Jean-Marie Saudubray
Matthias R. Baumgartner
John Walter *Eds.*

Inborn Metabolic Diseases

Diagnosis and Treatment

6th Edition

Springer

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With 81 figures

Springer

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Preface to the 6th edition

Inborn Metabolic Diseases: Diagnosis and Treatment, remains the standard textbook for professionals working in inherited metabolic medicine and biochemical genetics but it is also an essential resource for all specialities in this multidisciplinary field. The speciality of inherited metabolic disease is at the forefront of progress in medicine with new methods in metabolomics and genomics identifying the molecular basis for a growing number of conditions and syndromes that were previously unexplained. These powerful techniques allow us to link the clinical, biochemical and molecular characteristics of disorders and provide a basis for therapeutic interventions.

For this new edition all 43 chapters have been revised or newly written by authors with particular expertise in their subject areas. Since the previous edition published in 2011, two new categories of inborn errors of metabolism (IEM) and more than 300 'new' disorders have been described, 85% presenting with predominantly neurological manifestations. The chapters that encompass these have been considerably extended, including those involving complex lipids (phospholipids, triglycerides, sphingolipids) and non mitochondrial fatty acid homeostasis (including peroxisomal defects) (>60 disorders), congenital disorders of glycosylation (>90 disorders), purine metabolism (35 disorders), metal transport (>35 disorders), and disorders of oxidative phosphorylation (including mitochondrial transporters, iron-sulfur complex metabolism and mitochondrial tRNA synthetases) (>230 disorders). The newly described metabolic disorders affecting cytoplasmic tRNA synthetases and other factors related to cytoplasmic protein synthesis, transporters, channels and enzymes implicated in the logistics and regulation of the cell, challenge our current classification based on organelles and form a bridge between 'classic' metabolic diseases with metabolic markers and those caused by mutations in structural proteins without such markers, which are most often diagnosed by molecular techniques.

While this new edition highlights recent findings it continues to provide a comprehensive review of all IEM, with a particular focus on the clinical and biochemical approach to recognition, diagnosis and treatment at all ages. The clinical algorithms of chapters 1 and 2 incorporate both 'old' and 'new' disorders, and there are now more algorithms detailing neurological presentations. An updated listing of metabolic markers and profiles and a section on molecular techniques such as next generation sequencing and gene panels have been added. In order to keep the book to a reasonable size we have not included a chapter dedicated to newborn screening in this edition; instead this method of diagnosis is discussed for individual disorders in their relevant chapters.

As before, we continue to advocate referral to specialist centres for the diagnosis and treatment of inherited metabolic disorders. For countries in the European Union a list of such centres is compiled by the Society for the Study of Inborn Errors of Metabolism (SSIEM), while for the United States and Canada, Japan, Australia, South American and Middle East countries comparable lists are compiled by the American (SIMD), Japanese (JIMD), Australian (AIMD) South Latin America (SLEIMPN) and Middle East societies for the study of inherited metabolic diseases, respectively.

We pay tribute to our colleague George van den Berghe who has now retired from the editorial board. We welcome new authors and also thank those previous authors who, while not involved with this edition, have helped to lay the foundation for this book.

Jean-Marie Saudubray
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Section I
Diagnosis and Treatment: General Principles

Clinical Approach to Inborn Errors of Metabolism in Pediatrics

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Inborn errors of metabolism (IEM) are individually rare, but collectively numerous. The application of tandem mass spectrometry (tandem MS) to newborn screening and prenatal diagnosis has enabled presymptomatic diagnosis for some IEM. However, for most, neonatal screening tests are either too slow, expensive or unreliable and, as a consequence, a simple method of clinical screening is mandatory before initiating sophisticated biochemical investigations. The clinical diagnosis of IEM relies upon a limited number of principles:

- In the appropriate clinical context consider IEM in parallel with other more common conditions.
- Be aware of symptoms that persist and remain unexplained after the initial treatment and the usual investigations have been performed for more common disorders, may be due to an IEM.
- Suspect that any neonatal death may possibly be due to an IEM, particularly those that have been attributed to sepsis. Additionaly, true sepsis can trigger acute decompensation when there is an underlying IEM. Carefully review all autopsy findings.
- Do not confuse a symptom or a syndrome with aetiology-the underlying cause may be an IEM yet to be defined.
- Remember that IEM can present at any age, from fetal life to old age.
- Be aware that because most IEM have a recessive inheritance (although some have dominant, X-linked, or maternal inheritance), the majority of individual cases may appear sporadic.
- In the acute emergency situation first consider those IEM that are most amenable to treatment.
- Obtain help from specialized centers.

Until recently IEM were considered as a speciality of paediatricians. Indeed the term »inborn« in the mind of clinicians has meant for a long time, a disease which starts in the newborn period or at least in childhood. Although paediatricians have learned with time that in addition to severe neonatal forms most IEM can have mild forms with first clinical signs starting in adolescence or very late in adulthood, this concept of »adult onset IEM« has not reached the adult medical community until very recently (► Chapter 2). Since these late onset forms are often unrecognized, their exact prevalence is unknown. Based mainly upon personal experience over 40 years and on the literature analysis, this Chapter gives an overview of clinical clues to the diagnosis of IEM in pediatrics. In the following pages, inborn errors amenable to treatment are printed in **bold.**

Do not miss a treatable disorder!
First provide care for the patient (emergency treatment) and then the family (genetic counselling)!

1.1 Classification

1.1.1 Pathophysiology

From a pathophysiological perspective, metabolic disorders can be divided into the following three diagnostically useful groups.

Group 1: Disorders which give rise to intoxication This group includes inborn errors of intermediary metabolism (IEIM) that lead to an acute or progressive intoxication from the accumulation of small molecules proximal to the metabolic block. In this group are the inborn errors of amino acid (AA) catabolism (phenylketonuria, maple syrup urine disease, homocystinuria, tyrosinemia etc.), most organic acidurias (OA) (methylmalonic, propionic, isovaleric etc.), congenital urea cycle defects (UCD), sugar intolerances (galactosemia, hereditary fructose intolerance), metal intoxication (Wilson, Menkes, hemochromatosis…), and porphyrias. All the conditions in this group share clinical similarities: they do not interfere with the embryo-fetal development; they present with a symptom-free interval and clinical signs of »intoxication«, which may be acute (vomiting, coma, liver failure, thromboembolic complications etc.) or chronic (failure to thrive, developmental delay, ectopia lentis, cardiomyopathy etc.). Circumstances that can provoke acute metabolic attacks include catabolism, fever, intercurrent illness and food intake. Clinical expression is often both late in onset and intermittent. The diagnosis is straightforward and most commonly relies on plasma and urine AA, OA and acylcarnitine chromatography. Most of these disorders are treatable and require the emergency removal of the toxin by special diets, extra-corporeal procedures, or »cleansing« drugs (carnitine, sodium benzoate, penicillamine, etc.).

Although the pathophysiology is somewhat different the inborn errors of neurotransmitter synthesis and catabolism (monoamines, GABA and glycine) and the inborn errors of AA synthesis (serine, glutamine, proline/ornithine and asparagine) can also be included in this group since they share many characteristics: they are IEIM, their diagnosis relies on plasma, urine, and CSF investigations (AA, OA analyses, etc.), and some are amenable to treatment even when the disorder starts in utero, for example 3-phosphoglycerate dehydrogenase deficiency (► Chapter 24).

Group 2: Disorders involving energy metabolism These consist of IEM with symptoms due, at least partly, to a deficiency in energy production or utilization within liver, myocardium, muscle, brain or other tissues. This group can be divided into mitochondrial and cytoplasmic energy defects. Mitochondrial defects are the most severe and are generally untreatable. They encompass the congenital lactic acidemias (defects of pyruvate transporter, pyruvate carboxylase (PC), pyruvate dehydrogenase (PDH), and the Krebs cycle), mitochondrial respiratory chain disorders (disturbing the respiratory chain itself, a mitochondrial transporter or the coenzyme Q10 (CoQ) synthesis) and the fatty acid oxidation (FAO) and ketone body defects. Only the latter and CoQ defects are partly

treatable. Common symptoms in this group include hypoglycemia, hyperlactatemia, hepatomegaly, severe generalized hypotonia, myopathy, cardiomyopathy, failure to thrive, cardiac failure, circulatory collapse, sudden unexpected death in infancy, and brain involvement. Some of the mitochondrial disorders and pentose phosphate pathway (PPP) defects can interfere with the embryo-fetal development and give rise to dysmorphism, dysplasia and malformations [1]. Cytoplasmic energy defects are generally less severe. They include disorders of glycolysis, glycogen metabolism and gluconeogenesis, hyperinsulinisms (all treatable disorders), the disorders of creatine metabolism (partly treatable), and the PPP defects (untreatable). It has been recently found that vesicular glycolysis may be able to provide a constant intrinsic source of energy, independent of mitochondria, for the rapid axonal movement of vesicles over long distances [2]. Disturbances of these processes could be responsible for some still unexplained neurodegenerative disorders. We also emphasize the crucial role of the cytoplasmic citrate, glycolysis and the PPP in complex fatty acids and lipids synthesis through the timely provision of NADPH, glyceraldehyde 3-phosphate and dihydroxyacetone phosphate [3]. Diagnosis of group 2 disorders is difficult and relies on functional tests, enzymatic analyses requiring biopsies or cell culture, and on molecular analyses.

Group 3: Disorders involving complex molecules This group involves cellular organelles (lysosomes, peroxisomes, endoplasmic reticulum, Golgi apparatus and mitochondria) and includes diseases that disturb the synthesis, remodelling, recycling, trafficking and catabolism of complex molecules. Symptoms are most often permanent, progressive, independent of intercurrent events (even if an acute crisis may have occurred in the course of a disorder) and unrelated to food intake. All lysosomal storage disorders (LSD), peroxisomal disorders (PBD), disorders of intracellular trafficking and processing, such as alpha-1-antitrypsin,and congenital disorders of glycosylation (CDG) belong to this group.Beside these well known disorders a novel and rapidly expanding group of IEM involving the synthesis remodeling and recycling of complex lipids and fatty acids have recently been described [3]. This biochemical group encompasses metabolic defects of phospholipids, triglycerides, (▶ Chapter 34) sphingolipids (▶ Chapter 38), isoprenoids: cholesterol (▶ Chapter 32), ubiquinone (▶ Chapter 14), dolichol (▶ Chapter 41), plasmalogens and complex long chain fatty acids (very long chain fatty acids (VLCFA), fatty alcohol, branched chain fatty acids, eicosanoids derived from arachidonic acid: prostaglandins, leukotriens) (▶ Chapter 40).

Many other defects disturbing various systems implicated in the processing and trafficking of complex molecules can be hypothesised, as illustrated for example by

I. the CEDNIK syndrome due to a mutation in the *SNAP 29* gene coding for a SNARE protein involved in intracellular vesicle function and presenting as a neurocutaneous syndrome [4]
II. Mutations in *AP5Z1*, encoding a subunit of the AP-5 complex (that facilitates specialized cargo sorting in vesicular-mediated trafficking) which have been reported to cause hereditary spastic paraplegia, the cellular phenotype of which bears striking resemblance to features described in a number of LSDs [5]
III. mutations in *CHMP2B* which encodes the charged multivesicular body protein, and is characterised by neuronal lysosomal storage pathology presenting with familial frontotemporal dementia [6]
IV. a single point mutation in the gene for Rabenosyn-5 with evidence of defective endocytotic trafficking presenting with a complex phenotype including intractable seizures [7] and
V. mutations in *WDR45*, an autophagy-related gene, responsible for static encephalopathy of childhood with neurodegeneration in adulthood (SENDA syndrome), which presents with neurodegeneration with brain iron accumulation (NBIA) [8] that begins with early-onset spastic paraplegia and mental retardation, and then develops sudden-onset parkinsonism and dystonia during the patients late 20s to early 30s.

These new defects, all found by exome sequencing without obvious metabolic markers, raise the question of a broader definition of LSDs with the accumulation of undigestible material in the endosomal/lysosomal system. In addition mutations in two dually localised aminoacyl tRNA synthetases KARS and GARS, which act in both the mitochondria and the cytosol, as well as several cytosolic aminoacyl tRNA synthetases (LARS, HARS, MARS, YARS) have also been recently reported (▶ Chapter 14). They open a new field of IEM since cytoplasmatic tRNA synthetases are necessary for all proteins of the cell and therefore all organelles. The same is true for other factors related to cytosplasmic protein synthesis (e.g. regulatory factors like EIF2AK3, etc.) and furthermore also for nuclear factors related to gene expression and splicing.

The careful grouping of patients in well-defined clinical entities may provide algorithms for orientating metabolic (e.g., lipidomic approaches) and genetic (e.g., exome sequencing) investigations. Of note, these complex molecules synthesis and remodeling defects are at the crossroad of classical IEM due to an enzymatic block in a catabolic pathway and IEM affecting the synthesis and stability of structural molecules [9] [10]. The concept of complex molecules synthesis defects also opens the window to promising therapeutic trials, for example, by providing the distal missing compound, although only very few of such disorders are treatable acutely so far; however, enzyme replacement and substrate reduction therapy are available for several LSDs.

1.1.2 Clinical Presentation

Besides newborn screening in the general population (as for phenylketonuria) or in at-risk families, there are four groups of clinical circumstances in which physicians are faced with the possibility of a metabolic disorder:

- Early symptoms in the antenatal and neonatal period.
- Later-onset acute (and recurrent) attacks of symptoms such as coma, ataxia, vomiting, acidosis, exercise intolerance, cardiac, renal, liver or other visceral failure.
- Chronic and progressive neurological symptoms (developmental delay, mental retardation, epilepsy, neurological deterioration, psychiatric signs).
- Specific and permanent organ/system presentations that may concern all medical specialities (cardiology, dermatology, endocrinology, gastroenterology, hematology ... etc.).

1.2 Antenatal Symptoms

These can be classified in three major clinical categories:

1. True malformations (such as skeletal malformations, congenital heart disease, visceral aplasias and neural tube defects),
2. Dysplasias (like cortical heterotopias, cortical cysts, posterior fossa abnormalities, polycystic kidneys, liver cysts),
3. Functional signs (such as intrauterine growth retardation, hydrops foetalis, hepatosplenomegaly, microcephaly).

According to this classification true irreversible malformations are only observed in O-glycosylation disorders primary, or secondary to manganese transporter *SLC39A8* mutations (▶ Chapter 41), in cholesterol synthesis defects (▶ Chapter 32), in AA synthesis disorders, as with glutamine and asparagine synthetase deficiency (lissencephaly) (▶ Chapter 24), and rarely in severe energetic defects such as glutaric aciduria type II (▶ Chapter 12), some respiratory chain disorders and in the mitochondrial thiamine pyrophosphate carrier defect (*SLC25A19)* responsible for the Amisch lethal microcephaly (▶ Chapter 28) (◘ Table 1.1 and ◘ Table 1.2). Of note the congenital microcephaly observed in serine synthesis defects is partly reversible on early treatment in mild forms but not in the severe Neu Laxova presentation (▶ Chapter 24). Lysosomal, peroxisomal and N-glycosylation defects are responsible for dysplasia and functional abnormalities that are more or less reversible. The vast majority of »true intoxication« disorders (AA and OA catabolism disorders) do not interfere with the embryo-foetal development and do not give rise to dysmorphism and antenatal symptoms (although some severe OA may present with subtle congenital signs) (◘ Table 1.1 and ◘ Fig. 1.1). Coarse facies is present in many LSDs and is a highly diagnostic sign (◘ Table 1.2). Untreated maternal disturbances (such as PKU) can be responsible for foetal dysplasia (◘ Fig. 1.1).

◘ **Table 1.1** Inborn errors of metabolism with coarse facies or intrauterine growth retardation

Coarse Facies	
Age at onset: present at birth	Galactosialidosis (early infancy)
	I-cell disease
	GM1 gangliosidosis
	Sialidosis type II
	Sly (mucopolysaccharidosis (MPS) type VII) (rare)
Age at onset: early infancy	Multiple sulfatase deficiency (Austin disease)
	Fucosidosis type I
	Hurler disease (MPS type IH)
	Mannosidosis
	Maroteaux-Lamy disease (MPS type V)
	Salla disease
	Sialidosis type II
	Sly disease (MPS type VII)
Age at onset: childhood	Aspartylglucosaminuria
	Hunter disease (MPS type II)
	Pseudo-Hurler polydystrophy
	San Filippo disease (MPS type III)
Intrauterine Growth Retardation	
	Fetal alcohol syndrome
	Infants born to mothers with untreated phenylketonuria
	Cholesterol biosynthesis defects
	CDG, several types and N-Glycanase 1 deficiency (▶ Chapter 41)
	Lysosomal storage disorders
	Many non-metabolic polymalformative syndromes
	Peroxisomal disorders
	Respiratory chain disorders
	Transaldolase deficiency

Table 1.2 Dysplasia, dysmorphism and malformations

Dysplasia, dysmorphism	
Maternal metabolic disturbances	**PKU** (heart defect, microcephaly, specific facial dysmorphism)
	Alcohol (special face, hypotrophy)
	Diabetes (macrosomia)
	Drugs (specific facial dysmorphism, hypotrophy)
	Vitamin deficiencies (riboflavin)
Inborn errors affecting the fetus	Carnitine palmitoyl transferase II deficiency (renal cysts)
	N- and O-CDG many types, *SLC39A8* mutations (▶ Chapter 41)
	Phosphoglucomutase deficiency (cleft uvula and palate) (▶ Chapter 41)
	D-2-Hydroxyglutaric aciduria
	Glutaric aciduria II (MADD) (renal cysts)
	Inborn errors of collagen metabolism
	Hyperinsulinism (macrosomia, specific facial dysmorphism) (▶ Chapter 9)
	Hypoparathyroidism
	Hypophosphatasia
	Leprechaunism
	Lysosomal storage disorders (hydrops fetalis)
	Mevalonic aciduria (specific facial dysmorphism)
	Peroxisomal biogenesis defects (renal cysts, neuronal migration defects)
	Chondrodysplasia punctata types I, II, III
	Pyruvate dehydrogenase deficiency
	Phospholipids synthesis defects (several types, among them Lenz Majewski syndrome) (▶ Chapter 34)
	Respiratory chain defects
	Transaldolase deficiency (hydrops fetalis)
	Vici syndrome (*EPG5* mutations with agenesis of the corpus callosum)
Malformations	
Cholesterol synthesis defects: 8 types including Smith-Lemli-Opitz syndrome (▶ Chapter 32)	
Dolichol synthesis /recycling defects: Several types (▶ Chapter 41)	
O-glycosylation and related defects: Many types including Walker-Warburg syndrome and muscle eye brain disease and manganese carrier *SLC39A8* mutations (▶ Chapter 41)	
AA synthesis defects: glutamine and asparagine synthetase deficiency (lissencephaly) and serine synthesis (congenital microcephaly and Neu laxova syndrome) (▶ Chapter 24)	
Respiratory chain defects (ventricular septal, vertebral and limb defects, VACTERL association)	
3-OH-isobutyryl CoA deacylase deficiency (limbs & vertebral defects) (▶ Chapter 18)	
Non-ketotic hyperglycinemia (NKH)	
Mitochondrial thiamine pyrophosphate transporter (Amish microcephaly) (▶ Chapter 28)	

MPS Mucopolysaccharidoses, *CDG* congenital disorder of glycosylation

1.3 Neonatal and Early Infancy Presentation (<1 year) [11]

1.3.1 Clinical Presentation

The neonate has a limited repertoire of responses to severe illness. IEM may present with non-specific symptoms such as respiratory distress, hypotonia, poor sucking reflex, vomiting, diarrhoea, dehydration, lethargy, seizures; all problems that can easily be attributed to sepsis or some other common cause. Where a previously affected sibling has died, this may have been wrongly attributed to sepsis, heart failure, or intraventricular hemorrhage, and it is important to critically review clinical records and autopsy reports when they are available.

