, *20*, 876–913.
15. Drucker, D. J. (2005). Biologic actions and therapeutic potential of the proglucagon-derived peptides. *Nature Clin Pract Endo Metab*, *1*, 22–31.
16. Gotoh, K., Fukagawa, K., Fukagawa, T., et al. (2005). Glucagon-like peptide-1, corticotropin-releasing hormone, and hypothalamic neuronal histamine interact in the leptin-signaling pathway to regulate feeding behavior. *FASEB J*, *19*, 1131–1133.
17. Zhang, Y., Proenca, R., Maffei, M., et al. (1994). Positional cloning of the mouse obese gene and its human homologue. *Nature*, *393*, 372–425.
18. Lustig, R. H. (2006). Childhood obesity: behavioral aberration or biochemical drive? Reinterpreting the first law of thermodynamics. *Nature Clin Pract Endo Metab*, *2*, 447–458.
19. Chehab, F. F., Mounzih, K., Lu, R., Lim, M. E. (1997). Early onset of reproductive function in normal female mice treated with leptin. *Science*, *275*, 88–90.
20. Mantzoros, C. S., Flier, J. S., & Rogol, A. D. (1997). A longitudinal assessment of hormonal and physical alterations during normal puberty in boys: rising leptin levels may signal the onset of puberty. *J Clin Endocrinol Metab*, *82*, 1066–1070.
21. Mark, A. L., Rahmouni, K., Correia, M., & Haynes, W. G. (2003). A leptin-sympathetic-leptin feedback loop: potential implications for regulation of arterial pressure and body fat. *Acta Physiol Scand*, *177*, 345–349.
22. Boden, G., Chen, X., Mozzoli, M., & Ryan, I. (1996). Effect of fasting on serum leptin in normal human subjects. *J Clin Endocrinol Metab*, *81*, 454–458.
23. Keim, N. L., Stern, J. S., & Havel, P. J. (1998). Relation between circulating leptin concentrations and appetite during a prolonged, moderate energy deficit in women. *Am J Clin Nutr*, *68*, 794–801.
24. Flier, J. S. (1996). What's in a name? In search of leptin's physiologic role. *J Clin Endocrinol Metab*, *83*, 1407–1413.
25. Hassink, S. G., Sheslow, D. V., de Lancy, E., et al. (1996). Serum leptin in children with obesity: relationship to gender and development. *Pediatrics*, *98*, 201–203.
26. Guven, S., El-Bershawi, A., Sonnenberg, G. E., et al. (1999). Plasma leptin and insulin levels in weight-reduced obese women with normal body mass index: relationships with body composition and insulin. *Diabetes*, *48*, 347–352.
27. Barr, V. A., Malide, D., Zarnowski, M. J., et al. (1997). Insulin stimulates both leptin secretion and production by white adipose tissue. *Endocrinology*, *138*, 4463–4472.
28. Kolaczynski, J. W., Nyce, M. R., Considine, R. V., et al. (1996). Acute and chronic effects of insulin on leptin production in humans: studies *in vivo* and *in vitro*. *Diabetes*, *45*, 699–701.
29. Singhal, A., Farooqi, I. S., O'Rahilly, S., et al. (2002). Early nutrition and leptin concentrations later in life. *Am J Clin Nutr*, *75*, 993–999.
30. Lee, G. H., Proenca, R., Montez, J. M., et al. (1996). Abnormal splicing of the leptin receptor in diabetic mice. *Nature*, *379*, 632–635.
31. Spanswick, D., Smith, M. A., Groppi, V. E., et al. (1997). Leptin inhibits hypothalamic neurons by activation of ATP-sensitive potassium channels. *Nature*, *390*, 521–525.
32. Kishimoto, T., Taga, T., & Akira, S. (1994). Cytokine signal transduction. *Cell*, *76*, 252–262.
33. Banks, A. S., Davis, S. M., Bates, S. J., & Myers, M. G. (2000). Activation of downstream signals by the long form of the leptin receptor. *J Biol Chem*, *275*, 14563–14572.
34. Niswender, K. D., & Schwartz, M. W. (2003). Insulin and leptin revisited: adiposity signals with overlapping physiological and intracellular signaling capabilities. *Front Neuroendocrinol*, *24*, 1–10.
35. Porte, D., Baskin, D. G., Schwartz, M. W. (2005). Insulin signaling in the central nervous system: a critical role in metabolic homeostasis and disease from *C.* elegans to humans. *Diabetes*, *54*, 1264–1276.
36. Baskin, D. G., Wilcox, B. J., Figlewicz, D. P., & Dorsa, D. M. (1988). Insulin and insulin-like growth factors in the CNS. *Trends Neurosci*, *11*, 107–111.
37. Baura, G. D., Foster, D. M., Porte, D., et al. (1993). Saturable transport of insulin from plasma into the central nervous system of dogs in vivo: a mechanism for regulated insulin delivery to the brain. *J Clin Invest*, *92*, 1824–1830.
38. VanderWeele, D. A. (1994). Insulin is a prandial satiety hormone. *Physiol Behav*, *56*, 619–622.
39. McGowan, M. K., Andrews, K. M., & Grossman, S. P. (1992). Role of intrahypothalamic insulin in circadian patterns of food intake, activity, and body temperature. *Behav Neurosci*, *106*, 380–385.
40. Woods, S. C., Lotter, E. C., McKay, L. D., & Porte, D. (1979). Chronic intracerebroventricular infusion of insulin reduces food intake and body weight of baboons. *Nature*, *282*, 503–505.
41. Abusrewil, S. S., & Savage, D. L. (1989). Obesity and diabetic control. *Arch Dis Child*, *64*, 1313–1315.
42. VanderWeele, D. A. (1993). Insulin and satiety from feeding in pancreatic-normal and diabetic rats. *Physiol Behav*, *54*, 477–485.
43. Assimacopoulos-Jeannet, F., Brichard, S., Rencurel, F., et al. (1995). *In vivo* effects of hyperinsulinemia on lipogenic enzymes and glucose transporter expression in rat liver and adipose tissues. *Metabolism*, *44*, 228–233.
44. Cusin, I., Terrettaz, J., Rohner-Jeanrenaud, F., et al. (1990). Hyperinsulinemia increases the amount of GLUT4 mRNA in white adipose tissue and decreases that of muscles: a clue for increased fat depot and insulin resistance. Endocrinology, *127*, 3246–3248.
45. Woo, R., Kissileff, H. R., & Pi-Sunyer, F. X. (1984). Elevated post-prandial insulin levels do not induce satiety in normal-weight humans. *Am J Physiol*, *247*, R776–R787.
46. Niswender, K. D., Morton, G. J., Stearns, W. H., et al. (2001). Intracellular signalling: key enzyme in leptin-induced anorexia. *Nature*, *413*, 794–795.
47. Brüning, J. C., Gautam, D., Burks, D. J., et al. (2000). Role of brain insulin receptor in control of body weight and reproduction. *Science*, *289*, 2122–2125.
48. Schwartz, M. W., Figlewicz, D. P., Baskin, D. G., et al. (1994). Insulin and the central regulation of energy balance: update 1994. *Endocrinol Rev*, *2*, 109–113.
49. Lin, X., Taguchi, A., Park, S., et al. (2004). Dysregulation of insulin receptor substrate 2 in b-cells and brain causes obesity and diabetes. *J Clin Invest*, *114*, 908–916.
50. Plum, L., Ma, X., Hampel, B., et al. (2006). Enhanced PIP(3) signaling in POMC neurons causes K(ATP) channel activation and leads to diet-sensitive obesity. *J Clin Invest*, *116*, 1886–1901.
51. Bjorkbaek, C., Elmquist, J. K., Frantz, J. D., et al. (1998). Identification of SOCS-3 as a potential mediator of central leptin resistance. *Mol Cell*, *1*, 619–625.
52. Bence, K. K., Delibegovic, M., Xue, B., et al. (2006). Neuronal PTP1B regulates body weight, adiposity, and leptin action. *Nat Med*, *12*, 917–924.
53. Elmquist, J. K., Ahima, R. S., Elias, C. F., et al. (1998). Leptin activates distinct projections from the dorsomedial and ventromedial hypothalamic nuclei. *Proc Natl Acad Sci*, *95*, 741–746.
54. Thornton, J. E., Cheung, C. C., Clifton, D. K., & Steiner, R. A. (1997). Regulation of hypothalamic proopiomelanocortin mRNA by leptin in ob/ob mice. *Endocrinology*, *138*, 5063–5066.

55. Kristensen, P., Judge, M. E., Thim, L., et al. (1998). Hypothalamic CART is a new anorectic peptide regulated by leptin. *Nature*, *393*, 72–76.
56. Broberger, C., Johansen, J., Johasson, C., et al. (1998). The neuropeptide Y/agouti gene related protein (AGRP) brain circuitry in normal, anorectic, and monosodium glutamate-treated mice. *Proc Natl Acad Sci USA*, *95*, 15043–15048.
57. Liebowitz, S. F. (1995). Brain peptides and obesity: pharmacologic treatment. *Obes Res*, *3*, 573S–589S.
58. Kalra, S. P., & Kalra, P. S. (1996). Nutritional infertility: the role of the interconnected hypothalamic neuropeptide Y-galanin-opioid network. *Front Neuroendocrinol*, *17*, 371–401.
59. Beck, B., Stricker-Krongard, A., Nicolas, J. P., & Burlet, C. (1992). Chronic and continuous intracerebroventricular infusion of neuropeptide Y in Long-Evans rats mimics the feeding behavior of obese Zucker rats. *Int J Obes*, *16*, 295–302.
60. Stephens, T. W., Basinski, M., Bristow, P. K., et al. (1995). The role of neuropeptide Y in the antiobesity action of the obese gene product. *Nature*, *377*, 530–534.
61. Broberger, C., Landry, M., Wong, H., et al. (1997). Subtypes of the Y_1 and Y_2 of the neuropeptide Y receptor are respectively expressed in pro-opiomelanocortin and neuropeptide Y-containing neurons of the rat hypothalamic arcuate nucleus. *Neuroendocrinology*, *66*, 393–408.
62. Shutter, J. R., Graham, M., Kinsey, A. C., et al. (1997). Hypothalamic expression of ART, a novel gene related to agouti, is up-regulated in obese and diabetic mutant mice. *Genes Dev*, *7*, 454–467.
63. Graham, M., Shutter, J. R., Sarmiento, U., et al. (1997). Overexpression of Agrt leads to obesity in transgenic mice. *Nat Genet*, *17*, 273–274.
64. Wellman, P. J. (2005). Modulation of eating by central catecholamine systems. *Curr Drug Targets*, *6*, 191–199.
65. Leibowitz, S., Roosin, P., & Rosenn, M. (1985). Chronic norepinephrine injection into the hypothalamic paraventricular nucleus produces hyperphagia and increased body weight in the rat. *Pharmacol Biochem Behav*, *21*, 801–808.
66. Wellman, P. J., & Davies, B. T. (1992). Reversal of cirazoline-induced and phenylpropanolamine-induced anorexia by the alpha-1-receptor antagonist prazosin. *Pharmacol Biochem Behav*, *42*, 97–100.
67. Liebowitz, S. F., Alexander, J. T., Cheung, W. K., & Weiss, G. F. (1993). Effects of serotonin and the serotonin blocker metergoline on meal patterns and macronutrient selection. *Pharmacol Biochem Behav*, *45*, 185–194.
68. Wong, D. T., Reid, L. R., & Threlkeld, P. G. (1988). Suppression of food intake in rats by fluoxetine: comparison of enantiomers and effects of serotonin antagonists. *Pharmacol Biochem Behav*, *31*, 475–479.
69. Garattini, S., Bizzi, A., Caccia, S., & Mennini, T, (1992). Progress report on the anorectic effects of dexfenfluramine, fluoxetine, and sertraline. *Int J Obes*, *16*, S43–S50.
70. Calapai, G., Corica, F., Corsonello, A., et al. (1999). Leptin increases serotonin turnover by inhibition of nitric oxide synthesis. *J Clin Invest*, *104*, 975–982.
71. Nonogaki, K., Strack, A. M., Dallman, M. F., & Tecott, L. H. (1998). Leptin-independent hyperphagia and type 2 diabetes in mice with a mutated serotonin 5-HT_{2c} receptor gene. *Nat Med*, *4*, 1152–1156.
72. Simansky, K. J. (1996). Serotonergic control of the organization of feeding and satiety. *Behav Brain Res*, *73*, 37–42.
73. Pissios, P., Bradley, R. L., & Maratos-Flier, E. (2006). Expanding the scales: the multiple roles of MCH in regulating energy balance and other biological functions. *Endocr Rev*, *27*, 606–620.
74. Shimada, M., Tritos, N. A., Lowell, B. B., et al. (1998). Mice lacking melanin-concentrating hormone receptor are hypophagic and lean. *Nature*, *396*, 670–674.
75. Ludwig, D. S., Tritos, N. A., Mastaitis, J. W., et al. (2001). Melanin-concentrating hormone overexpression in transgenic mice leads to obesity and insulin resistance. *J Clin Invest*, *107*, 379–386.
76. Gomori, A., Ishihara, A., Ito, M., et al. (2003). Chronic intracerebroventricular infusion of MCH causes obesity in mice. *Am J Physiol*, *284*, E583–E588.
77. Taylor, M. M., & Samson, W. K. (2003). The other side of the orexins: endocrine and metabolic actions. *Am J Physiol*, *284*, E13–E17.
78. Hara, J., Beuckmann, C. T., Nambu, T., et al. (2001). Genetic ablation of orexin neurons in mice results in narcolepsy, hypophagia, and obesity. *Neuron*, *30*, 345–354.
79. Harris, G. C., & Aston-Jones, G. (2006). Arousal and reward: a dichotomy in orexin function. *Trends Neurosci*, *29*, 571–577.
80. Mieda, M., & Yanigasawa, M. (2002). Sleep, feeding, and neuropeptides: roles of orexins and orexin receptors. *Curr Opin Neurobiol*, *12*, 339–346.
81. Pagotto, U., Marsicano, G., Cota, D., et al. (2006). The emerging role of the endocannabinoid system in endocrine regulation and energy balance. *Endocr Rev*, *27*, 73–100.
82. Malcher-Lopes, R., Di, S., Marcheselli, V. S., et al. (2006). Opposing crosstalk between leptin and glucocorticoids rapidly modulates synaptic excitation via endocannabinoid release. *J Neurosci*, *26*, 6643–6650.
83. Chen, A. S., Marsh, D. J., Trumbauer, M. E., et al. (2000). Inactivation of the mouse melanocortin-3 receptor results in increased fat mass and reduced lean body mass. *Nat Genet*, *26*, 97–102.
84. Butler, A. A., & Cone, R. D. (2002). The melanocortin receptors: lessons from knockout models. *Neuropeptides*, *36*, 77–84.
85. Rahmouni, K., Haynes, W. G., Morgan, D. A., & Mark, A. L. (2003). Role of melanocortin-4 receptors in mediating renal sympathoactivation to leptin and insulin. *J Neurosci*, *23*, 5998–6004.
86. Collins, S., Kuhn, C. M., Petro, A. E., et al. (1996). *Role of leptin in fat regulation*. Nature, *380*, 677.
87. Muntzel, M., Morgan, D. A., Mark, A. L., & Johnson, A. K. (1994). Intracerebroventricular insulin produces non-uniform regional increases in sympathetic nerve activity. *Am J Physiol*, *267*, R1350–R1355.
88. Vollenweider, L., Tappy, L., Owlya, R., et al. (1995). Insulin-induced sympathetic activation and vasodilation in skeletal muscle: effects of insulin resistance in lean subjects. *Diabetes*, *44*, 641–645.
89. Blaak, E. E., Saris, W. H., & van Baak, M. A. (1993). Adrenoceptor subtypes mediating catecholamine-induced thermogenesis in man. *Int J Obesity*, *17*, S78–S81.
90. Viguerie, N., Clement, K., Barbe, P., et al. (2004). *In vivo* epinephrine-mediated regulation of gene express in human skeletal muscle. *J Clin Endocrinol Metab*, *89*, 2000–2014.
91. Navegantes, L. C., Migliorini, R. H., & do Carmo Kettelhut, I. (2002). Adrenergic control of protein metabolism in skeletal muscle. *Curr Opin Clin Nutr Metab Care*, *5*, 281–286.
92. Susulic, V. S., Frederich, R. C., Lawitts, J., et al. (1995). Targeted disruption of the beta 3-adrenergic receptor gene. *J Biol Chem*, *270*, 29483–29492.
93. Boss, O., Bachman, E., Vidal-Puig, A., et al. (1999). Role of the β_3-adrenergic receptor and/or a putative β_3-adrenergic receptor on the expression of uncoupling proteins and peroxisome proliferator-activated receptor-$_g$ coactivator-1. *Biochem Biophys Res Commun*, *261*, 870–876.
94. Lowell, B. B., & Spiegelman, B. M. (2000). Towards a molecular understanding of adaptive thermogenesis. *Nature*, *404*, 652–660.
95. Klingenberg, M., & Huang, S. G. (1999). Structure and function of the uncoupling protein from brown adipose tissue. *Biochem Biophys Acta*, *1415*, 271–296.
96. Powley, T. L., & Laughton, W. (1981). Neural pathways involved in the hypothalamic integration of autonomic responses. *Diabetologia*, *20*, 378–387.
97. Peles, E., Goldstein, D. S., Akselrod, S., et al. (1995). Interrelationships among measures of autonomic activity and cardiovascular risk factors during orthostasis and the oral glucose tolerance test. *Clin Auton Res*, *5*, 271–278.
98. Rohner-Jeanrenaud, F., & Jeanrenaud, B. (1985). Involvement of the cholinergic system in insulin and glucagon oversecretion of genetic preobesity. *Endocrinology*, *116*, 830–834.
99. Lustig, R. H. (2003). Autonomic dysfunction of the (beta-cell and the pathogenesis of obesity. *Rev Endocr Metabol Dis*, *4*, 23–32.
100. Kreier, F., Fliers, E., Voshol, P. J., et al. (2002). Selective parasympathetic innervation of subcutaneous and intra-abdominal fat-functional implications. *J Clin Invest*, *110*, 1243–1250.
101. Boden, G., & Hoeldtke, R. D. (2003). Nerves, fat, and insulin resistance. *N Engl J Med*, *349*, 1966–1967.
102. D'Alessio, D. A., Kieffer, T. J., Taborsky, G. J., & Havel, P. J. (2001). Activation of the parasympathetic nervous system is

necessary for normal meal induced-insulin secretion in rhesus macaques. *J Clin Endocrinol Metab*, *86*, 1253–1259.
103. Ahren, B., & Holst, J. J. (2001). The cephalic insulin response to meal ingestion in humans is dependent on both cholinergic and noncholingergic mechanisms and is important for postprandial glycemia. *Diabetes*, *50*, 1030–1038.
104. Gilon, P., & Henquin, J. C. (2001). Mechanisms and physiological significance of the cholinergic control of pancreatic β-cell function. *Endocr Rev*, *22*, 565–604.
105. Miura, Y., Gilon, P., & Henquin, J. C. (1996). Muscarinic stimulation increases Na^+ entry in pancreatic β-cells by a mechanism other than the emptying of intracellular Ca^{2+} pools. *Biochem Biophys Res Commun*, *224*, 67–73.
106. Zawalich, W. S., Zawalich, K. C., & Rasmussen, H. (1989). Cholinergic agonists prime the β-cell to glucose stimulation. *Endocrinology*, *125*, 2400–2406.
107. Nishi, S., Seino, Y., Ishida, H., et al. (1987). Vagal regulation of insulin, glucagon, and somatostatin secretion *in vitro* in the rat. *J Clin Invest*, *79*, 1191–1196.
108. Komeda, K., Yokote, M., & Oki, Y. (1980). Diabetic syndrome in the Chinese hamster induced with monosodium glutamate. *Experientia*, *36*, 232–234.
109. Rohner-Jeanrenaud, F., & Jeanrenaud, B. (1980). Consequences of ventromedial hypothalamic lesions upon insulin and glucagon secretion by subsequently isolated perfused pancreases in the rat. *J Clin Invest*, *65*, 902–910.
110. Berthoud, H. R., & Jeanrenaud, B. (1979). Acute hyperinsulinemia and its reversal by vagotomy following lesions of the ventromedial hypothalamus in anesthetized rats. *Endocrinology*, *105*, 146–151.
111. Tian, Y. M., Urquidi, V., & Ashcroft, S. J. H. (1996). Protein kinase C in β-cells: expression of multiple isoforms and involvement in cholinergic stimulation of insulin secretion. *Mol Cell Endocrinol*, *119*, 185–193.
112. Arbuzova, A., Murray, D., & McLaughlin, S. (1998). MARCKS, membranes, and calmodulin: kinetics of their interaction. *Biochim Biophys Acta*, *1376*, 369–379.
113. Blondel, O., Bell, G. I., Moody, M., et al. (1994). Creation of an inositol 1,4,5-triphosphate-sensitive Ca^{2+} store in secretory granules of insulin-producing cells. *J Biol Chem*, *269*, 27167–27170.
114. Rocca, A. S., & Brubaker, P. L. (1999). Role of the vagus nerve in mediating proximal nutrient-induced glucagon-like peptide-1 secretion. *Endocrinology*, *140*, 1687–1694.
115. Marin, P., Russeffé-Scrive, A., Smith, J., & Bjorntorp, P. (1988). Glucose uptake in human adipose tissue. *Metabolism*, *36*, 1154–1164.
116. Ramsay, T. G. (1996). Fat cells. *Endocrinol Metab Clin North Am*, *25*, 847–870.
117. Leibel, R. L. (2002). The role of leptin in the control of body weight. *Nutr Rev*, *60*, S15-S19.
118. Leibel, R. L., Rosenbaum, M., & Hirsch, J. (1995). Changes in energy expenditure resulting from altered body weight. *N Engl J Med*, *332*, 621–628.
119. Champigny, O., & Ricquier, D. (1990). Effects of fasting and refeeding on the level of uncoupling protein mRNA in rat brown adipose tissue: evidence for diet -induced and cold-induced responses. *J Nutr*, *120*, 1730–1736.
120. Aronne, L. J., Mackintosh, R., Rosenbaum, M., et al. (1995). Autonomic nervous system activity in weight gain and weight loss. *Am J Physiol*, *269*, R222–R225.
121. Kelley, A. E., Bakshi, V. P., Haber, S. N., et al. (2002). Opioid modulation of taste hedonics within the ventral striatum. *Physiol Behav*, *76*, 365–377.
122. Hommel, J. D., Trinko, R., Sears, R. M., et al. (2006). Leptin receptor signaling in midbrain dopamine neurons regulates feeding. *Neuron*, *51*, 801–810.
123. Shalev, U., Yap, J., & Shaham, Y. (2001). Leptin attenuates food deprivation-induced relapse to heroin seeking. *J Neurosci*, *21*, RC129, 121–125.
124. Carr, K. D., Tsimberg, Y., Berman, Y., & Yamamoto, N. (2003). Evidence of increased dopamine receptor signaling in food-restricted rats. *Neuroscience*, *119*, 1157–1167.
125. Farooqi, I. S., Bullmore, E., Keogh, J., et al. (2007). Leptin regulates striatal regions and human eating behavior. *Science*, *317*, 1355.
126. Figlewicz, D. P., Szot, P., Chavez, M., et al. (1994). Intraventricular insulin increases dopaminergic transporter mRNA in rat VTA/substantia nigra. *Brain Res*, *644*, 331–334.
127. Sipols, A. J., Bayer, J., Bennett, R., & Figlewicz, D. P. (2002). Intraventricular insulin decreases kappa opioid-mediated sucrose intake in rats. *Peptides*, *23*, 2181–2187.
128. Figlewicz, D. P. (2003). Adiposity signals and food reward: expanding the CNS roles of insulin and leptin. Am J Physiol Regul Integr Comp Physiol, 284, R882–R892.
129. Avena, N. M., Rada, P., & Hoebel, B. G. (2008). Evidence for sugar addiction: behavioral and neurochemical effects of intermittent, excessive sugar intake. *Neurosci Biobehav Rev*, *32*, 20–39.
130. Garber, A. K., & Lustig, R. H. (2011). Is fast food addictive? *Curr Drug Abuse Rev*, *4*, 146–162.
131. Ziauddeen, H., Farooqi, I. S., & Fletcher, P. C. (2012). Obesity and the brain: how convincing is the addiction model? *Nature Rev Neurosci*, *13*, 279–286.
132. Dallman, M. F., Pecoraro, N. C., La Fleur, S. E. (2005). Chronic stress and comfort foods: self-medication and abdominal obesity. *Brain Behav Immun*, *19*, 275–280.
133. La Fleur, S. E., Akana, S. F., Manalo, S. L., Dallman, M. F. (2004). Interaction between corticosterone and insulin in obesity: regulation of lard intake and fat stores. *Endocrinology*, *145*, 2174–2185.
134. Dallman, M. F., Pecoraro, N., Akana, S. F., et al. (2003). Chronic stress and obesity: a new view of "comfort food." *Proc Natl Acad Sci USA*, *100*, 11696–11701.
135. Tataranni, P. A., Larson, D. E., Snitker, S., et al. (1996). Effects of glucocorticoids on energy metabolism and food intake in humans. *Am J Physiol*, *271*, E317–E325.
136. Adam, T. C., & Epel, E. S. (2007). Stress, eating, and the reward system. *Physiol Behav*, *91*, 449–458.
137. Tomiyama, A. J., Dallman, M. F., & Epel, E. S. (2011). Comfort food is comforting to those most stressed: evidence of the chronic stress response network in high stress women. *Psychoneuroendocrinology*, *36*, 1513–1519.
138. Johnson, J. G., Cohen, P., Kasen, S., & Brook, J. S. (2002). Childhood adversities associated with risk for eating disorders or weight problems during adolescence or early adulthood. *Am J Psychiatry*, *159*, 394–400.
139. Roemmich, J. N., Wright, S. M., & Epstein, L. H. (2002). Dietary restraint and stress-induced snacking in youth. *Obes Res*, *10*, 1120–1126.
140. Heymsfield, S. B., Greenberg, A. S., Fujioka, K., et al. (1999). Recombinant leptin for weight loss in obese and lean adults: a randomized, controlled, dose-escalation trial. *JAMA*, *282*, 1568–1575.
141. Rosenbaum, M., Nicolson, M., Hirsch, J., et al. (1997). Effects of weight change on plasma leptin concentrations and energy expenditure. *J Clin Endocrinol Metab*, *82*, 3647–3564.
142. Caro, J. F., Kolaczynski, J. W., Nyce, M. R., et al. (1996). Decreased cerebrospinal fluid/serum leptin ratio in obesity: a possible mechanism for leptin resistance. *Lancet*, *348*, 159–161.
143. Banks, W. A., Kastin, A. J., Huang, W., et al. (1996). Leptin enters the brain by a saturable system independent of insulin. *Peptides*, *17*, 305–311.
144. Banks, W. A. (2008). The blood-brain barrier as a cause of obesity. *Curr Pharm Des*, *14*, 1606–1614.
145. Banks, W. A., Coon, A. B., Robinson, S. M., et al. (2004). Triglycerides induce leptin resistance at the blood-brain barrier. *Diabetes*, *53*, 1253–1260.
146. El-Haschimi, K., Pierroz, D. D., Hileman, S. M., et al. (2000). Two defects contribute to hypothalamic leptin resistance in mice with diet-induced obesity. *J Clin Invest*, *105*, 1827–1832.
147. Xu, Y., Hill, J. W., Fukuda, M., et al. (2010). PI3K signaling in the ventromedial hypothalamic nucleus is required for normal energy homeostasis. *Cell Metab*, *12*, 88–95.
148. Klöckener, T., Hess, S., Belgardt, B. F., et al. (2011). High-fat feeding promotes obesity via insulin receptor/PI3K-dependent inhibition of SF-1 VMH neurons. *Nat Neurosci*, *14*, 911–918.
149. Rosenbaum, M., Murphy, E. M., Heymsfield, S. B., et al. (2002). Low dose leptin administration reverses effects of sustained weight reduction on energy expenditure and circulating concentrations of thyroid hormones. *J Clin Endocrinol Metab*, *87*, 2391–2394.

150. Rosenbaum, M., Goldsmith, R., Bloomfield, D., et al. (2005). Low-dose leptin reverses skeletal muscle, autonomic, and neuroendocrine adaptations to maintenance of reduced weight. *J Clin Invest*, *115*, 3579–3586.
151. Lustig, R. H., Sen, S., Soberman, J. E., & Velasquez-Mieyer, P. A. (2004). Obesity, leptin resistance, and the effects of insulin suppression. *Int J Obes*, *28*, 1344–1348.
152. Isganaitis, E., & Lustig, R. H. (2005). Fast food, central nervous system insulin resistance, and obesity. *Arterioscler Thromb Vasc Biol*, *25*, 2451–2462.
153. Lustig, R. H., Mietus-Snyder, M. L., Bacchetti, P., et al. (2006). Insulin dynamics predict BMI and z-score response to insulin suppression or sensitization pharmacotherapy in obese children. *J Pediatr*, *148*, 23–29.
154. Mietus-Snyder, M. L., & Lustig, R. H. (2008). Childhood obesity: adrift in the "limbic triangle." *Ann Rev Med*, *59*, 119–134.
155. Ogden, C. L., Carroll, M. D., Kit, B. K., & Flegal K. M (2012). Prevalence of obesity and trends in body mass index among US children and adolescents, 1999–2010. *JAMA*, *307*, 483–483.
156. Dietz, W. H. (1998). Health consequences of obesity in youth: childhood predictors of adulthood disease. *Pediatrics*, *101*(Suppl. 3), 518–525.
157. Finkelstein, E. A., Khavjou, M. A., Thompson, H., et al. (2012). Obesity and severe obesity forecasts through 2030. *Am J Prev Med*, *42*, 563–570.
158. Must, A., & Strauss, R. S. (1999). Risks and consequences of childhood and adolescent obesity. *Int J Obes*, *23*, S2–S11.
159. Juonala, M., Magnussen, C. G., Berenson, G. S., et al. (2011). Childhood adiposity, adult adiposity, and cardiovascular risk factors. *N Engl J Med*, *365*, 1876–1885.
160. Hubbard, V. S. (2000). Defining overweight and obesity: what are the issues. *Am J Clin Nutr*, *72*, 1067–1068.
161. Calle, E. E., Thun, M. J., Petrelli, J. M., et al. (1999). Body-mass index and mortality in a prospective cohort of US adults. *N Engl J Med*, *341*, 1097–1105.
162. World Health Organization (1998). *Report of a WHO consultation on obesity*. *Obesity: preventing and managing the global epidemic.* Geneva: World Health Organization.
163. Troiano, R. P., Flegal, K. M., Kuczmarski, R. J., et al. (1995). Overweight prevalence and trends for children and adolescents. *Arch Pediatr Adolesc Med*, *149*, 1085–1091.
164. Whitaker, R. C., Wright, J. A., Pepe, M. S., et al. (1997). Predicting obesity in young adulthood from childhood and parental obesity. *N Engl J Med*, *337*, 869–873.
165. Dietz, W. H., & Robinson, T. N. (1998). Use of the body mass index (BMI) as a measure of overweight in children and adolescents. *J Pediatr*, *132*, 191–193.
166. BMI growth curves. (2001). Available at www.cdc.gov/growthcharts.
167. Fernández, J. R., Redden, D. T., Pietrobelli, A., & Allison, D. B. (2004). Waist circumference percentiles in nationally representative samples of African-American, European-American, and Mexican-American children and adolescents. *J Pediatr*, *145*, 439–444.
168. Gortmaker, S. L., Dietz, W. H., Sobol, A. M., & Wehler, C. A. (1987). Increasing pediatric obesity in the United States. *Am J Dis Child*, *141*, 535–540.
169. Ogden, C. L., Flegal, K. M., Carroll, M. D., & Johnson, C. L. (2002). Prevalence and trends in overweight among US children and adolescents, 1999–2000. *JAMA*, *288*, 1728–1732.
170. Ogden, C. L., Carroll, M. D., Curtin, L. R., et al. (2010). Prevalence of high body mass index in US children and adolescents, 2007–2008. *JAMA*, *303*, 242–249.
171. Brown, L. R. (2000). Obesity epidemic threatens health in exercise-deprived societies. Worldwatch Institute Internet release.
172. Ebbeling, C. B., Pawlak, D. B., & Ludwig, D. S. (2002). Childhood obesity: public-health crisis, common sense cure. *Lancet*, *360*, 473–482.
173. Gordon-Larsen, P., Adair, L. S., & Popkin, B. M. (2002). Ethnic differences in physical activity and inactivity patterns and overweight status. *Obes Res*, *10*, 141–149.
174. James, W. P. T., Nelson, M., Ralph, A., & Leather, S. (1997). Socioeconomic determinants of health: the contribution of nutrition to inequalities in health. *BMJ*, *314*, 1545–1549.
175. Drewnowski, A. (1998). Energy density, palatability, and satiety: implications for weight control. *Nutr Rev*, *56*, 347–353.
176. Martorell, R., Khan, L. K., Hughes, M. L., & Grummer-Strawn, L. M. (1998). Obesity in Latin-American women and children. *J Nutr*, *128*, 1464–1473.
177. Strauss, R. S., & Pollack, H. A. (2001). Epidemic increase in childhood overweight, 1986–1998. *JAMA*, *286*, 2845–2848.
178. Kimm, S. Y., Barton, B. A., Obarzanek, E., et al. (2002). Obesity development during adolescence in a biracial cohort: the NHLBI Growth and Health Study. *Pediatrics*, *110*, e54.
179. Crawford, P. B., Story, M., Wang, M. C., et al. (2001). Ethnic issues in the epidemiology of childhood obesity. *Pediatr Clin North Am*, *48*, 855–878.
180. Warner, M. L., Harley, K., Bradman, A., et al. (2006). Soda consumption and overweight status of 2-year-old Mexican-American children in California. *Obesity*, *14*, 1966–1974.
181. Dietz, W. H. (1998). Health consequences of obesity in youth: childhood predictors of adult disease. *Pediatrics*, *101*, 518–525.
182. Styne, D. M. (2001). Childhood and adolescent obesity: prevalence and significance. *Pediatr Clin North Am*, *48*, 823–854.
183. Guo, S. S., Huang, C., Maynard, L. M., et al. (2000). Body mass index during childhood, adolescence and young adulthood in relation to adult overweight and adiposity: the Fels Longitudinal Study. *Int J Obes*, *24*, 1628–1635.
184. Guo, S. S., Wu, W., Chumlea, W. C., & Roche, A. F. (2002). Predicting overweight and obesity in adulthood from body mass index values in childhood and adolescence. *Am J Clin Nutr*, *76*, 653–658.
185. Tirosh, A., Shai, I., Afek, A., et al. (2011). Adolescent BMI trajectory and risk of diabetes versus coronary disease. *N Engl J Med*, *364*, 1315–1325.
186. Whitaker, R. C., Pepe, M. S., Wright, J. A., et al. (1998). Early adiposity rebound and the risk of adult obesity. *Pediatrics*, *101*, 1–6.
187. Toschke, A. M., Vignerova, J., Lhotska, L., et al. (2002). Overweight and obesity in 6- to 14-year-old Czech children in 1991: protective effect of breast feeding. *J Pediatr*, *141*, 764–769.
188. von Kries, R., Koletzko, B., Sauerwald, T., et al. (1999). Breast feeding and obesity: cross sectional study. *BMJ*, *319*, 147–150.
189. Li, L., Law, C., Lo Conte, R., & Power, C. (2009). Intergenerational influences on childhood body mass index: the effect of parental body mass index trajectories. *Am J Clin Nutr*, *89*, 551–557.
190. Dorosty, A. R., Emmett, P. M., Cowin, S., & Reilly, J. J. (2000). Factors associated with early adiposity rebound. ALSPAC study team. *Pediatrics*, *105*, 1115–1118.
191. Li, S., Chen, W., Srinivasan, S. R., et al. (2003). Childhood cardiovascular risk factors and carotid vascular changes in adulthood. *JAMA*, *290*, 2271–2276.
192. Matsumoto, M., Han, S., Kitamura, T., & Accili, D. (2006). Dual role of transcription factor FoxO1 in controlling hepatic insulin sensitivity and lipid metabolism. *J Clin Invest*, *116*, 2464–2472.
193. Naïmi, M., Gautier, N., Chaussade, C., et al. (2007). Nuclear forkhead box O1 controls and integrates key signaling pathways in hepatocytes. *Endocrinol*, *148*, 2424–2434.
194. Lewis, G. F., Uffelman, K. D., Szeto, L. W., & Steiner, G. (1993). Effects of acute hyperinsulinemia on VLDL triglyceride and VLDL apoB production in normal weight and obese individuals. *Diabetes*, *42*, 833–842.
195. Brown, M. S., & Goldstein, J. L. (2008). Selective versus total insulin resistance: a pathogenic paradox. *Cell Metab*, *7*, 95–96.
196. Kim, S. P., Catalano, K. J., Hsu, I. R., et al. (2007). Nocturnal free fatty acids are uniquely elevated in the longitudinal development of diet-induced insulin resistance and hyperinsulinemia. *Am J Physiol Endocrinol Metab*, *288*, E454–E461.
197. Ginsberg, H. N., Zhang, Y. L., & Hernandez-Ono, A. (2005). Regulation of plasma triglycerides in insulin resistance and diabetes. *Arch Med Res*, *36*, 232–240.
198. Samuel, V. T., Petersen, K. F., & Shulman, G. I. (2010). Lipid-induced insulin resistance: unravelling the mechanism. *Lancet*, *375*, 2267–2277.
199. Large, V., & Arner, P. (1998). Regulation of lipolysis in humans. Pathophysiological modulation in obesity, diabetes, and hyperlipidaemia. *Diabetes Metab Res*, *24*, 409–418.
200. Gustafson, B., Hammarstedt, A., Andersson, C. X., & Smith, U. (2007). Inflamed adipose tissue: a culprit underlying the metabolic syndrome and atherosclerosis. *Arterioscler Thromb Vasc Biol*, *27*, 2276–2283.

201. Heilbronn, L. K., & Campbell, L. V. (2008). Adipose tissue macrophages, low grade inflammation and insulin resistance in human obesity. *Curr Pharm Des*, *14*, 1225–1230.
202. Bremer, A. A., Devaraj, S., Afify, A., & Jialal, I. (2011). Adipose tissue dysregulation in patients with metabolic syndrome. *J Clin Endocrinol Metab*, *96*, E1782–1788.
203. Boden, G. (1998). Free fatty acids (FFA), a link between obesity and insulin resistance. *Front Biosci*, *3*, 169–175.
204. Petersen, K. F., Dufour, S., Savage, D. B., et al. (2007). The role of skeletal muscle insulin resistance in the pathogenesis of the metabolic syndrome. *Proc Natl Acad Sci USA*, *104*, 12587–12594.
205. Levy-Marchal, C., Arslanian, S., Cutfield, W., et al. (2010). Insulin Resistance in Children Consensus Conference Group. Insulin resistance in children: consensus, perspective, and future directions. *J Clin Endocrinol Metab*, *95*, 5189–5198.
206. Whincup, P. H., Gilg, J. A., Papacosta, O., et al. (2002). Early evidence of ethnic differences in cardiovascular risk: cross sectional comparison of British South Asian and white children. *BMJ*, *324*, 635.
207. Weiss, R., Dufour, S., Taksali, S. E., et al. (2003). Prediabetes in obese youth: a syndrome of impaired glucose tolerance, severe insulin resistance, and altered myocellular and abdominal fat partitioning. *Lancet*, *362*, 951–957.
208. Bjorntorp, P. (1990). Portal adipose tissue as a generator of risk factors for cardiovascular disease and diabetes. *Arteriosclerosis*, *10*, 493–496.
209. Arner, P. (2002). Insulin resistance in type 2 diabetes: role of fatty acids. *Diab Metab Res Rev*, *18*(Suppl. 2), S5–S9.
210. Bonora, E. (2000). Relationship between regional fat distribution and insulin resistance. *Int J Obes*, *24*(Suppl. 2), S32–S35.
211. Abate, N., Garg, A., Peshock, R. M., et al. (1996). Relationship of generalized and regional adiposity to insulin sensitivity in men with NIDDM (1996). *Diabetes*, *45*, 1684–1693.
212. Wajchenberg, B. L. (2000). Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. *Endocr Rev*, *21*, 697–738.
213. Montague, C. T., & O'Rahilly, S. (2000). The perils of portliness: causes and consequences of visceral adiposity. *Diabetes*, *49*, 883–888.
214. Taksali, S. E., Caprio, S., Dziura, J., et al. (2008). High visceral and low abdominal subcutaneous fat stores in the obese adolescent: a determinant of an adverse metabolic phenotype. *Diabetes*, *57*, 367–371.
215. D'Adamo, E., Cali, A. M., Weiss, R., et al. (2010). Central role of fatty liver in the pathogenesis of insulin resistance in obese adolescents. *Diab Care*, *33*, 1817–1822.
216. Ravussin, E., Smith, S. R. (2002). Increased fat intake, impaired fat oxidation, and failure of fat cell proliferation result in ectopic fat storage, insulin resistance, and type 2 diabetes mellitus. *Ann NY Acad Sci*, *967*, 363–378.
217. Sung, K. C., & Kim, S. H. (2011). Interrelationship between fatty liver and insulin resistance in the development of type 2 diabetes. *J Clin Endocrinol Metab*, 96, 1093–1097.
218. Krssak, M., Falk Petersen, K., Dresner, A., et al. (1999). Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans: a ^{1}H NMR spectroscopy study. *Diabetologia*, *42*, 113–116.
219. Garg, A. (2004). Acquired and inherited lipodystrophies. *N Engl J Med*, *350*, 1220–1234.
220. Goodpaster, B. H., Thaete, F. L., Simoneau, J. A., & Kelley, D. E. (1997). Subcutaneous abdominal fat and thigh muscle composition predict insulin sensitivity independently of visceral fat. *Diabetes*, *46*, 1579–1585.
221. Perseghin, G., Scifo, P., De Cobelli, F., et al. (1999). Intramyocellular triglyceride content is a determinant of *in vivo* insulin resistance in humans: a ^{1}H-^{13}C nuclear magnetic resonance spectroscopy assessment in offspring of type 2 diabetic parents. *Diabetes*, *48*, 1600–1606.
222. Sinha, R., Dufour, S., Petersen, K. F., et al. (2002). Assessment of skeletal muscle triglyceride content by (1)H nuclear magnetic resonance spectroscopy in lean and obese adolescents: relationships to insulin sensitivity, total body fat, and central adiposity. *Diabetes*, *51*, 1022–1027.
223. Fabbrini, E., Magkos, F., Mohammed, B. S., et al. (2009). Intrahepatic fat, not visceral fat, is linked with metabolic complications of obesity. *Proc Natl Acad Sci USA*, *106*, 15430–15435.
224. Unger, R. H. (2003). Minireview: weapons of lean body mass destruction: the role of ectopic lipids in the metabolic syndrome. *Endocrinology*, *144*, 5159–5165.
225. Dobbins, R. L., Szczepaniak, L. S., Bentley, B., et al. (2001). Prolonged inhibition of muscle carnitine palmitoyltransferase-1 promotes intramyocellular lipid accumulation and insulin resistance in rats. *Diabetes*, *50*, 123–130.
226. Morino, K., Petersen, K. F., & Shulman, G. I. (2006). Molecular mechanisms of insulin resistance in humans and their potential links with mitochondrial dysfunction. *Diabetes*, *55*(Suppl. 2), S9-S15.
227. Samuel, V. T., Liu, Z. X., Qu, X., et al. (2004). Mechanism of hepatic insulin resistance in non-alcoholic fatty liver disease. *J Biol Chem*, *279*, 32345–32353.
228. Hirosumi, J., Tuncman, G., Chang, L., et al. (2002). A central role for JNK in obesity and insulin resistance. *Nature*, *420*, 333–336.
229. Calles-Escandon, J., & Cipolla, M. (2001). Diabetes and endothelial dysfunction: a clinical perspective. *Endocr Rev*, *22*, 36–52.
230. Moncada, S., Palmer R. M. J., & Higgs, E. A. (1991). Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol Rev*, *43*, 109–142.
231. Nakagawa, T., Tuttle, K. R., Short, R., & Johnson, R. J. (2006). Hypothesis: fructose-induced hyperuricemia as a causal mechanism for the epidemic of the metabolic syndrome. *Nat Clin Pract Nephrology*, *1*, 80–86.
232. Bonetti, P. O., Lerman, L. O., & Lerman, A. (2003). Endothelial dysfunction: a marker of atherosclerotic risk. *Arterio Thromb Vasc Biol*, *23*, 168–175.
233. Anderson, T. J., Uehata, A., Gerhard, M. D., et al. (1995). Close relation of endothelial function in the human coronary and peripheral circulations. *J Am Coll Cardiol*, *26*, 1235–1241.
234. Corretti, M. C., Anderson, T. J., Benjamin, E. J., et al. (2002). Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol*, *39*, 257–265.
235. Tounian, P., Aggoun, Y., Dubern, B. P., et al. (2001). Presence of increased stiffness of the common carotid artery and endothelial dysfunction in severely obese children: a prospective study. *Lancet*, *358*, 1400–1404.
236. Meyer, A. A., Kundt, G., Steiner, M., et al. (2006). Impaired flow-mediated vasodilation, carotid artery intima-media thickening, and elevated endothelial plasma markers in obese children: the impact of cardiovascular risk factors. *Pediatrics*, *117*, 1560–1567.
237. Berenson, G. S., Srinivasan, S. R., Bao, W., et al. (1998). Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults: the Bogalusa Heart Study. *N Engl J Med*, *338*, 1650–1656.
238. Raitakari, T. O., Juonala, M., Kahonen, M., et al. (2003). Cardiovascular risk factors in childhood and carotid artery intima-media thickness in adulthood. *JAMA*, *290*, 2277–2283.
239. PDoAiYPR Group (1990). Relationships in young men to serum lipoprotein cholesterol concentrations and smoking. *JAMA*, *264*, 3018–3024.
240. Davis, P. H., Dawson, J. D., Riley, W. A., & Lauer, R. M. (2001). Carotid intimal media thickness is related to cardiovascular risk factors measured from childhood through middle age. *Circulation*, *104*, 2815–2819.
241. Mahoney, L. T., Burns, T. L., Stanford, W., et al. (1996). Coronary risk factors measured in childhood and young adult life are associated with coronary artery calcification in young adults: the Muscatine Study. *J Am Coll Cardiol*, *27*, 277–284.
242. Saltiel, A. R. (2001). You are what you secrete. *Nat Med*, *7*, 887–880.
243. Unger, R. H. (2004). The hyperleptinemia of obesity-regulator of caloric surpluses. *Cell*, *117*, 145–146.
244. Arita, Y., Kihara, S., Ouchi, N., et al. (1999). Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Comm*, *257*, 79–83.
245. Shapiro, L., & Scherer, P. E. (1998). The crystal structure of a complement-1q family protein suggests an evolutionary link to tumor necrosis factor. *Curr Biol*, *8*, 335–338.
246. Kondo, H., Shimomura, I., Matsukawa, Y., et al. (2002). Association of adiponectin mutation with type 2 diabetes: a candidate gene for the insulin resistance syndrome. *Diabetes*, *51*, 2325–2328.

247. Pajvani, U. B., Du, X., Combs, T. P., et al. (2003). Structure-function studies of the adipocyte-secreted hormone Acrp30/adiponectin: implications for metabolic regulation and bioactivity. *J Biol Chem*, *278*, 9073–9085.
248. Kadowaki, T., & Yamauchi, T. (2005). Adiponectin and adiponectin receptors. *Endocr Rev*, *26*, 439–451.
249. Yamauchi, T., Kamon, J., Ito, Y., et al. (2003). Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. *Nature*, *423*, 762–769.
250. Ouchi, N., Kihara, S., Arita, Y., et al. (2000). Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF-kappaB signaling through a cAMP-dependent pathway. *Circulation*, *102*, 1296–1301.
251. Yatagai, T., Nagasaka, S., Taniguchi, A., et al. (2003). Hypoadiponectinemia is associated with visceral fat accumulation and insulin resistance in Japanese men with type 2 diabetes mellitus. *Metabolism*, *52*, 1274–1278.
252. Hotta, K., Funahashi, T., Bodkin, N. L., et al. (2001). Circulating concentrations of the adipocyte protein adiponectin: a decrease in parallel with reduced insulin sensitivity during the progression of type 2 diabetes in Rhesus monkeys. *Diabetes*, *50*, 1126–1133.
253. Al-Daghri, N. M., Al-Attas, O. S., Alokail, M. S., et al. (2011). Parent-offspring transmission of adipocytokine levels and their associations with metabolic traits. *PLoS One*, *6*, e18182.
254. Boström, P., Wu, J., Jedrychowski, M. P., et al. (2012). A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature*, *481*, 463–468.
255. Bordicchia, M., Liu, D., Amri, E. Z., et al. (2012). Cardiac natriuretic peptides act via p38MAPK to induce the brown fat thermogenic program in mouse and human adipocytes. *J Clin Invest*, *122*, 1022–1036; erratum in 1122, 1584.
256. Wang, T. J. (2012). The natriuretic peptides and fat metabolism *N Engl J Med*, *367*, 377–378.
257. Weisberg, S. P., McCann, D., Desai, M., et al. (2003). Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest*, *112*, 1673–1808.
258. Matsuzawa, Y., Funahashi, T., & Nakamura, T. (1999). Molecular mechanism of metabolic syndrome X: contribution of adipocytokines adipocyte-derived bioactive substances. *Ann N Y Acad Sci*, *892*, 146–154.
259. Cinti, S., Mitchell, G., Barbatelli, G., et al. (2005). Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. *J Lipid Res*, *46*, 2347–2355.
260. Yudkin, J. S., Kumari, M., Humphries, S. E., & Mohamed-Ali, V. (2000). Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? *Atherosclerosis*, *148*, 209–214.
261. Pickup, J. C., & Crook, M. A. (1998). Is type 2 diabetes mellitus a disease of the innate immune system? *Diabetologia*, *41*, 1241–1248.
262. Ridker, P. M., Hennekens, C. H., Buring, J. E., & Rifai, N. (2000). C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med*, *342*, 836–843.
263. Pradhan, A. D., Manson, J. E., Rifai, N., et al. (2001). C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *JAMA*, *286*, 327–334.
264. Cook, D. G., Mendall, M. A., Whincup, P. H., et al. (2000). C-reactive protein concentration in children: relationship to adiposity and other cardiovascular risk factors. *Atherosclerosis*, *149*, 139–150.
265. Ford, E. S., Galuska, D. A., Gillespie, C., et al. (2001). C-reactive protein and body mass index in children: findings from the Third National Health and Nutrition Examination Survey, 1988–1994. *J Pediatr*, *138*, 486–492.
266. Civitarese, A. E., & Ravussin, E. (2008). Mitochondrial energetics and insulin resistance. *Endocrinology*, *149*, 950–954.
267. Subasinghe, W., Syed, I., & Kowluru, A. (2011). Phagocyte-like NADPH oxidase promotes cytokine-induced mitochondrial dysfunction in pancreatic beta-cells: evidence for regulation by Rac1. *Am J Physiol Regul Integr Comp Physiol*, *300*, R12–R20.
268. Turrens, J. F. (2003). Mitochondrial formation of reactive oxygen species. *J Physiol*, *552*, 335–344.
269. Fan, X., Hussien, R., & Brooks, G. A. (2010). H2O2-induced mitochondrial fragmentation in C2C12 myocytes. *Free Radic Biol Med*, *49*, 1646–1654.
270. de Ferranti, S., & Mozaffarian, D. (2008). The perfect storm: obesity, adipocyte dysfunction, and metabolic consequences. *Clin, Chem*, *54*, 945–955.
271. Bremer, A. A., Mietus-Snyder, M. L., & Lustig, R. H. (2012). Toward a unifying hypothesis of metabolic syndrome. *Pediatrics*, *129*, 557–570.
272. Dirkx, R., Vanhorebeek, I., Martens, K., et al. (2005). Absence of peroxisomes in mouse hepatocytes causes mitochondrial and ER abnormalities. *Hepatology*, *41*, 868–878.
273. Schrader, M., & Fahimi, H. D. (2006). Peroxisomes and oxidative stress. *Biochim Biophys Acta*, *1763*, 1755–1766.
274. Reaven, G. M. (1988). Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes*, *37*, 1595–1607.
275. Haffner, S. M. (1999). Epidemiology of insulin resistance and its relation to coronary artery disease. *Am J Cardiol*, *84*, 11J–14J.
276. Hu, F. B., Stampfer, J. M., Haffner, S. M., et al. (2002). Elevated risk of cardiovascular disease prior to clinical diagnosis of type 2 diabetes. *Diab Care*, *25*, 1129–1134.
277. Alberti, K. G., & Zimmet, P. Z. (1998). Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med*, *15*, 539–553.
278. Zimmet, P., Alberti, G., Kaufman, F., et al. (2007). International Diabetes Federation Task Force on Epidemiology and Prevention of Diabetes. The metabolic syndrome in children and adolescents. *Lancet*, *369*, 2059–2061.
279. Ford, E. S., Giles, W. H., & Dietz, W. H. (2002). Prevalence of the metabolic syndrome among US adults: findings from the Third National Health and Nutrition Examination survey. *JAMA*, *287*, 356–359.
280. Angulo, P. (2002). Nonalcoholic fatty liver disease. *N Engl J Med*, *346*, 1221–1231.
281. Ludwig, J., Viggiano, T. R., McGill, D. B., & Ott, B. J. (1980). Nonalcoholic steatohepatitis: Mayo Clinic experience with a hitherto unnamed disease. *Mayo Clin Proc*, *55*, 434–438.
282. Meltzer, A. A., & Everhart, J. E. (1997). Association between diabetes and elevated serum alanine aminotransferase activity among Mexican Americans. *Am J Epidemiol*, *146*, 565–571.
283. Lavine, J. E., & Schwimmer, J. B. (2004). Nonalcoholic fatty liver disease in the pediatric population. *Clin Liver Dis*, *8*, 549–558.
284. Roberts, E. (2003). Nonalcoholic steatohepatitis in children. *Curr Gastroenterol Rep*, *5*, 253–259.
285. Burgert, T. S., Taksali, S. E., Dziura, J., et al. (2006). Alanine aminotransferase levels and fatty liver in childhood obesity: associations with insulin resistance, adiponectin, and visceral fat. *J Clin Endocrinol Metab*, *91*, 4287–4294.
286. Day, C. P., & James, O. F. (1998). Steatohepatitis: a tale of two "hits"? *Gastroenterology*, *114*, 842–845.
287. Shimomura, I., Shimano, H., Horton, J. D., et al. (1997). Differential expression of exons 1a an 1c in mRNAs for sterol regulatory element binding protein 1 in human and mouse organs and cultured cells. *J Clin Invest*, *99*, 838–845.
288. Shimomura, I., Matsuda, M., Hammer, R. E., et al. (2000). Decreased IRS2 and increased SREBP-1c lead to mixed insulin resistance and sensitivity in liver dystrophic and ob/ob mice. *Mol Cell*, *6*, 77–86.
289. Lim, J. S., Mietus-Snyder, M., Valente, A., et al. (2010). The role of fructose in the pathogenesis of NAFLD and the metabolic syndrome. *Nat Rev Gastroenterol Hepatol*, *7*, 251–264.
290. Schwimmer, J. B., Dunn, W., Norman, G. J., et al. (2010). SAFETY study: alanine aminotransferase cutoff values are set too high for reliable detection of pediatric chronic liver disease. *Gastroenterology*, *138*, 1357–1364.
291. Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group. (2004). Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Hum Reprod*, *19*, 41–47.
292. Ibanez, L., Potau, N., Francois, I., & de Zegher, F. (1999). Precocious pubarche, hyperinsulinism, and ovarian hyperandrogenism in girls: relation to reduced fetal growth. *J Clin Endocrinol Metab*, *84*, 2691–2695.
293. Azziz, R., Woods, K. S., Reyna, R., et al. (2004). The prevalence and features of the polycystic ovary syndrome in an unselected population. *J Clin Endocrinol Metab*, *89*, 2745–2749.