In Group 1 disorders (IEM that give rise to intoxication), an extremely suggestive clinical picture is that of a baby, born at full-term after a normal pregnancy and delivery, who, after an initial symptom-free period, relentlessly deteriorates for no apparent reason and does not respond to symptomatic therapy. The interval between birth and clinical symptoms may range from hours to weeks, depending on the nature of the metabolic block and the environment. Investigations, routinely performed in sick neonates, including a chest X-ray, CSF examination, septic screen, and cerebral ultrasound, yield normal results. This unexpected and »mysterious« deterioration after a normal initial period is the most important indication for this group of IEM. Careful re-evaluation of the child's condition is then warranted. In this context signs previously interpreted as non-specific manifestations of neonatal hypoxia, infection, or other common diagnoses take on a new significance. In energy deficiencies (Group 2 disorders), clinical presentation is often less evocative and displays variable severity. A clinical algorithm for screening for treatable IEM in neonates is presented in ◘ Fig. 1.1.

A careful reappraisal of the child is warranted for the following:

▪ Neurological deterioration (coma, lethargy): Metabolic encephalopathy

Most IEM that result in intoxication or energy deficiency are brought to a doctor's attention because of neurological deterioration. With intoxication, the initial symptom-free interval varies in duration depending on the condition. Typically, the first reported sign is poor sucking and feeding, after which the child sinks into an unexplained coma despite supportive measures. At a more advanced state, neurovegetative problems with respiratory abnormalities, hiccups, apneas, bradycardia, and hypothermia can appear. In the comatose state, characteristic changes in muscle tone and involuntary movements appear. In **maple syrup urine disease (MSUD)** generalized hypertonic episodes with opisthotonus, and slow boxing or pedalling movements are observed. Of note most non-metabolic causes of coma are associated with hypotonia, so that the presence of »normal« peripheral muscle tone in a comatose child reflects a relative hypertonia. Another neurological pattern observed in **OA** is axial hypotonia and limb hypertonia with fast large amplitude tremors and myoclonic jerks which are often mistaken for convulsions. An abnormal urine and body odor is present in some diseases in which volatile metabolites accumulate; the most important examples are the maple syrup odor of **MSUD** and the sweaty feet odor of **isovaleric acidemia** (IVA) and **type II glutaric acidemia (GAII)**. If any of the preceding signs or symptoms are present, metabolic disorders should be given a high diagnostic priority.

In energy deficiencies, the clinical presentation is less evocative and displays a more variable severity. In many conditions, there is no symptom-free interval. The most frequent findings are a severe generalized hypotonia, rapidly progressive neurological deterioration, and possible dysmorphism, or malformations. However, in contrast to the intoxication group, lethargy and coma are rarely initial signs. Hyperlactatemia with or without metabolic acidosis is very frequent. Cardiac and hepatic involvement are commonly associated (see below).

A few LSDs present in the neonatal period with neurological deterioration, hydrops foetalis or ichthyosis, such as Gaucher type II (collodion baby) and multiple steroid sulfatase deficiency. Most severe PBD present at birth with dysmorphism (Zellweger phenotype) and severe neurological dysfunction(neonatal adrenoleukodystrophy phenotype) (▶ Chapter 40). Severe forms of CDG involving N and O-glycosylation, glycosylphosphatidylinositol anchor and dolichol phosphate biosynthesis may also present with acute congenital neurological dysfunction although they more often present with hypotonia, seizures, dysmorphism, malformations and diverse visceral involvement (▶ Chapter 41).

▪ Seizures

Always consider the possibility of an IEM in a neonate with unexplained and refractory epilepsy [12]. Neonatal metabolic seizures are often a mixture of partial, erratic myoclonus of the face and extremities, or tonic seizures. Classically the term **»early myoclonic encephalopathy« (EME)** has been used if myoclonic seizures dominate the clinical pattern. The EEG often shows a burst-suppression pattern, however, myoclonic jerks may occur without EEG abnormalities.

A few treatable metabolic disorders can present in the neonatal period or early in infancy predominantly with »intractable« seizures:

- **pyridoxine responsive seizures (antiquitin deficiency),**
- **folinic acid responsive epilepsy** has been shown to be allelic to undiagnosed antiquitin deficiency (▶ Chapter 28),
- **pyridox(am)ine-5′-phosphate oxidase deficiency** (**pyridoxal phosphate responsive seizures**) (▶ Chapter 28),
- **3-phosphoglycerate dehydrogenase deficiency**,
- **other inborn errors of serine synthesis** responsive to serine supplementation (▶ Chapter 24),
- persistent congenital **hyperinsulinism**,
- **some forms of GPI anchor defects** that associate hyperphosphatasia may also respond to B6 (▶ Chapter 28 and 41),
- **biotin responsive holocarboxylase synthetase deficiency** can also rarely present predominantly with neonatal seizures,

Fig. 1.1 The »Sick« neonate: an algorithm for screening for treatable inborn errors of metabolism

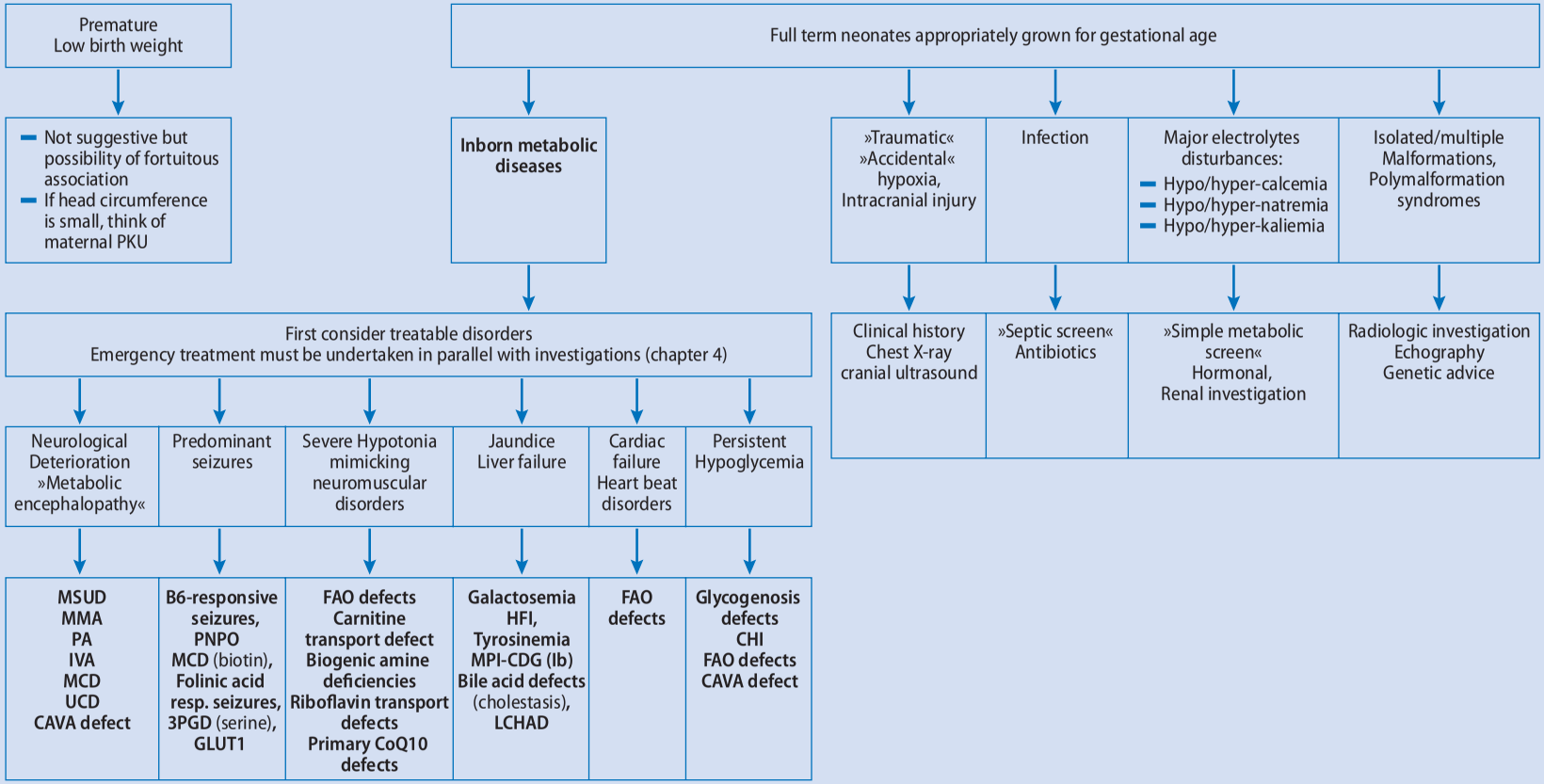

Fig. 1.1 The »Sick« neonate: an algorithm for screening for treatable inborn errors of metabolism. *CAVA*, carbonic anhydrase VA deficiency; *CDG*, congenital disorders of glycosylation; *FAO*, fatty acid oxidation disorders; *CoQ10*, coenzyme Q 10; *HFI*, hereditary fructose intolerance; *IVA*, isovaleric acidemia; *LCHAD*, 3-hydroxy long chain acylCoCa dehydrogenase; *MCD*, multiple carboxylase deficiency; *MMA*, methylmalonic aciduria; *MSUD*, maple syrup urine disease; *PA*, propionic acidemia; *CHI*, congenital hyperinsulinism ; *PKU*, phenylketonuria; *UCD*, urea cycle defects; *PNPO*, pyridox(am)ine-5′-phosphate oxidase; *3PGD*, 3-phosphoglycerate dehydrogenase

- **GLUT1 deficiency syndrome** (GLUT1-DS brain glucose transporter), responsive to a hyperketotic diet (▶ Chapter 10) and
- **biotin responsive biotinidase deficiency**, may rarely present in the first months of life as an epileptic encephalopathy (▶ Chapter 20).

Many other non treatable IEM can present in the neonatal period or early in infancy with severe epilepsy and encephalopathy:

- non ketotic hyperglycinemia (NKH),
- D-glyceric aciduria, mitochondrial glutamate transporter defect (SLC24A22) (▶ Chapter 29),
- hypoacetylaspartia and aspartate-glutamate carrier 1 deficiency (SLC25A12) (▶ Chapter 22),
- GABA transaminase deficiency,
- glutamine synthetase deficiency (▶ Chapter 24),
- the neonatal form of ceroid neuronal lipofuscinosis (cathepsin D deficiency) (▶ Chapter 38),
- peroxisome biogenesis defects (PBD) (▶ Chapter 40),
- mitochondrial disorders,
- sulfite oxidase deficiency (SO) (▶ Chapter 20),
- defects of purine metabolism,
- CDG (most of glycosylphosphatidylinositol (GPI) anchor synthesis defects with dysmorphic facial features of which Mabry syndrome with hyperphosphatasia) [13] (▶ Chapter 41),
- Menkes disease,
- GM3 synthetase deficiency,
- asparagine synthetase deficiency (▶ Chapter 24),
- hyperprolinaemia due to SLC24A22 mutations (▶ Chapters 16 and 29),
- adenosine kinase deficiency (patients may also present hypoglycaemia due to hyperinsulinism) (▶ Chapter 20 and 35),
- and the recently described inosine triphosphatase deficiency (▶ Chapter 35).

In most of these conditions, epilepsy is severe, with an early onset, and can present with spasms, myoclonus, partial or generalized tonic/clonic crises.

Hypotonia

Severe hypotonia is a common symptom in sick neonates. It is generally observed in non metabolic inherited diseases (mainly in severe fetal neuromuscular disorders). Only a few IEM present with isolated hypotonia in the neonatal period and only very few are treatable.

Discounting disorders in which hypotonia is part of a constellation of abnormalities, including, for example, major bone changes, dysmorphism, malformations, or visceral symptoms, the most severe metabolic hypotonias are observed in:

- hereditary hyperlactatemias,
- respiratory chain disorders,
- **urea cycle defects**,
- NKH,
- SO deficiency,
- PBD,
- CDG,
- trifunctional enzyme deficiency.

Central hypotonia is associated with lethargy, coma, seizures, and neurological symptoms in:

- NKH,
- SO deficiency,
- PBD.

Central hypotonia with characteristic metabolic changes is also observed in congenital lactic acidosis and **UCD** (hyperammonemia).

Severe global hypotonia and hypomotility mimicking neuromuscular diseases can appear in some treatable IEM such as

- **biogenic amine defects**,
- **primary carnitine deficiency** (not strictly in the neonatal period),
- **FAO defects**,
- **genetic defects of riboflavin transport** (▶ Chapter 12),
- **primary CoQ10 defects** (▶ Chapter 14).

Severe forms of Pompe disease and fatal congenital heart glycogenosis due to mutation in *PRKAG2* (▶ Chapter 5) can initially mimic respiratory chain disorders, or trifunctional enzyme deficiency when generalized hypotonia is associated with cardiomyopathy. However, Pompe disease does not strictly start in the neonatal period.

An emergent group of disorders to be considered in the differential diagnosis of the severe neonatal hypotonia are the IEM of the dolichols, which lead to hypoglycosylated proteins and belong to the CDG syndromes. Other than the severe hypotonia, they can associate different signs including dysmorphy, microcephaly, elevated CK, seizures and cardiac involvement although not all of them are constantly present.

A **pyridostigmine responsive congenital myasthenic syndrome** can be a presenting sign in ALG2, ALG14, DPAGT1, GFPT1, and GMPPB-CDGs (▶ Chapter 41).

Finally, one of the most frequent causes of neonatal hypotonia is Prader-Willi syndrome, where central hypotonia is apparently an isolated symptom at birth. This syndrome is mimicked by the hypotonia cystinuria syndrome an autosomal recessive disorder due to a deletion of 2 contiguous genes, *SLC3A1* coding for a cystine transporter and responsible for a massive cystinuria, and *PREPL* coding for a serine oligopeptidase responsible for the Prader Willi like phenotype (▶ Chapter 25).

These three neurological presentations are summarized in ◘ Table 1.3.

Hepatic and gastrointestinal presentations

Several clinical groups of hepatic signs can be identified:

- Massive hepatomegaly with hypoglycemia and seizures suggest **GSD type I or III**, or **gluconeogenesis defects**. **Severe hyperinsulinism** can sometimes display a moderate hepatomegaly.