294. Apter, D., Butzow, T., Laughlin, G. A., & Yen, S. S. (1995). Metabolic features of polycystic ovary syndrome are found in adolescent girls with hyperandrogenism. *J Clin Endocrinol Metab*, *80*, 2966–2973.
295. Lewy, V. D., Danadian, K., Witchel, S. F., & Arslanian, S. (2001). Early metabolic abnormalities in adolescent girls with polycystic ovarian syndrome. *J Pediatr*, *138*, 38–44.
296. Setji, T. L., Holland, N. D., Sanders, L. L., et al. (2006). Nonalcoholic steatohepatitis and nonalcoholic fatty liver disease in young women with polycystic ovary syndrome. *J Clin Endocrinol Metab*, *91*, 1741–1747.
297. Gambineri, A., Pelusi, C., Manicardi, E., et al. (2004). Glucose intolerance in a large cohort of Mediterranean women with polycystic ovary syndrome: phenotype and associated factors. *Diabetes*, *53*, 2353–2358.
298. Coviello, A. D., Legro, R. S., & Dunaif, A. (2006). Adolescent girls with polycystic ovary syndrome have an increased risk of the metabolic syndrome associated with increasing androgen levels independent of obesity and insulin resistance. *J Clin Endocrinol Metab*, *91*, 492–497.
299. Vryonidou, A., Papatheodorou, A., Tavridou, A., et al. (2005). Association of hyperandrogenemic and metabolic phenotype with carotid intima-media thickness in young women with polycystic ovary syndrome. *J Clin Endocrinol Metab*, *90*, 2740–2746.
300. Venkatsen, A. M., Dunaif, A., & Corbould, A. (2001). Insulin resistance in polycystic ovary syndrome: progress and paradoxes. *Recent Prog Horm Res*, *56*, 295–308.
301. Zhang, L. H., Rodriguez, H., Ohno, S., & Miller, W. L. (1995). Serine phosphorylation of human P450c17 increases 17,20-lyase activity: implications for adrenarche and the polycystic ovary syndrome. *Proc Natl Acad Sci USA*, *92*, 10619–10623.
302. Dunaif, A., Xia, J., Book, C. B., et al. (1995). Excessive insulin receptor serine phosphorylation in cultured fibroblasts and in skeletal muscle: a potential mechanism for insulin resistance in the polycystic ovary syndrome. *J Clin Invest*, *96*, 801–810.
303. Kaplowitz, P. B., Slora, E. J., Wasserman, R. C., et al. (2001). Earlier onset of puberty in girls: relation to increased body mass index and race. *Pediatrics*, *108*, 347–353.
304. Lustig, R. H., Hershcopf, R. J., & Bradlow, H. L. (1990). The effects of body weight and diet on estrogen metabolism and estrogen-dependent disease. In R. E. Frisch (Ed.), *Adipose tissue and reproduction*. (pp. 107–124). Basel: Karger.
305. Braunstein, G. D. (1999). Aromatase and gynecomastia. *Endocr Relat Cancer*, *6*, 315–324.
306. Kaplowitz, P. (1998). Delayed puberty in obese boys: comparison with constitutional delayed puberty and response to testosterone therapy. *J Pediatr*, *133*, 745–749.
307. Rose, S. R., Burstein, S., Burghen, G. A., et al. (1999). Caloric restriction for 24 hours increases mean night growth hormone. *J Pediatr Endocrinol Metab*, *12*, 175–183.
308. Coutant, R., Boux de Casson, F., Rouleau, S., et al. (2001). Body composition, fasting leptin, and sex steroid administration determine GH sensitivity in peripubertal short children. J *Clin Endocrinol Metab*, *86*, 5805–5812.
309. Olney, R. C., & Mougey, E. B. (1999). Expression of the components of the insulin-like growth factor axis across the growth plate. *Mol Cell Endocrinol*, *156*, 63–71.
310. Masuzaki, H., Paterson, J., Shinyama, H., et al. (2001). A transgenic model of visceral obesity and the metabolic syndrome. *Science*, *294*, 2166–2170.
311. Kesler, A., & Fattal-Valevski, A. (2002). Idiopathic intracranial hypertension in the pediatric population. *J Child Neurol*, *17*, 745–748.
312. de la Eva, R., Baur, L. A., Donaghue, K. C., & Waters, K. A. (2002). Metabolic correlates with obstructive sleep apnea in obese subjects. *J Pediatr*, *140*, 654–659.
313. Taylor, E. D., Theim, K. R., Mirch, M. C., et al. (2006). Orthopedic complications of overweight children and adolescents. *Pediatrics*, *117*, 2167–2174.
314. Kaechele, V., Wabitsch, M., Thiere, D., et al. (2006). Prevalence of gallbladder stone disease in obese children and adolescents: influence of the degree of obesity, sex, and pubertal development. *J Pediatr Gastroenterol Nutr*, *42*, 66–70.
315. Young-Hyman, D., Tanofsky-Kraff, M., Yanovski, S. Z., et al. (2006). Psychological status and weight-related distress in overweight or at-risk-for-overweight children. *Obesity*, *14*, 2249–2258.
316. Bell, L. M., Byrne, S., Thompson, A., et al. (2007). Increasing body mass index z-score is continuously associated with complications of overweight in children, even in the healthy weight range. *J Clin Endocrinol Metab*, *92*, 517–522.
317. Baier, L. J., & Hanson, R. L. (2004). Genetic studies of the etiology of type 2 diabetes in Pima Indians. *Diabetes*, *53*, 1181–1186.
318. Butte, N. F., Cai, G., Cole, S. A., Comuzzie, A. G. (2006). Viva la Familia Study: genetic and environmental contributions to childhood obesity and its comorbidities in the Hispanic population. *Am J Clin Nutr*, *84*, 646–654.
319. Neel, J. V. (1962). Diabetes mellitus: a thrifty genotype rendered detrimental by "progress" *Am J Hum Genet*, *14*, 353–362.
320. Mutch, D. M., & Clement, K. (2006). Genetics of human obesity. *Best Pract Res Clin Endocrinol Metab*, *20*, 647–664.
321. Cecil, J. E., Tavendale, R., Watt, P., et al. (2008). An obesity-associated FTO gene variant and increased energy intake in children. *N Engl J Med*, *359*, 2558–2566.
322. Loos, R. J. F., & Kilpelainen, T. O. (2010). Genome-wide association studies and human population obesity. In R. H. Lustig (Ed.), *Obesity before birth*. (pp. 95–112). New York: Springer.
323. Bradfield, J. P., Taal, H. R., Timpson, N. J., et al. (2012). A genome-wide association meta-analysis identifies new childhood obesity loci. *Nat Genet*, *44*, 526–531.
324. Barker, D. J. (2004). The developmental origins of chronic adult disease. *Acta Paediatr*, *93*(Suppl.), 26–33.
325. Godfrey, K. M., Sheppard, A., Gluckman, P. D., et al. (2011). Epigenetic gene promoter methylation at birth is associated with child's later adiposity. *Diabetes*, *60*, 1528–1534.
326. Roseboom, T. J., van der Meulen, J. H., Ravelli, A. C., et al. (2001). Effects of prenatal exposure to the Dutch famine on adult disease in later life: an overview. *Mol Cell Endocrinol*, *185*, 93–98.
327. Yajnik, C. S., Lubree, H. G., Rege, S. S., et al. (2002). Adiposity and hyperinsulnemia in Indians are present at birth. *J Clin Endocrinol Metab*, *87*, 5575–5580.
328. Arends, N. J., Boonstra, V. H., Duivenvoorden, H. J., et al. (2005). Reduced insulin sensitivity and the presence of cardiovascular risk factors in short prepubertal children born small for gestational age (SGA). *Clin Endocrinol*, *62*, 44–50.
329. Potau, N., Gussinye, M., Sanchez Ufarte, C., et al. (2001). Hyperinsulinemia in pre- and post-pubertal children born small for gestational age. *Horm Res*, *56*, 146–150.
330. Yajnik, C. S., Fall, C. H., Vaidya, U., et al. (1995). Fetal growth and glucose and insulin metabolism in four-year-old Indian children. *Diabet Med*, *12*, 330–336.
331. Silverman, B. L., Landsberg, L., & Metzger, B. E. (1993). Fetal hyperinsulinism in offspring of diabetic mothers: association with the subsequent development of childhood obesity. *Ann N Y Acad Sci*, *699*, 36–45.
332. Silverman, B. L., Rizzo, T. A., Cho, N. H., & Metzger, B. E. (1998). Long-term effects of the intrauterine environment. The Nortwestern University Diabetes in Pregnancy Center. *Diabetes Care*, *21*(Suppl. 2), B142–B149.
333. Boney, C. M., Verma, A., Tucker, R., & Vohr, B. R. (2005). Metabolic syndrome in childhood: association with birth weight, maternal obesity, and gestational diabetes. *Pediatrics*, *115*, e290–e296.
334. Knowler, W. C., Pettitt, D. J., Savage, P. J., & Bennett, P. H. (1981). Diabetes incidence in Pima Indians: contributions of obesity and parental diabetes. *Am J Epidemiol*, *113*, 144–156.
335. Dabelea, D., Hanson, R. L., Lindsay, R. S., et al. (2000). Intrauterine exposure to diabetes conveys risk for type 2 diabetes and obesity: a study of discordant sibships. *Diabetes*, *42*, 2208–2211.
336. Caughey, A. B. (2006). Obesity, weight loss, and pregnancy outcomes. *Lancet*, *368*, 1136–1138.
337. Ludwig, D. S., & Currie, J. (2010). The association between pregnancy weight gain and birth weight: a within family comparison. *Lancet*, *376*, 984–990.
338. Hofman, P. L., Regan, F., Jackson, W. E., et al. (2004). Premature birth and later insulin resistance. *N Engl J Med*, *351*, 2179–2186.
339. Harder, T., Bergmann, R., Kallischnigg, G., & Plagemann, A. (2005). Duration of breastfeeding and risk of overweight: a meta-analysis. *Am J Epidemiol*, *162*, 397–403.
340. Owen, C. G., Martin, R. M., Whincup, P. H., et al. (2005). The effect of breastfeeding on mean body mass index throughout life:

a quantitative review of published and unpublished observational evidence. *Am J Clin Nutr*, *82*, 1298–1307.
341. Miralles, O., Sanchez, J., Palou, A., & Pico, C. (2006). A physiological role of breast milk leptin in body weight control in developing infants. *Obesity*, *14*, 1371–1377.
342. Keith, S. W., Redden, D. T., Katzmaryk, P. T., et al. (2006). Putative contributors to the secular increase in obesity: exploring the roads less traveled. *Int J Obes*, *30*, 1585–1594.
343. Mozaffarian, D., Hao, T., Rimm, E. B., et al. (2011). Changes in diet and lifestyle and long-term weight gain in women and men. *N Engl J Med*, *364*, 2392–2404.
344. Ludwig, J., Sanbonmatsu, L., Gennetian, L., et al. (2011). Neighborhoods, obesity, and diabetes: a randomized social experiment. *N Engl J Med*, *365*, 1509–1519.
345. Andrew, R., Gale, C. R., Walker, B. R., et al. (2002). Glucocorticoid metabolism and the metabolic syndrome: associations in an elderly cohort. *Exp Clin Endocrinol Diab*, *110*, 284–290.
346. Kotelevtsev, Y. V., Holmes, M. C., Burchell, A., et al. (1997). 11β-hydroxysteroid dehydrogenase type 1 knockout mice show attenuated glucocorticoid inducible responses and resist hyperglycaemia on obesity and stress. *Proc Natl Acad Sci USA*, *94*, 14924–14929.
347. Chandola, T., Brunner, E., & Marmot, M. (2006). Chronic stress at work and the metabolic syndrome: prospective study. *BMJ*, *332*, 521–525.
348. Rosengren, A., Hawken, S., Ounpuu, S., et al. (2004). Association of psychosocial risk factors with risk of acute myocardial infarction in 11,119 cases and 13,648 controls from 52 countries (the INTERHEART study): case-control study. *Lancet*, *364*, 953–962.
349. Brunner, E. J., Hemingway, H., Walker, B. R., et al. (2002). Adrenocortical, autonomic, and inflammatory causes of the metabolic syndrome: nested case-control study. *Circulation*, *106*, 2659–2665.
350. Souverein, P. C., Berard, A., Van Staa, T. P., et al. (2004). Use of oral glucocorticoids and risk of cardiovascular and cerebrovascular disease in a population based case-control study. *Heart*, *90*, 859–865.
351. Bujalska, I. J., Kumar, S., & Stewart, P. M. (1997). Does central obesity reflect "Cushing's disease of the omentum"? *Lancet*, *349*, 1210–1213.
352. Spiegel, K., Sheridan, J. F., & Van Cauter, E. (2002). Effect of sleep deprivation on response to immunization. *JAMA*, *288*, 1471–1472.
353. Gangwisch, J. E., Malaspina, D., Boden-Albala, B., & Heymsfield, S. B. (2005). Inadequate sleep as a risk factor for obesity: analyses of the NHANES I. *Sleep*, *28*, 1217–1220.
354. Hasler, G., Buysse, D. J., Klaghofer, R., et al. (2004). The association between short sleep duration and obesity in young adults: a 13-year prospective study. *Sleep*, *27*, 602–603.
355. Taheri, S. (2006). The link between short sleep duration and obesity: we should recommend more sleep to prevent obesity. *Arch Dis Child*, *91*, 881–884.
356. Sekine, M., Yamagami, T., Hamanishi, S., et al. (2002). Parental obesity, lifestyle factors and obesity in preschool children: results of the Toyama birth cohort study. *J Epidemiol*, *12*, 33–39.
357. Reilly, J. J., Armstrong, J., Dorosty, A. R., et al. (2005). Early life risk factors for obesity in childhood: cohort study. *BMJ*, *330*, 1357.
358. Robinson, T. N. (2001). Television viewing and childhood obesity. *Pediatr Clin North Am*, *48*, 1017–1025.
359. Sgoifo, A., Braglia, F., Costoli, T., et al. (2003). Cardiac autonomic reactivity and salivary cortisol in men and women exposed to social stressors: relationship with individual ethological profile. *Neurosci Biobehav Rev*, *27*, 179–188.
360. Taras, H. L., Sallis, J. F., Patterson, T. L., et al. (1989). Television's influence on children's diet and physical activity. *J Dev Behav Pediatr*, *10*, 176–180.
361. DuRant, R. H., Baranowski, T., Johnson, M., & Thompson, W. O. (1994). The relationship among television watching, physical activity, and body composition of young children. *Pediatrics*, *94*, 449–455.
362. Coon, K. A., Goldberg, J., Rogers, B. L., & Tucker, K. L. (2001). Relationships between use of television during meals and children's food consumption patterns. *Pediatrics*, *107*, e7.
363. Effect of sedentary activities on resting metabolic rate (1994). [Computer program].
364. Crespo, C. J., Smit, E., Troiano, R. P., et al. (2001). Television watching, energy intake, and obesity in US children: results from the third National Health and Nutrition Examination Survey, 1988–1994. *Arch Pediatr Adolesc Med*, *155*, 360–365.
365. Robinson, T. N. (1999). Reducing children's television viewing to prevent obesity: a randomized controlled trial. *JAMA*, *282*, 1561–1567.
366. Parsons, T. J., Power, C., Logan, S., & Summerbell, C. D. (1999). Childhood predictors of adult obesity: a systematic review. *Int J Obes*, *23*(Suppl. 8), S1–S107.
367. Maffeis, C., Schutz, Y., Grezzani, A., et al. (2001). Meal-induced thermogenesis and obesity: is a fat meal a risk factor for fat gain in children? *J Clin Endocrinol Metab*, *86*, 214–219.
368. Jequier, E. (2001). Is fat intake a risk factor for fat gain in children? *J Clin Endocrinol Metab*, *86*, 980–983.
369. Tucker, L. A., Seljaas, G. T., & Hager, R. L. (1997). Body fat percentage of children varies according to their diet composition. *J Am Diet Assoc*, *97*, 981–986.
370. Pirozzo, S., Summerbell, C., Cameron, C., & Glasziou, P. (2002). Advice on low-fat diets for obesity. *Cochrane Database Syst Rev*, *2*, CD003640.
371. Kennedy, E., & Powell, R. (1997). Changing eating patterns of American children: a view from 1996. *J Am Coll Nutr*, *16*, 524–529.
372. Harnack, L., Stang, J., & Story, M. (1999). Soft drink consumption among US children and adolescents: nutritional consequences. *J Am Diet Assoc*, *99*, 436–441.
373. Center for Weight and Health. (2001). Pediatric overweight: a review of the literature June 2001. From www.cnr.berkeley.edu/cwh/news/announcements.shtml.
374. Foster, G. D., Wyatt, H. R., Hill, J. O., et al. (2003). A randomized trial of a low-carbohydrate diet for obesity. *N Engl J Med*, *348*, 2082–2090.
375. Stern, L., Iqbal, N., Seshadri, P., et al. (2004). The effects of low-carbohydrate versus conventional weight loss diets in severely obese adults: one-year follow-up of a randomized trial. *Ann Int Med*, *140*, 778–785.
376. Vining, E. P., Pyzik, P., McGrogan, J., et al. (2002). Growth of children on the ketogenic diet. *Dev Med Child Neurol*, *44*, 796–802.
377. Rasooly, R., Kelley, D. S., Greg, J., & Mackey, B. E. (2007). Dietary trans 10, cis 12-conjugated linoleic acid reduces the expression of fatty acid oxidation and drug detoxification enzymes in mouse liver. *Br J Nutr*, *97*, 58–66.
378. Chanmugam, P., Guthrie, J. F., Cecilio, S., et al. (2003). Did fat intake in the United States really decline between 1989–1991 and 1994–1996? *J Am Diet Assoc*, *103*, 867–872.
379. Dorfman, S. E., Laurent, D., Gounarides, J. S., et al. (2009). Metabolic implications of dietary trans-fatty acids. *Obesity*, *17*, 1200–1207.
380. Ludwig, D. S. (2002). The glycemic index: physiological mechanisms relating to obesity, diabetes, and cardiovascular disease. *JAMA*, *287*, 2414–2423.
381. Pawlak, D. B., Bryson, J. M., Denyer, J. S., & Brand-Miller, J. C. (2001). High glycemic index starch promotes hypersecretion of insulin and higher body fat in rats without affecting insulin sensitivity. *J Nutr*, *131*, 99–104.
382. Ludwig, D. S., Majzoub, J. A., Al-Zahrani, A., et al. (1999). High glycemic index foods, overeating, and obesity. *Pediatrics*, *103*, e261–e266.
383. Ebbeling, C. B., Leidig, M. M., Sinclair, K. B., et al. (2003). A reduced-glycemic load diet in the treatment of adolescent obesity. *Arch Ped Adolesc Med*, *157*, 773–779.
384. Ludwig, D. S., Pereira, M. A., Kroenke, C. H., et al. (1999). Dietary fiber, weight gain, and cardiovascular disease risk factors in young adults. *JAMA*, *282*, 1539–1546.
385. Liese, A. D., Schulz, M., Fang, F., et al. (2005). Dietary glycemic index and glycemic load, carbohydrate and fiber intake, and measures of insulin sensitivity, secretion, and adiposity in the Insulin Resistance Atherosclerosis Study. *Diab Care*, *28*, 2832–2838.
386. Pereira, M. A., & Ludwig, D. S. (2001). Dietary fiber and body weight regulation. Observations and mechanisms. *Pediatr Clin North Am*, *48*, 969–980.

387. Rigaud, D., Paycha, F., Meulemans, A., et al. (1998). Effect of psyllium on gastric emptying, hunger feeling and food intake in normal volunteers: a double blind study. *Eur J Clin Nutr*, *52*, 239–245.
388. Slavin, J. (2003). Why whole grains are protective: biological mechanisms. *Proc Nutr Soc*, *62*, 129–134.
389. Leach, J. D. (2007). Evolutionary perspective on dietary intake of fibre and colorectal cancer. *Eur J Clin Nutr*, *61*, 140–142.
390. Martlett, J. A., McBurney, M. I., & Slavin, J. L. (2002). Position of the American Dietetic Association: health implications of dietary fiber. *J Am Diet Assoc*, *102*, 993–1000.
391. Ludwig, D. S., Pereira, M. A., Kroenke, C. J., et al. (1999). Dietary fiber, weight gain, and cardiovascular disease risk factors in young adults. *JAMA*, *282*, 1539–1546.
392. Gross, L. S., Li, S., Ford, E. S., & Liu, S. (2004). Increased consumption of refined carbohydrates and the epidemic of type 2 diabetes in the United States: an ecologic assessment. *Am J Clin Nutr*, *79*, 774–779.
393. Ludwig, D. S., Peterson, K. E., Gortmaker, S. L. (2001). Relation between consumption of sugar-sweetened drinks and childhood obesity: a prospective, observational analysis. *Lancet*, *357*, 505–508.
394. Faith, M. S., Dennison, B. A., Edmunds, L. S., & Stratton, H. H. (2006). Fruit juice intake predicts increased adiposity gain in children from low-income families: weight status-by-environment interaction. *Pediatrics*, *118*, 2066–2075.
395. Committee on Nutrition, American Academy of Pediatrics (2001). The use and misuse of fruit juice in pediatrics. *Pediatrics*, *107*, 1210–1213.
396. Jurgens, H., Haass, W., Castaneda, T. R., et al. (2005). Consuming fructose-sweetened beverages increases body adiposity in mice. *Obes Res*, *13*, 1146–1156.
397. Havel, P. J. (2005). Dietary fructose: implications for dysregulation of energy homeostasis and lipid/carbohydrate metabolism. *Nutr Rev*, *63*, 133–157.
398. Le, K. A., & Tappy, L. (2006). Metabolic effects of fructose. *Curr Opin Nutr Metab Care*, *9*, 469–475.
399. Nagai, Y., Yonemitsu, S., Erion, D. M., et al. (2009). The role of peroxisome proliferator-activated receptor gamma coactivator-1 beta in the pathogenesis of fructose-induced insulin resistance. *Cell Metab*, *9*, 252–264.
400. Tuncman, G., Hirosumi, J., Solinas, G., et al. (2006). Functional in vivo interactions between JNK1 and JNK2 isoforms in obesity and insulin resistance. *Proc Natl Acad Sci U S A*, *103*, 10741–10746.
401. Stanhope, K. L., Schwarz, J. M., Keim, N. L., et al. (2009). Consuming fructose-, not glucose-sweetened beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. *J Clin Invest*, *119*, 1322–1334.
402. Teff, K. L., Grudziak, J., Townsend, R. R., et al. (2009). Endocrine and metabolic effects of consuming fructose- and glucose-sweetened beverages with meals in obese men and women: influence of insulin resistance on plasma triglyceride responses. *J Clin Endocrinol Metab*, *94*, 1562–1569.
403. Lee, O., Bruce, W. R., Dong, Q., et al. (2009). Fructose and carbonyl metabolites and endogenous toxins. *Chem Biol Interact*, *178*, 332–339.
404. Pickens, M. K., Yan, J. S., Ng, R. K., et al. (2009). Dietary sucrose is essential to the development of liver injury in the MCD model of steatohepatitis. *J Lipid Res*, *50*, 2072–2082.
405. Layman, D. K., & Walker, D. A. (2006). Potential importance of leucine in treatment of obesity and the metabolic syndrome. *J Nutr*, *136*(Suppl. 1), 319S–323S.
406. Newgard, C. B., An, J., Bain, J. R., et al. (2009). A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. *Cell Metab*, *9*, 311–326.
407. Higuchi, N., Kato, M., Miyazaki, M., et al. (2011). Potential role of branched-chain amino acids in glucose metabolism through the accelerated induction of the glucose-sensing apparatus in the liver. *J Cell Biochem*, *112*, 30–38.
408. Eaton, S. (2002). Control of mitochondrial beta-oxidation flux. *Prog Lipid Res*, *41*, 197–239.
409. Wang, T. J., Larson, M. G., Vasan, R. S., et al. (2011). Metabolic profiles and the risk of developing diabetes. *Nature Med*, *17*, 448–453.
410. Tai, E. S., Tan, M. L., Stevens, R. D., et al. (2010). Insulin resistance is associated with a metabolic profile of altered protein metabolism in Chinese and Asian-Indian men. *Diabetologia*, *53*, 757–787.
411. Steinberg, D., Pearson, T. A., & Kuller, L. H. (1991). Alcohol and atherosclerosis. *Ann Intern Med*, *114*, 967–976.
412. Suter, P. M., & Schutz, Y. (2008). The effect of exercise, alcohol or both combined on health and physical performance. *Int J Obes*, *32*(Suppl. 6), S48–S52.
413. Sozio, M., & Crabb, D. W. (2008). Alcohol and lipid metabolism. *Am J Physiol Endocrinol Metab*, *295*, E10–E16.
414. You, M., & Crabb, D. W. (2004). Molecular mechanisms of alcoholic fatty liver: role of sterol regulatory element-binding proteins. *Alcohol*, *34*, 39–43.
415. Guzmán, M., & Castro, J. (1990). Alterations in the regulatory properties of hepatic fatty acid oxidation and carnitine palmitoyltransferase I activity after ethanol feeding and withdrawal. *Alcohol Clin Exp Res*, *14*, 472–477.
416. Yokoyama, H., Hiroshi, H., Ohgo, H., et al. (2007). Effects of excessive ethanol consumption on the diagnosis of the metabolic syndrome using its clinical diagnostic criteria. *Intern Med*, *46*, 1345–1352.
417. Pereira, M. A., Jacobs, D. R., van Horn, L., et al. (2002). Dairy consumption, obesity, and the insulin resistance syndrome in young adults. *JAMA*, *287*, 2081–2089.
418. Zemel, M. B., Shi, H., Greer, B., et al. (2000). Regulation of adiposity by dietary calcium. *FASEB J*, *14*, 1132–1138.
419. Shi, H., Dirienzo, D., & Zemel, M. B. (2001). Effects of dietary calcium on adipocyte lipid metabolism and body weight regulation in energy-restricted aP2-agouti transgenic mice. *FASEB J*, *15*, 291–293.
420. Lin, Y. C., Lyle, R. M., McCabe, L. D., et al. (2000). Dietary calcium is related to changes in body composition during a two year exercise intervention in young women. *J Am Coll Nutr*, *19*, 754–760.
421. Yanoff, L. B., Parikh, S. J., Spitalnik, A., et al. (2006). The prevalence of hypovitaminosis D and secondary hyperparathyroidism in obese Black Americans. *Clin Endocrinol*, *64*, 523–529.
422. Heaney, R. P., Davies, K. M., & Barger-Lux, M. J. (2002). Calcium and weight: clinical studies. *J Am Coll Nutr*, *21*, 152S–155S.
423. Anderson, R. A. (2000). Chromium in the prevention and control of diabetes. *Diabetes Metab*, *26*, 22–27.
424. Fantus, I. G., & Tsiani, E. (1998). Multifunctional actions of vanadium compounds on insulin signaling pathways: evidence for preferential enhancement of metabolic versus mitogenic effects. *Mol Cell Biochem*, *182*, 109–119.
425. Ames, B. N. (2006). Low micronutrient intake may accelerate the degenerative diseases of aging through allocation of scarce micronutrients by triage. *Proc Natl Acad Sci U S A*, *103*, 17589–17594.
426. Pasarica, M., Shin, A. C., Yu, M., et al. (2006). Human adenovirus 36 induces adiposity, increases insulin sensitivity, and alters hypothalamic monoamines in rats. *Obesity*, *14*, 1905–1913.
427. Atkinson, R. L., Dhurandhar, N. V., Allison, D. B., et al. (2005). Human adenovirus-36 is associated with increased body weight and paradoxical reduction of serum lipids. *Int J Obes*, *29*, 281–286.
428. Turnbaugh, P. J., Ley, R. E., Mahowald, M. A., et al. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature*, *444*, 1027–1031.
429. Krajmalnik-Brown, R., Ilhan ZE, Kang, D. W., & Dibaise, J. K. (2012). Effects of gut microbes on nutrient absorption and energy regulation. *Nutr Clin Pract*, *27*, 201–214.
430. Rogers, P. C., Meacham, L. R., Oeffinger, K. C., et al. (2005). Obesity in pediatric oncology. *Pediatr Blood Cancer*, *45*, 881–891.
431. Foster, B. J., Shults, J., Zemel, B. S., & Leonard, M. B. (2006). Risk factors for glucocorticoid-induced obesity in children with steroid-sensitive nephrotic syndrome. *Pediatr Nephrol*, *21*, 973–980.
432. Bonny, A. E., Ziegler, J., Harvey, R., et al. (2006). Weight gain in obese and nonobese adolescent girls initiating depot medroxyprogesterone, oral contraceptive pills, or no hormonal contraceptive method. *Arch Pediatr Adol Med*, *160*, 40–45.
433. Quinn, M., Ficociello, L. H., & Rosner, B. (2003). Change in glycemic control predicts change in weight in adolescent boys with type 1 diabetes. *Pediatr Diabetes*, *4*, 162–167.

434. Vieweg, W. V., Sood, A. B., Pandurangi, A., & Silverman, J. J. (2005). Newer antipsychotic drugs and obesity in children and adolescents: how should we assess drug-associated weight gain? *Acta Psychiatr Scand*, *111*, 177–184.
435. Amamoto, T., Kumai, T., Nakaya, S., et al. (2006). The elucidation of the mechanism of weight gain and glucose tolerance abnormalities induced by chlorpromazine. *J Pharmacol Sci*, *102*, 213–219.
436. Draper, N., Echwald, S. M., Lavery, G. G., et al. (2002). Association studies between microsatellite markers with the gene encoding human 11β-hydroxysteroid dehydrogenase type 1 and body mass index, waist to hip ratio, and glucocorticoid metabolism. *J Clin Endocrinol Metab*, *87*, 4984–4990.
437. Tomlinson, J. W., Sinha, N., Bujalska, I., et al. (2002). Expression of 11beta-hydroxysteroid dehydrogenase type 1 in adipose tissue is not increased in human obesity. *J Clin Endocrinol Metab*, *87*, 5630–5635.
438. Kaartinen, J. M., Kaar, M. L., & Ohisalo, J. J. (1994). Defective stimulation of adipocyte adenylate cyclase, blunted lipolysis, and obesity in pseudohypoparathyroidism 1a. *Pediatr Res*, *35*, 594–597.
439. Farooqi, I. S. (2008). Mongenic human obesity. *Front Horm Res*, *36*, 1–11.
440. Farooqi, I. S., & O'Rahilly, S. (2006). Genetics of obesity in humans. *Endocr Rev*, *27*, 710–718.
441. Montague, C. T., Farooqi, I. S., Whitehead, J. P., et al. (1997). Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature*, *387*, 903–908.
442. Farooqi, I. S., Matarese, G., Lord, G. M., et al. (2002). Beneficial effects of leptin on obesity, T-cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. *J Clin Invest*, *110*, 1093–1103.
443. Farooqi, I. S., Keogh, J. M., Kamath, S., et al. (2001). Partial leptin deficiency and human adiposity. *Nature*, *414*, 34–35.
444. Clement, K., Vaisse, C., Lahlou, N., et al. (1998). A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature*, *392*, 398–401.
445. Farooqi, I. S., Wangensteen, T., Collins, S., et al. (2007). Clinical and molecular genetic spectrum of congenital deficiency of the leptin receptor. *N Engl J Med*, *356*, 237–247
446. Krude, H., Biebermann, H., Luck, W., et al. (1998). Severe early-onset obesity, adrenal insufficiency, and red hair pigmentation caused by POMC mutations in humans. *Nat Genet*, *19*, 155–157.
447. Farooqi, I. S., Drop, S., Clements, A., et al. (2006). Heterozygosity for a POMC-null mutation and increased obesity risk in humans. *Diabetes*, *55*, 2549–2553.
448. Jackson, R. S., Creemers, J. W., Ohagi, S., et al. (1997). Obesity and impaired prohormone processing associated with mutations in the prohormone convertase 1 gene. *Nat Genet*, *16*, 303–306.
449. Lee, Y. S., Poh, L. K. S., & Loke, K. Y. (2002). A novel melanocortin-3 receptor gene (MC3R) mutation associated with severe obesity. *J Clin Endocrinol Metab*, *87*, 1423–1426.
450. Vaisse, C., Clement, K., Durand, E., et al. (2000). Melanocortin-4 receptor mutations are a frequent and heterogeneous cause of morbid obesity. *J Clin Invest*, *106*, 253–262.
451. Farooqi, I. S., Keogh, J. M., Yeo, G. S., et al. (2003). Clinical spectrum of obesity and mutations in the melanocortin 4 receptor gene. *N Engl J Med*, *348*, 1085–1095.
452. Greenfield, J. R., Miller, J. W., Keogh, J. M., et al. (2009). Modulation of blood pressure by central melanocortinergic pathways. *N Engl J Med*, *360*, 44–52.
453. Kublaoui, B. M., Holder, J. L., Gemelli, T., & Zinn, A. R. (2006). Sim1 haploinsufficiency impairs melanocortin-mediated anorexia and activation of paraventricular nucleus neurons. *Mol Endocrinol*, *20*, 2483–2492.
454. Holder, J. L., Butte, N. F., Zinn, A. R. (2000). Profound obesity associated with a balanced translocation that disrupts the SIM1 gene. *Hum Mol Genet*, *9*, 101–108.
455. Hung, C. C., Luan, J., Sims, M., et al. (2006). Studies of the SIM1 gene in relation to human obesity and obesity-related traits. *Int J Obes*, *31*, 429–434.
456. Walters, R. G., Jacquemont, S., Valsesia A, et al. (2010). A new highly penetrant form of obesity due to deletions on chromosome 16p11.2. *Nature*, *463*, 671–675.
457. Han, J. C., Liu, Q. R., Jones, M., et al. (2008). Brain-derived neurotrophic factor and obesity in the WAGR syndrome. *N Engl J Med*, *359*, 918–927.
458. Gunay-Aygun, M., Cassidy, S. B., & Nicholls, R. D. (1997). Prader-Willi and other syndromes associated with obesity and mental retardation. *Behav Genet*, *27*, 307–324.
459. Cassidy, S. B. (1997). Prader-Willi syndrome. *J Med Genet*, *34*, 917–923.
460. Bekx, M. T., Carrel, A. L., Shriver, T. C., et al. (2003). Decreased energy expenditure is caused by abnormal body composition in infants with Prader-Willi syndrome. *J Pediatr*, *143*, 372–376.
461. Cummings, D. E., Clement, K., Purnell, J. Q., et al. (2002). Elevated plasma ghrelin levels in Prader-Willi syndrome. *Nat Med*, *8*, 643–644.
462. Carrel, A. L., Myers, S. E., Whitman, B. Y., & Allen, D. B. (2002). Benefits of long-term GH therapy in Prader-Willi syndrome: a 4-year study. *J Clin Endocrinol Metab*, *87*, 1581–1585.
463. Mykytyn, K., Nishimura, D. Y., Searby, C. C., et al. (2002). Identification of the gene (BBS1) most commonly involved in Bardet-Biedl, syndrome, a complex human obesity syndrome. *Nat Genet*, *31*, 435–438.
464. Mykytyn, K., Mullins, R. F., Andrews, M., et al. (2004). Bardet-Biedl syndrome type 4 (BBS4)-null mice implicate Bbs4 in flagella formation but not global cilia assembly. *Proc Natl Acad Sci USA*, *101*, 8664–8669.
465. Badano, J. L., Mitsuma, N., Beales, P. L., & Katsanis, N. (2006). The ciliopathies: an emerging class of human genetic disorders. *Ann Rev Genomics Hum Genet*, *22*, 125–148.
466. Yeo, G. S. H., Hung, C. C. C., Rochford, J., et al. (2004). A de novo mutation affecting human TrkB associated with severe obesity and developmental delay. *Nat Neurosci*, 7, 1187–1189.
467. Gray, J., Yeo, G. S., Cox, J. J., et al. (2006). Hyperphagia, severe obesity, impaired cognitive function, and hyperactivity associated with functional loss of one copy of the brain-derived neurotrophic factor (BDNF) gene. *Diabetes*, *55*, 3366–3371.
468. Chandler, K. E., Kidd, A., Al-Gazali, L., et al. (2003). Diagnostic criteria, clinical characteristics, and natural history of Cohen syndrome. *J Med Genet*, *40*, 233–241.
469. Collin, G. B., Marshall, J. D., Ikeda, A., et al. (2002). Mutations in ALMS1 cause obesity, type 2 diabetes, and neurosensory degeneration in Alstrom syndrome. *Nat Genet*, *31*, 74–78.
470. Lower, K. M., Turner, G., Kerr, B. A., et al. (2002). Mutations in PHF6 are associated with Borjeson-Forssman-Lehmann syndrome. *Nat Genet*, *32*, 661–665.
471. Ize-Ludlow, D., Gray, J. A., Sperling, M. A., et al. (2007). Rapid-onset obesity with hypothalamic dysfunction, hypoventilation, and autonomic dysregulation presenting in childhood. *Pediatrics*, *120*, e179–e188.
472. Jeanrenaud, B. (1985). An hypothesis on the aetiology of obesity: dysfunction of the central nervous system as a primary cause. *Diabetologia*, *28*, 502–513.
473. Satoh, N., Ogawa, Y., Katsura, G., et al. (1997). Pathophysiological significance of the obese gene product, leptin in ventromedial hypothalamus (VMH)-lesioned rats: evidence for loss of its satiety effect in VMH-lesioned rats. *Endocrinology*, *138*, 947–954.
474. Bray, G. A., Inoue, S., & Nishizawa, Y. (1981). Hypothalamic obesity. *Diabetologia* 20, 366–377.
475. Bray, G. A., & Nishizawa, Y. (1978). Ventromedial hypothalamus modulates fat mobilization during fasting. *Nature*, *274*, 900–902.
476. Sklar, C. A. (1994). Craniopharyngioma: endocrine sequelae of treatment. *Pediatr Neurosurg*, *21*, 120–123.
477. Bray, G. A. (1984). Syndromes of hypothalamic obesity in man. *Pediatr Ann*, *13*, 525–536.
478. Daousi, C., Dunn, A. J., Foy, P. M., et al. (2005). Endocrine and neuroanatomic predictors of weight gain and obesity in adult patients with hypothalamic damage. *Am J Med*, *118*, 45–50.
479. Lustig, R. H. (2002). Hypothalamic obesity: the sixth cranial endocrinopathy. *Endocrinologist*, *12*, 210–217.
480. Harz, K. J., Muller, H. L., Waldeck, E., et al. (2003). Obesity in patients with craniopharyngioma: assessment of food intake and movement counts indicating physical activity. *J Clin Endocrinol Metab*, *88*, 5227–5231.
481. Lustig, R. H., Post, S. M., Srivannaboon, K., et al. (2003). Risk factors for the development of obesity in children surviving brain tumors. *J Clin Endocrinol Metab*, *88*, 611–616.

482. Reeves, A. G., & Plum, F. (1972). Hyperphagia, rage, and dementia accompanying a ventromedial hypothalamic neoplasm. *Arch Neurol*, *20*, 616–624.
483. Schofl, C., Schleth, A., Berger, D., et al. (2002). Sympathoadrenal counterregulation in patients with hypothalamic craniopharyngioma. *J Clin Endocrinol Metab*, *87*, 624–629.
484. Coutant, R., Maurey, H., Rouleau, S., et al. (2003). Defect in epinephrine production in children with craniopharyngioma: functional or organic origin? *J Clin Endocrinol Metab*, *88*, 5969–5975.
485. Shaikh, M. G., Grundy, R. G., & Kirk, J. M. (2008). Reductions in basal metabolic rate and physical activity contribute to hypothalamic obesity. *J Clin Endocrinol Metab*, *93*, 2588–2593.
486. Lee, H. C., Curry, D. L., & Stern, J. S. (1989). Direct effect of CNS on insulin hypersecretion in obese Zucker rats: involvement of vagus nerve. *Am J Physiol*, *256*, E439–E444.
487. Preeyasombat, C., Bacchetti, P., Lazar, A. A., & Lustig, R. H. (2005). Racial and etiopathologic dichotomies in insulin secretion and resistance in obese children. *J Pediatr*, *146*, 474–481.
488. Tokunaga, K., Fukushima, M., Kemnitz, J. W., & Bray, G. A. (1986). Effect of vagotomy on serum insulin in rats with paraventricular or ventromedial hypothalamic lesions. *Endocrinology*, *119*, 1708–1711.
489. Inoue, S., & Bray, G. A. (1997). The effect of subdiaphragmatic vagotomy in rats with ventromedial hypothalamic lesions. *Endocrinology*, *100*, 108–114.
490. Smith, D. K., Sarfeh, J., & Howard, L. (1983). Truncal vagotomy in hypothalamic obesity. *Lancet*, *1*, 1330–1331.
491. Weiss, R., Dziura, J., Burgert, T. S., et al. (2004). Obesity and the metabolic syndrome in children and adolescents. *N Engl J Med*, *350*, 2362–2374.
492. Sakul, H., Pratley, R., Cardon, L., et al. (1997). Familiarity of physical and metabolic characteristics that predict the development of non-insulin-dependent diabetes mellitus in Pima Indians. *Am J Hum Genet*, *60*, 651–656.
493. Kissebah, A., & Krakower, R. (1994). Regional adiposity and mortality. *Physiol Rev*, *74*, 791–811.
494. Bodkin, N. L., Hannah, J. S., Ortmeyer, H. K., & Hansen, B. C. (1993). Central obesity in rhesus monkeys: association with hyperinsulinemia, insulin resistance, and hypertriglyceridemia? *Int J Obes*, *17*, 53–61.
495. Mukhtar, Q., Cleverly, G., Voorhees, R. E., & McGrath, J. W. (2001). Prevalence of acanthosis nigricans and its association with hyperinsulinemia in New Mexico adolescents. *J Adolesc Health*, *28*, 372–376.
496. Cruz, P., & Hud, J. (1992). Excess insulin binding to insulin-like growth factor receptors: proposed mechanism for acanthosis nigricans. *J Investig Dermatol*, *98*(Suppl.), 82S–85S.
497. Odeleye, O. E., de Courten, M., Pettitt, D. J., & Ravussin, E. (1997). Fasting hyperinsulinemia is a predictor of increased body weight gain and obesity in Pima Indian children. *Diabetes*, *46*, 1341–1345.
498. Maffeis, C., Moghetti, P., Grezzani, A., et al. (2002). Insulin resistance and the persistence of obesity from childhood into adulthood. *J Clin Endocrinol Metab*, *87*, 71–76.
499. Luepker, R. V., Perry, C. L., McKinlay, S. M., et al. (1996). Outcomes of a field trial to improve children's dietary patterns and physical activity. The Child and Adolescent Trial for Cardiovascular Health, CATCH collaborative group. *JAMA*, *275*, 768–776.
500. Davis, S. M., Going, S. B., Helitzer, D. L., et al. (1999). Pathways: a culturally appropriate obesity-prevention program for American Indian schoolchildren. *Am J Clin Nutr*, *64*, 796S–802S.
501. Sinaiko, A. R., & Caprio, S. (2012). Insulin resistance. *J Pediatr*, *1*, 11-15.
502. Epstein, L. H., Valoski, S., Wing, R., & McCurley, J. (1990). Ten-year follow-up of behavioral, family-based treatment for obese children. *JAMA*, *264*, 2519–2523.
503. Knowler, W. C., Barrett-Connor, E., Fowler, S. E., et al. (2002). Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med*, *346*, 393–403.
504. Oude Luttikhuis, H., Baur, L., Jansen, H., et al. (2009). Interventions for treating obesity in children. *Cochrane Database Syst Rev*, *1*, CD001872.
505. Flodmark, C. E., Marcus, C., & Britton, M. (2006). Interventions to prevent obesity in children and adolescents: a systematic literature review. *Int J Obes*, *30*, 579–589.
506. Sumithran, P., Prendergast, L. A., Delbridge, E., et al. (2011). Long-term persistence of hormonal adaptations to weight loss. *N Engl J Med*, *365*, 1597–1604.
507. Waters, E., de Silva-Sanigorski, A., Hall, B. J., et al. (2011). Interventions for preventing obesity in children. *Cochrane Database Syst Rev*, *12*, CD001871.
508. Assessment of childhood and adolescent obesity. Results from an international obesity task force workshop, Dublin, June 16, 1997. (1999). *Am J Clin Nutr*, *70*, 117S–175S.
509. Barlow, S. E., & Dietz, W. H. (1999). Obesity evaluation and treatment: expert committee recommendations. The Maternal and Child Health Bureau, Health Resources and Services Administration and the Department of Health and Human Services. *Pediatrics*, *102*, e29.
510. Baranowski, T., Mendelein, J., Resnicow, K., et al. (2000). Physical activity and nutrition in youthL an overview of obesity prevention. *Prev Med*, *31*, S1–S10.
511. Sothern, M. S., Schumacher, H., von Almen, T. K., et al. (2002). Committed to kids: an integrated, 4-level team approach to weight management in adolescents. *J Am Diet Assoc*, *102*, S81–S85.
512. Epstein, L. H., Roemmich, J. N., & Raynor, H. A. (2001). Behavioral therapy in the treatment of pediatric obesity. *Pediatr Clin North Am*, *48*, 981–993.
513. Resnicow, K., Davis, R., & Rollnick, S. (2006). Motivational interviewing for pediatric obesity: conceptual issues and evidence review. *J Am Diet Assoc*, *106*, 2024–2033.
514. Schwartz, R. P. (2010). Motivational interviewing (patient-centered counseling) to address childhood obesity. *Pediatr Annals*, *39*, 154–158.
515. James, J., Thomas, P., Cavan, D., & Kerr, D. (2004). Preventing childhood obesity by reducing consumption of carbonated drinks: cluster randomised controlled trial. *BMJ*, *328*, 1237.
516. Ebbeling, C. B., Feldman, H. A., Osganian, S. K., et al. (2006). Effects of decreasing sugar-sweetened beverage consumption on body weight in adolescents: a randomized, controlled pilot study. *Pediatrics*, *117*, 673–680.
517. Ebbeling, C. B., Leidig, M. M., Feldman, H. A., et al. (2007). Effects of a low-glycemic load vs low-fat diet in obese young adults: a randomized trial. *JAMA*, *297*, 2092–2102.
518. Ebbeling, C. B., Swain, J. F., Feldman, H. A., et al. (2012). Effects of dietary composition on energy expenditure during weight-loss maintenance. *JAMA*, *307*, 2627–2634.
519. Campbell, K., Waters, E., O'Meara, S., & Summerbell, C. (2001). Interventions for preventing obesity in childhood: a systematic review. *Obes Rev*, *2*, 149–157.
520. Dietz, W. H., & Robinson, T. N. (2005). Overweight children and adolescents. *N Engl J Med*, *352*, 2100–2109.
521. Nuutinen, O. (1991). Long-term effects of dietary counseling on nutrient intake and weight loss in obese children. *Eur J Clin Nutr*, *45*, 287–297.
522. Stice, E., Cameron, R., Killen, J., et al. (1999). Naturalistic weight reduction efforts prospectively predict growth in relative weight and onset of obesity among female adolescents. *J Consult Clin Psychol*, *67*, 967–974.
523. Klem, M. L., Wing, R. R., McGuire, M. T., et al. (1997). A descriptive study of individuals successful at long-term maintenance of substantial weight loss. *Am J Clin Nutr*, *66*, 239–246.
524. Ravussin, E., & Danforth, E. (1999). Beyond sloth-physical activity and weight gain. *Science*, *283*, 184–185.
525. Sothern, M. (2001). Exercise as a modality in the treatment of childhood obesity. *Pediatr Clin North Am*, *48*, 931–945.
526. Gutin, B., Barbeau, P., Owens, S., et al. (2002). Effects of exercise intensity on cardiovascular fitness, total body composition, and visceral adiposity of obese adolescents. *Am J Clin Nutr*, *75*, 818–826.
527. McMurray, R. G., Bauman, M. J., Harrell, J. S., et al. (2000). Effects of improvement in aerobic power on resting insulin and glucose concentrations in children. *Eur J Appl Physiol*, *81*, 132–139.
528. McKechnie, R., & Mosca, L. (2003). Physical activity and coronary heart disease: prevention and effect on risk factors. *Cardiol Rev*, *11*, 21–25.

529. Ferguson, M. A., Gutin, B., Le, N. A., et al. (1999). Effects of exercise training and its cessation on components of the insulin resistance syndrome in obese children. *Int J Obes Relat Metab Disord*, *22*, 889–895.
530. Nemet, D., Barkan, S., Epstein, Y., et al. (2005). Short- and long-term beneficial effects of a combined dietary-behavioral-physical activity intervention for the treatment of childhood obesity. *Pediatrics*, *115*, e443–e449.
531. McMurray, R. G., Harrell, J. S., Bangdiwala, S. I., et al. (2002). A school-based intervention can reduce body fat and blood pressure in young adolescents. *J Adolesc Health* 31, 125–132.
532. Trost, S. G., Pate, R. R., Ward, D. S., et al. (1999). Correlates of objectively measured physical activity in preadolescent youth. *Am J Prev Med*, *17*, 120–126.
533. McGovern, L., Johnson, J. N., Paulo, R., et al. (2007). Clinical review: treatment of pediatric obesity: a systematic review and meta-analysis of randomized trials. *J Clin Endocrinol Metab*, *93*, 4600–4605.
534. Yanovski, S. Z., & Yanovski, J. A. (2002). Drug therapy: obesity. *N Engl J Med*, *346*, 591–602.
535. Bray, G. A., & Greenway, F. L. (1992). Current and potential drugs for treatment of obesity. *Endocr Rev*, *20*, 805–875.
536. Abenhaim, L., Moride, Y., Brenot, F., et al. (1996). Appetite-suppressant drugs and risk of primary pulmonary hypertension. International pulmonary hypertension study group. *N Engl J Med*, *335*, 609–616.
537. Jick, H., Vasilakis, C., Weinrauch, L. A., et al. (1998). A population-based study of appetite-suppressant drugs and the risk of cardiac valve regurgitation. *N Engl J Med*, *339*, 719–724.
538. Heal, D. J., Gosden, J., & Smith, S. L. (2012). What is the prognosis for new centrally-acting anti-obesity drugs? *Neuropharmacology*, *63*, 132–146
539. Morgan, J. P., & Funderburk, F. R. (1992). Phenylpropanolamine and blood pressure: a review of prospective studies. *Am J Clin Nutr*, *55*, 206S–210S.
540. Mittendorfer, B., Ostlund, R. J., Patterson, B., & Klein, S. Z. (2001). Orlistat inhibits dietary cholesterol absorption. *Obes Res*, *9*, 599–604.
541. Guerciolini, R., Radu-Radulescu, L., Boldrin, M., et al. (2001). Comparative evaluation of fecal fat excretion induced by orlistat and chitosan. *Obes Res*, *9*, 364–367.
542. Zhi, J., Melia, A. T., Guercioloni, R., et al. (1994). Retrospective population-based analysis of the dose-response (fecal fat excretion) relationship of orlistat in normal and obese volunteers. *Clin Pharmcol Therap*, *56*, 82–85.
543. Reitman, J. B., Castro-Cabezas, M., de Bruin, T. W., & Erkelens, D. W. (1994). Relationship between improved postprandial lipemia and low-density lipoprotein metabolism during treatment with tetrahydrolipstatin, a pancreatic lipase inhibitor. *Metabolism*, *43*, 293–298.
544. Maahs, D., de Serna, D. G., Kolotkin, R. L., et al. (2006). Randomized, double-blind, placebo-controlled trial of orlistat for weight loss in adolescents. *Endocr Pract*, *12*, 18–28.
545. Chanoine, J. P., Hampl, S., Jensen, C., et al. (2005). Effects of orlistat on weight and body composition in obese adolescents: a randomized controlled trial. *JAMA*, *293*, 2873–2883.
546. McDuffie, J. R., Calis, K. A., Booth, S. L., et al. (2002). Effects of orlistat on fat-soluble vitamins in obese adolescents. *Pharmacotherapy*, *22*, 814–822.
547. Davidson, M. B., & Peters, A. L. (1997). An overview of metformin in the treatment of type 2 diabetes mellitus. *Am J Med*, *102*, 99–110.
548. Stumvoll, M., Nurjhan, N., Perriello, G., et al. (1995). Metabolic effects of metformin in non-insulin-dependent diabetes mellitus. *N Engl J Med*, *333*, 550–554.
549. DeFronzo, R. A., & Goodman, A. M. (1995). Efficacy of metformin in patients with non-insulin-dependent diabetes mellitus. *N Engl J Med*, *333*, 541–549.
550. Jones, K. L., Arslanian, S., Peterokova, V. A., et al. (2002). Effect of metformin in pediatric patients with type 2 diabetes. *Diabetes Care*, *25*, 89–94.
551. Diabetes Prevention Program Research Group (2002). Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med*, *346*, 393–403.
552. Mogul, H. R., Peterson, S. J., Weinstein, B. I., et al. (2001). Metformin and carbohydrate-modified diet: a novel obesity treatment protocol: preliminary findings from a case series of nondiabetic women with midlife weight gain and hyperinsulinemia. *Heart Dis*, *3*, 285–292.
553. Lee, A., & Morley, J. E. (1998). Metformin decreases food-consumption and induces weight-loss in subjects with obesity with type-II non-insulin-dependent diabetes. *Obes Res*, *6*, 47–53.
554. Paolisso, G., Amato, L., Eccellente, R., et al. (1998). Effect of metformin on food intake in obese subjects. *Eur J Clin Invest*, *28*, 441–446.
555. Bailey, C. J., & Turner, R. C. (1996). Metformin. *N Engl J Med*, *334*, 574–579.
556. Lenhard, J. M., Kliewer, S. A., Paulik, M. A., et al. (1997). Effects of troglitazone and metformin on glucose and lipid metabolism: alterations of two distinct molecular pathways. *Biochem Pharmacol*, *54*, 801–808.
557. Zhou, G., Myers, R., Li, Y., et al. (2001). Role of AMP-activated protein kinase in mechanism of metformin action. *J Clin Invest*, *108*, 1167–1174.
558. Kumar, N., & Dey, C. S. (2002). Metformin enhances insulin signalling in insulin-dependent and -independent pathways in insulin resistant muscle cells. *Br J Pharmacol*, *137*, 329–336.
559. Mannucci, E., Ognibene, A., Cemasco, F., et al. (2001). Effect of metformin on glucagon-like peptide-1 (GLP-1) and leptin levels in obese nondiabetic subjects. *Diabetes Care*, *24*, 489–494.
560. Freemark, M., & Bursey, D. (2001). The effects of metformin on body mass index and glucose tolerance in obese adolescents with fasting hyperinsulinemia and a family history of type 2 diabetes. *Pediatrics*, *107*, e55.
561. Wilson, D. M., Abrams, S. H., Aye, T., et al. (2010). Metformin extended release treatment of adolescent obesity: a 48-week randomized, double-blind, placebo-controlled trial with 48-week follow-up. *Arch Pediatr Adol Med*, *164*, 116–123.
562. Velasquez, E. M., Mendoza, S., Hamer, T., et al. (1994). Metformin therapy in polycystic ovarian syndrome reduces hyperinsulinemia, insulin resistance, hyperandrogenemia, and systolic blood pressure, while facilitating normal menses and pregnancy. *Metabolism*, *43*, 647–654.
563. Pasquali, R., Gambineri, A., Biscotti, D., et al. (2000). Effect of long-term treatment with metformin added to hypocaloric diet on body composition, fat distribution, and androgen and insulin levels in abdominally obese women with and without the polycystic ovary syndrome. *J Clin Endocrinol Metab*, *85*, 2767–2774.
564. Jamieson, M. A. (2002). The use of metformin in adolescents with polycystic ovary syndrome: for and against. *J Pediatr Adolesc Gynecol*, *15*, 109–114.
565. Ibanez, L., Valls, C., Potau, N., et al. (2000). Sensitization to insulin in adolescent girls to normalize hirsutism, hyperandrogenism, oligomenorrhea, dyslipidemia, and hyperinsulinism after precocious adrenarche. *J Clin Endocrinol Metab*, *85*, 3526–3530.
566. Marchesini, G., Brizi, M., Bianchi, G., et al. (2001). Metformin in non-alcoholic steatohepatitis. *Lancet*, *358*, 893–894.
567. Schwimmer, J. B., Middleton, M. S., Deutsch, R., & Lavine, J. E. (2005). A phase 2 trial of metformin as a treatment for non-diabetic paediatric non-alcoholic steatohepatitis. *Aliment Pharmacol Ther*, *21*, 871–879.
568. Morrison, J. A., Cottingham, E. M., & Barton, B. A. (2002). Metformin for weight loss in pediatric patients taking psychotropic drugs. *Am J Psychiatr*, *159*, 655–657.
569. Silverman, B., Franklin, G. M., & Bolin, R. (1997). Lactic acidosis traced to thiamine deficiency related to nationwide shortage of multivitamins for total parenteral nutrition. *MMWR*, *46*, 523–528.
570. Bray, G. A., & Gallagher, T. F. (1975). Manifestations of hypothalamic obesity in man: a comprehensive investigation of eight patients and a review of the literature. *Medicine*, *54*, 301–333.
571. Velasquez-Mieyer, P. A., Cowan, P. A., Buffington, C. K., et al. (2003). Suppression of insulin secretion promotes weight loss and alters macronutrient preference in a subset of obese adults. *Int J Obes*, *27*, 219–226.
572. Hsu, W. H., Xiang, H. D., Rajan, A. S., et al. (1991). Somatostatin inhibits insulin secretion by a G-protein-mediated decrease in Ca^{2+} entry through voltage-dependent Ca^{2+} channels in the beta-cell . *J Biol Chem*, *266*, 837–843.

573. Mitra, S. W., Mezey, E., Hunyady, B., et al. (1993). Colocalization of somatostatin receptor sst5 and insulin in rat pancreatic β-cells. *Endocrinology*, *140*, 3790–3796.
574. Bertoli, A., Magnaterra, R., Borboni, P., et al. (1998). Dose-dependent effect of octreotide on insulin secretion after OGTT in obesity. *Horm Res*, *49*, 17–21.
575. Krentz, A. J., MacDonald, L. M., & Schade, D. S. (1994). Octreotide: a long-acting inhibitor of endogenous hormone secretion for human metabolic investigations. *Metabolism*, *43*, 24–31.
576. Lamberts, S. W. J., Van Der Lely, A. J., De Herder, W. W., & Hofland, L. J. (1996). Drug therapy: octreotide. *N Engl J Med*, *334*, 246–254.
577. Tauber, M. T., Harris, A. G., & Rochiccioli, P. (1994). Clinical use of the long-acting somatostatin analog octreotide in pediatrics. *Eur J Pediatr*, *153*, 304–310.
578. Farooqi, I. S., Jebb, S. A., Langmack, G., et al. (1999). Effects of recombinant leptin therapy in a child with congenital leptin deficiency. *N Engl J Med*, *341*, 913–915.
579. Gibson, W. T., Farooqi, I. S., Moreau, M., et al. (2004). Congenital leptin deficiency due to homozygosity for the d1333G mutation: report of another case and evaluation of response to four years of leptin therapy. *J Clin Endocrinol Metab*, *89*, 4821–4826.
580. Shi, Z. Q., Chinookoswong, N., Wang, J. L., et al. (2002). Additive effects of leptin and oral anti-obesity drugs in treating diet-induced obesity in rats. *Diabetes*, *51*(Suppl. 2), 1707.
581. Carrel, A. L., Moerchen, V., Myers, S. E., et al. (2004). Growth hormone improves mobility and body composition in infants and toddlers with Prader-Willi syndrome. *J Pediatr*, *145*, 744–749.
582. Kaplowitz, P. B., Rundle, A. C., & Blethen, S. L. (1998). Weight relative to height before and during growth hormone therapy in prepubertal children. *Horm Metab Res*, *30*, 565–569.
583. Simsolo, R. B., Ezzat, S., Ong, J. M., et al. (1995). Effects of acromegaly treatment and growth hormone on adipose tissue lipoprotein lipase. *J Clin Endocrinol Metab*, *80*, 3233–3238.
584. Williams, T., Berelowitz, M., Joffe, S. N., et al. (1984). Impaired growth hormone responses to growth hormone-releasing factor in obesity: a pituitary defect reversed with weight reduction. *N Engl J Med*, *311*, 1403–1407.
585. Jorgensen, J. O., Pedersen, S. B., Borglum, J., et al. (1994). Fuel metabolism, energy expenditure, and thyroid function in growth hormone-treated obese women: a double-blind, placebo-controlled study. *Metabolism*, *43*, 872–877.
586. Torekov, S. S., Madsbad, S., & Holst, J. J. (2011). Obesity—an indication for GLP-1 treatment? Obesity pathophysiology and GLP-1 treatment potential. *Obes Rev*, *12*, 593–601.
587. Teter, C. J., Early, J. J., & Gibbs, C. M. (2000). Treatment of affective disorder and obesity with topiramate. *Ann Pharmacother*, *34*, 1262–1265.
588. Wilkes, J. J., Nelson, E., Osborne, M., et al. (2005). Topiramate is an insulin-sensitizing compound in vivo with direct effects on adipocytes in female ZDF rats. *Am J Physiol Endocrinol Metab*, *288*, E617–E624.
589. Wilding, J., Van Gaal, L., Rissanen, A., et al. (2004). A randomized double-blind placebo-controlled study of the long-term efficacy and safety of topiramate in the treatment of obese subjects. *Int J Obesity*, *28*, 1399–1410.
590. Wynne, K., Park, A. J., Small, C. J., et al. (2005). Subcutaneous oxyntomodulin reduces body weight in overweight and obese subjects: a double-blind, randomized, controlled trial. *Diabetes*, *54*, 2390–2395.
591. Michalsky, M., Kramer, R. E., Fullmer, M. A., et al. (2011). Developing criteria for pediatric/adolescent bariatric surgery programs. *Pediatrics*, *128*(Suppl. 2), S65–S70.
592. Strauss, R. (2002). Perspectives on childhood obesity. *Curr Gastroenterol Rep*, *4*, 244–250.
593. Inge, T. H., Krebs, N. F., Garcia, V. F., et al. (2004). Bariatric surgery for overweight adolescents: concerns and recommendations. *Pediatrics*, *114*, 217–223.
594. Whitaker, R. C., Wright, J. A., Pepe, M. S., et al. (1997). Predicting obesity in young adulthood from childhood and parental obesity. *N Engl J Med*, *337*, 869–873.
595. Inge, T. H., Xanthakos, S. A., & Zeller, M. H. (2007). Bariatric surgery for pediatric extreme obesity: now or later? *Int J Obes*, *31*, 1–14.
596. Apovian, C. M., Baker, C., Ludwig, D. S., et al. (2005). Best practice guidelines in pediatric/adolescent weight loss surgery. *Obes Res*, *13*, 274–282.
597. Mun, E. C., Blackburn, G. L., & Matthews, J. B. (2001). Current status of medical and surgical therapy for obesity. *Gastroenterology*, *120*, 669–681.
598. O'Brien, P. E., Sawyer, S. M., Laurie, C., et al. (2010). Laparoscopic adjustable gastric banding in severely obese adolescents: a randomized trial. *JAMA*, *303*, 519–526.
599. Boza, C., Muñoz, R., Salinas, J., et al. (2011). Safety and efficacy of Roux-en-Y gastric bypass to treat type 2 diabetes mellitus in non-severely obese patients. *Obes Surg*, *21*, 1330–1336.
600. Sjostrom, C. D., Peltonen, M., & Sjostrom, L. (2002). Effects of 2 and 10 years weight loss retention on cardiovascular risk factors. *Int J Obes*, *26*, S218, 865.
601. Shah, M., Simha, V., & Garg, A. (2006). Review: long-term impact of bariatric surgery on body weight, co-morbidities, and nutritional status. *J Clin Endocrinol Metab*, *91*, 4223–4231.
602. Yanovski, J. A. (2001). Intensive therapies for pediatric obesity. *Pediatr Clin North Am*, *48*, 1041–1053.
603. Strauss, R. S., Bradley, L. J., & Brolin, R. E. (2001). Gastric bypass surgery in adolescents with morbid obesity. *J Pediatr*, *138*, 499–504.
604. Sugerman, H. J., Sugerman, E. L., DeMaria, E. J., et al. (2003). Bariatric surgery for severely obese adolescents. *J Gastrointest Surg*, *7*, 102–107.
605. Lawson, M. L., Kirk, S., Mitchell, T., et al. (2006). One-year outcomes of Roux-en-Y gastric bypass for morbidly obese adolescents: a multicenter study from the Pediatric Bariatric Study Group. *Ped Surg*, *41*, 137–143.
606. Kohli, R., Stefater, M. A., Inge, & T. H. (2011). Molecular insights from bariatric surgery. *J Rev Endocrino Metab Disord*, *12*, 211–217.
607. Towbin, S., Inge, T. H., Garcia, V. F., et al. (2004). Beriberi after gastric bypass surgery in adolescence. *J Pediatr*, *145*, 263–267.
608. Franco, J. V., Ruiz, P. A., Palermo M, & Gagner, M. (2011). A review of studies comparing three laparoscopic procedures in bariatric surgery: sleeve gastrectomy, Roux-en-Y gastric bypass and adjustable gastric banding. *Obes Surg*, *21*, 1458–1468.
609. Mason, E. E., Scott, D. H., Doherty, C., et al. (1995). Vertical banded gastroplasty in the severely obese under age twenty-one. *Obes Surg*, *5*, 23–33.
610. Greenstein, R. J., & Rabner, J. G. (1995). Is adolescent gastric-restrictive anti-obesity surgery warranted? *Obes Surg*, *5*, 138–144.
611. Breaux, C. W. (1995). Obesity surgery in children. *Obes Surg*, *5*, 279–284.
612. Nguyen, N. T., Paya, M., Stevens, M., et al. (2004). The relationship between hospital volume and outcome in bariatric surgery at academic medical centers. *Ann Surg*, *240*, 586–594.
613. Zingmond, D. S., McGory, M. L., & Ko, C. Y. (2005). Hospitalization before and after gastric bypass surgery. *JAMA*, *294*, 1918–1924.
614. Ray, R. M., & Senders, C. W. (2001). Airway management in the obese child. *Pediatr Clin North Am*, *48*, 1055–1063.
615. DeBoer, M. D., & Marks, D. L. (2006). Therapy insight: use of melanocortin antagonists in the treatment of cachexia in chronic disease. *Nat Clin Pract Endocrinol Metab*, *2*, 459–466.
616. Walsh, D., & Nelson, K. A. (2002). Autonomic nervous system dysfunction in advanced cancer. *Support Care Cancer*, *10*, 523–528.
617. Barber, M. D., Ross, J. A., & Fearon, K. C. (2000). Disordered metabolic response with cancer and its management. *World J Surg*, *24*, 681–689.
618. Gahagan, S., & Holmes, R. (1998). A stepwise approach to evaluation of undernutrition and failure to thrive. *Ped Clin North Am*, *45*, 169–187.
619. Krugman, S. D., & Dubowitz, H. (2003). Failure to thrive. *Am Fam Phys*, *68*, 879–884.
620. Schmitt, B. D., & Mauro, R. D. (1989). Non-organic failure to thrive: an outpatient approach. *Child Abuse Negl*, *13*, 235–248.
621. Kuhnle, U., Lewicka, S., & Fuller, P. J. (2004). Endocrine disorders of sodium regulation: role of adrenal steroids in genetic defects causing sodium loss or sodium retention. *Horm Res*, *61*, 68–83.
622. Sills, R. H. (1978). Failure to thrive. The role of clinical and laboratory evaluation. *Am J Dis Child*, *132*, 967–969.