Table 1.3 Neurological Presentations

Predominant clinical symptom	Main clinical signs	Major biological signs	Most likely diagnoses (disorder/enzyme deficiency)
Neurological deterioration: Metabolic encephalopathy Mostly metabolic and treatable	Lethargy, coma, hiccups Poor sucking, Hypothermia Hypotonia, hypertonia Abnormal movements Large amplitude tremor Myoclonic jerks »Burst suppression« Abnormal odor	Ketosis, acidosis	**MSUD** (odor)
		Ketoacidosis,bone marrow suppression	**OA: MMA, PA, IVA** (odor)
		Hyperlactatemia	**MCD**
		Hyperammonemia	**Urea cycle defects,OAs, CAVA deficiency, GA type II** (odor)
		Characteristic changes of AAC or OAC	**all disorders (MSUD, OA, UVD, CAVA, GAII)**
Seizures Some metabolic, Some treatable	Mostly repetitive seizures unresponsive to antiepileptic drugs,only responsive to specific treatments	Metabolic ketoacidosis, abnormal organic acids	**MCD**
		Elevated pipecolic acid (CSF, P, U) and alpha-aminoadipic semialdehyde (U) Hyperphosphatasia	**Pyridoxine responsive Folinic acid responsive**
		Elevated CSF glycine, threonine, 3-orthomethyldopa, lactate, low dopamine and serotonin	**Pyridoxal phosphate responsive**
		Hypocalcemia Hypomagnesaemia	**Congenital magnesium malabsorption**
		Severe hypoglycemia Low serine (P/CSF)	**PHHI, Adenosine kinase deficiency 3PGD deficiency**
	Severe encephalopathy with associated diverse neurological signs	Low copper (P)	Menkes disease
		Hyperglycinemia	NKH
		Sulphite test (U) S-sulfocysteine (U)	Molybdenum cofactor disease/SO deficiency
		GABA in the CSF	GABA transaminase
		Low glutamine (P, U, CSF)	Glutamine synthetase deficiency
		Glutamate oxidation in fibroblasts	Glutamate transporter deficiency
		High proline (P), low glutamate (CSF), accumulation of lipids in fibroblasts	Hyperprolinaemia due to SLC25A22 defect
		Diverse complex lipid and fatty acid profile abnormalities (P) Glycosylated transferrin	GM3 synthetase deficiency, mutations in FAH2, ELOVL4, GPI anchor defects, Peroxisomal biogenesis defects
		Purines (U)	Adenylosuccinate lyase deficiency
	Facial dysmorphia Malformations Severe hypotonia	Abnormal VLCFA, phytanic, Plasmalogen (P and fibroblasts)	Peroxisomal defects
		Glycosylated transferrin	CDG syndrome
		None/Hyperphosphatasia (P)	GPI anchor synthesis defects
		Sterols (P)	Cholesterol biosynthesis

Table 1.3 (continued)

Predominant clinical symptom	Main clinical signs	Major biological signs	Most likely diagnoses (disorder/enzyme deficiency)
Severe Hypotonia Rarely metabolic, rarely treatable	Isolated mimicking neuro-muscular diseases	None	Prader Willi syndrome PFK deficiency (severe form)
		Glycosylated transferrins	IEM of the Dolichols
		Massive cystinuria	Hypotonia/Cystinuria
		Hyperlactacidaemia	Mitochondrial disorders
		Abnormal CSF biogenic amines/ pterines, glycine	**Neurotransmitter disorders**
		Acylcarnitines (P) organic acids, (U) low carnitine (P)	**Riboflavin transporter defects, FAO, carnitine transporter**
		CoQ10 fibroblasts, muscle	**Primary CoQ10 defects**
	Fetal distress Hydramnios Arthrogryposis Respiratory failure	None	Severe fetal neuromuscular diseases Steinert, Myasthenia Congenital myopathy Sensitivo-motor neuropathy
		Hyperlactacidaemia	Mitochondrial disorders
		Hyperglycinaemia, abnormal plasma BCAA,ketoglutarate (U)	Lipoilation defects
	Predominant dysmorphia Malformations	VLCFA,phytanic, plasmalogen (P and fibroblasts)	Peroxisomal defects
		Sterols (P)	Cholesterol defects
		Tubulopathy	Lowe syndrome
		Glycosylated transferrin	N Glycosylation defects
		APO-B glycosylation (P)	O Glycosylation defects
		None/hyperphosphatasia (P)	GPI anchor synthesis defects
		Chromosome analyses	Chromosomal abnormalities
	Cataract Tubulopathy	Hyperlactatemia Enzyme / DNA analyses	Lowe syndrome Respiratory chain defects
	Cardiomyopathy Macroglossia	Vacuolated lymphocytes	Pompe, PRKAG2
		Hyperlactatemia	Respiratory chain defects
		Acylcarnitine (P)	Trifunctional enzyme deficiency

AAC, amino acid chromatography; *CDG*, congenital disorders of glycosylation; *CSF* cerebrospinal fluid; CoQ coenzyme Q; *GA II*, glutaric aciduria type II; HVA, homovanillic acid; *IVA*, isovaleric acidemia; *MCD*, mutliple carboxylase deficiency; *MMA*, methylmalonic aciduria; *MSUD*, maple syrup urine disease; *NKH*, non ketotic hyperglycinemia; *OA*, organic acidurias; *OAC*, organic acid chromatography; *P* plasma; *PA*, propionic acidemia; *PFK*, phosphofructokinase; *PHHI*, primary hyperinsulinemic hypoglycemia of infancy; *PNPO*, pyridox-(am)ine-5′-phosphate oxidase; *SO* sulfite oxidase; *U* Urine; *VLCFA*, very long chain fatty acids; *3PGD*, 3-phosphoglycerate dehydroge-nase; *5HIAA* hydroxy indol acetic acid

- Liver failure (jaundice, coagulopathy, hepatocellular necrosis with elevated transaminases, hypoglycaemia, ascites and edema) suggests:
  - **fructosemia** (now very rare since infant formulas are fructose free),
  - **galactosemia,**
  - **tyrosinemia type I** (after 3 weeks),
  - neonatal hemochromatosis,
  - respiratory chain disorders (mostly mitochondrial DNA depletion, TRMU and mitochondrial translation factor deficiency),
  - transaldolase deficiency.

The recently described mutations in *LARS* (coding for cytoplasmic leucyl-tRNA synthetase) present with hypoalbuminemia, recurrent acute infantile liver failure (RALF), anemia, seizures and encephalopathic crisis [14]. Recently *NBAS* mutations were also identified as a new molecular cause of fever-dependent episodes of RALF with onset in infancy. NBAS protein is a component of SNAREt complex mediating the docking and fusion of transport vesicles with target membranes. The first episode of ALF presented with recurrent vomiting and increasing lethargy 1 or 2 days after the onset of fever. ALF episodes usually started with massively elevated ALAT and ASAT, succeeded by severe coagulopathy and mild to moderate jaundice [15]. *CCDC 115* mutations present with a storage-disease-like phenotype involving hepatosplenomegaly, highly elevated bone-derived alkaline phosphatase, elevated aminotransferases, and elevated cholesterol, in combination with abnormal copper metabolism, abnormal glycosylation type II and neurological symptoms . Two individuals died of liver failure (▶ Chapter 41).

GRACILE syndrome linked to *BCSL1* mutations [16] displays severe fetal growth retardation, lactic acidosis, failure to thrive, hyperaminoaciduria, very high serum ferritin, hemosiderosis of the liver and early death.

One must emphasize that there are frequent difficulties in investigating patients with severe hepatic failure. At an advanced state, many non specific abnormalities secondary to liver damage can be present: melituria (galactosuria, glycosuria, fructosuria), hyperammonemia, hyperlactatemia, hypoglycemia after a short fast, hypertyrosinemia (>200 µmol/l), and hypermethioninemia (sometimes higher than 500 µmol/l).

- Cholestatic jaundice with failure to thrive is a predominant finding in:
  - alpha-1-antitrypsin deficiency,
  - Byler disease,
  - **inborn errors of bile acid metabolism**,
  - PBD, Niemann-Pick disease type C,
  - CDG syndromes (PMM2-CDG, MPI-CDG, COG1 and 7-CDG),
  - hepatocerebral syndrome due to mitochondrial DNA depletion,
  - citrin deficiency.

Cerebrotendinous xanthomatosis, citrin deficiency, arginase deficiency [17] and Niemann-Pick C can present as a transient asymptomatic jaundice before neurological signs appear later in life. Two new complex lipid synthesis disorders, the MEGDHEL syndrome (*SERAC* mutation) that can mimick Niemann-Pick C with a positive filipin test and the spastic paraparesis type 5 due to oxysterol 7-hydroxylase deficiency [18] may also present with such a transient cholestatic liver disease.

- Liver steatosis: Hepatic presentations of **FAO disorders** and **UCD** consist of acute steatosis or Reye syndrome with normal bilirubin rather than true liver failure.
  Long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency is an exception which may present early in infancy (but not strictly in the neonatal period) as cholestatic jaundice, liver failure and hepatic fibrosis (▶ Chapter 12). Chanarin-Dorfman syndrome (*ABHD5* mutations) presents early in infancy with liver steatosis, cataract, deafness, congenital ichthyosis, and myopathy while the newly described cytoplasmic glycerol 3 phosphate dehydrogenase 1 deficiency displays an asymptomatic early infantile hepatomegaly and steatosis with transient hypertriglyceridemia (▶ Chapter 34).
- Hepatosplenomegaly (HSM) with storage signs (coarse facies, macroglossia, hydrops foetalis, ascitis, oedema, dysostosis multiplex, vacuolated lymphocytes) are observed in:
  - GM1 gangliosidosis
  - Sialidosis type II
  - I-cell disease
  - Niemann-Pick disease type A
  - MPS type VII, Galactosialidosis (▶ Chapter 38 and 39)
  - ALG1-CDG (Ik) (▶ Chapter 41)
  - *CCDC 115* mutations (▶ Chapter 41)
  - congenital erythropoietic porphyria (▶ Chapter 36)
- HSM with inflammatory syndrome, haematological or immunologic features can be seen in:
  - Lysinuric protein intolerance (LPI) (macrophage activating syndrome, leucopenia)
  - Mevalonic aciduria (inflammatory syndrome and recurrent severe anemia)
  - Transaldolase deficiency (hydrops foetalis with severe anemia) (see also ▶ Section 1.6.7 and ▶ Section 1.6.8)

Congenital diarrhoeal disorders (CDD) are rare heterogeneous enteropathies, often with severe clinical manifestations. Affected genes include those related to disaccharidase deficiency, ion or nutrient transport defect like *SLC26A3* mutations causing congenital secretory chloride diarrhoea, pancreatic insufficiency, lipid trafficking or **MPI-CDG (Ib**) and ALG8-CDG(Ih). A disorder presenting with CDD linked to *DGAT1* mutations has been recently described. Affected neonates present with vomiting, colicky pain, and non bloody, watery diarrhea, protein-losing enteropathy, hypoalbuminemia and hyperlipidemia (▶ Chapter 34).

Cardiac presentation

Some metabolic disorders can present predominantly with cardiac disease. Cardiac failure and a dilated hypertrophic cardiomyopathy (pure dilated cardiomyopathies are very rare), most often associated with hypotonia, muscle weakness, and failure to thrive, suggests:

- **FAO disorders** (with hypoglycaemia)
- respiratory chain disorders (with severe lactic acidosis)
- **Pompe disease** (with suggestive ECG and vacuolated lymphocytes)
- fatal congenital heart glycogenosis due to mutation in *PRKAG2* (▶ Chapter 5)

Methylglutaconic aciduria is found in Barth syndrome, Sengers syndrome (▶ Chapter 34), DNAJC19 and TMEM 70

(▶ Chapter 18) and ketoglutarate excretion in ketoglutarate dehydrogenase deficiency (▶ Chapter 11).

Several observations suggest that some respiratory chain disorders are tissue specific and are only expressed in the myocardium while many others are ubiquitous like the Mitochondrial Translation Elongation Factor defect (▶ Chapter 14). PMM2-CDG (Ia) can sometimes present in infancy with cardiac failure due to pericardial effusions, cardiac tamponnade, and cardiomyopathy.

Dolichol kinase 1 deficiency (DOLK-CDG) may present with progressive dilated cardiomyopathy resulting in death within 1 year. Other clinical manifestations include microcephaly, »parchment-like« ichthyosis with loss of hair, eyebrows and eyelashes, intractable seizures, severe hypotonia with elevated creatine kinase (CK) and severe liver dysfunction (▶ Chapter 41).

Many defects of **long-chain FAO** can present with cardiomyopathy and/or arrhythmias and conduction defects (auriculoventricular block, bundle branch blocks, ventricular tachycardia) which may lead to cardiac arrest [19] (▶ Chapter 12).

1.3.2 Metabolic Derangements and Diagnostic Tests

▪ Initial approach and protocol for investigation

As soon as there is clinical suspicion of an IEM, general supportive measures and laboratory investigations should be undertaken concurrently (◘ Table 1.4). Abnormal urine odors can be detected on a drying filter paper or by opening a container of urine which has been closed at room temperature for a few minutes. Although serum ketone bodies reach 0.5-1 mmol/l in early neonatal life, acetonuria, if observed in a newborn, is always abnormal and an important sign of a metabolic disease. The dinitrophenylhydrasine (DNPH) test screens for the presence of alpha-keto acids as occur in MSUD. However, it has now largely been abandoned because of its poor specificity and because AA chromatography has become much more readily available. Hypocalcemia and elevated or reduced blood glucose are frequently present in metabolic diseases and the physician should be wary of attributing marked neurological dysfunction purely to these findings.

The metabolic acidosis of OA is usually accompanied by an elevated anion gap. Urine pH should be below 5; otherwise, renal acidosis is a consideration. Metabolic acidosis resulting from IEM may develop as result of accumulation of fixed anion (lactate, ketone bodies, organic acid or a combination of both) or loss of bicarbonate, which is usually due to tubular dysfunction. In metabolic acidosis resulting from fixed anion, the plasma chloride concentration is normal and the anion gap, a reflection of the concentration of unmeasured anions, is increased. In patients with metabolic acidosis caused by loss of bicarbonate, the plasma chloride is elevated and the anion gap (the difference between the plasma sodium and the sum of the chloride and bicarbonate) is generally normal (ie, 10–15 mmol/L). In metabolic acidosis with high anion gap the presence or absence of ketonuria is the major clinical clue to the diagnosis.

A normal blood pH does not exclude hyperlactatemia, as neutrality is usually maintained until serum levels reach 6 mmol/l (as long as bicarbonate levels remain >18 mmol/). Ammonia and lactic acid should be determined systematically in newborns at risk. An elevated ammonia level in itself can induce respiratory alkalosis; hyperammonemia with ketoacidosis suggests an underlying OA, but an isolated hyperammonemia can occur. Elevated lactic acid levels in the absence of infection or tissue hypoxia are a significant finding. Moderate elevations (hyperlactatemia: 3–6 mmol/l) are often observed in organic acidemias and in the hyperammonaemias; levels greater than 6 mmol/l (lactic acidosis) are frequent in hypoxia (see below ▶ Section 1.4.2). PA, MMA and IVA may induce granulocytopenia and thrombocytopenia (bone marrow suppression), which may be mistaken for sepsis. Transaldolase deficiency and early onset forms of mevalonate kinase deficiency present with severe recurrent hemolytic anemia.

The storage of adequate amounts of plasma, urine, blood on filter paper, and CSF, is an important element in reaching a diagnosis. The utilization of these precious samples should be carefully planned after taking advice from specialists in IEM.

▪ Identification of five major types of metabolic distress

Once the above clinical and laboratory data have been collected, specific therapeutic recommendations can be made. This process is completed within 2–4 h and often precludes waiting long periods for the results of sophisticated diagnostic investigations. On the basis of this evaluation, most patients can be classified into one of five types (◘ Table 1.5). The experienced clinician will, of course, have to carefully interpret the metabolic data, particularly in relation to time of collection and ongoing treatment. At the same time, it is important to collect all the biologic data listed in ◘ Table 1.5. Some very significant symptoms (such as metabolic acidosis and especially ketosis) can be moderate and transient, largely depending on the symptomatic therapy. Conversely, at an advanced state, many non-specific abnormalities (such as respiratory acidosis, severe hyperlactatemia, secondary hyperammonemia) can disturb the original metabolic profile. This applies particularly to IEM with a rapid fatal course such by the urea cycle defects, in which the initial characteristic presentation of hyperammonemia with respiratory alkalosis shifts rapidly to a rather non-specific picture of acidosis and hyperlactatemia.

In our experience, types I and II **(MSUD, OA)**, type IVa **(UCD, FAO disorders)**, NKH, and respiratory chain defects account for more than 80% of newborn infants with inborn errors of intermediary metabolism.

Table 1.4 Protocol for emergency investigations

	Immediate investigations	Storage of samples
Urine	Smell (special odor) Look (special color) Acetone (Acetest, ketostick Ames) Reducing substances (Clinitest, Clinistick Ames) Keto acids (DNPH) pH (pHstix Merck) Sulfitest (Merck) Electrolytes (Na, K), urea, creatinine Uric acid	Urine collection: collect fresh samples before and after treatment and freeze at –20°C. Do not use the samples without expert metabolic advice. Specialist metabolic investigation include: OAC, AAC, orotic acid, porphyrins
Blood	Blood cell count Electrolytes (search for anion gap) Glucose, Calcium Blood gases (pH, pCO_2, HCO_3, pO_2) Uric acid Prothrombin time Transaminases (and other liver tests) Ammonia Lactic acid 3-hydroxybutyrate* Free fatty acids (FFA)*	Plasma (5 ml) heparinized at -20°C Blood on filter paper: 2 spots (as »Guthrie« test) Whole blood (10-15 ml) collected on EDTA and frozen (for molecular biology studies) Specialist metabolic investigations include: - Total homocysteine, AAC (P) - Acylcarnitine (tandem MS) (P) - OAC (U) - Porphyrins (U) - Neurotransmitters (P,CSF,U) (HPLC, Tandem MS)
Miscellaneous	Lumbar puncture Chest X-ray Cardiac echography, ECG Cerebral ultrasound, EEG	Skin biopsy (fibroblast culture) CSF (1 ml), frozen (neurotransmitters, AA) Postmortem: liver, muscle biopsies (▶ Chapter 3)

AA, amino acid; *AAC*, amino acid chromatography; *CSF*, cerebrospinal fluid; *DNPH*, dinitrophenylhydrazine; *ECG*, electrocardiogram; *EDTA*, ethylenediaminetetra-acetic acid; *EEG*, electroencephalogram; *MS*, mass spectrometry; *HPLC*, high performance liquid chromatography; *OAC*, organic acid chromatography; P, plasma; U, urine;
* 3-hydroxybutyrate and FFA data are generally not obtained in an emergency but are useful for interpreting the metabolic profile. Similarly, pyruvate and acetoacetate are not included in this emergency protocol

Table 1.5 Classification of inborn errors presenting in the neonatal period and in early infancy

Types	Clinical type	Acidosis/ Ketosis	Other signs	Most likely diagnosis (disorder/enzyme deficiency)	Diagnostic investigations
I	**Neurological deterioration,** »Intoxication« type, 4–10 days of »well« period Slow abnormal movements Hypertonia	Acidosis 0/± DNPH +++ Acetest 0/±	NH_3 N or ↑± Lactate N Blood count N Glucose N Calcium N	**MSUD** (abnormal odour)	Aminoacid chromatography (P, U) Blood spot for tandem MS-MS
II	**Neurological deterioration,** »Intoxication« type 1–3 days of »well«period Fast abnormal movements Dehydration	Acidosis ++ Acetest ++ DNPH 0/± Ketoacidosis	NH_3 ↑ +/++ Lactate N or ↑± Blood count: leucopenia, thrombopenia Glucose/Calcium N or ↓+	**Organic acidurias** (MMA, PA, IVA, MCD) **Ketolysis defects** (3-ketothiolase, SCOT)	OAC by GLCMS (U, P) Carnitine (P) Carnitine esters tandem MS (U, P) Blood spot for tandem MS-MS
	Neurological deterioration, »energy deficiency« type, with liver or cardiac symptoms	Acidosis ++/± Acetest 0 DNPH 0 No ketosis	NH_3↑±/++ Lactate ↑±/++ Blood count N Glucose ↓ +/++ Nonketotic hypoglycemia	**Fatty acid oxidation** and **ketogenesis defects (GAII, CPTII, VLCAD, CAT, MCKAT,** HMGCoA lyase)	Idem above Metabolic profile (see ▶ Chapter 3) Fatty acid oxidation studies on lymphocytes or fibroblasts

Table 1.5 (continued)

Types	Clinical type	Acidosis/ Ketosis	Other signs	Most likely diagnosis (disorder/enzyme deficiency)	Diagnostic investigations
III	**Neurological deterioration,** »energy deficiency« type, Polypnea Hypotonia Lactic acidosis sometimes well tolerated	Acidosis +++/+ Acetest ++/0 Lactate +++/+ Lactic acidosis	NH_3 N or ↑± Blood count: anemia or N Glucose N or ↓± Calcium N	Congenital lactic acidoses (pyruvate carrier, PC, **PDH, MCD**, Krebs cycle, respiratory chain, lipoilation defects)	Plasma redox states ratios (L:Pyr, 3OHB:AA) OAC (U), AAC (P) Polarographic studies Enzyme assays (muscle, lymphocytes, fibroblasts)
IV a)	**Neurological deterioration,** »intoxication« type, 0–3 days »well« period Moderate hepatocellular disturbances Hypotonia, seizures, coma	Acidosis 0 (alkalosis) Acetest 0/+ DNPH 0	NH_3 ↑ +/+++ Lactate N or ↑ + Blood count N Glucose N (low in CAVA def) Calcium N	**Urea cycle defects CAVA deficiency HHH syndrome Fatty acid oxidation defects (GAII, CPTII, VLCAD, LCHAD, CAT) PA, MMA, IVA**	AAC, OAC (P,U) Orotic acid (U) Liver or intestine enzyme studies (CPS, OTC)
b)	**Neurological deterioration** Seizures Myoclonic jerks Severe hypotonia	Acidosis 0 Acetest 0 DNPH 0 **No major metabolic disturbance**	NH_3 N Lactate N or ↑ + Blood count N Glucose N	NKH, SO plus XO	AAC (plasma, CSF)
				Asparagine, glutamine, serine synthesis defects	AAC (plasma, CSF)
				B6-dependency	OAC (AASA, pipecolic)
				Neurotransmitters and **PNPO defects**	OAC, Neurotransmitters (P, U, CSF)
				Peroxisomal defects	VLCFA, phytanic acid (P)
				Trifunctional enzyme	Acylcarnitine (P), OAC (U)
				Respiratory chain	Lactate (P), OAC (U)
				CDG syndrome	Glycosylated transferrin (P)
				Cholesterol biosynthesis	Sterols (P)
				Phospholipids synthesis/ recycling	Lipidomics: P/CSF/fibroblasts
				Dolichol defects	DNA analysis
				Vici syndrome	DNA analysis
Va)	**Recurrent hypoglycemia with hepatomegaly**	Acidosis ++/0 Acetest +/0	Lactate ↑ +/++ NH_3 ↑ + (in FAO)	**Glycogenosis type I** (acetest +/–) **Glycogenosis type III** (acetest ++) **FBPase** (acetest + or +/–)	Fasting test, Loading test DNA analyses, enzyme studies (liver, lymphocytes, fibroblasts)
				FAO defects (acetest-)	Organic acids, acylcarnitine
			intractable hypoglycemia	**PHHI** (acetest-)	Insulin plasma levels
b)	**Hepatomegaly** Jaundice Liver failure Hepatocellular necrosis +/- tubulopathy **(galactosemia, HFI, Tyr I)**	Acidosis +/0 Acetest +/0	NH_3 N or ↑ + Lactate ↑ +/++ Glucose N or ↓ ++	**HFI (only on fructose)**	DNA analyses, Enzyme studies
				Galactosemia	Galactose spot test (Blood)
				Tyrosinemia type I	Organic acids (succinyl acetone) (U)
				Hemochromatosis	Iron in tissues
				OXPHOS defects Mito DNA depletion Mito translation factor (*TRMU, LARS*)	Organic acids (U), enzyme/ DNA analyses

Table 1.5 (continued)

Types	Clinical type	Acidosis/ Ketosis	Other signs	Most likely diagnosis (disorder/enzyme deficiency)	Diagnostic investigations
b)				TALDO	Polyols (tandem MS) (U)
				Mevalonic aciduria	Organic acids (U), enzyme, DNA
c)	**Hepatomegaly** **Cholestatic Jaundice** ± Failure to thrive ± Chronic diarrhea ± osteoporosis ± rickets	Acidosis 0 Ketosis 0	NH_3 N Lactate N Glucose N	Alpha-1-antitrypsin	Protein electrophoresis
				Inborn errors of **bile acid metabolism**	Bile acids (P, U) bile by tandem MS
				Peroxisomal defects	VLCFA, phytanic pipecolic acid (P)
				CDG syndrome I and II	Glycosylated transferrin (P)
				Niemann-Pick type C	Filipin test (fibroblasts), Oxysterols (P)
				MEGDHEL syndrome	Filipin test, OAC (U) (methylglutaconic)
				LCHAD	OAC (U) acylcarnitine profile (P)
				Mevalonic aciduria	OAC (U)
				Oxysterol 7-hydroxylase deficiency	Sterols (P)
				Cerebrotendinous Xanthomatosis	Sterols (P)
				Citrin deficiency	AAC (P) (citrulline can be normal)
d)	**Hepatosplenomegaly** »**Storage**« **signs** (coarse facies, ascites, hydrops fetalis, macroglossia, bone changes, cherry red spot, vacuolated lymphocytes) ± Failure to thrive ± Chronic diarrhea ± Hemolytic anemia	Acidosis 0 Acetest 0 Ketosis 0 DNPH 0	NH_3 N Lactate N or ↑ Glucose N Hepatic signs ±/++	**Congenital erythropoietic porphyria**	Porphyrins
				GM1 gangliosidosis, Sialidosis type II	Oligosaccharides, sialic acid, MPS (U)
				I-cell disease, Niemann-Pick type A/C, MPS VII, Galactosialidosis	Enzyme studies (lymphocytes, fibroblasts) DNA analysis
				CDG syndrome	Glycosylated transferrin (P)
				Mevalonic aciduria	OAC (U)
				TALDO	Polyols (U) (tandem MS)

L, lactate; *P*, pyruvate; *3OHB*, 3-hydroxybutyrate; *AA*, acetoacetate; *GLCMS*, gas liquid chromatography mass spectrometry; *VLCFA*, very-long-chain fatty acids.
N, normal (normal values = NH_3<80 µM; lactate <1.5 mM; glucose 3.5-5.5 mM); ±, slightly modified; +, moderate; ++, marked; +++, significant/massive; ↑ elevated; ↑ decreased; 0, absent (acidosis) or negative (acetest, dinitrophenylhydrazine, DNPH).
AASA, α-aminoadipic acid semialdehyde; *AAC*, amino acid chromatography; *CAT*, carnitine acylcarnitine translocase; CAVA, carbonic anhydrase Va; *CPS*, carbamyl phosphate synthetase; *CPT II*, carnitine palmitoyltransferase II; *GA II*, glutaric aciduria type II; HFI, hereditary fructose intolerance; *HMGCoA*, 3-OH-3-methylglutaryl coenzyme A; *ISSD*, infantile sialic acid storage disease; *IVA*, isovaleric acidemia; *LARS* encodes a cytoplasmic leucyl-tRNA synthetase enzyme; *LCHAD*, 3-OH long-chain acyl CoA dehydrogenase; *MCD*, multiple carboxylase; *MCKAT*, medium-chain 3-ketoacylCoA A thiolase; *MMA*, methylmalonic acidemia; *MPS VII*, mucopolysaccharidosis type VII; *MS*, mass spectrometry; *MSUD*, maple syrup urine disease; *NKH*, nonketotic hyperglycinemia; *OAC*, organic acid chromatography; *OTC*, ornithine transcarbamylase; *P*, plasma; *PA*, propionic acidemia; *PC*, pyruvate carboxylase; *PDH*, pyruvate dehydrogenase; *PNPO*, pyridox(am)ine-5'-phosphate oxidase; *PZO*, peroxisomal disorders; *SO*, sulfite oxidase; *SCOT*, succinyl CoA trasferase ;*TALDO*, transaldolase; *U*, urine; *TRMU*, involved in thio-modified mitochondrial tRNAs; *VLCAD*, very-long-chain acyl CoA dehydrogenase; *XO*, xanthine oxidase; *3PGD*, 3-phosphoglycerate dehydrogenase.

1.4 Later Onset Acute and Recurrent Attacks (Late Infancy and Beyond)

1.4.1 Clinical Presentations

Consider the possibility of an IEM in a child or adult (▶ Chapter 2) with an acute unexplained, recurrent or refractory attack at any age. Indeed, in about 50% of the patients with IEM, disease onset is later. The symptom-free period is often longer than 1 year and may extend into late childhood, adolescence, or even late adulthood. Each attack can follow a rapid course ending either in spontaneous improvement or unexplained death, despite supportive measures in the intensive care unit. Between attacks the patient may appear normal. Onset of acute disease may be precipitated by an intercurrent event or may occur without overt cause. Excessive protein intake, prolonged fasting, prolonged exercise, and all conditions that enhance protein catabolism, may exacerbate such decompensations.

▪ Coma, Strokes and Attacks of Vomiting with Lethargy (◘ Table 1.6)

Acute encephalopathy is a common problem in infants and children with IEM. All types of coma can be indicative of an IEM, including those presenting with focal neurological signs. Neither the age at onset, the accompanying clinical signs (hepatic, gastrointestinal, neurological, psychiatric etc.), the mode of evolution (improvement, sequelae, death), nor the routine laboratory data, allow an IEM to be ruled out a priori. Two categories can be distinguished:

1. Metabolic coma without focal neurological signs.

The main varieties of metabolic comas may all be observed in these late-onset, acute diseases: coma with predominant metabolic acidosis, coma with predominant hyperammonemia, coma with predominant hypoglycemia, and combinations of these three major abnormalities. A rather confusing finding in some OA and ketolytic defects is ketoacidosis with hyperglycemia and glycosuria that mimics diabetic coma. The diagnostic approach to these metabolic derangements is developed below (see ▶ Section 1.3.2).

2. Neurological coma with focal signs, seizures, severe intracranial hypertension, strokes or stroke-like episodes.

Although most recurrent metabolic comas are not accompanied by neurological signs other than encephalopathy, some patients with **OA** and **UCD** present with focal neurological signs or cerebral edema. These patients can be mistakenly diagnosed as having a cerebrovascular accident or cerebral tumor. In fact, IEM can lead to »classic strokes« (either ischemic or hemorraghic; they follow a well-defined anatomic vascular territory), and »stroke-like« episodes, where the areas involved do not follow these precise anatomic vascular territories and mostly produce high intensity images in the basal ganglia and the cortex.

In most of these disorders, stopping the protein intake, delivering high glucose infusion rate and giving »cleansing drugs« can be life saving.Another treatable condition is **thiamine-biotin-responsive basal ganglia disease (TBBGD)** which presents from childhood to adulthood with a subacute encephalopathic picture of undefined origin including confusion, vomiting, movement disorders, and a vague history of febrile illness (▶ Chapter 28). MRI study shows Leigh-like changes involving the caudate, putamen and the medial thalami. **PDH and biotinidase deficiency** are other treatable causes that may present in a similar manner. Arterial tortuosity syndrome (*GLUT 10* mutations) characterized by generalized tortuosity and elongation of all major arteries may result in acute infarction due to ischaemic strokes or an increased risk of thrombosis (▶ Chapter 10).

All severe forms of **homocystinuria** (total homocysteine >100 μM) can cause an acute cerebrovascular accident from late childhood to adulthood. These include **cystathionine-β-synthase deficiency** (usually B6-responsive in the late onset presentations), the severe **MTHFR** defects (folate/betaine responsive) and **CblC, CblD** defects (hydroxocobalamin responsive) (▶ Chapter 20 and 27). Patients with **MMA** may, after first presenting with metabolic decompensation, have acute extrapyramidal and corticospinal tract involvement as a result of bilateral destruction of the globus pallidus with variable involvement of the internal capsule. Cerebellar hemorrhage has also been observed in IVA, PA, and MMA (▶ Chapter 18).

EPEMA syndrome due to *ETHE1* mutations starts in general early in infancy and is characterized by the association of progressive encephalopathy and recurrent attacks of metabolic decompensation with lactic acidosis and bilateral lesions in the striatum, resembling Leigh syndrome, with evocative relapsing petechiae and orthostatic acrocyanosis (▶ Chapter 20). Aicardi Goutiéres syndrome due to mutations in different genes involved in interferon biology may present with strokes, inflammatory syndrome, chronic arthropathy and chilblains (see ▶ Section 1.6.8 and ▶ Section 1.6.16).

Two patients with 3-hydroxyisobutyric aciduria presenting with recurrent episodes of vomiting and ketoacidotic coma have been described (▶ Chapter 18). Monocarboxylate transporter type 1 deficiency (MCT1) has been reported as a cause of recurrent episodies of severe ketoacidosis often associated with cycling vomiting but without consciousness depression (▶ Chapter 13). Patients with mitochondrial DNA mutations have presented with cyclical vomiting associated with intermittent lactic acidosis. GA type I frequently presents with an encephalopathic episode, mimicking encephalitis, in association with an intercurrent gastrointestinal or viral infection (▶ Chapter 22). Mitochondrial encephalopathy lactic acidosis stroke-like episodes (MELAS) syndrome is another important diagnostic consideration in such late-onset and recurrent comas (▶ Chapter 14). Early episodic central nervous system problems, possibly associated with liver insufficiency or cardiac failure, have been the initial findings in some cases of CDG syndrome (▶ Chapter 41). **Wilson disease** can rarely present with an acute episode of encephalopathy with extrapyramidal signs. Late onset forms of **PDH** can present in childhood with recurrent attacks of ataxia, sometimes described by the patient as recurrent episodes of pain or muscular weakness (due to dystonia or to peripheral neuropathy) (▶ Chapter 11). **Riboflavin transporter defects** can present as brainstem en-

Table 1.6 Diagnostic approach to recurrent attacks of coma and vomiting with lethargy

Clinical Presentation	Metabolic derangements or other important signs		Most frequent diagnosis (disorder/enzyme deficiency)	Differential diagnosis
Metabolic Coma (without focal neurological signs)	Acidosis (metabolic) - pH <7.20 - HCO_3^-<10 mmol/l - PCO_2 <25 mmHg	Ketosis + (acetest ++)	Mitochondrial disorder **MCD**, PC **MMA, PA, IVA, GAI, MSUD*** **Ketolysis defects** **Gluconeogenesis defects**	Diabetes Intoxication Encephalitis
		Ketosis -	**PDH, Ketogenesis defects** **FAO, FBP, EPEMA**	
	Hyperammonemia - NH_3> 100 µmol/l - Resp alkalosis - pH >7.45 - pCO_2 <25 mmHg	Normal glucose	**Urea cycle defects*** **HHH syndrome** **LPI**	Reye syndrome Encephalitis Intoxication
		Hypoglycemia	**FAO (MCAD*)** **HMGCoA lyase** **CAVA deficiency**	
	Hypoglycemia (<2 mmol/l)	Acidosis +	**Gluconeogenesis defects** **MSUD** **HMGCoA lyase** **FAO**	Drugs and toxin Ketotic hypoglycemia Adrenal insufficiency GH deficiency Hypopituitarism
	Hyperlactatemia (>4 mmol/l)	Normal glucose	PC, **MCD**, Krebs cycle Respiratory chain*, **PDH*** (without ketosis) EPEMA syndrome	Hypoxia Sepsis
		Hypoglycemia	**Gluconeogenesis defects** (ketosis variable) **FAO** (moderate hyperlactatemia, no ketosis)	
Neurological coma (with focal signs, seizures, or intracranial hypertension)	Biological signs are very variable, can be absent or moderate; see »Metabolic coma«	Cerebral edema Hemiplegia (hemianopsia)	**MSUD, OTC** **MSUD, OTC, MMA, PA,** PGK	Cerebral tumor Migraine Encephalitis
		Basal ganglia involvement/ extrapyramidal signs	**TBBGD,** Leigh syndrome of diverse etiologies, **MMA, GA1, Wilson, homocystinurias*,** **Manganese transporter defect**	Acute encephalitis (infectious, immunomediated), metal intoxication
		Cranial nerve involvement, bulbar palsy	**Riboflavin transporter defects**	Brainstem encephalitis
		»Stroke-like«	**UCD, MMA, PA, IVA*** Respiratory chain (MELAS*) Homocystinurias* CDG syndrome ***THTR1* mutations** **Fabry*** Maltase acid* (rare) Racemase deficiency (rare)	Moya Moya syndrome Vascular hemiplegia Cerebral thrombophlebitis, Cerebral tumor
	Abnormal coagulation, (not constant alterations) Hemolytic anemia	»Classic strokes« Thromboembolic events	AT III, Protein C,S deficiencies **Homocystinurias*** CDG, PGK Menkes and GAI can produce subarachnoidal hemorrhage	Sickle cell anemia

1

Table 1.6 (continued)

Clinical Presentation	Metabolic derangements or other important signs		Most frequent diagnosis (disorder/enzyme deficiency)	Differential diagnosis
Hepatic coma (hepatomegaly, cytolysis or liver failure) Reye syndrome	Normal bilirubin Slight elevation of Transaminases	Steatosis and fibrosis	**FAO, UCD**	
	Very high transaminases Severe coagulopathy	Recurrent acute liver failure (RALF) triggered by fever	*NBAS* mutations	
	Hypoalbuminemia, anemia, seizures and encephalopathic crisis	Recurrent acute liver failure (RALF)	*LARS* (coding for cytoplasmic leucyl-tRNA synthetase)	
	Hyperlactatemia	Liver failure	Respiratory chain defects	Reye syndrome Hepatitis, intoxication
	Hemolytic jaundice	Cirrhosis Chronic hepatic dysfunction	**Wilson*, Manganese transporter defect**	
	Hypoglycemia	Exudative enteropathy	Hepatic fibrosis with enteropathy **(MPI-CDG)**	

AT III, antithrombin III; *CAVA*, carbonic anhydrase Va; *CDG*, carbohydrate-deficient glycoprotein syndrome; *EPEMA*, encephalopathy, petechiae, ethylmalonic aciduria syndrome; *FAO*, fatty acid oxidation; *FBP*, fructose 1-6 biphosphatase; *GA*, glutaric aciduria;*GH*, growth hormone; *HMG-CoA*, 3-hydroxy-3-methylglutaryl coenzyme A; *IVA*, isovaleric acidemia; *LPI*, lysinuric protein intolerance; *MCD*, multiple carboxylase deficiency; *MELAS*, mitochondrial encephalopathy lactic acidosis stroke-like episodes; *MMA*, methylmalonic acidemia; *MSUD*, maple syrup urine disease; *OTC*, ornithine transcarbamylase; *PA*, propionic acidemia; *PC*, pyruvate carboxylase; *PDH*, pyruvate dehydrogenase; *PGK*, phosphoglycerate kinase; *RALF*, recurrent acute liver failure;*TBBGD*, thiamine-biotin-responsive basal ganglia disease;*THTR1*, Thiamine transporter (thiamine responsive megaloblastic anemia);*UCD*, urea cycle disorders
Bold face, treatable disorders; * Cases reported in adults as presenting or predominant symptom

cephalitis since the symptoms can be triggered by viral infections followed by progressive weakness and cranial nerve involvement including bulbar palsy. Treatment with riboflavin reverts the clinical picture (▶ Chapter 12).