623. Berwick, D. M., Levy, J. C., & Kleinerman, R. (1982). Failure to thrive: diagnostic yield of hospitalization. *Arch Dis Child*, *57*, 347–351.
624. Gahagan, S. (2006). Failure to thrive: a consequence of undernutrition. *Pediatr Rev*, *27*, e1–e11.
625. Gabay, M. P. (2002). Galactogogues: medications that induce lactation. *J Hum Lact*, *18*, 274–279.
626. Maggioni, A., & Lifshitz, F. (1995). Nutritional management of failure to thrive. *Ped Clin North Am*, *42*, 791–810.
627. Hershkovitz, E., Printzman, L., Segev, Y., et al. (1999). Zinc supplementation increases the level of serum insulin-like growth factor-I but does not promote growth in infants with nonorganic failure to thrive. *Horm Res*, *52*, 200–204.
628. Lozoff, B., Jimenez, E., & Wolf, A. W. (1991). Long-term developmental outcome of infants with iron deficiency. *N Engl J Med*, *325*, 687–694.
629. Davis, M. P. (2007). Anorexia and cachexia in cancer. In S. Yeung, C. Escalante, R. F. Gagel (Eds.), *Internal medicine care of cancer patients.* New York: Decker.
630. Kelly, J. F., Elias, C. F., Lee, C. E., et al. (2004). Ciliary neurotrophic factor and leptin induce distinct patterns of immediate early gene expression in the brain. *Diabetes*, *53*, 911–920.
631. Argiles, J. M., Busquets, S., & Lopez-Soriano, F. J. (2002). The role of uncoupling proteins in pathophysiological states. *Biochem Biophys Res Comm*, *293*, 1145–1152.
632. Makino, T., Noguchi, Y., Yoshikawa, T., et al. (1998). Circulating interleukin 6 concentrations and insulin resistance in patients with cancer. *Br J Surg*, *85*, 1658–1662.
633. Yoshikawa, T., Noguchi, Y., Doi, C., et al. (1999). Insulin resistance is connected with the alterations of substrate utilization in patients with cancer. *Cancer Lett*, *141*, 93–98.
634. Yoshikawa, T., Noguchi, Y., Doi, C., et al. (2001). Insulin resistance in patients with cancer: relationships with tumor site, tumor stage, body-weight loss, acute-phase response, and energy expenditure. *Nutrition*, *17*, 590–593.
635. Tayek, J. A., & Katz, J. (1997). Glucose production, recycling, Cori cycle, and gluconeogenesis in humans: relationship to serum cortisol. *Am J Physiol*, *272*, E476–E484.
636. Russell, A. (1951). A diencephalic syndrome of emaciation in infancy and childhood. *Arch Dis Child*, *26*, 274.
637. Fleischman, A., Brue, C., Poussaint, T. Y., et al. (2005). Diencephalic syndrome: a cause of failure to thrive and a model of partial growth hormone resistance. *Pediatrics*, *115*, e742–e748.
638. Brauner, R., Trivin, C., Zerah, M., et al. (2006). Diencephalic syndrome due to hypothalamic tumor: a model of the relationship between weight and puberty onset. *J Clin Endocrinol Metab*, *91*, 2467–2473.
639. Vlachopapadopoulou, E., Tracey, K. J., Capella, M., et al. (1993). Increased energy expenditure in a patient with diencephalic syndrome. *J Pediatr*, *122*, 922–924.
640. Hoek, H. W., & van Hoeken, D. (2003). Review of the prevalence and incidence of eating disorders. *Int J Eating Dis*, *34*, 383–396.
641. Johnson, J. G., Cohen, P., Kasen, S., & Brook, J. S. (2002). Eating disorders during adolescence and the risk for physical and mental disorders during early adulthood. *Arch Gen Psychiatry*, *59*, 545–552.
642. Rigotti, N. A., Neer, R. M., Skates, S. J., et al. (1991). The clinical course of osteoporosis in anorexia nervosa: a longitudinal study of cortical bone mass. *JAMA*, *265*, 1133–1138.
643. Bulik, C. M., Sullivan, P. F., Fear, J. L., et al. (1999). Fertility and reproduction in women with anorexia nervosa: a controlled study. *J Clin Psychiatry*, *60*, 130–135.
644. Lambe, E. K., Katzman, D. K., Mikulis, D. J., et al. (1997). Cerebral gray matter volume deficits after weight recovery from anorexia nervosa. *Arch Gen Psychiatry*, *54*, 537–542.
645. Strober, M., Freeman, R., & Morrell, W. (1997). The long-term course of severe anorexia nervosa in adolescents: survival analysis of recovery, relapse, and outcome predictors over 10–15 years in a prospective study. *Int J Eating Dis*, *22*, 339–360.
646. Tamai, H., Mori, K., Matsubayashi, S., et al. (1986). Hypothalamic-pituitary-thyroidal dysfunctions in anorexia nervosa. *Psychother Psychosom*, *46*, 127–131.
647. Gianotti, L., Arvat, E., Valetto, M. R., et al. (1998). Effects of beta-adrenergic agonists and antagonists on the growth hormone response to growth hormone-releasing hormone in anorexia nervosa. *Biol Psychiatry*, *43*, 181–187.
648. Licinio, J., Wong, M. L., & Gold, P. W. (1996). The hypothalamic pituitary adrenal axis in anorexia nervosa. *Horm Res*, *62*, 75–83.
649. Misra, M., & Klibanski, A. (2006). Anorexia nervosa and osteoporosis. *Rev Endo Metab Dis*, *7*, 91–99.
650. Misra, M., Miller, K. K., Tsai, P., et al. (2006). Elevated peptide YY levels in adolescent girls with anorexia nervosa. *J Clin Endocrinol Metab*, *91*, 1027–1033.
651. Welt, C. K., Chan, J. L., Bullen, J., et al. (2004). Recombinant human leptin in women with hypothalamic amenorrhea. *N Engl J Med*, *351*, 987–997.
652. Balligand, J. L., Brichard, S. M., Brichard, V., et al. (1998). Hypoleptinemia in patients with anorexia nervosa: loss of circadian rhythm and unresponsiveness to short-term refeeding. *Eur J Endocrinol*, *138*, 415–420.
653. Monteleone, P., Fabrazzo, M., Tortorella, A., et al. (2002). Opposite modifications in circulating leptin and soluble leptin receptor across the eating disorder spectrum. *Mol Psychiatry*, *7*, 641–646.
654. Chan, J. L., & Mantzoros, C. S. (2005). Role of leptin in energy-deprivation states: normal human physiology and clinical implications for hypothalamic amenorrhoea and anorexia nervosa. *Lancet*, *366*, 74–85.
655. Brichard, S. M., Delporte, M. L., & Lambert, M. (2003). Adipocytokines in anorexia nervosa: a review focusing on leptin and adiponectin. *Horm Metab Res*, *35*, 337–342.
656. Tagami, T., Satoh, N., Usui, T., et al. (2004). Adiponectin in anorexia nervosa and bulimia nervosa. *J Clin Endocrinol Metab*, *89*, 1833–1837.
657. Bosy-Westphal, A., Brabant, G., Haas, V., et al. (2005). Determinants of plasma adiponectin levels in patients with anorexia nervosa examined before and after weight gain. *Eur J Nutr*, *44*, 355–359.
658. American Psychiatric Association (2006). Treatment of patients with eating disorders, third edition. *Am J Psychiatr*, *163*, 4–54.
659. Klibanski, A., Biller, B. M., Schoenfeld, D. A., et al. (1995). The effects of estrogen administration on trabecular bone loss in young women with anorexia nervosa. *J Clin Endocrinol Metab*, *80*, 898–904.
660. Robinson, E., Bachrach, L. K., & Katzman, D. K. (2000). Use of hormone replacement therapy to reduce the risk of osteopenia in adolescent girls with anorexia nervosa. *J Adolesc Health*, *26*, 343–348.
661. Ross MG, Huber, I., & Desai, M. (2010). Intrauterine growth restriction, small for gestational age, and experimental obesity. In R. H. Lustig (Ed.), *Obesity before birth: maternal and prenatal effects on the offspring* (pp. 215–239). New York: Springer.
662. Lustig, R. H. (2010). Fructose: metabolic, hedonic, and societal parallels with ethanol. *J Am Diet Assoc*, *110*, 1307–1321.

QUESTIONS

1. Which correlates best with metabolic dysfunction?
   a. Visceral fat
   b. Intrahepatic fat
   c. Body mass index
   d. Subcutaneous fat

Answer: b

Fabbrini, E., Magkos, F., Mohammed, B. S., et al. (2009). Intrahepatic fat, not visceral fat, is linked with metabolic complications of obesity. *Proc Natl Acad Sci USA*, *106*, 15430–15435.

2. As caloric intake has increased since the 1980s, how has dietary fat intake been affected?
   a. Increased proportionately (i.e., percentage remained the same)
   b. Increased disproportionately (i.e., percentage increased)
   c. Increased because trans-fat intake increased
   d. Remained the same

Answer: d

Chanmugam, P., Guthrie, J. F., Cecilio, S., et al. (2003). Did fat intake in the United States really decline between 1989-1991 and 1994-1996? *J Am Diet Assoc*, *103*, 867–872.

3. Hypothalamic obesity occurs due to defective hypothalamic leptin signaling, resulting in which of the following?
   a. Autonomic dysfunction
   b. Increased caloric intake
   c. Decreased physical activity
   d. Decreased resting energy expenditure

Answer: a

Lustig, R. H. (2008). Hypothalamic obesity: causes, consequences, treatment. *Ped Endocr Rev*, *6*, 220–227.

4. It what way does hepatic fructose metabolism differ from hepatic glucose metabolism?
   a. Increases glycogen formation
   b. Promotes de novo lipogenesis
   c. Decreases forkhead protein (Foxo1) phosphorylation
   d. Increases mitochondrial β oxidation

Answer: b

Faeh, D., Minehira, K., Schwarz, J. M., et al. (2005). Effect of fructose overfeeding and fish oil administration on hepatic de novo lipogenesis and insulin sensitivity in healthy men. *Diabetes*, *54*, 1907–1913.

5. A 5-year-old is seen for obesity. Which feature suggests a classic endocrinopathy?
   a. Mental retardation
   b. Early adiposity rebound
   c. Family history
   d. Growth attenuation

Answer: d

6. A 7-year-old obese male is referred for evaluation. Which of the following suggest an epigenetic cause for the obesity?
   a. Acanthosis nigricans
   b. Normal weight parents
   c. Low Apgar scores
   d. Small for gestational age

Answer: d

Cutfield, W. S., Hofman, P. L., Mitchell, M., & Morison, I. M. (2007). Could epigenetics play a role in the developmental origins of health and disease? *Pediatr Res*, *61*(5 Pt. 2), 68R–75R.

7. A proven effective lifestyle strategy for stabilizing weight in children includes which of the following?
   a. Bringing lunch to school
   b. Reducing television time
   c. Increasing sleep duration
   d. Participating in physical education at school

Answer: b

Robinson, T. N. (1999). Reducing children's television viewing to prevent obesity: a randomized controlled trial. *JAMA*, *282*, 1561–1567.

8. Aside from liver biopsy, what is the most accurate for diagnosing nonalcoholic fatty liver disease?
   a. ALT
   b. Liver ultrasound
   c. MRI
   d. MRS

Answer: c

Mazhar, S. M., Shiehmorteza, M., & Sirlin, C. B. (2009). Noninvasive assessment of hepatic steatosis. *Clin Gastroenterol Hepatol*, 7, 135–140; Schwenzer, N. F., Springer, F., Schraml, C., et al. (2009). Non-invasive assessment and quantification of liver steatosis by ultrasound, computed tomography and magnetic resonance. *J Hepatol*, *51*, 433–445.

9. What is the current mean daily fiber intake?
   a. 6 g/day
   b. 12 g/day
   c. 18 g/day
   d. 24 g/day

Answer: b

Martlett, J. A., McBurney, M. I., & Slavin, J. L. (2002). Position of the American Dietetic Association: health implications of dietary fiber. *J Am Diet Assoc*, *102*, 993–1000.

10. Which subcellular organelle is thought to be responsible for most forms of obesity with mental retardation?
    a. Cilia
    b. Mitochondria
    c. Endoplasmic reticulum
    d. Peroxisomes

Answer: a

Sen Gupta, P., Prodromou, N., & Chapple, J. (2009). Can faulty antennae increase adiposity? The link between cilia proteins and obesity. *J Endocrinol*, *203*, 327–336.

CHAPTER 23

Lipid Disorders in Children and Adolescents

Stephen R. Daniels, MD, PhD • Sarah C. Couch, PhD, RD

CHAPTER OUTLINE

INTRODUCTION

Cardiovascular disease (CVD) is a major cause of morbidity and mortality among adults in industrialized countries. Dyslipidemia (specifically elevated low-density lipoprotein [LDL] cholesterol, low high-density lipoprotein [HDL] cholesterol, high non-HDL cholesterol, and triglycerides) has been identified as an independent risk factor in the development of CVD. There is strong evidence that lipoprotein levels track from childhood into adulthood and that abnormal levels of LDL cholesterol and perhaps other lipoproteins are associated with atherosclerosis and therefore with related adverse outcomes.

This chapter reviews the evidence for the role of cholesterol abnormalities in the early natural history of atherosclerosis. In addition, a general overview of lipoprotein metabolism is provided—followed by a review of genetic disorders in the metabolism of lipoproteins. Secondary causes of high cholesterol are explained, including the increasing prevalence of obesity and metabolic syndrome as a cause of cholesterol abnormalities in the pediatric population. Standards and approaches to screening for hyperlipidemia in children are reviewed, as well as current approaches to the dietary and pharmacologic management of pediatric lipid disorders.

METABOLISM

Lipid disorders in children and adolescents can result from defects in the production, transport, or degradation of lipoproteins. To understand the diverse causes of lipoprotein abnormalities, a brief review of lipoprotein structure, function, and metabolism is provided. Table 23-1 summarizes the lipoprotein subclasses, the source of each one, and the constituent lipids and apolipoproteins associated with each particle.

Triglycerides, cholesterol esters, phospholipids, and plant sterols within food postingestion are digested to fatty acids, 2-monoglycerides, lysophospholipids, unesterified cholesterol, and plant sterols. Absorption of these digestive end products occurs through two mechanisms: passive diffusion and carrier-mediated transport. In passive diffusion, nonpolar lipids are solubilized with the aid of bile acids and lysophospholipids into mixed micelles that can diffuse through the apical surface of the enteric membrane. Carrier-mediated transport involves several different transport proteins for fatty acids and sterols. CD 36, a fatty acid translocase, promotes long chain fatty acid and cholesterol absorption in the proximal small intestine.[1] At least two additional transporters, Niemann-Pick C1-like 1 protein (NPC1L1) and scavenger receptor B1 (SR-B1),

TABLE 23-1 **Lipoprotein Subclasses and Associated Apolipoproteins and Lipid Constituents**

Lipoprotein	Apolipoprotein	Source	Lipid Constituents
Chylomicrons	ApoB-48, apoC-II,* apoC-III, apoE*	Intestine	Dietary triglycerides
VLDL	ApoB-100, C-II,* C-III, apoE*	Liver	Endogenous cholesterol and triglyceride
IDL	ApoB-100, apoE	VLDL metabolism	Cholesterol and triglyceride
LDL	ApoB-100	VLDL metabolism	Cholesterol
HDL	ApoA-I, apoA-II, apoC-II, apoE	Liver and intestine	Cholesterol and phospholipid

*Transferred from HDL.
VLDL, very low density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

play a role in sterol uptake.[2,3] As such, NPC1L1 and SR-B1 are targets for the cholesterol-lowering medication ezetimibe, a potent inhibitor of cholesterol and plant sterol absorption.

Most of the plant sterol ingested and about half of the absorbed cholesterol are excreted from the intestinal cell back into the lumen by two adenosine triphosphate (ATP)-binding cassette (ABC) half-transporters, G5 and G8, thus limiting the amount of these sterols that is absorbed.[4,5] A rare mutation of either ABCG5 or ABCG8, known as sitosterolemia, results in abnormally high plant sterol levels in plasma and tissues and deposition of sterols in the skin and arteries. Individuals with this disorder are at an increased risk of premature atherosclerosis.[6]

Within the enterocyte, lipids are aggregated into lipoproteins through the action of a microsomal triglyceride transfer protein (MTP). MTP conjugates triglycerides and cholesterol ester with apolipoprotein B-48 (apoB-48) on the luminal side of the endoplasmic reticulum (ER) membrane to create a mature chylomicron.[7,8] A similar process is used to aggregate triglyceride and cholesterol with apoB-100 in the liver to form very low density lipoprotein (VLDL) particles. In the genetic disorder abetalipoproteinemia, mutations in the gene encoding MTP result in an inability to produce chylomicrons and VLDL, suggesting the essential nature of MTP in chylomicron and VLDL biogenesis.[8]

Chylomicrons once formed are too large to penetrate the capillary membrane. Consequently, they are secreted into the lymphatic system and enter the venous plasma compartment through the thoracic lymph duct. As the nascent particles are released into the plasma, several apolipoproteins (including apoC-II, C-III, and apoE) are preferentially transferred to the chylomicrons from circulating high-density lipoproteins (HDLs).[9] Figure 23-1 depicts chylomicron metabolism.

Chylomicrons transport dietary triglyceride and cholesterol to sites of storage or metabolism.[10] They are rapidly cleared from the circulation through the action of lipoprotein lipase (LPL). LPL is a triglyceride hydrolase found on the capillary endothelium of various tissues, with its highest concentration in muscle and adipose tissues.[11] LPL is activated by apoC-II and inhibited by apoC-III on the chylomicron. As the triglyceride contained within the chylomicron is hydrolyzed, the particle decreases in size. When approximately 80% of the initial triglyceride has been removed, apoC-II dissociates from its surface.[11] The triglyceride-depleted chylomicrons, now considered chylomicron remnants, are taken up by the liver through the LDL receptor (LDLR), a receptor that recognizes apoE on the chylomicron surface and apoB100 on the surface of liver-derived lipoproteins.[9] A smaller fraction of remnants may also be internalized via an LDLR-related protein-1 (LRP1)-mediated endocytosis.[12,13]

Very low density lipoproteins (VLDLs) originate from the liver, and like chylomicrons they are triglyceride-rich particles (Figure 23-2). In contrast to the intestinally

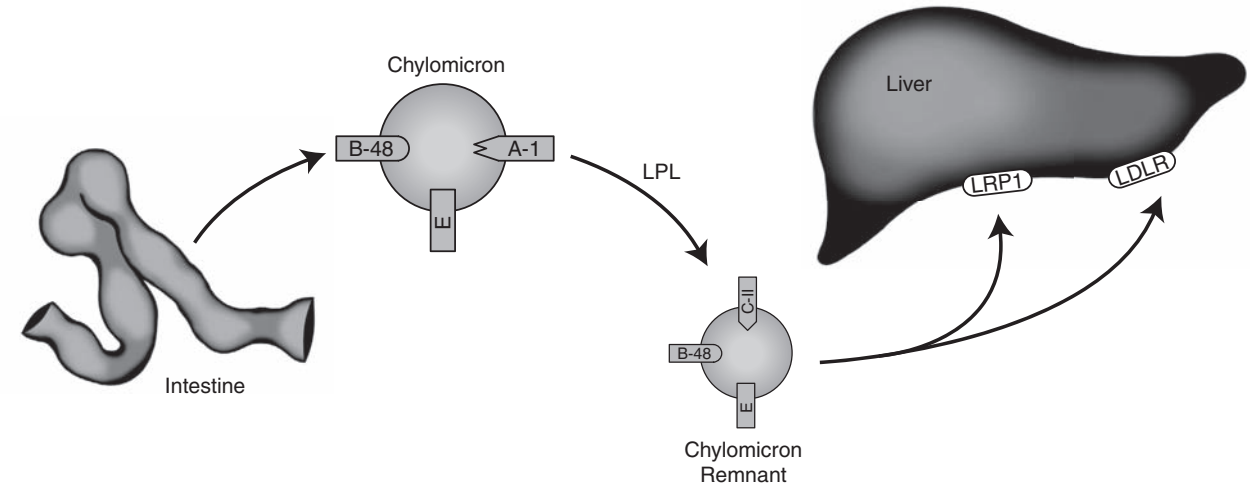


FIGURE 23-1 ■ Endogenous lipoprotein metabolism. See text for details. (Courtesy of Emilie Graham, University of Cincinnati.)

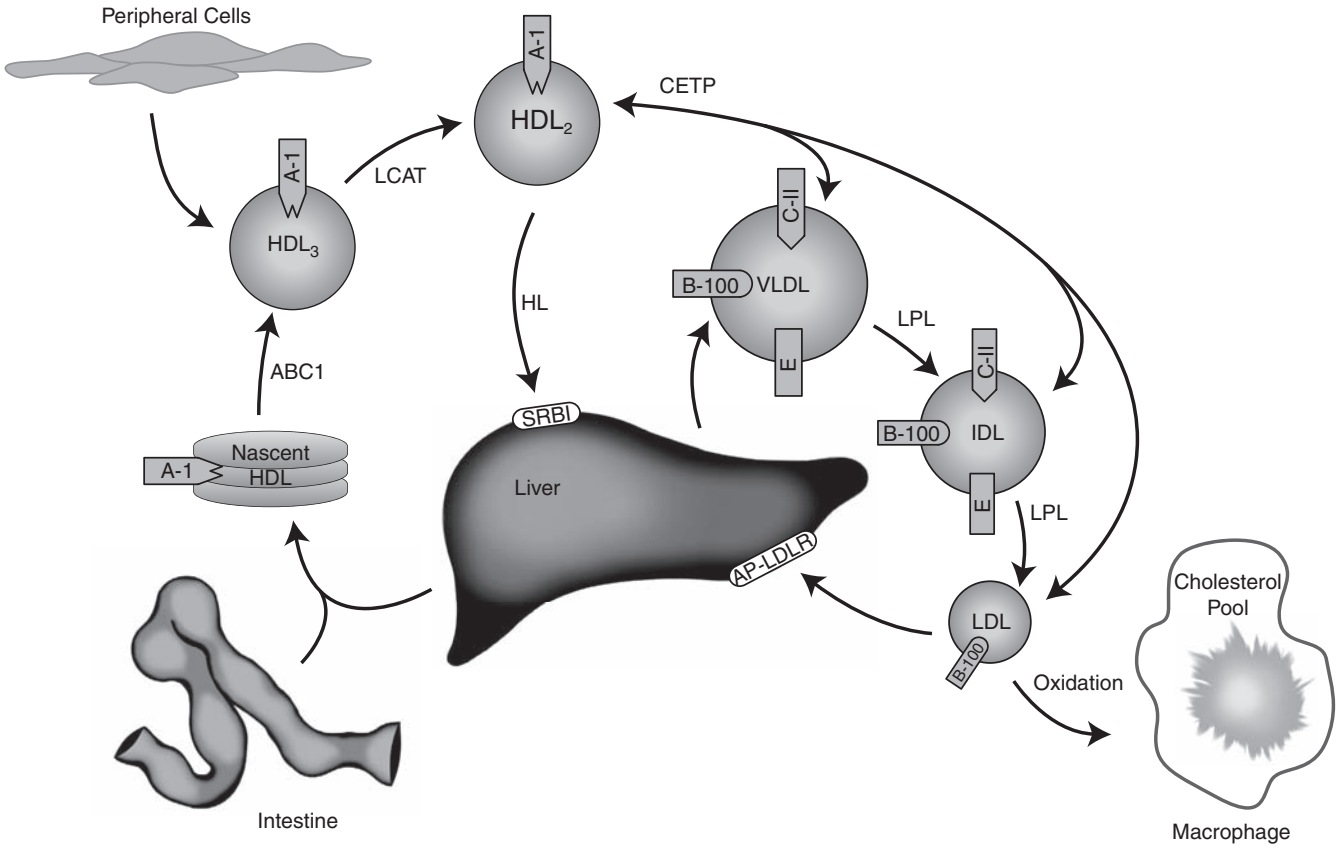


FIGURE 23-2 ■ Exogenous lipoprotein metabolism. See text for details. (Courtesy of Emilie Graham, University of Cincinnati.)

derived chylomicrons, the fatty acids contained within the VLDL triglyceride come from de novo synthesis from dietary carbohydrate, lipoprotein remnants, or circulating fatty acids internalized by the liver from plasma.[9] Within the hepatocyte, triglyceride and cholesterol ester are assembled by an MTP and surrounded with a phospholipid membrane associated with apoB-100.[8] The mature VLDL particles are released into the lymph and ultimately into the vascular space, where other apolipoproteins (including apoC-II, apoC-III, and apoE) adsorb to the VLDL surface. The metabolism of the VLDL particle follows a route similar to that of the chylomicron: apoC-II on its surface activates LPL, LPL hydrolyzes the VLDL triglyceride, the particle decreases in size (after an 80% loss of triglyceride), and ultimately apoC-II dissociates—resulting in the formation of VLDL remnants (also known as intermediate-density lipoproteins [IDLs]). Approximately half of the IDL is then removed from plasma through the interaction of apoB with the LDLR on the surface of liver cells.[9] The rest of the IDL is converted to LDL through further hydrolysis of core triglycerides by hepatic triglyceride lipase (HL).[14] ApoE is transferred from IDL to HDL during the transition of the remnant to LDL.[15]

LDL, the major carrier of cholesterol in plasma, is taken up into peripheral tissues and liver cells by the LDLR assisted by an adaptor protein (AP). The AP binds to the LDLR and clathrin, suggesting a role for AP in the recruitment and retention of LDLR in clathrin-coated pits.[16] Upon receptor binding, the LDL particle bound to LDLR/AP is rapidly internalized into clathrin-coated pits by endocytosis. Within the cell, the newly formed endosome becomes acidified through the action of an ATP-dependent proton pump.[17] Acidification causes degradation of the clathrin coat, dissociation of the LDLR from LDL, and subdivision of the endosomal membranes. The endosome containing the LDLR recirculates back to the cell membrane for additional LDL uptake. Alternatively, proprotein convertase subtilisin/kexin type 9 (PCSK9), binds LDLR, and short-circuits recycling of LDLR from the endosome, leading to its degradation.[18] The remaining LDL-containing endosome fuses with a lysosome, where hydrolytic enzymes digest the lipoprotein into its component parts: unesterified cholesterol, fatty acids, and free amino acids.[17]

The amount of cholesterol released from endosomal uptake regulates hepatic synthesis of LDLR and cholesterol. When cellular concentration of cholesterol is low, sterol receptor binding protein (SREBP) is released from the Golgi. SREBP translocates to the nucleus, where it serves as a nuclear factor that enhances the transcription of LDLR and hydroxymethylglutaryl (HMG) CoA reductase, the rate-limiting enzyme of cholesterol biosynthesis.[19] In this way, intracellular hepatic cholesterol concentration regulates the amount of cholesterol internalized and synthesized by the cell.

When excess LDL and other small apoB-containing lipoproteins (chylomicron remnants and IDL) are present in the plasma, the capacity of the LDLR to remove them is exceeded and these particles become more susceptible to oxidation. Oxidized apoB-containing lipoproteins can be taken up by scavenger receptors on macrophages in

the subendothelium of arteries and may contribute to the formation of atherosclerotic lesions.[20]

HDL transfers cholesterol and other lipids from peripheral tissues (including arterial atheroma) back to the liver. The particles are synthesized predominantly in the liver (and to a lesser extent in the intestine) as lipid-poor precursor particles (prebeta HDL) containing apoA-I (Figure 23-2).[9] Nascent HDL interacts with the plasma membrane of cells, collecting lipid through an ATP-binding cassette transporter-A1 (ABCA1) mechanism.[3,4] The cholesterol and phospholipids transferred through this process adsorb to the HDL, forming a disk-shaped particle referred to as HDL3. Within the plasma, HDL3 interacts with the enzyme lecithin cholesterol acyl transferase (LCAT)—which catalyzes the esterification of particle-associated cholesterol. ApoA-I on the HDL surface activates LCAT. Once formed, the cholesterol ester is more hydrophobic and moves to the interior of the particle—creating a sphere-shaped HDL particle known as HDL2.[21]

As HDL2 increases in size, the particle becomes substrate for cholesterol ester transfer protein (CETP). This enzyme promotes the exchange of esterified cholesterol within HDL2 for triglyceride contained within apoB-100–associated lipoproteins.[22] This lipid exchange is the primary mechanism whereby HDL participates in reverse cholesterol transport from tissues back to the liver. The rest of the cholesterol ester is selectively taken up from HDL by hepatocytes via a SRB1, without concomitant uptake of the entire HDL particle. This latter process may require the action of HL.[23] The lipid-poor prebeta HDL resulting from this process is released for recycling.[16]

PRIMARY DYSLIPIDEMIAS

Lipoprotein synthesis, transport, and metabolism occur in many steps and involve many specialized proteins. A number of genetic defects have been identified in these processes and are referred to as primary dyslipidemias. Most of these genetic defects present in childhood. Table 23-2 summarizes pediatric lipoprotein disorders with reference to the characteristic lipoprotein profile of each one. The genetic and metabolic etiologies of these disorders are detailed in the following material.