In summary, all these disorders should be considered in the differential diagnosis of acute cerebral injury and clinical pictures mimicking infectious/inflammatory encephalitis, strokes or stroke-like episodes. Vaguely defined and/or undocumented diagnoses such as encephalitis, basilar migraine, intoxication, poisoning, or cerebral thrombophlebitis should therefore be questioned, particularly when even moderate ketoacidosis, hyperlactatemia, or hyperammonemia is present. In fact, these apparent initial acute manifestations are frequently preceded by other premonitory symptoms, which may be unrecognized or misinterpreted. Such symptoms include acute ataxia, persistent anorexia, chronic vomiting, failure to thrive, hypotonia, and progressive developmental delay – symptoms that are often observed in **UCD**, respiratory chain defects, and **OA**.

Certain features or symptoms are characteristic of particular disorders. For example, macrocephaly is a frequent finding in **GAI**; unexplained episodes of dehydration may occur in **OA**; and hepatomegaly at the time of coma is an important although inconsistent finding in **fructose bisphosphatase deficiency**. Severe hematologic manifestations and recurrent infections are common in **IVA, PA, and MMA**. Macrocytic anemia may be an important clue indicating a **cobalamin or folate disorder**.

When coma is associated with hepatic dysfunction, Reye syndrome secondary to disorders of **FAO** or **UCD** should be considered.In adults both conditions may mimick an alcoholic hepatic encephalopathy. Hepatic coma with liver failure and hyperlactatemia can be the presenting sign of respiratory chain disorders (▶ Chapter 14). Finally, hepatic coma with cirrhosis, chronic hepatic dysfunction, hemolytic jaundice, and various neurological signs (psychiatric, extrapyramidal) is a classic, but underdiagnosed manifestation of **Wilson disease.** A similar clinical scenario can be found at advanced stages of **manganese transporter deficiency** characterized by dystonia/parkinsonism, hypermanganesemia, polycythemia and chronic liver disease (▶ Chapter 37).

■ Recurrent Attacks of Ataxia (◘ Table 1.7)

Intermittent acute ataxia and disturbed behavior can be the presenting signs of late-onset intermittent **MSUD** and **OA**, where they are associated with ketoacidosis and sometimes with hyperglycemia which can mimic diabetic ketoacidosis (▶ Chapter 18). Late onset **OTC** and **ASS** deficiency can

Table 1.7 Ataxias

Clinical presentation	Metabolic derangements or other important signs	Additional symptoms	Most frequent diagnosis (disorder/enzyme deficiency)	Differential diagnosis
Acute ataxia	Ketoacidosis Characteristic AAC (P) and OAC (U) profiles	Special odor Neutropenia Thrombopenia Hyperglycemia	**Late onset MSUD** **MMA, PA, IVA**	Diabetes
	Hyperammonemia (sometimes slight elevation) AAC (P), orotic acid (U)	Respiratory alkalosis Hepatomegaly	**Urea cycle defects** (OTC, ASS)	Intoxication Encephalitis Brain tumor
	Hyperlactatemia (sometimes very moderate and only in post-prandial state)	Normal L/P ratio No ketosis Peripheral neuropathy	**PDH**	Migraine Cerebellitis (varicella) Polymyoclonia Acetazolamide responsive polymyoclonia
		High L/P ratio Ketosis Cutaneous signs	**MCD** Respiratory chain defects	Ataxia Acute exacerbation in chronic ataxias Channelopathies
	AAC (U)(neutral AA in urines)	Skin rashes, Pellagra, sun intolerance	Hartnup	
	Low glucorrachia (CSF) compared to blood glucose	Worse with fasting, may be associated with abnormal movements	**GLUT-1**	
	Low plasma biotinidase		Biotinidase deficiency	

AAC, amino acid chromatography; *ASA*, arginosuccinic aciduria; *IVA*, isovaleric acidemia; *L*, lactate; *LPI*, lysinuric protein intolerance; *MMA*, methymalonic acidemia; *MCD*, Multiple carboxylase deficiency;*MSUD*, maple syrup urine disease; *OAC*, organic acid chromatography; *OTC*, ornithine transcarbamylase; *P*, pyruvate; *PA*, propionic acidemia; *PDH*, pyruvate dehydrogenase. **Bold face**, treatable disorders;

present with recurrent attacks of ataxia. Energy defects such as **GLUT1 deficiency** may present with intermittent ataxia (or gait dyspraxia) as the only finding. In these cases ataxia is usually worse before meals (▶ Chapter 10). Mild forms of **biotinidase deficiency** and Hartnup disease (▶ Chapter 25) may also cause intermittent ataxia. Acute ataxia associated with peripheral neuropathy is a frequent presenting sign of **PDH** deficiency; moderate hyperlactatemia with a normal L/P ratio supports this diagnosis (▶ Chapter 11). In general, most episodic ataxias belong to the channelopathies, which are not classical IEM but as other channel disorders in the nervous system, contribute to modulate brain neurotransmission.

Acute psychiatric symptoms (Table 1.8)

Late-onset forms of **UCD,** mainly partial **OTC** deficiency, can present late in childhood or in adolescence with psychiatric symptoms. Because hyperammonemia and liver dysfunction can be mild even at the time of acute attacks, these intermittent late-onset forms of **UCD** can easily be misdiagnosed as hysteria, schizophrenia, or alcohol or drug intoxication (▶ Chapter 19). **Acute intermittent porphyria** and **hereditary coproporphyria** present classically with recurrent attacks of vomiting, abdominal pain, neuropathy, and psychiatric symptoms (▶ Chapter 36). Finally, patients with **homocysteine remethylation defects** may present with schizophrenia-like, betaine and sometimes folate-responsive episodes. In view of **these possible diagnoses, it is justified to systematically measure ammonia, porphyrins and plasma homocysteine in every patient presenting with unexplained acute psychiatric symptoms**. Episodes of acute psychosis mostly in adulthood also occur in the autosomal dominant disorder neuroferritinopathy which is associated with low serum ferritin, in **cerebrotendinous**

Table 1.8 Diagnostic approach to recurrent attacks of psychiatric symptoms

Clinical presentation	Metabolic derangements or other important signs	Additional symptoms	Most frequent diagnosis (disorder/enzyme deficiency)
Psychiatric symptoms, particularly if atypical (hallucinations, delirium, dizziness, aggressivity, anxiety, schizophrenic-like behaviour, agitation)	Hyperammonemia (sometimes moderate), AAC, orotic acid	Slight liver dysfunction Vomiting Failure to thrive	**Urea cycle defects (OTC, ASA, arginase)** **LPI**
	Ketoacidosis AAC, OAC	Ataxia, neutropenia	**Organic acid defects, MSUD**
	Port-wine urine Porphyrins in plasma/urine	Abdominal pain All kinds of neuropathy Vomiting	**Acute intermittent porphyria** **Hereditary coproporphyria**
	Homocystinuria (total homocysteine >100 µM)	Stroke, seizures Myelopathy	**Methylene tetrahydrofolate reductase deficiency**
	AAC (neutral AA in urines)	Skin rashes, pellagra	Hartnup disease
	Low copper,ceruloplasmin Low serum ferritin	Dystonia, Parkinsonism, Kayser Fleisher ring Pallidal necrosis	**Wilson** Neuroferritinopathy PKAN 2
	Foam cells in bone marrow, Cholestanol (plasma sterols)	Vertical ophtalmoplegia Dentate nuclei hyperintensity	**Niemann-Pick type C** **Cerebrotendinous xanthomatosis**
	None	Epilepsy, retinitis pigmentosa	Ceroid Lipofuscinosis

AAC, amino acid chromatography; *ASA*, arginosuccinic aciduria; MSUD, maple syrup urine disease; *LPI*, lysinuric protein intolerance; *PKAN*, panthothenate kinase associated degeneration;*OAC*, organic acid chromatography; *OTC*, ornithine transcarbamylase; **bold face**, treatable disorders

xanthomatosis and in several late-onset complex molecule disorders (such as LSD and NBIA syndromes) (▶ Chapter 2) [20].

Dehydration (Table 1.9)

In pediatrics, dehydration is a common consequence of diarrhea caused by a variety of enteral or parenteral acute infections. However, these common infectious diseases can occasionally trigger acute decompensation of an IEM. Moreover, aside from dehydration due to gastrointestinal losses, some IEM can present as recurrent attacks of dehydration secondary to polyuria (in **OA**), hyperventilation (in severe acidosis or alkalosis) or excessive sweating. The main accompanying findings (severe diarrhoea, salt wasting, ketoacidosis, failure to thrive, Fanconi syndrome) can be used to classify dehydration due to IEM. Carbonic anhydrase 12 deficiency has been recently decribed in a consanguineous Israeli Bedouin kindred with autosomal recessive hyperchlorhydrosis whose only symptoms were visible salt precipitates after sweating, a preponderance of hyponatremic dehydration, poor feeding and slow weight gain in infancy [21].

Reye Syndrome, sudden unexpected death in infancy (SUDI) and near-miss

A large number of IEM has been described that produce episodes fitting the criteria originally used to define Reye syndrome. There is now considerable evidence that many of the disorders responsible for Reye syndrome were misdiagnosed in the past because of inadequate investigations for IEM. Another important reason for this underestimation is the necessity of collecting blood and urine specimens for metabolic investigations at an appropriate time in relation to the illness since most disorders affecting the mitochondrial pathway, **UCD, HHH** syndrome and **FAO disorders** may produce only intermittent abnormalities. In addition, in contrast to the usual belief, a normal or non specific urinary OA and acylcarnitine pattern, even at the time of an acute attack, does not exclude an inherited FAO disorder. True SUDI due to an IEM is, however, a rare event despite the large number of publications on the topic and despite the fact that >30 metabolic defects are possible causes [22]. This assertion is not true in the first week of life in which unexpected death (SIDS or near-miss) **is a priori due to a FAO disorder** until proven otherwise. The recently described LARS and NBAS mutations (the latter triggered by fever) presenting with recurrent episodes of acute liver failure (RALF) may have been mistaken with Reye syndrome [14][15].

Exercise Intolerance and Recurrent Myoglobinuria

Exercise intolerance and recurrent myoglobinuria syndrome is defined as myalgias, cramping, and/or limb weakness associated with elevated serum levels of creatine phosphokinase (CK) and other sarcoplasmic enzymes (usually >100 times upper limit of normal), recurrent pigmenturia, and sometimes

Table 1.9 Attacks of Dehydration

Leading Symptoms	Other signs	Age at onset	Diagnosis (disorder/enzyme deficiency)
Severe diarrhea: »gastrointestinal causes«	Severe watery acidic diarrhea Glycosuria	Neonatal	**Glucose galactose malabsorption** **Lactase deficiency**
	Hydramnios, no meconium Severe watery nonacidic diarrhea Metabolic alkalosis Low K^+, Cl^-	Congenital	**Congenital chloride diarrhea**
	Severe watery diarrhea	Neonatal After weaning or when sucrose or starch dextrins are added to the diet	**Diacylglycerol acyl transferase** **Sucrase isomaltase**
	Anorexia, failure to thrive Weight loss (before cutaneous lesions and alopecia)	2-4 weeks or after weaning	**Acrodermatitis enteropathica**
Ketoacidosis: »organic acidurias«	Polyuria Polypnea Hyperglycemia Glycosuria	Infancy to early childhood	**Diabetic coma** **MMA, PA, IVA** **3-Ketothiolase** Hydroxyisobutyric aciduria
Failure to thrive, anorexia, poor feeding, polydipsia, polyuria: »renal tubular dysfunction«	Photophobia Renal Fanconi syndrome	Infancy (3-6 months)	**Cystinosis**
	Hypernatremia, vomiting Psychomotor retardation Spasticity	Neonatal to first month	**Nephrogenesis diabetes insipidus** (X-linked)
	Hyperchloremia Metabolic acidosis Alkaline urine pH	Early in infancy	**RTA type I (distal)** **RTA type II (proximal)** **RTA type IV**
	Hypoglycemia Hepatic glycogenosis Fanconi syndrome	Early in infancy	**Fanconi Bickel syndrome** (GLUT II deficiency)
	Pulmonary infections Chronic diarrhea Salted sweet	Infancy to early childhood	**Cystic fibrosis**
Salt-losing syndrome: »adrenal dysfunctions«	Severe hyponatremia Ambiguous genitalia	End of first week of life	**Congenital adrenal hyperplasias**
	Unambiguous genitalia	End of first week	**Hypoaldosteronism**
	Ambiguous genitalia	Infancy to early childhood	**Congenital adrenal hypoplasia** **Congenital adrenal hyperplasia,** late-onset forms
	Unambiguous genitalia		Hypo & pseudohypoaldosteronism
	Hypoketotic hypoglycemia		**FAO (CPT I and II)**

CPT, carnitine palmitoyl transferase; *FAO*, fatty acid oxidation; *GLUT*, glucose transporter; *IVA*, isovaleric acidemia; *MMA*, methylmalonic aciduria; *PA*, propionic aciduria; *RAT*, renal tubular acidosis. **Bold face**, treatable disorders

acute renal failure. In the last instance, or when the patient is in a comatose state, clinical muscular symptoms can be missed. An important rule is to check serum CK and for myoglobinuria in such conditions. The disorders of muscle energy metabolism present in two ways:

In the glycolytic disorders, exercising muscle is most vulnerable during the initial stages of exercise and during intense exercise when carbohydrates are the main energy source. A »second-wind« phenomenon sometimes develops. Clinically, the glycolytic disorders are mostly observed in late childhood,

adolescence, or adulthood. Their diagnosis is based largely on the absence of hyperlactatemia during a physical exercise test, and the muscle magnetic resonance spectroscopy pattern. The CK level remains elevated in most patients. The most frequent and typical disorder in this group is McArdle disease due to myophosphorylase deficiency (▶ Chapter 5).

In the FAO disorders, attacks of myoglobinuria occur typically after mild to moderate prolonged exercise and are particularly likely when patients are additionally stressed by fasting, cold, or infection. This group is largely dominated by muscle **CPT II, VLCAD, LCHAD and trifunctional (TF)** deficiencies, which may occur in childhood, in adolescence, or later (▶ Chapter 12). Recurrent rhabdomyolysis has been described in a child with **GAI** (▶ Chapter 22) and recently in one patient with an **FAD transporter defect** (▶ Chapter 12). **Deficiencies of VLCAD** and SCHAD may also present with a myopathy.

Mutations in *TANGO2* encoding transport and Golgi organization 2 homolog have been recently described in infants and children with episodic rhabdomyolysis, hypoglycemia, hyperammonemia, and susceptibility to life-threatening cardiac tachyarrhythmias mimicking a fatty acid oxidation defect [23][24].

Mutations in RYR 1 encoding the ryanodine receptor present with muscle rigidity and rhabdomyolysis when affected individuals are exposed to general anesthesia from infancy (recessive mutations) to adults (dominant mutations) [25].

LPIN1 gene mutations have recently been found in 60% of a series of patients presenting with unexplained recurrent myoglobinuria after exclusion of primary FAO disorder. This suggests that lipin1 deficiency should be regarded as a major cause of severe myoglobinuria in infancy (▶ Chapter 34). Recurrent rhabdomyolisis and stroke like episodes has been described in a single adult patient with racemase deficiency (*AMACR*).

Adenylate deaminase deficiency has been suspected to cause exercise intolerance and cramps in a few patients, but the relationship between clinical symptoms and the enzyme defect is uncertain (▶ Chapter 35). Respiratory chain disorders (RCD) can present with recurrent muscle pain and myoglobinuria from neonatal period to adolescence. RCD should be suspected when hyperlactatemia is accompanied by an elevated L:P ratio, either permanently or after meals. Sometimes the lactate abnormality will be found only after an exercise test. In RCD, muscle symptoms are often associated with cardiomyopathy or diverse neurologic signs (encephalomyopathy) (▶ Chapter 14). A case of lipoamide dehydrogenase deficiency presenting with recurrent myoglobinuria has been described in an adult. It is not clear whether normo- and hyperkalemic paralysis due to sodium channel gene mutations may present with attacks of exercise intolerance.

Abdominal Pain (Recurrent Attacks)

Table 1.10 contains all pertinent information on recurrent attacks of abdominal pain. Mevalonic aciduria due to mevalonate kinase deficiency can present as recurrent attacks of abdominal pain with fever, skin rashes, arthralgias, and inflammatory syndrome and hyper IgD.