Disorders of Cholesterol Metabolism

Familial Hypercholesterolemia

Familial hypercholesterolemia (FH) is the most common single gene disorder of lipoprotein metabolism. FH is inherited as an autosomal-dominant trait with relatively low prevalence in Western countries. The prevalence has been reported to be 10 times higher in certain populations with a presumed founder effect, such as the Lebanese, the French Canadians, and the South Afrikaners.[24] The heterozygous form is found in 1 in 500 persons, and the homozygous form is found in 1 in 1 million persons. The disorder is caused by a mutation in the LDLR gene.[25] More than 1100 mutations in this gene have been identified, including those that affect receptor synthesis, intracellular transport, ligand binding, internalization, and recycling.[26] In the heterozygous form, inheritance of one defective LDLR gene results in plasma LDL cholesterol levels two to three times higher than normal.[25]

Individuals with heterozygous FH are at an increased risk of developing early-onset coronary artery disease (CAD), usually between the ages of 30 and 60 years.[24] In the homozygous form, individuals inherit a mutant allele for FH from both parents, resulting in plasma LDL cholesterol concentrations that are four to six times higher than normal.[27] A more severe phenotype is found in individuals with receptor-negative mutations (those with <5% residual LDL receptor activity) compared to those with receptor-defective mutations (5% to 30% of normal LDL receptor activity).[28] Due to the excessively high plasma cholesterol levels in individuals with homozygous FH, cholesterol deposits are common in

TABLE 23-2 **Pediatric Lipoprotein Disorders***

Lipoprotein Disorder	Lipoprotein Analysis	Blood Lipids	Genetic Defect
Familial hypercholesterolemia	↑↑LDL	↑↑Cholesterol	LDL receptor (LDLR)
Autosomal recessive hypercholesterolemia	↑↑LDL	↑↑ Cholesterol	LDLRAP
Autosomal dominant hypercholesterolemia	↑↑LDL (with increase in function mutations)	↑↑ Cholesterol	PCSK9
Familial ligand-defective apoB-100	↑↑ LDL	↑↑Cholesterol	ApoB-100
Sitosterolemia	↑ LDL	↑ Cholesterol	ABCG5 or ABCG8
Familial combined hyperlipidemia	↑ VLDL, ↑ LDL, ↓ HDL	↑ Cholesterol, ↑ triglycerides	Unknown
Familial hypertriglyceridemia	↑↑ VLDL, ↓ HDL	↑ Triglycerides	Unknown
Familial chylomicronemia syndrome	↑↑ Chylomicrons ↑ VLDL	↑↑Triglycerides	Lipoprotein lipase (LPL), ApoC-II, Apo A-V, GP1HBP1
Hypoalphalipoproteinemia	↓ HDL	Normal	ApoA-1
Dysbetalipoproteinemia	↑↑ Chylomicron remnants, ↑↑ IDL	↑↑ Cholesterol, ↑↑ triglycerides	ApoE

*↑↑ Very high; ↑ moderately elevated; and ↓ decreased.
LDL, low-density lipoprotein; VLDL, very low density lipoprotein; HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein. See text for details.

the tendons (xanthomas) and eyelids (xanthelasmas)—generally by the age of 5 years.[29] In the heterozygous form, xanthomas occur less frequently and generally not until one reaches older adulthood. Children with homozygous FH have early-onset atherosclerosis and often have myocardial infarction in the first decade of life, and death from CAD in the second decade. [29]

Autosomal Dominant and Autosomal-Recessive Hypercholesterolemia

Autosomal-dominant hypercholesterolemia (ADH) is another inherited disorder resulting in a phenotype that is expressed as marked elevations or low levels of LDL cholesterol. ADH is caused by mutations in proprotein convertase subtilisin/kexin type 9 (PCSK9).[30] This protein binds and favors degradation of the LDLR and thereby modulates the plasma levels of LDL cholesterol. Some of the naturally occurring PCSK9 mutations favor an increase in the function of the protein and cause hypercholesterolemia, whereas some favor a loss of function and are associated with low LDL cholesterol. The latter mutations appear to confer protection from developing CAD.

Autosomal recessive hypercholesterolemia (ARH) is caused by mutations in the ARH gene, which encodes the adaptor protein required for normal LDLR-mediated endocytosis in hepatocytes.[31] Several different mutations in this protein have been identified, all leading to a lack of or suboptimal internalization of the LDLR.[32] Cholesterol levels in individuals with ARH are five to six times higher than normal. Children with this disorder are clinically similar to those with homozygous FH. However, their parents usually have normal lipoprotein profiles. [31]

Familial Ligand-Defective ApoB-100

Familial ligand-defective apoB-100 (FDB) is a monogenic disorder that clinically resembles heterozygous FH. The disease is characterized by moderate to markedly high plasma LDL cholesterol levels, normal triglycerides, and tendon xanthomas. The disorder is caused by poor binding of the LDL particle to the LDLR due to a mutation in apoB-100.[33] Deficient LDLR binding results in a decreased clearance of LDL from plasma. The disorder is most common in individuals of European descent (1 per 1000).[9] Patients with FDB are at moderate to high risk of developing CAD.[34]

Sitosterolemia

Sitosterolemia is a rare autosomal-recessive disease caused by a mutation in either of two genes (ABCG5 or ABCG8) encoding the ABC half-transporters.[35] These genes are expressed in enterocytes and hepatocytes. The ABC half-transporters limit the absorption of cholesterol and plant sterols (and possibly shellfish sterols) in the gut. They also promote biliary and fecal excretion of cholesterol and phytosterols.[36,37] Defective proteins result in an abnormally high absorption of plant sterols (and, to a lesser extent, cholesterol) into the enterocyte and decreased excretion of these sterols from the liver into the bile. Plasma cholesterol can be mildly, moderately, or markedly elevated, whereas plant sterol concentrations in the plasma are markedly increased. Patients with sitosterolemia develop premature CAD and xanthomas in childhood, and may develop aortic stenosis.[35]

Disorders of Overproduction of VLDL

Familial combined hyperlipidemia (FCHL) is an autosomal-dominant disorder with a prevalence of 1% to 2% in Western populations.[38] Individuals with FCHL generally share the same metabolic defect, which is overproduction of hepatic VLDL. Families with FCHL have multiple patterns of hyperlipidemia, including hypercholesterolemia, hypertriglyceridemia, and elevated apoB levels. A diagnosis of FCHL is based on the presence of increased levels of cholesterol, triglyceride, or apoB in patients and their first-degree relatives.[38] Veerkamp and colleagues have developed a nomogram to calculate the probability that a person is likely to be affected by FCHL.[39] A classic feature of FCHL is that the lipoprotein profile is variable in the same individual over time and may depend on factors such as diet, exercise, and weight. FCHL can manifest in childhood but is usually not fully expressed until adulthood.[38] Patients with FCHL often have concurrent problems with insulin resistance, central obesity, and hypertension and are at an increased risk of premature CAD. [40]

Syndromes with a similar phenotype are hyperapobetalipoproteinemia, LDL subclass pattern B, and the clustering of CVD risk factors known as metabolic syndrome in adults.[38] Of the three, the latter syndrome is much more prevalent in children. Rates of metabolic syndrome are continuing to rise with the prevalence of obesity in the pediatric population.[41] There appears to be a mechanistic link between central obesity, insulin resistance, and dyslipidemia—with central obesity generally preceding both glucose and lipid abnormalities.

Disorders of Marked Hypertriglyceridemia

Familial Hypertriglyceridemia

Familial hypertriglyceridemia (FHTG) follows an autosomal-dominant inheritance pattern expressed predominantly in adulthood, with a population prevalence of ~5% to 10%.[38] The prevalence in children is increasing. Obesity is an important factor that can expedite the expression of FHTG, and patients often have concurrent glucose intolerance. The phenotype for FHTG is moderate to markedly high serum triglycerides (200 to 500 mg/dL range) and low to normal LDL and HDL cholesterol levels. The metabolic cause of the disorder is hepatic secretion of large triglyceride-rich VLDL particles that are catabolized slowly.[42] The fundamental genetic defect for FHTG has not been identified.

Familial Chylomicronemia Syndrome

Chylomicronemia syndrome is a compilation of rare monogenetic disorders that cause marked impairment of

lipoprotein lipase (LPL) activity. These disorders are phenotypically expressed as hypertriglyceridemia (usually triglycerides [TGs] > 1000 mg/dL) due to diminished or absent hydrolysis of chylomicron and VLDL-associated triglycerides by LPL.[43] Impairment of LPL activity may be related to LPL deficiency, apoC-II (cofactor for LPL) deficiency, or the more recently described apoA5 and glycosylphosphatidylinositol-anchored HDL-binding protein 1 (GPIHBP1) loss-of-function mutations that result in poor hydrolysis of chylomicron and VLDL-associated triglycerides.[44]

In homozygous chylomicronemia, fasting plasma has a viscous, creamy appearance due to the presence of large numbers of chylomicron particles. Risks for pancreatitis and hepatosplenomegaly are increased due to the markedly elevated serum triglycerides.[43] In addition, eruptive xanthomas and neurologic symptoms may be apparent. Individuals heterozygous for the syndrome may have a mild to moderate elevation in plasma triglycerides. Environmental factors such as weight gain may exacerbate hypertriglyceridemia. Premature CAD is generally not a feature of chylomicronemia, but cases have been reported.[44]

HYPOLIPIDEMIAS

Low HDL Cholesterol

In clinical practice, patients with low HDL cholesterol levels commonly have concurrent high triglycerides, with or without elevations in small dense LDL cholesterol.[38] These patients are usually obese, and the mechanistic explanation for this dyslipidemic triad is VLDL overproduction. Less common are familial disorders of HDL, including familial hypoalphalipoproteinemia, mutations of the apoA-1 protein, Tangier disease, and LCAT deficiency.[45] These disorders are characterized by a low HDL cholesterol level with no other lipid abnormality. Familial hypoalphalipoproteinemia follows an autosomal-dominant inheritance pattern.[46] ApoA-1 levels are also often low due to decreased production of HDL.

A number of mutations have been described in the apoA-1 gene and are associated with low HDL cholesterol and low apoA-1.[45,47] Tangier disease is due to mutations in the ABCA1 gene.[47] Patients affected by this disease are not able to actively withdraw cholesterol from cells onto nascent HDL particles, causing rapid degradation of the nascent HDL. ApoA-1 is rapidly cleared before it is able to acquire cholesterol. In Tangier disease, HDL cholesterol levels are close to zero and the apoA-1 levels are less than 5 mg/dL. The risk of premature CAD in these patients is mild to moderate.[46,47] LCAT deficiency is a very rare autosomal recessive disorder cause by mutations in LCAT, an enzyme synthesized by the liver and secreted into the plasma, where it associates with lipoproteins.[48] LCAT esterifies free cholesterol on the surface of HDL and enables the accumulation of cholesteryl esters in the core of HDL. In LCAT deficiency, lack of normal cholesterol esterification impairs formation of mature HDL particles, which are readily catabolized along with apoA-1. Remarkably, despite the extremely low levels of plasma HDL cholesterol (usually < 10 mg/dL) and apoA-1, premature CAD is not a consistent feature of this disorder.

Abetalipoproteinemia

Abetalipoproteinemia is associated with low serum cholesterol (< 50 mg/dL) and triglycerides (~2 to 45 mg/dL). Patients with this disorder present with steatorrhea and fatty liver. Without treatment, ataxia follows (with acanthocytosis and retinitis pigmentosa). Abetalipoproteinemia is caused by a defect in MTP.[49] Without MTP, no chylomicrons, VLDL, or LDL appear in the plasma. In these patients, HDL takes over as the primary cholesterol carrier. Thus, the defect is not fatal. Because of significant fat malabsorption, fat-soluble vitamin status is impaired.

In particular, because vitamin E absorption and cellular uptake require chylomicron and LDL transport, high doses of vitamin E are required to prevent retinal and sensory neuron degeneration. Additional dietary considerations include restricting long-chain dietary triglycerides to less than 15 g/day to alleviate the steatorrhea. Medium-chain triglycerides (MCT oils) can be used as an alternative source of energy.[44]

Hypobetalipoproteinemia

Hypobetalipoproteinemia is an autosomal-dominant disorder resulting from a defect in the apoB gene that produces a truncated apolipoprotein B.[50] Cholesterol levels in patients with heterozygous hypobetalipoproteinemia are usually 50% of those of an unaffected family member. The heterozygous form of this condition is benign. However, homozygous hypobetalipoproteinemia is associated with severe hypocholesterolemia, significant steatorrhea, fatty liver, acanthocytosis retinopathy, and peripheral neuropathy.[51]

Disorders with Lipoprotein Clearance via ApoE Pathways

Dysbetalipoproteinemia is characterized by elevated cholesterol and triglyceride levels.[38] The disorder results from the presence of a polymorphism of the apoE allele (apoE2, rather than the more common apoE3 or less common apoE4).[52] Metabolically, this defect results in a poor uptake of remnant particles and abnormal remnant catabolism because of the abnormal apoE. Increased remnants, VLDL, chylomicrons, and apoE are all present. Xanthomas may occur, and premature CAD has been reported. This lipoprotein disorder is rare in children and often presents in young adulthood.[38]

SECONDARY CAUSES

Secondary dyslipidemias can result from a variety of diseases and conditions (see Box 23-1). In the United States, the most prevalent cause of secondary dyslipidemia is overweight and obesity.[53] The dyslipidemic triad (namely, elevated triglycerides and small dense LDL and low HDL cholesterol) is commonly associated with overweight (in particular, with central adiposity).[53,54] In addition to dyslipidemia, insulin resistance and elevated blood pressure may be present. This cluster of abnormalities is

BOX 23-1 Selected Secondary Causes of Pediatric Hyperlipoproteinemia

Endocrine
- Hypothyroidism
- Diabetes
- Pregnancy

Exogenous
- Drugs
- Obesity
- Alcohol

Renal
- Nephrotic syndrome
- Chronic renal failure

Hepatic
- Cholestasis
- Biliary atresia
- Hepatitis
- Biliary cirrhosis

Immunologic
- HIV infection/AIDS

known in adults as the metabolic syndrome. Empirical evidence in children also indicates that obesity during childhood is associated with the same risk factor clustering seen in adults, that it continues into adult life, and that it is associated with an increased risk for accelerated early atherosclerosis.[55] The primary approach to treating this disorder in both adults and children is weight management. Improvement in weight status and a decrease in body fatness have been shown to be associated with improvements in the dyslipidemia and other comorbidities associated with obesity.[56]

Metabolic lipid perturbations in adult patients with types 1 and 2 diabetes mellitus are similar to those found in patients with the metabolic syndrome, but often are more severe.[57] Generally in adults with diabetes, triglycerides are elevated and HDL cholesterol is low—and LDL cholesterol can be normal or mildly or moderately elevated. Diabetes in adults is considered a CAD risk equivalent according to the National Cholesterol Education Program (NCEP). This means that the risk for developing CAD in patients with poorly controlled diabetes is equivalent to those with established CAD.[58] For this reason, the NCEP recommends aggressive treatment of dyslipidemia in adult patients with diabetes.

Although type 1 diabetes is currently the main form of diabetes seen in children, in the United States a growing number of patients with type 2 diabetes are under the age of 18 years.[59] Change in the prevalence of type 2 diabetes in youth is likely related to the growing obesity epidemic occurring in the pediatric population.[59,60] Data on lipid concentrations in children and adolescents with diabetes are few, particularly in those with type 2 diabetes.

The Search for Diabetes in Youth Study assessed the prevalence of serum lipid abnormalities among a representative sample of U.S. children and adolescents with type 1 and type 2 diabetes.[61] Findings from this study showed a substantial number of diabetic children over the age of 10 years with abnormal serum lipids: nearly 50% had an LDL cholesterol level above the optimal level of 100 mg/dL. For children with type 2 diabetes, 37% had elevated triglyceride levels and 44% had low HDL cholesterol. These data highlight the importance of serum lipid screening in children with diabetes. A growing body of literature also shows early vascular dysfunction in children with diabetes, regardless of type.[62] This is thought to be due to glycemic and lipid abnormalities associated with poorly managed diabetes. For this reason, new treatment guidelines recommend intensive glucose and lipid management for children with diabetes.[56] These guidelines are discussed later in the chapter.

Other causes of secondary dyslipidemia include hypothyroidism, nephrotic syndrome, other renal diseases, liver diseases, and infection.[63] The risk of development of atherosclerosis with these conditions is unknown but is likely proportionate to the length of exposure and extent of elevation in serum LDL cholesterol levels. Cardiovascular disease is common in patients with chronic renal insufficiency.[64] Dyslipidemias can also result from the ingestion of a variety of medications. These medications include progestins, estrogens, androgens, anabolic steroids, corticosteroids, cyclosporine, and retinoids. Secondary causes of dyslipidemias should be identified by patient historical data and a careful physical examination.[63] Laboratory tests (including thyroid, renal, and liver function panels) can confirm the diagnosis.

The treatment of dyslipidemia in patients with secondary causes is focused on managing the underlying disease. Diet and physical activity changes may also be recommended to reduce elevated LDL cholesterol and triglyceride levels.

VASCULAR CHANGES AND DYSLIPIDEMIA

It is well established that elevated concentrations of total cholesterol and LDL cholesterol in adult life are strong and reversible risk factors for CAD.[58] Whether dyslipidemia during childhood contributes to atherosclerotic lesions in coronary and other arteries has been a subject of debate, but accumulating evidence from pathology and in vivo imaging studies favors a relationship. Atherosclerotic lesions result from deposits of lipid and cholesterol in the intima of the arterial wall.[65] Early lesions, called fatty streaks, are formed from the accumulation of macrophages filled with lipid droplets (foam cells).

Fatty streaks do not disorganize the normal structure of the intima, do not deform or obstruct the artery, and are in and of themselves not considered harmful.[66] However, some continue to accumulate macrophage foam cells and extracellular lipid and smooth muscle cells—forming raised plaques. From these, more advanced lesions may develop—with further deposition of extracellular lipid, cholesterol crystals, collagen, and potentially calcium.[67] It is these raised lesions that result in a myocardial infarction because of their increasing size and obstruction of the arterial lumen or because of rupture of

the fibrous plaque, which results in the release of thrombogenic substances from the necrotic core.[67]

Pathobiologic studies of the coronary arteries of young individuals who died from causes unrelated to heart disease have been useful in documenting the progression of atherosclerosis by age and risk factor determinants. Stary and colleagues studied more than 500 postmortem samples of coronary arteries from persons younger than 30 years of age and found the presence of fatty streaks in the majority of children younger than 9 years of age, raised lesions in about half of adolescents, and more advanced lesions in about a third of the young adults studied.[68] In 93 autopsies of young adults for whom childhood risk factor data were available, Berenson and colleagues found that the extent of the surface of arteries covered with fatty streaks and fibrous plaques was positively associated with LDL cholesterol, triglycerides, blood pressure, and body mass index and negatively associated with HDL cholesterol levels in childhood.[69]

The Pathobiological Determinants of Atherosclerosis in Youth (PDAY) study reached similar conclusions from examination of more than 3000 postmortem samples of coronary arteries of young adults who died from noncardiovascular events and who likewise had a variety of surrogates for antimortem risk factor measures available.[70] In general, pathology studies have made important contributions to the identification of risk factors for early aspects of the atherosclerotic process. In conjunction with findings from longitudinal studies such as the Framingham Heart Study (in which risk factor assessments of participants preceded the development of cardiovascular disease),[71] a group of risk factors, often referred to as the traditional risk factors for CAD, has been established. A complete list of pediatric risk factors for CAD is found in Box 23-2.

Advances in vascular imaging technology have provided a means of measuring early pathologic changes and functional abnormalities against coronary and other arteries in response to adverse changes in cardiovascular disease risk factors. The advantage in using this technology is that walls of superficial arteries can be imaged noninvasively in real time at high resolution, and changes to the arterial wall can be measured as a continuous variable from childhood to adulthood in patients with and without the presence of risk factors for CVD.[72] Computed tomography (CT) scanning is considered one of the most sensitive noninvasive tools for imaging the extent and location of coronary artery calcium present in atheroma.[73]

The presence of coronary artery calcium has been associated with adverse cardiovascular disease outcomes in adults.[74] In adolescents, small studies have shown associations among hypercholesterolemia, body mass index (BMI), and significant coronary artery calcium. In the Muscatine Study in which participants were assessed for CVD risk factors during their school-age years and later assessed for cardiovascular changes by CT scan, 31% of men and 10% of women aged 29 to 37 years had significant coronary artery calcification.[75] In this study, childhood risk factors associated with calcification were obesity, increased blood pressure, and low HDL cholesterol. Gidding and colleagues showed significant coronary calcium by electron bean CT in 7 of 29 young adults with heterozygous familial hypercholesterolemia.[76] Overweight was found to increase the likelihood of calcium being present in individuals already at high risk.

Vascular ultrasound imaging has been utilized to assess alterations in brachial artery flow-mediated dilation, which is a measure of endothelial function, and carotid intima-media thickness (IMT).[73] In adults, both measures have been associated with adverse changes in traditional cardiovascular disease risk factors,[77] respond to normalization of risk factors,[78] and are considered important early markers for the progression of atherosclerotic disease.[77-80] Although few studies have used ultrasound technology to evaluate coronary arteries in the young, children with hypercholesterolemia have been assessed using measures of the carotid and brachial arteries and have been found to have abnormalities of carotid IMT and brachial artery vasodilation.[81-83]

Further, in young adults aged 33 to 42 years who had comprehensive risk factor assessments performed some 25 years prior, Davis and colleagues found an association between mean carotid IMT and elevations in total cholesterol and triglyceride concentrations during childhood.[84] Lavrencic and associates found that the mean carotid IMT was significantly greater in youth with FH compared with those in a control group—as well as being significantly greater in all subjects in regard to total cholesterol, LDL cholesterol, and systolic blood pressure.[85] Similar risk factors have been associated with impaired vasodilation, indicating endothelial dysfunction.[86-88]

In summary, these studies confirm the utility of vascular imaging for detecting early pathologic and functional changes to coronary vessels and associations with modifiable CVD risk factors in the young. Clinically, vascular imaging by ultrasound may be a valuable means of estimating the benefit of treating multiple CVD risk factors in children and adolescents. However, the collection of more normative data across age, race, and gender groups and longitudinal studies to determine age and puberty-related

BOX 23-2 Pediatric Risk Factors for Coronary Artery Disease

Positive Risk Factors

- Elevated LDL cholesterol (≥ 130 mg/dL)
- Family history of premature (aged > 55 years) coronary heart disease, CVD, or peripheral vascular disease
- Smoking
- Hypertension
- Obesity (≥ 95th percentile weight for height on National Center for Health Statistics [NCH] growth chart)
- Physical inactivity
- Diabetes

Negative Risk Factors

- High HDL cholesterol (> 60 mg/dL)

changes in these measures is needed before these methods could be adopted in clinical evaluation.[89] In general, CT scans may be less useful in younger patients because calcium depositions are uncommon before young adulthood.

SCREENING FOR LIPID DISORDERS

Routine Screening

The approach to pediatric screening for dyslipidemia has been controversial. Since the 1990s, pediatric guidelines established by the National Cholesterol Education Program (NCEP) have provided the standard of care with respect to lipid screening and treatment of dyslipidemia in children.[90] These guidelines recommend selective blood cholesterol screening in children based on a positive family history of premature CVD (prior to age 55 years), presence of dyslipidemia in a parent (total cholesterol > 240 mg/dL), or presence of additional CVD risk factors in the child such as hypertension, diabetes, and obesity. If family history is unknown, recommendations suggest that lipid screening of a child be done at the discretion of the primary care provider. These guidelines have served as the basis for related recommendations from the American Academy of Pediatrics,[91] the American Heart Association,[92] and the American Diabetes Association.[60]

The controversy regarding the NCEP targeted screening approach stems largely from studies showing that the NCEP family history–based approach compared to a universal or general approach to childhood screening for dyslipidemia missed many children with moderate dyslipidemia (as many as 30% to 60%) and failed to detect a substantial number who likely had genetic dyslipidemia who might require more intensive therapy.[93] Familial hypercholesterolemia (FH) is a relatively common problem, with the heterozygous form occurring in 1 in 500 individuals.[26,27] FH has been clearly associated with an increased lifetime risk of atherosclerotic CAD, and earlier treatment is associated with reduced subclinical evidence of atherosclerosis.[27] To increase the likelihood of detecting young patients with FH and other genetic dyslipidemias, the National Lipid Association[94] and more recently the National Heart, Lung, and Blood Institute (NHLBI) Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents[56] recommended universal lipid screening for children ages 9 to 10 years.

These newer recommendations recognized that there is considerable variation in LDL cholesterol with age during growth and development, especially during puberty.[95,96] Total and LDL cholesterol levels tend to decline during puberty, meaning that some adolescents will appear normal when in fact they will have elevated levels after puberty.[95] For this reason, 9 to 10 years of age was selected as a good age to screen, before the effect of puberty lowers LDL cholesterol levels, but closer to an age when drug therapy may be appropriate.[56] Additionally, the National Lipid Association (NLA)[94] and the expert panel[56] agreed that lipid screening should occur earlier (≥ 2 years of age) in the following conditions: presence of a positive family history for hypercholesterolemia or premature coronary heart disease (CHD) or the presence of major CHD risk factors such as hypertension, diabetes, and obesity.

The expert panel[56] also added nonfasting HDL cholesterol as a new screening tool for the identification of dyslipidemia in children. Non-HDL cholesterol is calculated by subtracting the HDL cholesterol from the total cholesterol. This measure reflects the amount of cholesterol carried by atherogenetic apolipoprotein B–containing lipoproteins (VLDL, IDL, and LDL). In both adults and children, non-HDL cholesterol has been determined to be more predictive of persistent dyslipidemia and therefore atherosclerosis and future CVD events than total cholesterol, LDL cholesterol, or HDL cholesterol alone.[97] A major advantage of non-HDL cholesterol is that it can be accurately calculated in a nonfasting state and is therefore practical to obtain in a primary care setting. Percentiles for non-HDL cholesterol have been established (Table 23-3) and are based on data from the Bogalusa Heart Study,[98] where non-HDL cholesterol ≥ the 95th percentile is

TABLE 23-3 **Normal Plasma Lipid and Lipoprotein Concentrations (mg/dL) for Children and Adolescents***

Category	Low	Acceptable	Borderline	High
Total cholesterol	—	< 170	170-199	≥ 200
LDL cholesterol	—	< 110	110-129	≥ 130
Non-HDL cholesterol	—	< 120	120-144	≥ 145
Apolipoprotein B	—	< 90	90-109	≥ 110
Triglycerides	—			
0-9 y		< 75	75-99	≥ 100
10-19 y		< 90	90-129	≥ 130
HDL cholesterol	< 40	> 45	40-45	
Apolipoprotein A-1	< 115	> 120	115-120	—

*Values for plasma lipids and lipoproteins are from the Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents (2011). Summary report. Pediatrics 128:S1-S44.

LDL, low-density lipoprotein; HDL, high-density lipoprotein.

considered "abnormal/high" and between the 75th to 95th percentile is considered "borderline."

If a nonfasting non-HDL cholesterol is found to be abnormal, a fasting (12-hour) lipoprotein analysis is recommended[56] to allow quantification of total cholesterol, HDL cholesterol, triglycerides, and calculation of LDL cholesterol. The Friedewald equation—LDL cholesterol = total cholesterol − (HDL cholesterol + triglycerides/5)—can be used to calculate LDL cholesterol as long as the serum triglyceride level is < 400 mg/dL.[99] Direct measurement of LDL cholesterol concentration is available through some commercial laboratories and is indicated for individuals whose fasting triglyceride level is ≥ 400 mg/dL.

The lipid/lipoprotein cut points to be used in screening are presented in Table 23-3. Cut points for total cholesterol and LDL cholesterol in children are from the NCEP report.[90] The 75th and 95th percentiles for lipid values from this report were used to define "borderline" and "high" risk categories, respectively. If a lipoprotein analysis reveals LDL cholesterol to be borderline or high, there is consensus among guidelines that the test should be repeated and the average value of the two tests be considered for clinical decision making.[56]

Although the NCEP report provided population percentiles for triglyceride and HDL-C concentrations, recommended cut points for these variables were not established back in 1992.[90] Measurement of these variables has become more important because they are part of the clustering of risk factors associated with the growing epidemic of pediatric obesity in the United States.[54] Data from pathology and imaging studies show adverse changes in vascular structure and function in adulthood related to low HDL cholesterol and high triglyceride levels in childhood.[89] Therefore, the latest guideline[56] recommends cut points for triglycerides and HDL cholesterol based on percentiles provided by the NCEP.[90] The 75th and 95th percentiles for triglyceride values were used to define "borderline" and "high" risk categories, respectively, and the 25th and the 10th percentiles were used to define "borderline" and "high" risk groups for HDL cholesterol (see Table 23-3).

Although well-standardized immunochemical methods are available for the determination of apoB and apoA-1 measurements, guidelines suggest that measurements of apolipoproteins for universal screening provide no additional advantage over measuring non-HDL cholesterol, LDL cholesterol, and HDL cholesterol levels, except in youth with premature CVD in parents.[100,101] Cut points for apoB and apoA-1 from the National Health and Nutrition Examination Survey (NHANES) should be used in these cases[100] (see Table 23-3). Measurement of lipoprotein subclasses and their sizes in children and adolescents by advanced lipoprotein testing has been found to have no sufficient clinical utility to warrant routine use.[56]

Clinical evaluation of children and adolescents at high risk for CVD based on abnormal lipid levels should include a careful review of the patient's medical and family history and physical examination to identify additional risk factors and secondary causes of dyslipidemia. Assessment should include the following: review of past medical or family history for hypertension, diabetes mellitus, medication use, obesity, poor dietary habits (including excessive intake of saturated fat), sedentary behavior, and tobacco use; measurement of height, weight, and calculation of BMI; Tanner staging to assess pubertal growth; blood pressure measurement; physical inspection of skin, eyes, and tendons for lipid deposition and palpitation of the thyroid gland and liver for signs of enlargement; and laboratory tests (including thyroid, renal, and liver function panels). Glucose and insulin levels should be measured to assess for the presence of metabolic syndrome or diabetes.

Genetic Testing

Increasingly, DNA-based tests are being used to confirm the diagnosis of FH in patients with a family member who has a mutation or in a young patient with high LDL cholesterol with tendon xanthomas or atherosclerotic disease.[102] Currently, three genes (LDLR, apoB, and PCSK9) have been identified in association with mutations that cause this disorder.[103] In addition to LDLR, most laboratories test for the apolipoprotein B gene r500Q mutation. Rapid and relatively inexpensive methods have been developed to test a selected subset of the LDLR mutations. However, more expensive "complete gene scans" are needed for mutation-negative samples.[103] Once a mutation is identified, relatives can be tested rapidly and cheaply.