Table 1.10 Abdominal pain

With flatulence, diarrhea, lose stools	Diacylglycerol acyltransferase 1 deficiency **Lactose malabsorption** **Congenital sucrase isomaltase deficiency**
With vomiting, lethargy, ketoacidosis	**Urea cycle defects (OTC, ASA)** **Organic acidurias** (MMA, PA, IVA) **Ketolysis defects** Respiratory chain disorders **Diabetes**
With neuropathy, psychiatric symptoms	**Porphyrias** **Tyrosinemia type I** **OTC** (late onset) MNGIE syndrome (intestinal obstruction)
With fatigue,weakness	Hemochromatosis
With hepatomegaly and splenomegaly	Cholesterol ester storage disease Lipoprotein lipase deficiency **Lysinuric protein intolerance** **MPI-CDG (protein losing enteropathy)** Hemochromatosis Mevalonate kinase deficiency
With pain in extremities	**Fabry disease** δ-aminolevulinate dehydratase deficiency **Sickle cell anemia**
With hemolytic anemia	**Coproporphyria** Hereditary spherocytosis **Sickle cell anemia** Nocturnal paroxysmal hemoglobinuria
With Crohn/Pseudo Crohn disease	Trifunctional enzyme? **Carnitine transporter** deficiency (?) **Glycogenosis type Ib**
With inflammatory syndrome (fever rash, IC reactive protein)	Hyper IgD syndrome (mevalonate kinase deficiency)

Cardiac failure and heart beat disorders (Table 1.11)

Acute cardiac failure and disorders of heart rhythm can be the first signs in IEM. The important metabolic causes of cardiomyopathy are Pompe and Danon disease (▶ Chapter 5), all **long-chain FAO disorders** except CPT I deficiency, MADD, RCD, Barth and Sengers syndromes (▶ Chapter 34) (▶ overview »Cardiomyopathies«). Fatal congenital heart glycogenosis due to mutation in *PRKAG2* can initially mimic RCD, or trifunctional enzyme deficiency when generalized hypotonia is associated with cardiomyopathy. In adults *PRKAG2/AMPK* clinical phenotype displays a hypertrophic cardiomyopathy and preexcitation syndrome (Wolf Parkinson White and

atrioventricular block) (▶ Chapter 5). Dilated cardiomyopathy is a major sign in several dolichol synthesis/recycling defects and in COG 7-CDG (▶ Chapter 41). PMM2-CDG may at times present in infancy as tamponade with pericardial effusion, multiorgan failure, and characteristic cutaneous and neurologic features. Pericardial effusion associated with severe fatty liver has been observed in late onset type II glutaric aciduria. Isolated isobutyryl CoA dehydrogenase deficiency presenting with dilated cardiomyopathy has been recently described.

Alternatively, heart failure may result from disturbed cardiac rhythm. In congenital hypoparathyroidism and pseudohypoparathyroidism, cardiac failure can be the consequence of severe hypocalcemia with a prolonged QT interval on ECG. In *PRKAG2/AMPK* (▶ Chapter 5) and in Kearns-Sayre syndrome (KSS) (▶ Chapter 14) atrioventricular block with syncope is a classic sign, usually associated in KSS with progressive external ophthalmoplegia and retinal degeneration (see »Hyperlactatemia« below). In the rare disorder triosephosphate isomerase deficiency, which presents early in infancy as hemolytic anemia and progressive neurologic dysfunction, arrhythmia may cause sudden cardiac death (▶ Chapter 7). A hyperkinetic hemodynamic state with sinus tachycardia, a classic finding in hyperthyroidism is also an early presenting sign in **thiamine-deficient and dependent states** associated with lactic acidosis which can be dramatically relieved by thiamine administration (▶ Chapter 28). Finally, all **long-chain FAO disorders** except CPT I deficiency can present in early infancy, even in the neonatal period, with cardiac arrest or hypotension, which is readily misdiagnosed as toxic shock or malignant hyperthermia. Disorders of heart rhythm (premature ventricular complexes, atrioventricular block, and ventricular tachycardia) are frequent features and could be due to accumulation of long-chain acylcarnitines. In a series of 107 patients with FAO disorders, 37 patients displayed disorders of heart rhythm where it was the presenting symptom in 20 [19].

Table 1.11 Arrhythmias, conduction defects (heart beat disorders)

Primary dysrhythmias	**Adrenal dysfunction** (hyperkalemia)
	AMP activated protein kinase (*PRKAG2*: WPW)
	Cardiac glycogenosis (Wolf Parkinson White: WPW)
	Triose phosphate isomerase deficiency
	D2-hydroxyglutaric aciduria (AV block)
	Fatty acid oxidation disorders (CPT II, carnitine translocase, LCAD, LCHAD, TF, VLCAD), *TANGO2* mutations
	Carnitine transporter defect (only in adults)
	Hypoparathyroidism (hypocalcemia)
	Kearns-Sayre syndrome (respiratory chain disorders)
	Propionic acidemia (prolonged QTc)
	Thiamine deficiency/dependent states
With cardiac/ multiorgan failure	PMM2-CDG (Ia) (with tamponade)

WPW, Wolf Parkinson White; *AV*, auriculoventricular; *CPT*, carnitine palmitoyl transferase; *LCAD/LCHAD/VLCAD*, long/3hydroxy long/very long chain acyl-CoA dehydrogenase; *TF*, trifunctional enzyme; *CDG*, congenital disorders of glycosylation

Cardiomyopathies

- AMP activated protein kinase (presenting sign)
- Barth syndrome (3 methyl glutaconic aciduria)
- PMM2-CDG (with pericardial effusion, can be the presenting sign)
- Congenital muscle dystrophies
- Conserved Oligomeric Golgi (COG7-CDG) defects (CDG IIe: dilated cardiomyopathy)
- Dolichol synthesis/recycling disorders (SRD5A3, DOLK, and DPM3-CDG): dilated cardiomyopathy
- D-2hydroxyglutaric aciduria
- *DNAJC19* mutations
- **Fabry disease** (may be presenting sign with myocardial infarction)
- **Fatty acid oxidation disorders** (presenting sign)
- Friedreich ataxia (presenting sign)
- **Glycogenosis type III,** and IV (where it can be presenting sign)
- GM1 gangliosidosis
- Hemochromatosis type 2 (adults)
- 3-Ketothiolase deficiency
- Isobutyryl-CoA dehydrogenase
- Lipin 1 deficiency
- **Methylmalonic aciduria (Cbl C) and rarely MMCoA mutase,** Malonic aciduria
- Mucopolysaccharidosis
- Muscular glycogen synthetase (presenting sign)
- Neutral lipid storage myopathy
- Pompe disease, Danon disease (presenting sign)
- **Propionic acidemia** (can be presenting sign)
- Respiratory chain disorders (presenting sign)
- *RBCK1* mutations (E3 ubiquitin ligase)
- **Selenium deficiency**
- Sengers syndrome (3-methylglutaconic aciduria)
- Steinert disease myotonic dystrophy
- **Thiamine deficiency**
- **Thiamine responsive anemia**
- *TMEM70* mutations
- **Tyrosinemia type 1**

Table 1.12 Liver Failure (Ascites, Edema): see ▶Section 1.4 for congenital and neonatal presentation

Age at onset	Disorder
infancy	*ACAD 9* mutations Alpha-1 antitrypsin deficiency Congenital disorder of glycosylation Cholesterol ester storage disease Cystic fibrosis Familial hepatic fibrosis with exsudative enteropathy: **MPI-CDG (Ib)** and ALG8-CDG (Ih) **Ketogenesis defects** Mitochondrial tRNA translation disorders (*LARS, MARS, HARS)* *NBAS* mutations *TRMU* mutations(transient acute liver failure) Pyruvate carboxylase deficiency **S-adenosylhomocystine hydrolase deficiency** **Urea cycle defects** Wolman disease
childhood to adolescence	Wilson disease

Liver failure, ascitis, oedema

Table 1.12 presents the disorders with liver failure according to age of onset. Recent literature highlights the disorders that involve the mitochondrial tRNA translation (*TRMU*, *LARS*, *HARS* and MARS mutations) (▶ Chapter 14).

Pain in extremities and Bone Crisis

Pain in extremities can be due to bone, vascular, neurologic, autonomic or complex mechanisms. Painful crisis can be observed as presenting or major symptom in four groups of metabolic/genetic disorders. Bone pain is a frequent symptom in vitamin D deficiency and hereditary hypophosphatemic rickets where it is associated with bone changes (rickets).

Bone pain can occur in sickle-cell anemia and various porphyrias in association with other signs. One particularly important form of painful crisis that occurs early in infancy in sickle-cell anemia is the hand-foot syndrome, which is a dactylitis characterized by sudden onset of painful swelling of the dorsum of the hands and feet. In various porphyrias, bone crisis is associated with abdominal pain, constipation, vomiting, and neuropathy. These crises may also occur in tyrosinemia type I.

Bone pain can occur in three progressive neurologic disorders:

- In the infantile form of Krabbe disease, bone crises are expressed by irritability and incessant crying, which may precede by a few weeks the characteristic psychomotor deterioration with peripheral neuropathy.
- Similar crises have been observed in defects of biopterin synthesis and in aromatic amino acid decarboxyase deficiency.
- In late infantile metachromatic leukodystrophy, bone crises are associated with limb hypertonia, muscle weakness, and progressive neurologic deterioration. In Gaucher disease type III, bone crises can precede, accompany, or follow a large variety of neurologic symptoms; they appear in late childhood or adolescence and are associated with splenomegaly.

Finally, bone pain may occur as an isolated symptom. Bone crisis is frequently the presenting symptom in hemizygotic Fabry disease, non neuronopathic Gaucher disease (type I) and in hereditary sensory autonomic neuropathy (HSAN). The »Fabry crisis« can last from minutes to several days. It consists of agonizing burning pain commencing in the palms and soles and radiating to the proximal extremities. Because it is often associated with fever and elevated erythrocyte sedimentation, it may be confused with rheumatic fever, neurosis, or erythromelalgia. Another item in the differential diagnosis may be metabolic crisis in mevalonic aciduria, which causes arthralgias, morbilliform rash, fever, lymphadenopathy, and hepatosplenomegaly (see also below ▶ Section 1.6.8). Painful crises are usually triggered by exercise, fatigue, emotional stress, or rapid changes in temperature and humidity. A »Fabry crisis« is rarely observed in female heterozygotes.

Chronic recurrent multifocal osteomyelitis (CRMO) is an inflammatory disorder that primarily affects children. Its hallmark is recurring episodes of sterile osteomyelitis. The clinical presentation is with an insidious onset of bone pain with or without fever. Two genetic syndromes have CRMO as a prominent phenotype - Majeed syndrome (with inflammatory dermatosis and congenital dyserythropoietic anaemia) and deficiency of the interleukin-1 receptor antagonist (▶ Chapter 34) [26].

Many patients with Gaucher disease type I (chronic non-neuronopathic) experience episodic pain lasting for days to months in the hips, legs, back, and shoulders. Although osteopenia and osteolysis are common, some patients have neither radiographic, scintigraphic, nor scanographic evidence of bone involvement at the time of the first attack. Rarely, bone crisis precedes hepatosplenomegaly and is the presenting sign of the disease (▶ Chapter 38).

1.4.2 Metabolic Derangements and Diagnostic Tests

Initial approach, protocol of investigation

The initial approach to the late-onset acute forms of IEM, as with the approach to acute neonatal distress, is based on the proper use of a few screening tests. As with neonates, the laboratory data listed in Table 1.6 must be collected at the same time during the acute attack and both before and after treatment.

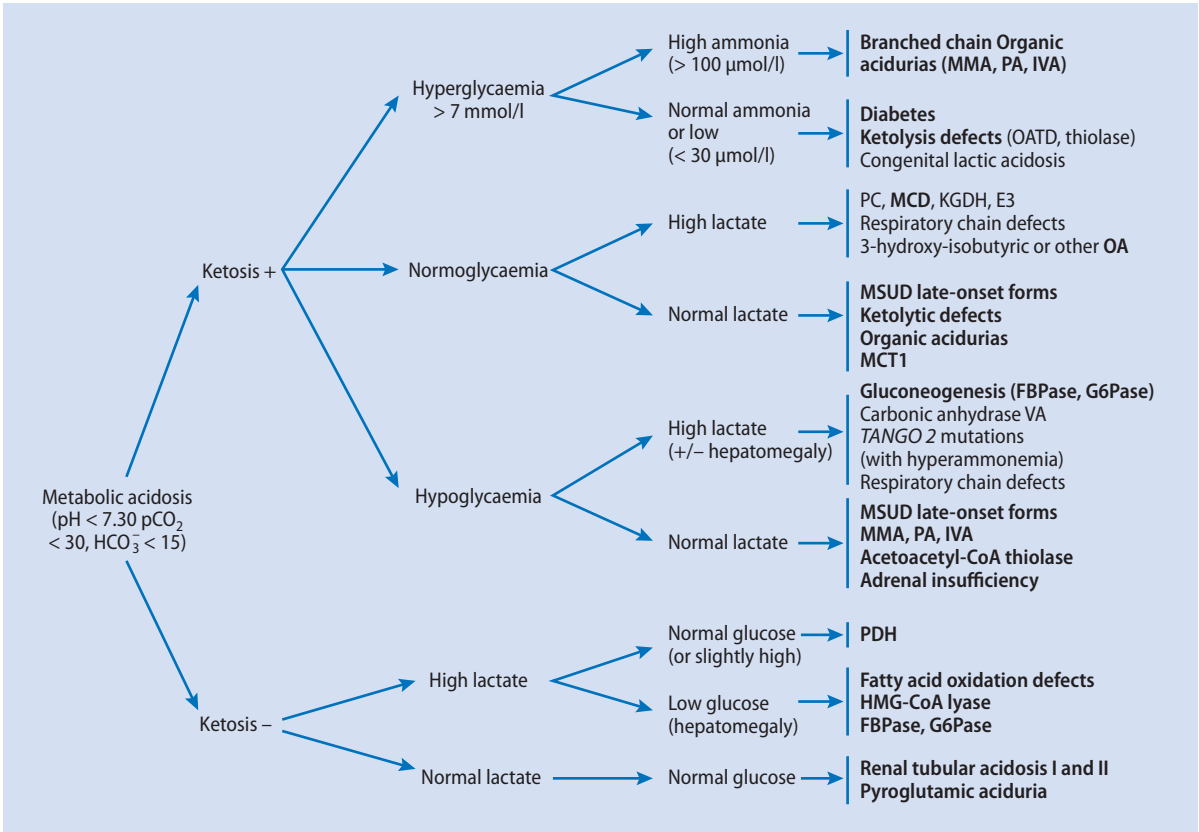

Fig. 1.2 Metabolic acidosis. *E3*, lipoamido oxido reductase; *FBP*, fructose bisphosphatase; *G6P*, glucose-6-phosphatase; *HMG-CoA*, 3-hydroxy-3-methylglutaryl coenzyme A; *IVA*, isovaleric acidemia; *KGDH*, alpha-ketoglutarate dehydrogenase; *MCD*, multiple carboxylase deficiency;*MCT1*, monocarboxylate transporter1; *MMA*, methylmalonic aciduria; *MSUD*, maple syrup urine disease; *OATD*, oxoacid CoA transferase; *PA*, propionic acidemia; *PC*, pyruvate carboxylase; *PDH*, pyruvate dehydrogenase; *SCAD*, short chain acyl-CoA dehydrogenase; **bold face**, treatable disorders

Metabolic acidosis (Fig. 1.2)

Metabolic acidosis is a very common finding in paediatrics and is defined by a plasma bicarbonate level < 18 mmol/l while the pH is <7.38. It can be observed in a large variety of acquired conditions, including infections, severe catabolic states, tissue anoxia, severe dehydration, and intoxication, all of which should be ruled out. However, these can also trigger an acute decompensation of an unrecognized IEM. The presence or absence of ketonuria associated with metabolic acidosis is the major clinical clue to the diagnosis.

When metabolic acidosis is NOT associated with ketosis, **PDH deficiency**, **FAO disorders**, and some **disorders of gluconeogenesis** should be considered, particularly when there is moderate to severe hyperlactatemia. **FAO and gluconeogenesis defects** display a concomitant fasting hypoglycemia. Although **fructose bisphosphatase** deficiency is classically considered to give rise to ketoacidosis, some patients have had relatively low concentrations of ketone bodies during hypoglycaemia (▶ Chapter 8). When metabolic acidosis occurs with a normal anion gap and without hyperlactatemia or hypoglycemia, the most frequent cause **is renal tubular acidosis (RTA) type I or II**. Pyroglutamic aciduria also can present early in life with permanent, isolated metabolic acidosis, which can be mistaken for RTA type II (▶ Chapter 30).

A number of IEM cause a metabolic acidosis with an associated ketosis. The range of serum ketone body concentration varies with age and nutritional state. **Insulin-dependent diabetes**, inborn errors of **branched-chain amino acid metabolism** (▶ Chapter 18) congenital lactic acidoses such as **multiple carboxylases** (▶ Chapter 26) and pyruvate carboxylase deficiencies (▶ Chapter 11), some forms of **glucose-6-phosphatase** (▶ Chapter 5) and **fructose biphosphatase deficiencies** (▶ Chapter 8) and **ketolytic defects** (▶ Chapter 13) are the main groups of metabolic disorders. The glucose level which can be high, normal, or low is the first parameter to be considered in order to classify these disorders.

In the case of hyperglycemia, the classic diagnosis is diabetic ketoacidosis. However, **OA** such as **PA, MMA, or IVA and ketolytic defects** can also be associated with hyperglycemia and glycosuria, mimicking diabetes. Hyperglycemia resolves easily within few hours after insulin infusion. The distinction between OA and ketolytic defects is based on ammonia and lactate levels (which are generally increased in OA and normal or low in ketolytic defects) and on the organic acid profile.