At present, it is unclear whether knowledge of specific mutations will lead to improved treatment. Several studies have shown that the degree of cholesterol lowering achieved by statins is influenced by the type of mutation (e.g., individuals with the APOB r3500Q mutation showed a strong positive response to statin therapy).[104,105] In addition, the diagnosis of FH based on genetic testing improved uptake and adherence to treatment in several studies.[106,107] Concerns remain about the long-term benefits and potential side effects from lifelong treatment with statins for identified children. As progress in this area continues, current treatment algorithms may need modification to describe the role of genetic testing in clinical practice.

DIET THERAPY IN MANAGING DYSLIPIDEMIA

New pediatric nutrition recommendations from the expert panel[56] support a two-pronged dietary approach to managing pediatric hypercholesterolemia: one geared to the population in general and the second focused on an individualized treatment of dyslipidemia, including an approach to elevated LDL-C and an approach to elevated triglycerides. On the population level for healthy infants, the expert panel[56] has recommended breastfeeding as optimal to 12 months of age if possible with supplementation of complementary foods when appropriate; the introduction of iron-fortified formula is recommended if breastfeeding is stopped or reduced before 1 year of age. Previous nutrition recommendations from the NCEP[90] did not include recommendations for

infants; however evidence in favor of the sustained cardiovascular benefits of breastfeeding, including lower total cholesterol, BMI, and carotid IMT into adulthood, is now strong.[108-110] These guidelines are in accordance with the Surgeon General's Office, the World Health Organization, the American Academy of Pediatrics, and the American Academy of Family Physicians.

The 2010 Dietary Guidelines for Americans (DGA)[111] provide optimal nutrition guidance for primary care providers to use in promoting nutrient adequacy and CVD risk reduction in children > 2 years of age. In keeping with the goal of reducing chronic disease risk among all Americans, the DGA support a dietary fat composition of 25% to 35% of calories, with less than 10% of calories from saturated fat, fewer than 300 mg of cholesterol per day, and no or minimal trans fats included. The DGA also emphasizes setting an appropriate daily calorie goal for healthy weight stability. This dietary pattern closely matches the former NCEP Step 1 diet[90] and is viewed as a primary preventive measure to optimize serum lipids, thereby reducing the risk of CVD in the pediatric population at large. Similar food-based dietary recommendations are available through the World Health Organization (www.fao.org/docrep/X0243E/x0243e00.htm).

Within appropriate gender- and age-specific requirements for growth and development in normal children and children with dyslipidemia, the expert panel[56] has recommended an early transition (between 1 and 2 years of age) to reduced fat, unflavored milk (from whole to 2%), and a dietary composition that matches that recommended by the DGA.[111] Advice to transition early to a low-fat diet (minimum of 30% of total calories to age 2 years) is supported by data from the ongoing Special Turku Risk Intervention Program (STRIP) study,[112-114] which showed that limiting total fat, saturated fat, and dietary cholesterol to DGA levels could be instituted safely after 6 months of age under medical supervision and lowered total cholesterol, LDL cholesterol, and BMI more than a usual higher-fat diet. The expert panel[56] has cautioned that any dietary changes initiated during childhood should be tailored to the child to ensure optimal growth and development and administered under the guidance of the child's primary care physician. Estimated calorie requirements based on age and gender at three levels of physical activity from the Institute of Medicine[115] are found in Table 23-4.

The expert panel's[56] population approach for youth ages 4 years and older encourages consumption of a plant-based diet comparable to the Dietary Approaches to Stop Hypertension (DASH)[116] dietary pattern. This dietary pattern recommends 7 to 10 servings/day of fruits and vegetables and 6 to 10 servings of breads, cereals, and grains. Whole fruits and vegetables rather than juice and whole grains rather than processed grains are encouraged. Key sources of saturated fat and cholesterol are moderated on the DASH dietary plan. For example, red meats are limited to modest amounts of lean cuts (e.g., 5 to 6 oz per day) and dairy products are limited to skim or low-fat varieties (24 to 32 oz/day). Plant-based foods should constitute the largest proportion of energy in children's diets. Most choices within these food groups are low in fat, cholesterol free, and high in fiber—and will help displace energy sources containing saturated fat.[117-118] The major health benefits of a DASH style of eating are lowered blood pressure, improved lipids, and improved weight status.[119,120] Box 23-3 highlights some practical dietary strategies for lowering saturated fat and cholesterol in the diets of the young.

For the individual child with identified dyslipidemia, a positive family history of premature CVD, obesity, or hypertension, the expert panel[56] has recommended initiating the same dietary approach recommended for the general population (as described previously). The expert panel has deemed this plan the Cardiovascular Health Integrated Lifestyle Diet (CHILD-1) and considers this the first line of therapy to remediate CVD risk factors with a primary target of lowering LDL cholesterol levels. If LDL cholesterol levels remain abnormal (LDL cholesterol ≥ 130 mg/dL) after 3 to 6 months of dietary adherence to the CHILD-1, further restriction of dietary saturated fat (< 7% of total calories) and cholesterol (< 200 mg/day), referred to as the CHILD-2 (LDL), is recommended. Repeated studies have shown that a stepwise dietary saturated fat reduction to 7% of total calories and cholesterol to 200 mg/day is safe and efficacious in lowering LDL cholesterol in children with hypercholesterolemia.[121-123]

TABLE 23-4 **Estimated Calorie Needs per Day by Age, Gender, and Physical Activity Level***

Gender	Age (Years)	Calorie Requirements (kcals) by Activity Level		
		SEDENTARY	MODERATELY ACTIVE	ACTIVE
Child	2-3	1000-1200	1000-1400	1000-1400
Female	4-8	1200-1400	1400-1600	1400-1800
	9-13	1400-1600	1600-2000	1800-2200
	14-18	1800	2000	2400
Males	4-8	1200-1400	1400-1600	1600-2000
	9-13	1600-2000	1800-2200	2000-2600
	14-18	2000-2400	2400-2800	2800-3200

*Estimated amounts of calories needed to maintain caloric balance for various age and gender groups at three different levels of physical activity are from the Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents (2011). Summary report. Pediatrics 128:S1-S44.

BOX 23-3 Practical Dietary Strategies for Lowering Saturated Fat and Cholesterol

- Eat 7 to 10 servings of fresh, frozen, or canned fruits and vegetables daily.
- Use vegetable oils and soft margarines low in saturated fat and trans fatty acids instead of butter or most other animal fats in the diet.
- Eat whole-grain breads and cereals rather than processed grain products.
- Use nonfat (skim) or low-fat milk and dairy products daily.
- Eat more fish, especially oily fish (broiled or baked).
- Eat lean cuts of meat, and trim any obvious fat from red meat before cooking.
- Take the skin off chicken or turkey and eat only the white meat.
- Avoid processed meats such as hotdogs, sausage, and bologna.
- Avoid creams or sauces made with butter or whole-milk dairy products.
- Choose low-fat snacks such as ginger snaps, graham crackers, pretzels, plain popcorn, animal crackers, and vanilla wafers.
- Use recommended portion sizes on food labels when preparing and serving food.

Low-fat diets should be designed to be nutritionally adequate and consultation with a registered dietitian is particularly helpful in that regard. In some studies, lower intakes of calcium, zinc, vitamin E, and phosphorus on low-fat diets were reported for diets initiated without medical supervision.[123] To prevent overzealous implementation of a very low fat diet for children with dyslipidemia, which could lead to failure to thrive,[124] the expert panel[56] has recommended 25% as the lower end of the range for total fat calories, consistent with the acceptable macronutrient distribution range stipulated by the DGA[111] for children ≥ 2 years of age. In most cases, dietary compliance to the CHILD-2 diet should normalize borderline-high LDL cholesterol levels. The reported response to diets with this composition of total fat, saturated fat, and cholesterol in children with LDL cholesterol levels > 130 mg/dL has ranged from 3% to 10%.[125,126]

For children older than 2 years of age with persistently high LDL cholesterol levels, additional dietary adjuncts can be used. These may include margarines containing plant stanol/sterol esters or water-soluble fibers, such as psyllium. About three servings of stanol containing margarine daily (equivalent to approximately 2 g/day), consumed as part of a low-fat diet, can reduce LDL cholesterol by 5% to 15%.[127-129] Psyllium at a dose of 6 g/day added to a low-fat diet may provide an additional 5% to 10% reduction in LDL cholesterol.[129,130] The extent to which LDL cholesterol is lowered may depend on previous dietary intake and baseline LDL cholesterol levels. Dietary supplements are covered in more detail later in the chapter (dietary additives and supplements).

For children with elevated triglycerides or low HDL cholesterol, the expert panel[56] has recommended following the CHILD-2 (TG) dietary pattern, which includes reducing one's intake of simple sugars from processed desserts, snacks, and sugared-sweetened beverages. Foods that are high in simple sugar generally add excess calories to the diet, which may contribute to weight gain and elevate serum triglycerides.[131] Weight management should be a goal of diet therapy for children with a BMI ≥ the 85th percentile. Weight-reduction approaches should focus on decreasing the child's weight-for-height percentile while maintaining linear growth.[132] Although weight loss may temporarily lower HDL cholesterol, weight stabilization at a new lower level will lead to a gradual increase in HDL cholesterol over time.[133] Fish oils from fatty fish, such as salmon and tuna, contain omega 3 fatty acids, which may also help to lower serum triglycerides.[134] Increased physical activity should be encouraged with a goal of 1 hour per day of moderate to vigorous activity, and sedentary activities (such as watching television viewing and playing computer and video games) should be limited to no more than 2 hours per day.[56]

PHARMACOLOGIC MANAGEMENT

For many children with moderately or severely elevated LDL cholesterol, which usually results from a genetic dyslipidemia, diet alone will not lower their cholesterol levels to the acceptable or even borderline range. In these cases, lipid-lowering drug therapy will be required to achieve LDL cholesterol treatment goals in children age 10 years and older. Long-term drug therapy is associated with a decreased incidence of heart disease and overall mortality in adults.[58] Although no studies directly demonstrate the efficacy of administering lipid-lowering drug therapy in children to prevent CVD, there is evidence from vascular imaging studies that statins may delay the atherosclerotic disease process.[135-137]

The expert panel[56] has recommended using medication in patients who are at least 10 years of age and whose postdietary (e.g., CHILD-1 → CHILD-2 after 6 months) LDL cholesterol level is ≥ 190 mg/dL or whose LDL cholesterol level is ≥ 160 mg/dL and there is a family history of early CVD, with at least one high-level or at least two moderate-level risk factors for CVD. In children with FH who have substantially elevated LDL cholesterol, medication management may need to be started earlier than age 10.[137] A lipid specialist should be consulted in these cases. A follow-up LDL cholesterol assessment is recommended 6 weeks after starting drug therapy and every 3 months thereafter until LDL cholesterol goals are met. Thereafter, follow-up can be less frequent.

For children in general, the goal for LDL cholesterol is < 130 mg/dL. For children with diabetes, the goal for LDL cholesterol is < 100 mg/dL.[60,138] The lower LDL cholesterol goal for children with diabetes reflects a synthesis of pediatric guidelines and treatment recommendations for adults with diabetes that now consider the presence of diabetes a coronary heart disease risk equivalent.[58] Table 23-5 summarizes the recommendations of the expert panel[56] regarding lipid screening

TABLE 23-5 **Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents Recommendations for Lipid Management of Children and Adolescents with Diabetes***

	Type 1 and Type 2 Diabetes Mellitus
Screening	After glycemic control > 2 years at diagnosis if other CVD risk factors; otherwise at 10 years; if normal, rescreen every 5 years At diagnosis regardless of age; if normal, rescreen every 2 years
Lipid goals	LDL-C ≤ 100 mg/dL Non-HDL-C < 120 mg/dL Triglycerides < 90 mg/dL
Treatment strategies	Glycemic control Diet (CHILD-1→CHILD-2) Physical activity Weight reduction if appropriate Medication indications if initial management fails: • Age ≥ 10 years • If LDL > 160 mg/dL • If LDL 130-159 mg/dL consider based on CVD risk profile • Statins with or without resins • Fibrates if TG > 1000 mg/dL • Manage other CVD risk factors (see Table 23-4) if appropriate

*Recommendations are from the Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents (2011). Summary report. Pediatrics 128:S1-S44.
CVD, cardiovascular disease; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

and management of children and youth with diabetes. Medications used in the treatment of specific lipid abnormalities are summarized in Table 23-6 and are reviewed in the following material.

Bile Acid–Binding Agents

Bile acid–binding agents lower serum cholesterol indirectly by binding with bile acids in the gastrointestinal tract. This action prevents their reabsorption into the enterohepatic circulation, resulting in their loss from the body and removal from the cholesterol pool.[139] To compensate for this loss, the liver increases endogenous cholesterol synthesis and up-regulates LDL-receptor synthesis—thereby lowering LDL cholesterol levels in the blood. In bile-acid resin trials in children, a dose of 8 g/day while on a cholesterol-lowering diet resulted in a decrease in LDL cholesterol ranging from 10% to 20%.[140,141]

Bile-acid resins come in powder and tablet form. The powder is usually taken twice daily (4-g scoop) mixed with water or juice. Resins in this form tend to be gritty in texture and children complain that they are unpleasant to drink. Tablets are more palatable but are large and difficult for some children to swallow. Overall, studies report poor to fair compliance with the medication.[139,142] Side effects are few and are mainly gastrointestinal in nature, including constipation and gas. These can be minimized with increased intake of water and fiber. Resins may increase triglyceride levels and may interfere with the absorption of certain medications and fat-soluble vitamins.[143] Supplementation with a multivitamin and folate (1 mg daily) is usually recommended.[139]

HMG-CoA Reductase Inhibitors

HMG-CoA reductase inhibitors (also known as statins) lower LDL cholesterol in the blood by blocking hepatic HMG Co-A reductase, the rate-limiting enzyme in cholesterol biosynthesis.[137] This action depletes the intracellular cholesterol pool, leading to an up-regulation of LDL receptors and a decrease in serum cholesterol. Doses ranging from 5 to 40 mg/day in children with familial hypercholesterolemia have resulted in a 23% to 40% lowering of LDL cholesterol.[135-137] Statins may be taken in combination with bile acid–binding agents for patients who fail to meet LDL cholesterol targets with statin monotherapy. No adverse effects of the combined therapies were noted in a pediatric trial of the medications, and the efficacy of the two medications taken together was additive.[94]

In vivo imaging studies have demonstrated improvements in surrogate markers for atherosclerosis with statin therapy. Reversal of endothelial dysfunction and regression of carotid IMT have been reported in children treated with statins over a 2-year period.[136,137] This latter finding suggests that the initiation of lipid-lowering statins in childhood may inhibit progression or might even lead to a regression of atherosclerosis.

Adverse effects of the medication are few but have included gastrointestinal (GI) complaints, elevated liver transaminase and creatine kinase (CK) levels, and myositis.[139,143,144] For this reason, statins would not be recommended for patients with liver disease. Follow-up of patients treated with statins should include a laboratory assessment of liver transaminase and CK levels and identification of adverse physical symptoms such as muscle cramps.

TABLE 23-6 **Drugs Used in the Treatment of Pediatric Lipid Disorders**

Class of Medication	Common Name	Mechanism of Action	Change in Lipid Profile	Adverse Effects
Bile acid–binding agents	Cholestyramine Colestipol Colesevelam	Binds intestinal bile acids; more cholesterol converted into bile acids; decrease hepatic cholesterol pool	↓ LDL ↑ TG	Constipation Abdominal cramping
HMG Co-A reductase inhibitors	Atorvastatin Simvastatin Pravastatin Rosuvastatin Lovastatin Fluvastatin	Inhibits cholesterol synthesis in hepatic cells; decreases cholesterol pool resulting in up-regulation of LDLR	↓ LDL ↓ TG ↑ HDL	Dyspepsia ↑ Liver transaminases ↑ CK Myositis
Fibrates*	Fenofibrate	Increases degradation of VLDL cholesterol and TG; hepatic synthesis of VLDL may be decreased	↓ TG ↑ HDL	Constipation Myositis Anemia
	Gemfibrozil			
Niacin*	Niacin extended release	Inhibits release of free fatty acids from adipose tissue; decreases VLDL and LDL cholesterol production and HDL cholesterol degradation	↓ LDL ↓ TG ↑ HDL	Flushing Headache ↑ Liver transaminases
Cholesterol absorption blocker*	Ezetimibe	Inhibits intestinal absorption of cholesterol and plant sterols	↓ LDL	Not reported in children or adolescents

*Not FDA approved for youths (as of this writing).
CK, creatine kinase; LDLR, low-density lipoprotein receptor; VLDL, very low density lipoprotein; LDL, low-density lipoprotein; TG, triglycerides; HDL, high-density lipoproteins.

Because statins are potentially teratogenic, it is essential that physicians determine that adolescent girls are not pregnant or likely to become pregnant before initiating therapy.[139] The longest statin trials have been 2 years in length. Thus, whether statins adversely affect long-term growth and development and are safe for lifelong use has not been ascertained. Longer-term safety and efficacy studies are needed, particularly with a follow-up of vascular end points.

Inhibitors of Cholesterol Absorption

Ezetimibe is a cholesterol-lowering agent that prevents the absorption of cholesterol and plant sterols by inhibiting the passage of sterols across the intestinal wall.[145] The reduction in cholesterol absorption leads to a decrease in hepatic cholesterol uptake and availability. As a result, there is a compensatory increase in hepatic cholesterol biosynthesis, an up-regulation of LDL receptor expression, and an overall decrease in blood LDL cholesterol levels. In children and adolescents, a daily intake of 10 mg/day reduced LDL cholesterol by approximately 20%.[78] Currently ezetimibe has not been systematically studied, and the Food and Drug Administration (FDA) has not approved it for use in children.

Fibric Acid Derivatives

Fibric acid derivatives (also known as fibrates) lower blood triglyceride levels by increasing degradation and reducing the hepatic production of VLDL.[139,145] The drug also increases the production of apoA-I, resulting in higher HDL cholesterol levels. Side effects are similar to those for statins and include GI complaints, elevated liver transaminase activity, and myopathy.[139] For this reason, fibrates are not recommended for use with statins. These medications are not FDA approved for use with children and adolescents and should be used only in consultation with a lipid specialist for adolescents with genetic hypertriglyceridemia.[56]

Niacin

Niacin decreases hepatic VLDL production, leading to decreased production of LDL cholesterol.[139,145] Children with heterozygous FH treated with 1000 to 2250 mg of niacin daily over an average of 8 months showed a 23% to 30% reduction in LDL cholesterol.[146] However, 76% of the children had adverse effects from therapy (e.g., flushing, headache, nausea, glucose intolerance, myopathy, abnormal liver function) and 38% discontinued the drug. Niacin is not recommended for children younger than 2 years of age and is generally not used to treat children with FH unless LDL cholesterol is persistently elevated or unusual hypertriglyceridemia and low HDL cholesterol are present.[56] Niacin given in combination with statins has been used to treat homozygous FH. Niacin is available in immediate and slow-release forms (Niaspan, Slo-Niacin).

Dietary Additives and Supplements

Plant sterols and stanols consumed at levels of 2 g per day have been shown to reduce LDL cholesterol levels by 9% to 20% in adults.[58] Foods containing these additives (e.g., margarines and salad dressings) lower serum cholesterol

by preventing dietary cholesterol absorption in the gastrointestinal tract.[127] In children with familial hypercholesterolemia, use of 2 g/day of plant sterols decreased LDL cholesterol by 5% to 15% but did not improve endothelial function.[128] This suggests that LDL cholesterol must be reduced to a certain threshold level before improvement of endothelial function can occur.

More studies examining the long-term effects of plant sterols on the vascular endothelium are warranted. Concern has been raised about the potential for the malabsorption of fat and fat-soluble vitamins in children consuming plant sterols chronically.[58] The expert panel[56] recommends reserving the use of foods supplemented with plant sterols/stanols to children with moderate to severe elevation in cholesterol, and monitoring fat-soluble vitamin status.[123]

Dietary supplements containing soluble fiber, garlic, and fish oils have been used to treat pediatric dyslipidemia with limited to moderate efficacy. In a randomized controlled trial, Davidson and colleagues studied the effect of psyllium fiber versus placebo on change in blood cholesterol levels of 6- to 18-year-old children with hypercholesterolemia.[130] Psyllium fiber (6 g/day) and the placebo were added to a ready-to-eat cereal. Compliance to treatment was excellent; consumption of the enriched cereal resulted in a modest 7% reduction in LDL cholesterol concentrations compared to the control cereal.

Garlic extract therapy was studied in a randomized controlled trial for effects on serum lipoproteins in 8- to 18-year-old children with FH.[147] The extract was given in three daily doses of 300 mg versus a placebo for 8 weeks. No significant effects of garlic treatment versus placebo were noted on total, LDL, or HDL cholesterol.

Omega-3 fatty acids, as found in fish oils, are known to reduce serum triglyceride levels in adults.[58] In a randomized crossover study, children with FH and FCHL were supplemented with either 1.2 g/day of docosahexaenoic acid (DHA) or a placebo for 6 weeks.[134] All children were given dietary counseling to reduce saturated fat intake to $< 7\%$ of calories. Outcomes studied included triglycerides, LDL and HDL cholesterol, and endothelial function as measured by flow-mediated dilation (FMD) of the brachial artery. Findings showed that DHA supplementation was associated with increased levels of total, LDL, and HDL cholesterol but no change in triglycerides compared to the placebo group. FMD improved significantly after DHA supplementation compared to baseline in both groups, and the change was greater in the DHA-treated group versus the controls. This finding suggests that in children DHA may not be effective for positively modifying serum lipids. However, the endothelium may be a therapeutic target for DHA in hyperlipidemic children.

LDL Apheresis

For children with homozygous familial hypercholesterolemia and extremely elevated LDL cholesterol (> 500 mg/dL), where LDL cholesterol cannot be reduced through the combination of diet therapy and multiple medication management, biweekly LDL apheresis has been utilized successfully to achieve significant lipid lowering under the care of a lipid specialist. In this process, apoB-containing particles are selectively removed from the circulation through extracorporeal precipitation.[148] Apheresis reduced LDL concentrations up to 72% compared with maximal drug therapy in patients with homozygous FH. The procedure is FDA approved, and qualified medical sites that perform this procedure are listed on the National Lipid Association website (www.lipid.org).

CONCLUSIONS AND FUTURE DIRECTIONS

Increasing evidence indicates that extreme elevation in LDL cholesterol is associated with vascular pathology in youth. With new recommendations for universal screening of pediatric patients, more patients with significant hypercholesterolemia are likely to be identified. Fortunately, appropriate therapy is available to modify and potentially reverse vascular abnormalities. Pediatric clinical trials of lifestyle and drug therapy to treat dyslipidemia suggest similar effectiveness and safety to that observed in adults. Most studies, however, have been of short or medium term in design. Longer-term safety and efficacy studies are needed, particularly with follow-up of vascular end points. Also, few clinical data are available to document at what age drug therapy should be appropriately and safely started. More work is needed in this regard to support clinical judgments on what age and dosage should be used in children at high risk for CHD.

REFERENCES

1. Nassir, F., Wilson, B., Han, X., et al. (2007). CD36 is important for fatty acid and cholesterol uptake by the proximal but not distal intestine. *J Biol Chem*, *282*, 19493–19501
2. Betters, J. L., & Yu, L. (2010). NPC1L1 and cholesterol transport. *FEBS Lett*, *584*, 2740–2747.
3. Hui, D. Y., Labonte, E. D., & Howles, P. N. (2008). Development and physiological regulation of intestinal lipid absorption III. Intestinal transporters and cholesterol absorption. *Am J Physiol Gastrointest Liver Physiol*, *294*, G839–G843.
4. Choudhuri, S., & Klaassen, C. D. (2006). Structure, function, expression, genomic organization, and single nucleotide polymorphisms of human ABCB1 (MDR1), ABCC (MRP), and ABCG2 (BCRP) efflux transporters. *Int J Toxicol*, *25*, 231–259.
5. Cavelier, C., Lorenzi, I., Rohrer, L., & von Eckardstein, A. (2006). Lipid efflux by the ATP-binding cassette transporters ABCA1 and ABCG1. *Biochim Biophys Acta*, *1761*, 655–666.
6. Berge, K. E., Tian, H., Graf, G. A., et al. (2000). Accumulation of dietary cholesterol in sitosterolemia caused by mutations in adjacent ABC transporters. *Science*, *290*, 1771–1775.
7. Mansbach, C. M., & Cameron, S. J. (2010). The biogenesis of chylomicrons. *Ann Rev Physiol*, 72, 315–333.
8. Shoulders, C. C., & Shelness, G. S. (2005). Current biology of MTP: implications for selective inhibition. *Curr Top Med Chem*, *5*, 283–300.
9. Sook Sul, H., & Storch, J. (2012). Cholesterol and lipoproteins: synthesis, transport and metabolism. In M. H. Stipanuk (Ed.), *Biochemical, physiological, and molecular aspects of human metabolism* (pp. 393–415) (3rd ed.). St. Louis: Saunders/Elsevier.
10. Daniels, S. R. (2003). Lipid metabolism and secondary forms of dyslipoproteinemia in children. *Prog Ped Card*, *17*, 135–140.
11. Wang, H., & Eckel, R. H. (2009). Lipoprotein lipase: from gene to obesity. *Am J Physiol*, *297*, E271–E288.
12. Twinckler, T. B., Dallinga-Thie, G. M., Cohn, J. S., & Chapman, M. J. (2004). Elevated remnant-like particle cholesterol concentration: a characteristic feature of the atherogenic lipoprotein phenotype. *Circulation*, *109*, 1918–1925.

13. Yu, K. C.-W., Chen, W., & Cooper, A. D. (2001). LDL receptor-related protein mediates cell-surface clustering and hepatic sequestration of chylomicron remnants in LDLR-deficient mice. *J Clin Invest*, *107*, 1387–1394.
14. Olivecrona, G., & Olivecrona, T. (2010). Triglyceride lipases and atherosclerosis. *Curr Opin Lipidol*, *21*, 409–415.
15. Zambon, A., Bertocco, S., Vitturi, N., et al. (2003). Relevance of hepatic lipase to the metabolism of triacylglycerol-rich lipoproteins. *Biochem Soc Trans*, *31*, 107–1074.
16. Hegele, R. A. (2009). Plasma lipoproteins: genetic influences and clinical implications. *Nat Rev Genet*, *10*, 109–121.
17. Beglova, N., & Blacklow, S. C. (2005). The LDL receptor: how acid pulls the trigger. *Trends Biochem Sci*, *30*, 309–317.
18. Tibolla, G., Norata, G. D., Artali, R., et al. (2011). Proprotein convertase subtilisin/kexin type 9 (PCSK9): from structure-function relation to therapeutic inhibition. *Nutr Metab Card Dis*, *21*, 835–843.
19. Walker, A. K., & Naar, A. M. (2012). SREBPs: regulators of cholesterol/lipids as therapeutic targets in metabolic disorders, cancers and viral diseases. *Clin Lipidol*, *7*, 27–36.
20. Riggoti, A. (2000). Scavenger receptors and atherosclerosis. *Biol Res*, *33*, 97–103.
21. Krimbou, L., Marcil, M., & Genest, J. (2006). New insights into the biogenesis of human high-density lipoproteins. *Curr Opin Lipidol*, *17*, 258–267.
22. Schaefer, E. J., & Azstalos, B. F. (2006). Cholesteryl ester transfer protein inhibition, high-density lipoprotein metabolism and heart disease risk reduction. *Curr Opin Lipidol*, *17*, 394–398.
23. Ohashi, R., Mu, H., Wang, X., et al. (2005). Reverse cholesterol transport and cholesterol efflux in atherosclerosis. *QJM*, *98*, 845–856.
24. Goldstein, J. L., & Brown, M. S. (2009). History of discovery: the LDL receptor. *Arterioscler Thromb Vasc Biol*, *29*, 431–438.
25. Familial hypercholesterolemia from children to adults. (2009). *Card Drugs Ther*, *16*, 289–293.
26. Benn, M. (2009). Apolipoprotein B levels, APOB alleles, and risk of ischemic cardiovascular disease in the general population, a review. *Atherosclerosis*, *206*, 17–30.
27. Fahed, A. C., Nemer, G. M. (2011). Familial hypercholesterolemia: the lipids or the genes? *Nutr Metab 8*, *23*, 1–12.
28. Pisciotta, L., Oliva, P., Pes, G. M., et al. (2006). Autosomal recessive hypercholesterolemia (ARH) and homozygous familial hypercholesterolemia (FH): a phenotypic comparison. *Atherosclerosis*, *188*, 398–405.
29. Naoumova, R. P., Thompson, G. R., & Soutar, A. K. (2004). Current management of severe homozygous hypercholesterolemia. *Curr Opin Lipidol*, *15*, 413–422.
30. Van der Graaf, A., Avis, H. J., Kusters, D. M., et al. (2011). Molecular basis of autosomal dominant hypercholesterolemia: assessment in a large cohort of hypercholesterolemic children. *Circulation*, *22*, 1167–1173
31. Arca, M. (2002). Autosomal recessive hypercholesterolemia in Sardinia, Italy, and mutations in ARH: a clinical and molecular genetic analysis. *Lancet*, *359*, 841–847.
32. Lind, S., Olsson, A. G., & Erickson, M. (2004). Autosomal recessive hypercholesterolemia: normalization of plasma LDL cholesterol by ezetimibe in combination with statin treatment. *J Inter Med*, *256*, 406–412.
33. Imes, C. C., & Austin, M. A. (2012). Low density lipoprotein cholesterol, apolipoprotein B and risk of coronary heart disease: from familial hyperlipidemia to genomics. *Biol Res Nurs*, *1*, 1–17.
34. Liyanage, K. E., Burnett, J. R., Hooper, A. J., & Van Bockxmeer, F. M. (2011). Familial hypercholesterolemia: epidemiology, Neolithic origins and modern geographic distribution. *Critical Rev Clin Lab Sci*, *48*, 1–18.
35. Patel, M. D., & Thompson, P. D. (2006). Phytosterols and vascular disease. *Atherosclerosis*, *186*, 12–19.
36. Von Bergmann, K., Sudhop, M. D., & Lütjohann, D. (2005). Cholesterol and plant sterol absorption: recent insights. *Am J Cardiol*, *96*, 10D–14D.
37. Bartnikowska, E. (2009). Biological activities of phytosterols with particular attention to their effects on lipid metabolism. *Polish J Food Nutr Sci*, *59*, 105–112.
38. Miller, M., Stone, N. J., Ballantyne, C., et al. (2011). Triglycerides and cardiovascular disease: a scientific statement from the American Heart Association. *Circulation*, *123*, 2292–2333.
39. Veerkamp, M. J., deGraaf, J., Hendricks, J. C., et al. (2004). Nomogram to diagnose familial combined hyperlipidemia on the basis of results of a 5-yar follow-up study. *Circulation*, *109*, 2980–2985.
40. Wiesbauer, F., Blesssberger, H., & Azar, D. (2009). Familial combined hyperlipidemia in very young myocardial infarction survivors (< 40 years of age). *Eur Heart J*, *30*, 1073–1079.
41. Eckel, R. H., Grundy, S. M., & Zimmet, P. Z. (2005). The metabolic syndrome. *Lancet*, *365*, 1415–1428.
42. Hopkins, P. N., Heiss, G., Ellison, R. C., et al. (2003). Coronary artery disease risk in familial combined hyperlipidemia and familial hypertriglyceridemia: a case control comparison from the National Heart, Lung and Blood Institute Family Heart Study. *Circulation*, *108*, 519–523.
43. Leaf, D. A. (2008). Chylomicronemia and the chylomicronemia syndrome: a practical approach to management. *Am J Med*, *121*, 10–12.
44. Goldberg, I. J. (2009). Hypertriglyceridemia: impact and treatment. *Endocrinol Metab Clin North Am*, *38*, 137–149.
45. Hovingh, G. K. (2005). Inherited disorders of HDL metabolism and atherosclerosis. *Curr Opin Lipidol*, *16*, 139–145.
46. Clauss, S. B., & Kwiterovich, P. O. (2003). Genetic disorders of lipoprotein transport in children. *Prog Ed Card*, *17*, 123–133.
47. Assmann, G., Von Ekardstein, A., & Brewer, H. B., Jr. (2001). Familial analphalipoproteinemia: Tangier disease. In C. Scriver, A. Beaudet, W. Sly, & D. Valle (Eds.), The metabolic and molecular bases of inherited disease (pp. 2937–2960) (8th ed.). New York: McGraw-Hill.
48. Asztalos, B. F., Schaefer, E. J., & Horvath, K. V. (2007). Role of LCAT in HDL remodeling: investigation of LCAT deficiency states. *J Lipid Res*, *48*, 592–599.
49. DiFilippo, M., Crehalet, H., Samson-Boumas, M. E., et al. (2012). Molecular and functional analysis of two new *MTTP* gene mutations in an atypical case of abetalipoproteinemia. *J Lipid Res*, *53*, 548–555.
50. Schonfeld, G. (2003). Familial hypobetalipoproteinemia: a review. *J Lipid Res*, *44*, 878–883.
51. Targui, P., & Averna, M. (2011). Hypobetalipoproteinemia: genetics, biochemistry, and clinical spectrum. *Adv Clin Chem*, *54*, 81–107.
52. Smelt, A. H., & de Beer, F. (2004). Apolipoprotein E and familial dysbetalipoproteinemia: clinical, biochemical and genetic aspects. *Semin Vasc Med*, *4*, 249–257.
53. Rosenson, R. S. (2012). *Secondary causes of dyslipidemia*. Retrieved from www.uptodate.com/contents/secondary-causes-of-dyslipidemia
54. Daniels, S. R., Arnett, D. K., Eckel, R. H., et al. (2005). Overweight in children and adolescents; pathophysiology, consequences, prevention and treatment. *Circulation*, *111*, 1999–2012.
55. Brambilla, P., Lissau, I., Flodmark C.-E., et al. (2007). Metabolic risk factor clustering examination in children; to draw a line across pediatric metabolic syndrome. *Int J Obes*, *31*, 591–600.
56. Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents (2011). Summary Report. *Pediatrics*, *128*, S1–S44.
57. Chahil, T. J., & Ginsberg, H. N. (2006). Diabetic dyslipidemia. *Endocrinol Metab Clin North Am*, *35*, 491–510.
58. Expert Panel on Detection, Evaluation, and Treatment of High Cholesterol in Adults (2001). Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA*, *285*, 2486–2497.
59. Kaufman, F. R. (2002). Type 2 diabetes mellitus in children and youth: a new epidemic. *J Pediatr Endocrinol Metab*, *15*, 737–744.
60. American Diabetes Association (2003). Management of dyslipidemia in children and adolescents with diabetes. *Diabetes Care*, *26*, 2194–2197.
61. Kershnar, A. K., Daniels, S. T., Imperatore, G., et al. (2006). Lipid abnormalities are prevalent in youth with type 1 and type 2 diabetes: the Search for Diabetes in Youth Study. *J Pediatr*, *149*, 314–319.