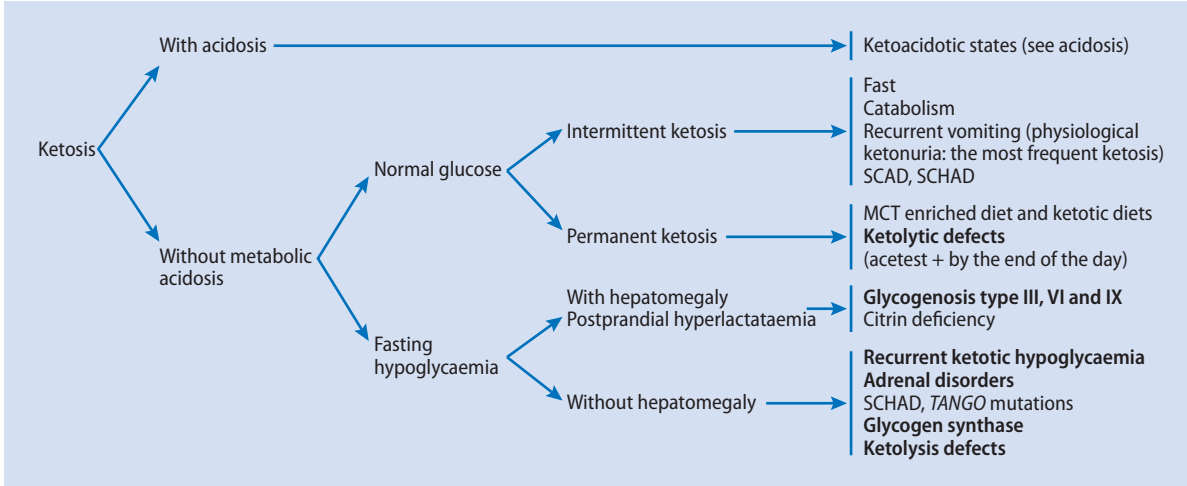

Fig. 1.3 Ketosis. (see also Fig. 1.2) *MCAD*, medium chain acyl coenzyme A dehydrogenase; *MCT*, medium chain triglycerides; *SCAD*, short-chain acyl coenzyme A dehydrogenase; *SCHAD*, hydroxy short-chain acyl coenzyme A dehydrogenase. **Bold face**, treatable disorders

In the case of hypoglycemia, the first group of disorders to be considered is the **gluconeogenesis** defects and **GSD type Ia and b**. The main findings suggestive of this group are hepatomegaly and hyperlactatemia, although they are not constant. When there is no significant hepatomegaly, late-onset forms of **MSUD** and **OA** deficiency should be considered. A classic differential diagnosis is **adrenal insufficiency**, which can cause a ketoacidotic attack with hypoglycemia. **GSD III and glycogen synthetase deficiency** don't present with severe keto acidosis but rather with recurrent ketotic hypoglycaemia (see below).

If the glucose level is normal, congenital lactic acidosis must be considered in addition to the disorders discussed above. Severe ketoacidosis recurrent crisis with normal glucose and lactate is very suggestive of ketolytic defects and the recently described monocarboxylate 1 transporter deficiency (▶ Chapter 13). According to this schematic approach to inherited ketoacidotic states, a simplistic diagnosis of fasting ketoacidosis or ketotic hypoglycemia should be questioned when there is a concomitant severe metabolic acidosis.

Ketosis (Fig. 1.3)

While ketonuria should always be considered abnormal in neonates, it is a physiological result of catabolism in late infancy, childhood, and even adolescence. However, as a general rule, hyperketosis> 6 mmoles/l of total plasma ketone bodies that produces metabolic acidosis (serum bicarbonate <18 mmol/l) is not physiological. Ketosis which is not associated with acidosis, hyperlactatemia, or hypoglycemia, is likely to be a normal physiological reflection of the nutritional state (fasting, catabolism, vomiting, medium-chain triglyceride-enriched or other ketogenic diets). Of interest, severe forms of ketolytic defects (**succinyl-CoA transferase** and **3-ketothiolase** deficiencies) can present with persistent moderate ketonuria occurring mainly in the fed state at the end of the day (▶ Chapter 13). Paradoxical post prandial ketosis suggests a deficient anaplerosis of the Krebs cycle that is actually observed in pyruvate carboxylase deficiency (▶ Chapter 11).

Significant fasting ketonuria without acidosis is often observed in **GSD type III** in childhood (with marked hepatomegaly) and in **glycogen synthase** defect in infancy (with normal liver size). In both disorders there is a characteristic metabolic profile with fasting ketotic hypoglycaemia and normal lactate, and postprandial hyperlactatemia and hyperglycemia (▶ Chapter 5).

Ketosis without acidosis is observed in ketotic hypoglycemias of childhood (a frequent condition) and in association with hypoglycemias due to **adrenal insufficiency**. Absence of ketonuria in hypoglycemic states, as well as in fasting and catabolic circumstances (such as vomiting, anorexia, or intercurrent infections), is an important observation**,** suggesting a **FAO or ketogenesis disorder** (▶ Chapters 12 and 13). It can also be observed in hyperinsulinemic states at any age and in growth hormone deficiency in infancy. However, **SCHAD**, **SCAD**, and **MCAD** deficiencies can present as recurrent attacks of ketotic hypoglycemia as these enzymes are both sufficiently far down the β-oxidation pathway to be able to generate some ketones from long chain fatty acids (▶ Chapter 12).

Hyperlactatemia

Lactate and pyruvate are normal metabolites. Their plasma levels reflect the equilibrium between their cytoplasmic production from glycolysis and their mitochondrial consumption by different tissues. The reversible cytoplasmic enzyme lactate dehydrogenase catalyses lactate synthesis from pyruvate by using NADH as a hydrogen donor (Pyruvate+NADH+H = Lactate+NAD or Lactate = Pyruvate+NADH/NAD+H). Accordingly, the blood lactate and pyruvate levels and the L/P ratio reflects the redox state of the cells (NADH/NAD ratio). Hyperlactatemia can be due to an elevation of the pyruvate, the NADH/NAD ratio, H+ (acidosis) or both.

Blood lactate accumulates due to elevation of NADH/NAD ratio in circulatory collapse, in hypoxia, and in other conditions involving failure of cellular respiration and all severe acidotic states. These conditions must be excluded before an inborn error of lactate-pyruvate oxidation is sought. Persistent hyperlactatemias can also result from many acquired conditions, such as diarrhoea, persistent infections (mainly of the urinary tract), hyperventilation, and hepatic failure. Ketosis is absent in most hyperlactatemias secondary to tissue hypoxia, while it is a nearly constant finding in most IEM (except in **PDH deficiency**, **GSD type I** and **FAO disorders** see above ▶ Section 1.4.2). On the other hand, the level of lactate is not discriminating; some acquired disorders are associated with very high levels, whereas it is only moderately raised in some inborn errors of lactate-pyruvate metabolism. Nutritional state also influences the levels of lactate and pyruvate.

Once the **OA**, **UCD** (mainly **citrullinemia**), and **FAO defects** that can cause secondary moderate hyperlactatemia have been excluded as possible diagnoses, four types of IEM remain to be considered:

The cytoplasmic defects present in a context of hypoglycaemia:

- disorders of liver glycogen metabolism (▶ Chapter 5),
- disorders of liver gluconeogenesis (▶ Chapter 8).

The mitochondrial defects present in a context of neurological disturbances

- abnormalities of lactate-pyruvate oxidation (mitochondrial pyruvate transporter *MPC*, PDH, PC and Krebs cycle defects) (▶ Chapter 11),
- deficient activity in one of the components of the respiratory chain (▶ Chapter 14).

The diagnosis of hyperlactatemias is further based upon two metabolic criteria:

- *Time of occurrence of hyperlactatemia relative to feeding:* in disorders of gluconeogenesis (**fructose bisphosphatase** and **glucose-6-phosphatase deficiencies**), hyperlactatemia reaches its maximum level (up to 15 mmol/l) when the patient is fasting, acidotic and hypoglycemic. By contrast, **GSD types III and VI** and in **glycogen synthetase deficiency**, hyperlactatemia is observed only in the postprandial period in patients on a carbohydrate-rich diet. Here, hyperlactatemia never exceeds 6 mmol/l and therefore there is no acidosis (bicarbonates >18 mmol/l). In PC deficiency severe hyperlactatemia (>7 mmol/l) is present in both the fed and the fasted state, but tends to decrease in post prandial period. In disorders of MPC, **PDH**, alpha-ketoglutarate dehydrogenase, and respiratory chain function, maximum lactate levels are observed in the fed state (although all hyperlactatemias exceeding 7 mmol/l appear more or less persistent). In these disorders, there is a real risk of missing a moderate (although significant) hyperlactatemia if the level is checked only before breakfast after an overnight fast (as it is usual for laboratory determinations).
- *Determinations of L/P and ketone bodies ratios before and after meals.* These ratios are useful only in »mitochondrial« hyperlactatemias in a neurologic context. They indirectly reflect cytoplasmic (L/P) and mitochondrial (3OHB/AA) redox potential states. They must be measured in carefully collected blood samples. Three abnormal hyperlactatemia/hyperpyruvicemia profiles are nearly pathognomonic of an inborn error of lactate-pyruvate metabolism:
  - When hyperpyruvicemia (>0.3 mmol/l) is associated with a normal or low L/P ratio (<12) without hyperketonemia, **PDH deficiency** or MPC are highly probable, regardless of the lactate level.
  - When the L/P ratio is very high (>30) and is associated with a paradoxical postprandial hyperketonemia and with a normal or low 3OHB/AA ratio (<1.5), a diagnosis of PC deficiency (isolated or secondary to **biotinidase** or **holocarboxylase synthetase deficiency**) or alpha-ketoglutarate dehydrogenase deficiency is virtually certain. In PC deficiency, there is also a very characteristic AA profile with hyperammonemia, high citrulline and low glutamine.
  - When both L/P and 3OHB/AA ratios are elevated and associated with a significant postprandial hyperketonemia, RCD should be suspected.

All other situations, especially when the L/P ratio is high without hyperketonemia, are compatible with RCD, but acquired anoxic conditions should also be ruled out (see above).

The association of other biomarkers Hyperglycinemia and abnormal concentration of plasma branched chain AA (not constant) can be found in lipoylation defects (▶ Chapter 23). Certain OA profiles are also very helpful such as in the case of 3-methylglutaconic acid for the »3-methylglutaconic aciduriasˆ« (▶ Chapter 18), and Krebs cycle metabolites (as examples high excretion of alpha-ketoglutarate can point towards, alpha-ketoglutarate dehydrogenase deficiency but it is also found in lipoilation and thiamine transport defects). Low thiamine concentration in the CSF is also a marker for thiamine transporter defects [27] (▶ Chapter 28).

Hypoglycemia

In children the preponderant glucose source is

- food in the postprandial insulinemic period (<2.5 hours after a meal)
- hepatic glycogenolysis during a short fast (>2.5 hours to 8 hours after meal)
- hepatic glycogenolysis (progressively decreasing) and gluconeogenesis (progressively increasing) during a short to moderate fast (>8 hours to 12-15 hours),and
- hepatic gluconeogenesis during a long fast (>15 hours).

This timing may vary with age and nutritional state [28][29].

The clinical approach to hypoglycemia is based on four major clinical criteria:

- the liver size
- the characteristic timing of hypoglycaemia
- the association with lactic acidosis (suggesting gluconeogenesis impairement), and
- the association with hyperketosis or hypoketosis (the latter suggesting FAO or ketogenesis disorders).

Crucial information comes from the timing of hypoglycaemia which can be:

- unpredictable and only postprandial:<2.5 hours after meal (suggests hyperinsulinism or Munchausen by proxy)
- only after a short fast > 2.5 hours to 8 hours (suggests glycogenosis type I, III or 0)
- after a moderate to long fast >8 hours to 24 hours (suggests **gluconeogenesis defects**: »enzymatic« causes like FBPase deficiency or of »energetic« causes, mostly **FAO and ketogenesis** defects and RCD) [30]. Other clinical findings of interest are hepatic failure, vascular hypotension, dehydration, short stature, neonatal body size (head circumference, weight and height), and evidence of encephalopathy, myopathy, or cardiomyopathy.

Based on the liver size, hypoglycemias can be classified into two major groups:

- *Hypoglycemia with permanent hepatomegaly*. Hypoglycemia associated with permanent hepatomegaly is usually due to an IEM. When hepatomegaly is the most prominent feature without liver failure, **GSD type I** and **type III** are the most likely diagnoses. **FBPase deficiency and mitochondrial FAO defects** may present with a major to moderate hepatomegaly during hypoglycemic attacks**.** Disorders presenting with hepatic fibrosis and cirrhosis, such as hereditary **tyrosinemia type I**, also can give rise to hypoglycemia. The late-onset form of **HFI** rarely, if ever, presents with isolated postprandial fructose induced hypoglycemic attacks (▶ Chapter 8). S-adenosyl homocysteine hydrolase deficiency presents with fasting hypoglycemia and liver failure, often triggered by high protein or methionine ingestion, and is associated with hepatic fibrosis, mental retardation, and marked hypermethioninemia (▶ Chapter 20). RCD can present with liver failure, hypoglycaemia and fasting lactic acidosis which can mimic FBPase deficiency [30]. PMM2 and **MPI-CDG** (**phosphomannose isomerase** deficiency) with hepatic fibrosis and exsudative enteropathy can cause hypoglycemia early in infancy (▶ Chapter 41)
- *Hypoglycemia without permanent hepatomegaly*. It is important to determine the timing of hypoglycemia and to look for metabolic acidosis and ketosis when the patient is hypoglycemic. Unpredictable hypoglycaemic attacks occurring postprandial or after a very short fast and without ketosis are mostly due to **hyperinsulinism (congenital or Munchausen by proxy)** (▶ Chapter 9) at any age**,** or to **growth hormone deficiency** or related disorders in early infancy. Adenosine kinase deficiency may also present early in infancy with hyperinsulinemic hypoglycaemia (▶ Chapter 20).

Most episodes of hypoglycemia, due to IEM that are not accompanied by permanent hepatomegaly, appear after at least 8 h of fasting. This is particularly true for inherited **FAO disorders** except in the neonatal period. Severe fasting hypoglycemia without ketosis, strongly suggests **FAO disorders** (without severe acidosis) (▶ Chapter 12), **HMG-CoA lyase deficiency**, or **HMG-CoA synthetase deficiency** (with acidosis) (▶ Chapter 13). When ketoacidosis is present at the time of hypoglycemia, **ketolytic defects** (▶ Chapter 13), **OA**, **late-onset MSUD** (▶ Chapter 18), and glycerol kinase deficiencies (▶ Chapter 7) should be considered but hypoglycemia is very rarely the presenting metabolic abnormality in these disorders. **Adrenal insufficiencies** should be systematically considered in the differential diagnosis, especially when vascular hypotension, dehydration, and hyponatremia are present. Fasting hypoglycemia with ketosis occurring mainly in the morning and in the absence of metabolic acidosis suggests recurrent functional ketotic hypoglycemia, which presents mostly in late infancy or childhood in those who were small for gestational age or with macrocephaly. This pattern is rarely associated with IEM. All types of **adrenal insufficiencies** (peripheral or central) can share this presentation. SCHAD and **MCAD** deficiency can rarely present as recurrent attacks of ketotic hypoglycemia (▶ Chapter 12) as can **glycogen synthetase deficiency** (▶ Chapter 5). ◘ Fig. 1.4 summarizes the simplified diagnostic approach to hypoglycaemia. Although this is not a constant finding, some neurotransmitter defects (amino-acid decarboxylase deficiency (AADC) and dopamine beta-hydroxylase deficiency) can also present hypoketotic hypoglycaemias, especially in stressful situations. Additionnaly pyridoxine-dependent epilepsy can present with profound hypoglycaemia associated with hyperlactacidaemia [31].

▪ Hyperammonaemia

Many IEM can give rise to hyperammonaemia. In the context of acute neonatal encephalopathy, severe hyperammonaemia (>500 µmoles/l) is generally caused either by a **UCD** (with respiratory alkalosis, no ketosis and no bone marrow suppression) or by an **OA (PA, MMA, IVA** with metabolic acidosis, ketosis and leuco-thrombocytopenia) (▶ Chapter 18). Plasma glutamine is generally elevated in **UCD** (>1000 µmoles/l) **and LPI** while it is close to normal or low (<500 µmoles/l) in **OA**s. Plasma citrulline levels further allows the distinction between mitochondrial and cytoplasmic **urea cycle defects** (▶ Chapter 19). Severe neonatal forms of ornithine aminotransferase defect may mimick ornithine carbamyl transferase deficiency, before ornithine elevation occurs (▶ Chapter 21). Hyperammonaemia with hyperornithinemia and homocitrullinuria is diagnostic for the mitochondrial ornithine transporter defect (**HHH syndrome**) (▶ Chapter 21).

Neonatal hyperammonaemia associated with lactic acidosis (>6 mmol/l) and hyperketosis suggests PC (with low glutamine and high citrulline) (▶ Chapter 11), **MCD** (▶ Chapter 26) or carbonic anhydrase VA deficiencies (▶ Chapter 19), both with characteristic organic acid profile.