62. Naylor, L. H., Green, D. J., Jones, T. W., et al. (2011). Endothelial function and carotid intima medial thickness in adolescents with type 2 diabetes mellitus. *J Ped*, *159*, 971–974.
63. Daniels, S. R. (2003). Lipid metabolism and secondary forms of dyslipoproteinemia in children. *Progr Pediatr Cardiol*, *17*, 135–140.
64. Galley, R. (2006). Improving outcomes in renal disease. *JAAPA*, *19*, 20–25.
65. Badimon, L., Martinez-Gonzalez, J., Llorente-Cortes, V., et al. (2006). Cell biology and lipoproteins in atherosclerosis. *Curr Mol Med*, *6*, 439–456.
66. Moore, M. J., & Freeman, M. W. (2006). Scavenger receptors in atherosclerosis: beyond lipid uptake. *Arterioscler Thromb Vasc Biol*, *26*, 1702–1711.
67. Kher, N., & Marsh, J. D. (2004). Pathobiology of atherosclerosis: a brief review. *Semin Thromb Hemost*, *30*, 665-672.
68. Stary, H. C., Stoll, J. D., Yin, J., et al. (1996). The natural history of atherosclerosis in the aorta in the first forty years of life. In Fuster, V (Ed.), *Syndromes of atherosclerosis: correlations of clinical imaging and pathology* (pp. 225–238). Armonk, NY: Futura Publishing.
69. Berenson, G. S., Srinivasan, S. R., Bao, W., et al. (1998). Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults: the Bogalusa Heart Study. *N Eng J Med*, *338*, 1650–1656.
70. McGill, H. C., McMahan, C. A., Herderick, E. E., et al. (2002). Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group. *Circulation*, *105*, 2712–2718.
71. Lloyd-Jones, D. M., Wilson, P. W., Larson, M. G., et al. (2004). Framingham risk score and prediction of lifetime risk of coronary heart disease. *Am J Cardiol*, *94*, 20–24.
72. de Groot, E., Hovingh, K., Wiegman, A., et al. (2004). Measurement of arterial wall thickness as a surrogate marker for atherosclerosis. *Circulation*, *109*, III33–III38.
73. Sankatsing, R. R., de Groot, E., Jukema, J. W., et al. (2005). Surrogate markers for atherosclerosis disease. *Curr Opin Lipidol*, *16*, 434–441.
74. Keelan, P. C., Bielak, L. F., Ashai, K., et al. (2001). Long term prognostic value of coronary calcification detected by electron-beam computed tomography in patients undergoing coronary angiography. *Circulation*, *104*, 412–417.
75. Mahoney, L. T., Burns, T. L., Staford, W., et al. (1996). Coronary risk factors measured in childhood and young adults are associated with coronary artery calcification in young adults: the Muscatine Study. *J Am Coll Cardiol*, *27*, 277–284.
76. Gidding, S. S., Bookstein, L. C., & Chomka, E. V. (1998). Usefulness of electron beam tomography in adolescents and young adults with heterozygous familial hypercholesterolemia. *Circulation*, *98*, 2580–2583.
77. Kobayashi, K., Akishita, M., Yu, W., et al. (2004). Interrelationship between non-invasive measurements of atherosclerosis: flow mediated dilation of brachial artery, carotid intima-media thickness and pulse wave velocity. *Atherosclerosis*, *173*, 13–18.
78. Watanabe, K., Sugiyama, S., Kugiyama, K., et al. (2005). Stabilization of carotid atheroma assessed by quantitative ultrasound analysis in nonhypercholesterolemic patients with coronary artery disease. *J Am Coll Cardiol*, *46*, 2022–2030.
79. Teregawa, H., Kato, M., Kurokawa, J., et al. (2001). Usefulness of flow-mediated dilation of the brachial artery and/or the intima-media thickness of the carotid artery in predicting coronary narrowing in patients suspected of having coronary artery disease. *Am J Cardiol*, *88*, 1147–1151.
80. Furomoto, T., Fujii, S., Saito, N., et al. (2002). Relationships between brachial artery flow mediated dilation and carotid artery intima-media thickness in patients with suspected coronary artery disease. *Jpn Heart J*, *43*, 117–125.
81. Raitakari, O. T., Juonala, M., Kahonen, M., et al. (2003). Cardiovascular risk factors in childhood and carotid artery intima-media thickness in adulthood: the Cardiovascular Risk in Young Finns Study. *JAMA*, *290*, 2277–2283.
82. Sanchez, A., Barth, J. D., & Zhang, L. (2000). The carotid artery wall thickness in teenagers is related to their diet and the typical risk factors of heart disease among adults. *Atherosclerosis*, *152*, 265–266.
83. Tonstad, S., Jokimsen, O., Stensland-Bugge, E., et al. (1996). Risk factors related to carotid intima-media thickness and plaque in children with familial hypercholesterolemia and control subjects. *Arteriosclero Thromb Vasc Biol*, *16*, 984–991.
84. Davis, P. H., Dawson, J. D., Riley, W. A., & Lauer, R. M. (2001). Carotid intima-media thickness is related to cardiovascular risk factors measured from childhood through middle age. *Circulation*, *104*, 2815–2819.
85. Lavrencic, A., Kosmina, B., Keber, I., et al. (1996). Carotid intima-media thickness in young patients with familial hypercholesterolemia. *Heart*, *76*, 321–325.
86. Leeson, C. P., Kattenhorn, M., Morley, R., et al. (2001). Impact of low birth weight and cardiovascular risk factors on endothelial function in early adult life. *Circulation*, *103*, 1264–1268.
87. Meyer, A. A., Kundt, G., Lenschow, U., et al. (2006). Improvement of early vascular changes and cardiovascular risk factors in obese children after a six-month exercise program. *J Am Coll Cardiol*, *48*, 1865–1870.
88. Meyer, A. A., Kundt, G., Steiner, M., et al. (2006). Impaired flow-mediated vasodilation, carotid artery intima-media thickening, and elevated endothelial plasma markers in obese children: the impact of cardiovascular risk factors. *Pediatrics*, *117*, 1560–1567.
89. Urbina, E. M., Williams, R. V., Alpert, B. S., et al. (2009). Noninvasive assessment of subclinical atherosclerosis in children and adolescents. *Hypertension*, *54*, 919–950.
90. National Cholesterol Education Program (NCEP) (1992). Highlights of the report of the expert panel on blood cholesterol levels in children and adolescents. *Pediatrics*, *89*, 495–501.
91. Daniels, S. R., Greer, F. R., & Committee on Nutrition (2008). Lipid screening and cardiovascular health in childhood. *Pediatrics*, *122*, 198–208.
92. McCrindle, B. W., Urbina, E. M., Dennison, B. A. et al. (2007). American Heart Association Atherosclerosis, Hypertension, Obesity in Youth Committee: American Heart Association Council of Cardiovascular Disease in the Young; American Heart Association Council on Cardiovascular Nursing; Drug therapy of high-risk lipid abnormalities in children and adolescents: a scientific statement from the American Heart Association Atherosclerosis, Hypertension, and Obesity in Youth Committee Council of Cardiovascular Disease in the Young, with the Council on Cardiovascular Nursing. *Circulation*, *115*, 1948–1967.
93. Rifai, S. K., Neufeld, E., Ahlstrom, P., et al. (1996). Failure of current guidelines for cholesterol screening in urban African-American adolescents. *Pediatrics*, *98*, 383–388.
94. Daniels, S. R., Gidding, S. S., & deFerranti, S. D. (2011). National Lipid Association Expert Panel on Familial Hypercholesterolemia. Pediatric aspects of familial hypercholesterolemia: recommendations from the National Lipid Association Expert Panel on Familial Hypercholesterolemia. *J Clin Lipidol*, *3*(Suppl.), S30–S37.
95. Morrison, J. A. (2003). A longitudinal evaluation of the NCEP-Peds guidelines for evaluated total and LDL cholesterol in adolescent girls and boys. *Prog Pediatr Cardiol*, *17*, 159–168.
96. Labarthe, D. R., Dai, S., & Fulton, J. E. (2003). Cholesterol screening in children: insights from Project HeartBeat! and NHANES III. Prog Pediatr Cardiol, *17*, 169–178.
97. Srinivasan, S. R., Frontini, M. G., Xu, J., & Berenson, G. S. (2006). Utility of childhood non-high density lipoprotein cholesterol levels in predicting adult dyslipidemia and other cardiovascular risks: the Bogalusa Heart Study. *Pediatrics*, *118*, 201–206.
98. Srinivasan, S. R., Myers, L., & Berenson, G. S. (2002). Distribution and correlates of non-high density lipoprotein cholesterol in children: the Bogalusa Heart Study. *Pediatrics*, *110*, e29.
99. Warnick, G. R., Knopp, R. H., Fitzpatrick, V., & Branson, L. (1990). Estimating low-density lipoprotein cholesterol by the Friedewald equation is adequate for classifying patients on the basis of nationally recommended cutpoints. *Clin Chem*, *36*, 15–19.
100. Bachorik, P. S., Lovejoy, K. L., Carroll, M. S., & Johnson, C. L. (1997). Apolipoprotein B and A1 distributions in the United States, 1988–1991; results of the National Health and Nutrition Examination Survey III (NHANES III). *Clin Chem*, *43*, 2364–2378.
101. Mudd, J. O., Borlaug, B. A., Johnston, P. V., et al. (2007). Beyond low density lipoprotein cholesterol; defining the role of low-density lipoprotein heterogeneity in coronary artery disease. *J Am Coll Cariol*, *50*, 1735–1741.

102. Vergopoulos, A., Knoblauch, H., & Schuster, H. (2002). DNA testing for familial hypercholesterolemia: improving disease recognition and patient care. *Am J Pharmacogenomics*, *2*, 253–262.
103. Hadfield, S. G., & Humphries, S. E. (2005). Implementation of cascade testing for the detection of familial hypercholesterolemia. *Curr Opin Lipidol*, *16*, 428–433.
104. Miltiadous, G., Xenophontas, S., Bairaktarik, K., et al. (2005). Genetic and environmental factors affecting the response to statin therapy in patients with molecularly defined familial hypercholesterolemia. *Pharmacogenet Genomics*, *15*, 219–225.
105. Humphries, S. E., Whittall, R. A., Hubbart, C. S., et al. (2006). Genetic cause of familial hypercholesterolemia in patients in the UK: relation to plasma lipids and coronary heart disease risk. *J Med Genetics*, *43*, 943–949.
106. Umans-Eckenhausen, M. A., Defesche, J. C., & Sijbrands, E. J. (2001). Review of the first 5 years of screening for familial hypercholesterolemia in the Netherlands. *Lancet*, *357*, 165–168.
107. Leren, T. P., Manshaus, T., Skovholt, U., et al. (2004). Application of molecular genetics for diagnosing familial hypercholesterolemia in Norway: results from a family based screening program. *Semin Vasc Med*, *4*, 75–85.
108. Demmers, T. A., Jones, P. J., Wang, Y., et al. (2005). Effects of early cholesterol intake on cholesterol biosynthesis and plasma lipids among infants until 18 months of age. *Pediatrics*, *115*, 1594–1601.
109. Bayley, T. M., Alasmi, M., Thorkelson, T., et al. (2002). Longer term effects of early dietary cholesterol level on synthesis and circulating cholesterol concentrations in human infants. *Metabolism*, *51*, 25–33.
110. Mize, C. E., Uauy, R., Kramer, R., et al. (1995). Lipoprotein cholesterol responses in healthy infants fed defined diets from ages 1 to 12 months: comparison of diets predominant in oleic acid versus linoleic acid, with parallel observations in infants fed a human milk-based diet. *J Lipid Res*, *36*, 1178–1187.
111. US Department of Agriculture & US Department of Health and Human Services, (2011). *Dietary guidelines for Americans 2010* (7th ed.). Washington, DC: US Government Printing Office.
112. Simell, O., Ronnermaa, T., Lapinleimu, H., et al. (2000). Special Turku Coronary Risk Factor Intervention Project for Babies (STRIP). *Am J Clin Nutr*, *72*, 1316S–1331S.
113. Salo, P., Viikari, J., Rask-Nissila, L., et al. (1999). Effect of low saturated fat, low cholesterol dietary intervention on fatty acid compositions in serum lipid fractions in 5-year old children. The STRIP project. *Eur J Clin Nutr*, *53*, 927–932.
114. Talvia, S., Lagstrom, H., Rasanen, M., et al. (2004). A randomized intervention since infancy to reduce intake of saturated fat; calorie (energy) and nutrient intakes up to the age of 10 years in the Special Turku Coronary Risk Factor Intervention Project. *Arch Pediatric Adolesc Med*, *158*, 41–47,
115. Institute of Medicine (2002). *Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids*. Washington, DC: The National Academies Press.
116. Appel, L. J., Moore, T. J., Obarzanek, E., et al. (1997). A clinical trial of the effects of dietary patterns on blood pressure. DASH Collaborative Research Group. *N Engl J Med*, *336*, 1117–1124.
117. Kronsberg, S. S., Obarzanek, E., Affenito, S. G., et al. (2003). Macronutrient intake of black and white adolescent girls over 10 years: the NHLBI Growth and Health Study. *J Am Diet Assoc*, *103*, 852–860.
118. Ritchie, L. D., Spector, P., Stevens, M. J., et al. (2007). Dietary patterns in adolescence are related to adiposity in young adulthood in black and white females. *J Nutr*, *137*, 399–406.
119. Obarzanek, E., Sacks, F. M., Vollmer, W. M., et al. (2001). Effects on blood lipids of a blood-pressure lowering diet: the Dietary Approaches to Stop Hypertension (DASH) trial. *Am J Clin Nutr*, *74*, 80–89.
120. Sacks, F. M., Svetkey, L. P., Vollmer, W. M., et al. (2001). DASH Collaborative Research Group. *N Engl J Med*, *344*, 3–10.
121. Lauer, R. M., Obarzanek, E., Hunsberger, S., et al. (2000). Efficacy and safety of lowering dietary intake of total fat, saturated fat and cholesterol in children with elevated LDL-cholesterol: the Dietary Intervention Study in Children. *Am J Clin Nutr*, *72*, 1332S–1342S.
122. Obarzanek, E., Kimm, S. Y., Barton, B. A., et al. (2001). Long-term safety and efficacy of a cholesterol lowering diet in children with elevated low-density lipoprotein cholesterol: seven year results of the Dietary Intervention Study in Children. *Pediatrics*, *107*, 256–264.
123. Jacobson, M. S., Tomopoulus, S., Williams, C. L., et al. (1998). Normal growth in high risk hyperlipidemic children and adolescents with dietary intervention. *Prev Med*, *27*, 775–780.
124. Lifshitz, F., & Moses, N. (1989). A complication of dietary treatment of hypercholesterolemia. *Am J Dis Child*, *143*, 537–542.
125. Williams, C. L., Hayman, L. L., Daniels, S. R., et al. (2002). Cardiovascular health in childhood: a statement of health professionals from the Committee on Atherosclerosis, Hypertension, and Obesity in the Young (AHOY) of the Council on Cardiovascular Disease in the Young, American Heart Association. *Circulation*, *106*, 143–160.
126. Tershakovec, A. W., Shannon, B. M., Achterberg, C. L., et al. (1998). One-year follow-up of nutrition education for hypercholesterolemic children. *Am J Public Health*, *88*, 258–261.
127. Gylling, H., Siimes, M. A., & Miettinen, T. A. (1995). Sitostanol ester margarine in dietary treatment of children with familial hypercholesterolemia. *J Lipid Res*, *36*, 1807–1812.
128. Amundsen, A. L., Ose, L., Nenseter, M. S., & Ntanios, F. Y. (2002). Plant sterol ester-enriched spread lowers plasma total and LDL cholesterol in children with familial hypercholesterolemia. *Am J Clin Nutr*, *76*, 338–344.
129. Williams, C. L., Bollella, M., Spark, A., & Puder, D. (1995). Soluble fiber enhances the hypocholesterolemic effect of the step 1 diet in childhood. *J Am Coll Nutr*, *14*, 251–257.
130. Davidson, M. H., Dugan, L. D., Burns, J. H., et al. (1996). A psyllium-enriched cereal for treatment of hypercholesterolemia in children: a controlled, double-blind, cross-over study. *Am J Clin Nutr*, *63*, 96–102.
131. Starc, T. J., Shea, S., Cohn, L. C., et al. (1998). Greater dietary intake of simple carbohydrate is associated with lower concentrations of high-density lipoprotein cholesterol in hypercholesterolemic children. *Am J Clin Nutr*, *67*, 1147–1154.
132. Gidding, S. S., Dennison, B. A., Birch, L. L., et al. for the American Heart Association (2006). Dietary recommendations for children and adolescents: a guide for practitioners. *Pediatrics*, *117*, 544–559.
133. Reinehr, R., de Sousa, G., Toschke, A. M., & Andler, W. (2006). Long-term follow-up of cardiovascular disease risk factors in children after an obesity intervention. *Am J Clin Nutr*, *84*, 490–496.
134. Engler, M. M., Engler, M. B., Malloy, M., et al. (2004). Docosahexaenoic acid restores endothelial function in children with hyperlipidemia: results from the EARLY study. *Int J Clin Pharmacol Ther*, *42*, 672–679.
135. Wiegman, A., Hutten, B. A., deGroot, E., et al. (2004). Efficacy and safety of statin therapy in children with familial hypercholesterolemia: a randomized controlled trial. *JAMA*, *292*, 331–337.
136. DeJongh, S., Lilien, M. R., & Roodt, J. (2002). Early statin therapy restores endothelial function in children with familial hypercholesterolemia. *J Am Coll Cardiol*, *40*, 2117–2121.
137. Rodenberg, J., Vissers, M. N., & Wiegman, A. (2007). Statin treatment in children with familial hypercholesterolemia: the younger the better. *Circulation*, *116*, 664–668.
138. American Diabetes Association (2005). Standards of medical care in diabetes. *Diabetes Care*, *28*, S4–S36.
139. McCrindle, B. W. (2003). Drug therapy of hyperlipidemia. *Prog Pediatr Cardiol*, *17*, 141–150.
140. McCrindle, B. W., O'Neill, M. B., Cullen-Dean, G., & Helden, E. (1997). Acceptability and compliance with two forms of cholestyramine in the treatment of hypercholesterolemia in children: a randomized, crossover trial. *J Pediatr*, *130*, 266–273.
141. Tonstad, S., Knudtzon, J., Sivertsen, M., et al. (1996). Efficacy and safety of cholestyramine therapy in peripubertal and prepubertal children with familial hypercholesterolemia. *J Pediatr*, *129*, 42–49.
142. McCrindle, B. W. (2006). Hyperlipidemia in children. *Thromb Res*, *118*, 49–58.
143. McCrindle, B. W., Helden, E., Cullen-Dean, G., & Conner, W. T. (2002). A randomized crossover trial of combination pharmacologic therapy in children with familial hyperlipidemia. *Pediatr Res*, *51*, 715–721.
144. Arambepola, C., Farmer, A. J., Perera, R., & Neil, H. A. (2007). Statin treatment for children and adolescents with heterozygous

familial hypercholesterolemia: a systematic review and meta-analysis. *Atherosclerosis*, *195*, 339–347.
145. Rodenburg, J., Vissers, M. N., Daniels, S. R., et al. (2004). Lipid-lowering medications. *Pediatr Endocrinol Rev*, *2*, 171–180.
146. Colletti, R. B., Neufeld, E. J., Roff, N. K., et al. (1993). Niacin treatment of hypercholesterolemia in children. *Pediatrics*, *92*, 78–82.
147. McCrindle, B. W., Helden, E., & Conner, W. T. (1998). Garlic extract therapy in children with hypercholesterolemia. *Arch Pediatr Adolesc Med*, *152*, 1089–1094.
148. Lee, W. P., Datta, B., Ong, B. B., et al. (2011). Defining the role of lipoprotein apheresis in the management of familial hypercholesterolemia. *Am J Cardiovasc Drugs*, *11*, 363–370.

QUESTIONS

1. A 4-year-old child is diagnosed with the autosomal recessive disease sitosterolemia with a mutation in two genes encoding the ABC half-transporter. In assessing the patient, all of the following are likely to be present *except* which one?
   a. High plant sterol levels in plasma
   b. Tendon xanthomas
   c. Normal plasma cholesterol
   d. Aortic stenosis

Answer: c

2. Which of the following statements commonly describes individuals with heterozygous familial hypercholesterolemia (FH) of the receptor-defective type?
   a. Have plasma LDL-cholesterol that is two to three times higher than normal
   b. Experience early onset atherosclerosis
   c. Have xanthomas in the tendons
   d. Require treatment with LDL apheresis

Answer: a

3. A mutation in proprotein convertase subtilisin/kexin type 9 (PCSK9) of the increase in function type will generally result in which of the following?
   a. Marked elevations in LDL-cholesterol
   b. Low levels of LDL-cholesterol
   c. High levels of VLD particles
   d. Low levels of HDL-cholesterol

Answer: a

4. Which of the following statements is (are) true regarding familial combined hyperlipidemia (FCHL)?
   a. The metabolic defect is overproduction of VLDL.
   b. A diagnosis of the disorder is based on the presence of increased levels of cholesterol, triglyceride or apo B in patients and their first-degree relatives.
   c. The disorder can manifest in childhood but is usually not fully expressed until adulthood.
   d. All the statements above are true about FCHL.

Answer: d

5. All *except* which of the following could cause chylomicronemia syndrome?
   a. Lipoprotein lipase deficiency
   b. Apo CII deficiency
   c. Apo A5 loss of function mutations
   d. LDL receptor deficiency

Answer: d

6. The NHLBI Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents has recommended universal lipid screening for which of the following groups?
   a. All children
   b. Children ages 9 to 10 years
   c. Adolescents ages 12 years and above
   d. Adolescents ages 16 years and above

Answer: b

7. The expert panel added a nonfasting HDL cholesterol as a new screening tool for the identification of dyslipidemia in childhood. What is (are) the advantage(s) of this assessment tool over other methods?
   a. It reflects the amount of cholesterol carried by all atherogenic apolipoprotein B containing lipoproteins.
   b. Is more predictive of persistent dyslipidemia than total cholesterol, LDL-cholesterol or HDL cholesterol alone.
   c. It can be accurately calculated in a nonfasting state.
   d. All of the statements above are considered advantages of nonfasting HDL cholesterol measurement.

Answer: d

8. Which of the following is true of the Cardiovascular Health Integrated Lifestyle Diet (CHILD-1)?
   a. Emphasizes reducing dietary saturated fat to less than 7% of total calories
   b. Is considered the first line of therapy to lower LDL-cholesterol levels in children
   c. Recommends the use of plant sterols at a 2-gram dose per day
   d. Recommends a dietary cholesterol reduction to 200 mg/day

Answer: b

9. To lower triglycerides, all of the following are encouraged as part of the expert panel's CHILD-2 (TG) dietary pattern *except* which one?
   a. Reduced intake of simple sugars
   b. Weight management/reduction as necessary
   c. Reduced intake of dairy foods to no more than two servings per day.
   d. Increased fatty fish consumption (e.g., salmon and tuna) to increase intake of omega-3 fatty acids

Answer: c

10. Which of the following medications may be useful for a child with high triglycerides that cannot be lowered with the CHILD-2 (TG) diet in consultation with a lipid specialist?
    a. Ezetimibe
    b. Fibrates
    c. Statins
    d. Bile acid resins

Answer: b

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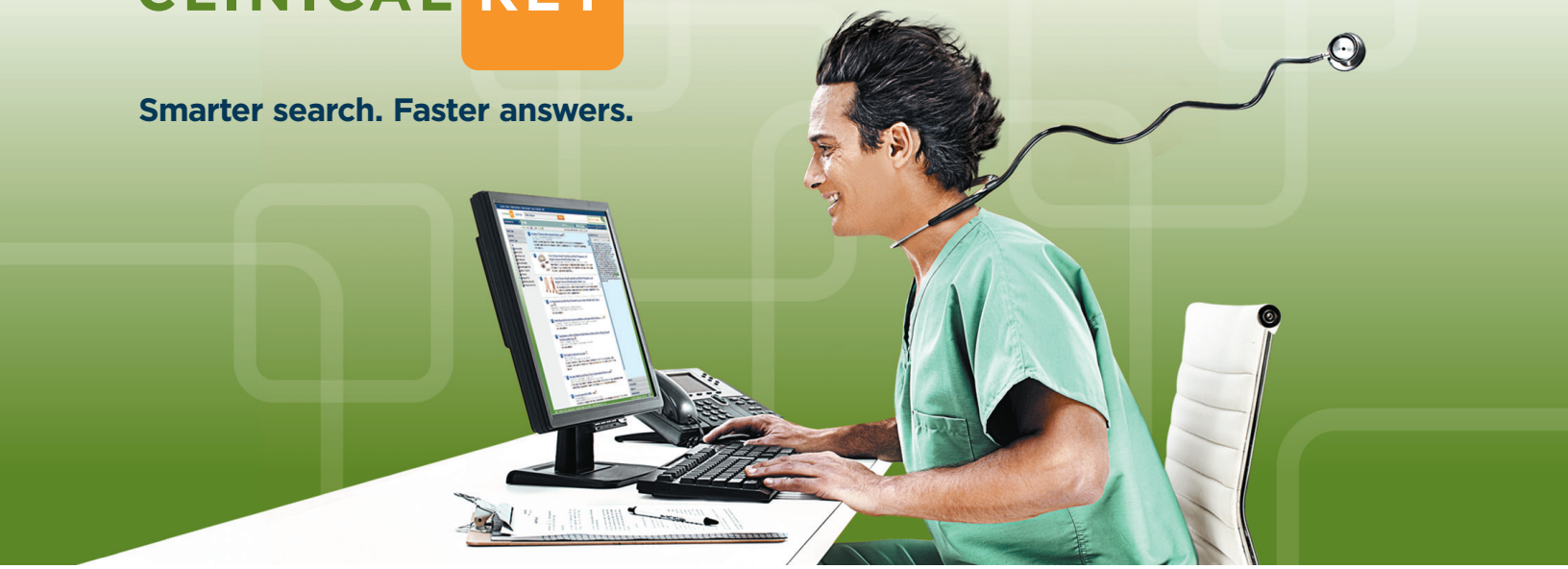

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