In a context of severe hypoketotic hypoglycaemia, hyperammonaemia (in general NH_3 <250 µmoles/l) suggests a

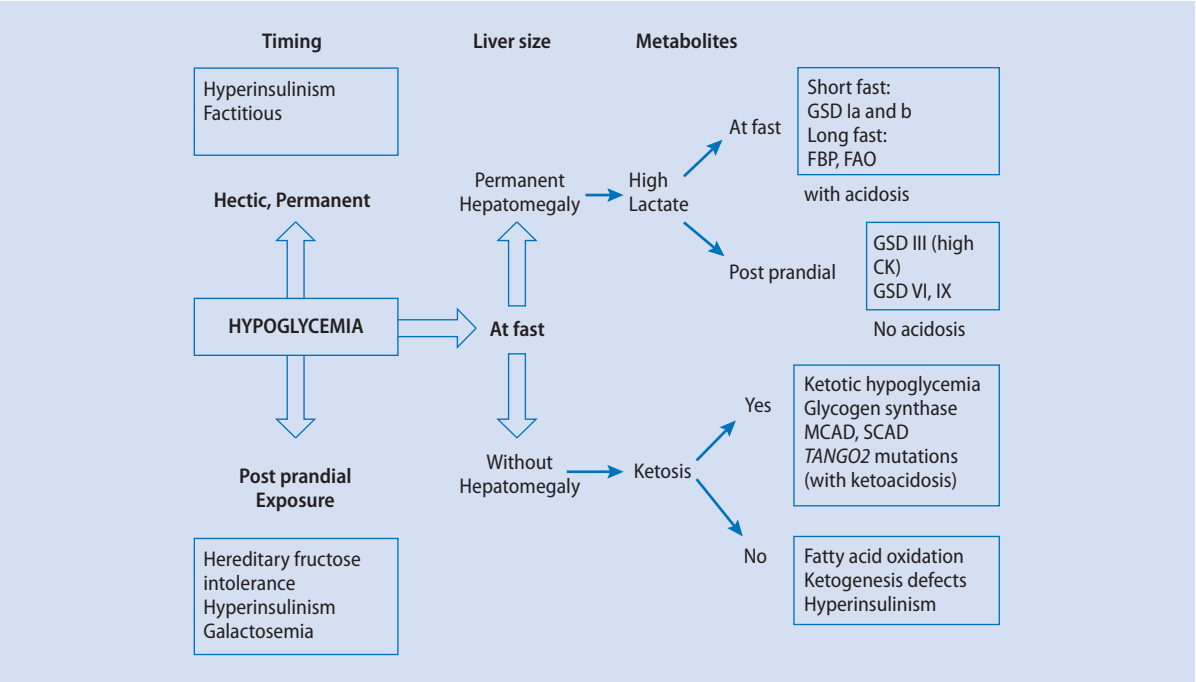

Fig. 1.4 Diagnostic approach to hypoglycaemia in paediatrics based on the timing of hypoglycaemia, the size of the liver and the metabolic profile. *PHHI*, Persistent hyperinsulinism; *HFI*, Hereditary fructose intolerance; *GAL*, Galactosemias; *GSD*, Glycogen storage disease; *FBP*, Fructose biphosphatase; *FAO*, Fatty acid oxidation; *MCAD*, Medium chain acyl CoA dehydrogenase; *SCAD*, Short chain acyl CoA dehydrogenase

hyperinsulism/hyperammonaemia syndrome linked to activating mutations in the glutamate dehydrogenase gene (▶ Chapter 9), or a **fatty oxidation defect** with cardiac involvement (▶ Chapter 12). Transient hyperammonemia with hypoglycaemia may also be observed in premature babies with respiratory distress syndrome. Plasma lysine is elevated in most patients with hyperammonemia. This is due to impaired lysine metabolism secondary to depletion of 2-ketoglutarate [32]. A low plasma lysine level with low ornithine and arginine contrasting with a high urinary excretion of these dibasic aminoacids is diagnostic for lysinuric protein intolerance (▶ Chapter 25). Mild elevations of NH_3 (<150 µmol/l) may be also a concomitant and accessory finding in MSUD, PDH deficiency and in patients treated by sodium valproate. A paradoxical and moderate elevation of NH_3 only with fasting may be observed in some forms of pyrroline-5-carboxylate synthetase deficiency (▶ Chapter 21).

Hyperuricaemias and hypouricaemias

Normal uricaemia is about 50 mg/l in men, 40 mg/l in women and 30-40 mg/l in children. In children a plasma uric acid level >60 mg/l must be always considered abnormal. Hyperuricaemia can result from excessive input, decreased output or both, with regard to the uric acid (UA) pool. Input derives from cellular catabolism of the nucleic acids, purine synthesis and degradation of purines in food. Output results from bacterial intestinal destruction and renal elimination. UA filtered by glomeruli is reabsorbed in the proximal tubule; urinary UA comes from distal secretion which is competitive with OA (lactic, MMA, PA ...). Several tubular UA transporters have been already described, *SLC22A12* (type I), *SLC2A9* (type II) and GLUT9 acting probably in a multimolecular complex »transportosome» allowing cooperation between multiple transporters [33][34].

Secondary hyperuricaemias with low to very low UA excretion are observed in transient neonatal hyperuricaemia and in renal failure from all causes, and can be caused by a variety of other disorders: hyperlactatemias, **GSD1**, **OAs such as MMA** (in which gout crisis and hyperuricaemic nephropathy can be a presenting sign), muscular GSD and **FAO defects** in acute crisis and during treatment with dichloroacetate, and after a fructose load. Hyperuricaemia is a prominent finding in the recently described HUPRA syndrome [35].

Primary hyperuricaemias with high UA excretion are seen in primary **classic gout** and in the rare disorders PRPP synthetase superactivity and Lesch-Nyhan syndrome (HGPRT deficiency).

Primary hypouricaemias can result from decreased UA production, as observed in xanthine oxidase and molybdenum co-factor deficiency (with almost no UA in urine) and purine nucleoside phosphorylase, PRPP-synthetase and guanine desaminase deficiency (with low UA excretion), but is more commonly due to decreased renal tubular UA reabsorbtion. Cytosolic 5' nucleotidase superactivity results in marked hypouricosuria (▶ Chapter 35). Renal hypouricaemia due to renal UA transporter defects is characterised by blood uric

acid <20 mg/l with high UA excretion. It is usually asymptomatic but may present with acute renal injury and nephrolithiasis (▶ Section 1.6.10) and predispose to Parkinson disease.

Glycosuria

Glucosuria should be separated from other meliturias (galactose, fructose).

Permanent glucosuria is found in the asymptomatic renal glucosuria (*SGLT2* and *MAP 17* mutations) (▶ Chapter 10), untreated (decompensated) diabetes mellitus with hyperglycemia, Fanconi Bickel syndrome (GLUT II deficiency) where it is associated with hypoglycaemia, and more generally in all IEM causing a Fanconi tubulopathy where glycosuria is more marked in postprandial period (◘ Table 1.35).

Intermittent glucosuria is found in glycogen synthetase deficiency (with ketotic hypoglycaemia), in glucose galactose malabsorption (with severe diarrhoea), in GLUTII deficiency (mostly in postprandial state) and in some acute attacks of OA (with ketoacidosis) where it can be mistaken for insulin dependent diabetes.

1.5 Chronic and Progressive Neurological Symptoms (Mental Retardation, Developmental Delay, Epilepsy, Neurological Deterioration and Psychiatric Symptoms)

Many acute presentations of inherited disorders that are apparently of delayed onset are preceded by insidious premonitory symptoms which may have been ignored or misinterpreted. Chronic nutritional and gastrointestinal symptoms (such as failure to thrive, anorexia, chronic vomiting, see ▶ Section 1.6.6), or muscular symptoms (muscle weakness, hypotonia, poor muscle mass, see ▶ Section 1.6.9) can precede or accompany subtle neurological signs.

Neurological symptoms are very frequent in IEM and encompass progressive psychomotor retardation, seizures, and a number of neurological abnormalities, in both the central and peripheral system, sensorineural defects and psychiatric symptoms.

Many IEIM present with an early and non-specific progressive developmental delay, poor feeding, hypotonia, some degree of ataxia, and frequent autistic features. The list has lengthened rapidly as new laboratory techniques have been applied. The relationship between clinical and biochemical abnormalities is not always firmly established. This is the case for example for histidinemia, hyperlysinemia, hyperprolinemia, alpha-amino-adipic aciduria, saccharopinuria, cystathioninuria, Hartnup »disease« and acetyl amino aciduria due to amino acylase I deficiency (▶ Chapter 22).

A similar picture has also emerged with organic acidurias making it important to link clinical symptoms and metabolic disturbances. It becomes more and more difficult to screen patients on clinical grounds when the clinical findings are rather non specific, such as developmental delay, microcephaly, hypotonia or convulsions. Among the new categories of IEIM that can present with uninformative clinical manifestations are, for example, adenylosuccinase deficiency, dihydropyrimidine dehydrogenase deficiency, 4-hydroxybutyric aciduria, D-2-hydroxyglutaric acidurias, late onset NKH, creatine deficiency, some CDG syndromes and peroxisomal defects and a number of other inborn errors. These disorders rarely, if ever, cause true development arrest; rather, they cause progressive subacute developmental delay. Conversely, the important gap between neurological descriptions and their molecular basis tends to disappear with the use of molecular technics (exome sequencing, gene panels) (▶ Chapter 3). Many well-known heritable neurological or polymalformative syndromes have been identified by using these technics. This is illustrated by the recent individualisation of the defects in phospholipids, sphingolipids and long chain fatty acids biosynthesis and remodelling, a novel and rapidly expanding group of IEM with neurological/muscular presentations (▶ Chapters 35, 38, 40) [3].

1.5.1 Diagnostic Approach to Neurological and Mental Deterioration Related to Age

◘ Table 1.13 to ◘ Table 1.29 present a general approach to IEM involving neurological and/or mental deterioration. Diseases are classified according to their age at onset, the presence or absence of associated extraneurological signs, and the neurological presentation itself. IEM with neurological signs presenting in the neonate (birth to 1 month; ◘ Table 1.13, ◘ Table 1.14) and those presenting intermittently as acute attacks of coma, lethargy, ataxia, or acute psychiatric symptoms (◘ Table 1.6, ◘ Table 1.7, ◘ Table 1.8), were discussed earlier. Brain development is a dynamic, complex and changing process that determines which category of neurological signs are more likely to appear at a particularage range. According to this idea, in early infancy, IEM tend to produce a global neurological involvement, affecting mostly all functions of the brain (motor, cognitive, behavioural), therefore, the suspicion of an IEM at this period of time should be very high regardless the precise description of the neurological symptoms. By contrast, as children grow up, the brain is more capable to express single or predominant symptoms, meaning that it is easier to organize the differential diagnosis based on well-defined neurological manifestations. Finally, psychiatric manifestations are more common at late stages of neurodevelopment, being especially relevant in adolescents and adults. This approach is especially important not only to make a correct diagnosis but also to avoid missing **treatable disorders** (▶ Section 1.5.3, ◘ Table 1.29).

Early Infancy

Three general categories can be identified:

Category 1: Neurological diseases associated with Extraneurological Symptoms (◘ Table 1.13). Visceral signs appear in lysosomal disorders. A cardiomyopathy (associated with early neurological dysfunction, failure to thrive, and

Table 1.13 Neurological disease with extraneurological symptoms (1–12 months)

Leading symptoms	Other signs	Diagnosis (disorder/enzyme deficiency)
Visceral signs	Hepatosplenomegaly Storage signs, coarse facies	Lysosomal disorders in general GM1, I-Cell disease Sialidosis type II, Niemann-Pick A Lactosyl ceramidosis Sandhoff, Salla disease
	Hepatosplenomegaly Opisthotonos, spasticity	Gaucher type II
	Hepatomegaly Retinitis pigmentosa	Peroxisomal defects CDG Diverse complex lipid synthesis defects
Hair and cutaneous symptoms	Steely brittle hair. Neurotrichosis (hair-shaft abnormalities: trichorrexis nodosa, monilethrix, pili torti)	Menkes (X-linked): trichothiodystrophy **Argininosuccinic aciduria** (trichorrexis nodosa) CDG, mitochondrial disorders
	Alopecia, cutaneous rashes	**Biotinidase deficiency** Respiratory chain defects
	Cutis laxa	CDG, diverse complex lipid synthesis defects
	Ichthyosis and spastic paraplegia	Sjögren-Larsson syndrome Fatty acid elongase 4 (*ELOVL4)*
	Ichthyosis and optic atrophy	**Serine deficiency syndrome** Multiple sulfatase deficiency Gaucher type II Diverse complex lipid synthesis defects MPDU1-CDG (If), DK1-CDG (Im), SRD5A3-CDG (1q)
	Peculiar fat pads on buttocks	CDG
	Cyanosis, hypertonicity	Cytochrome b-5 reductase
	Kernicterus, athetosis	Crigler-Najjar disease
	Acrocyanosis, petechiae	EPEMA syndrome (*ETHE1)*
Hematological disorders	Megaloblastic anemia with failure to thrive, RP	**Folate and cobalamin defects** **UMP synthetase**
	Pancytopenia	Gaucher disease type III Niemann-Pick disease type A
	Neutropenia	Barth syndrome, Aspartylglucosaminuria
	Thrombocytopenia	Niemann-Pick disease type B **Cobalamin defects** **Holocarboxylase synthetase deficiency**
Cardiac symptoms	Cardiomyopathy Heart failure, heart rythm disorders Cardiomyopathy, cataract	D2-hydroxyglutaric acidemia Respiratory chain defects, CDG Sengers syndrome (*AGK* mutations)
Ocular symptoms	Cherry red spot, hydrops fetalis	GM1-gangliosidosis, Galactosialidosis, Sialidosis type I
	Myoclonic jerks, macrocephaly	Tay-Sachs, Sandhoff disease
	Optic Atrophy, macrocephaly Optic atrophy, Coloboma	Canavan disease MPDU1-CDG (If),Iq
	Nystagmus, dystonia, stridor	Pelizaeus-Merzbacher (X-linked)

Table 1.13 (continued)

Leading symptoms	Other signs	Diagnosis (disorder/enzyme deficiency)
Ocular symptoms	Retinitis pigmentosa	See ▶ Section 1.6.11
	Abnormal ocular movements (oculogyric crises, dystonic ocular movements)	**Neurotransmitter defects**
	Strabismus	PMM2-CDG (1a)
	Supranuclear paralysis	Gaucher, Niemann-Pick disease type C

CDG, congenital disorder of glycosylation; *RP*, retinitis pigmentosa; *UMP*, uridine mono phosphate.
Bold face, treatable disorders

hypotonia), sometimes responsible for cardiac failure, is suggestive of respiratory-chain disorders, D-2-hydroxyglutaric aciduria (with atrioventricular block), or CDG (see ▶ Section 1.6.1). Abnormal hair and cutaneous signs appear in Menkes disease, Sjögren-Larsson syndrome, elongase 4 deficiency, **biotinidase deficiency**, and respiratory-chain disorders. Congenital ichthyosis is frequently observed in many complex lipids synthesis/remodeling disorders (see Table 1.31). Peculiar fat pads of the buttocks and thick and sticky skin (like tallow, peau d'orange), and inverted nipples are highly suggestive of CDG. A generalized cyanosis, unresponsive to oxygen, suggests methaemoglobinemia, which is associated with severe hypertonicity in cytochrome-b5-reductase deficiency. Kernicterus and athetosis are complications of Criggler-Najjar syndrome. The EPEMA syndrome due to *ETHE*1 mutations (▶ Chapter 20) is characterized by an orthostatic acrocyanosis, relapsing petechiae, pyramidal signs, mental retardation, and recurrent attacks of lactic acidosis (▶ Section 1.6.2). The presence of megaloblastic anemia suggests an IEM of **folate and cobalamin** (Cbl) metabolism (▶ Section 1.6.6). Ocular abnormalities can be extremely helpful diagnostic signs, for example cherry-red spot, optic atrophy, nystagmus, abnormal eye movements, and retinitis pigmentosa (▶ Section 1.5.2).

Category 2: disorders with specific or suggestive neurological signs (Table 1.14). Predominant extrapyramidal symptoms are associated with IEM of **biopterin** and **neurotransmitters, pyridox(am)ine phosphate oxidase deficiency**, Lesch-Nyhan syndrome, cytochrome-b5-reductase deficiency, Criggler-Najjar syndrome, the early-onset form of GA type I, **cerebral creatine deficiency (GAMT)** and different causes of Leigh syndrome. Dystonia can also be observed as a subtle but presenting sign in X-linked Pelizaeus-Merzbacher syndrome. It can be also associated with psychomotor retardation, spastic paraplegia and ataxia in the **cerebral folate deficiency syndrome** which is more likely to produce a cerebral palsy-like clinical picture. Other than folate receptor auto-antibodies, which is not a constant finding, very low folate concentration in the CSF can be due to *FOLR1* mutations, severe MTHFR deficiency, DHFR and Kearns-Sayre syndrome (▶ Chapter 27) [36].

Macrocephaly with a startle response to sound, incessant crying, and irritability are frequent early signs in GM-2 gangliosidosis, Canavan disease (▶ Chapter 22), Alexander leukodystrophy, infantile Krabbe disease, all disorders with a severe early neurological regression. Macrocephaly can be also an initial sign in glutaric aciduria type 1, L-2-hydroxyglutaric aciduria and in respiratory-chain disorders due to complex-I deficiency (association with hypertrophic cardiomyopathy) (▶ Section 1.5.2).

Recurrent attacks of seizures unresponsive to anticonvulsant drugs occurring in the first year of life is the usual presenting manifestation of the **blood brain-barrier glucose-transporter (GLUT-1)** defect (▶ Chapter 10). Recurrent attacks of neurological crisis associated with progressive neurological and mental deterioration suggest Leigh syndrome, which can present at any age from early in infancy to late childhood. Leigh syndrome is not a specific disorder but, rather, the clinical phenotype of any of several IEM involving energy metabolism (Table 1.22) (▶ Chapter 14). Recurrent stroke-like episodes often associated with anorexia, failure to thrive, and hypotonia can be presenting symptoms in **urea-cycle defects** (mostly **OTC deficiency**), late-onset **MSUD**, **OA**, **GA type I**, CDG, and respiratory-chain disorders. Thromboembolic events can be the presenting sign of **classical homocystinuria** and CDG (see above ▶ Section 1.4.1). Angelman syndrome sometimes displays a very suggestive picture, with early-onset encephalopathy, happy-puppet appearance, and epilepsy with a highly suggestive EEG pattern.

Category 3: disorders with non-specific developmental delay (Table 1.14). A large number of IEM present with non-specific early progressive developmental delay, poor feeding, hypotonia, some degree of ataxia, frequent autistic features, and seizures. Many IEM can masquerade as a cerebral palsy by presenting as a permanent impairment of movement or posture. Consequently, it is mandatory to systematically screen such children for those IEM which are **at least partly treatable**. In this context, late-onset **subacute forms of hyperammonemia** (usually **OTC** deficiency in girls) can present with an apparently non-specific early encephalopathy (▶ Chapter 19) and **IEM of neurotransmitter synthesis**, especially **dopa-**

Table 1.14 Prominent Neurological Involvement (1 to 12 months)

Leading symptoms/signs	Other signs	Diagnosis (disorder/enzyme deficiency)
With suggestive neurological signs		
Extrapyramidal signs	Major parkinsonism Dystonia-Parkinsonism Abnormal neurotransmitters	**Inborn errors of biopterin metabolism and neurotransmitters** (dopamine, serotonin) AADC, TH, DTD, PNPO Mitochondrial disorders
	Dystonia, generalized (dystonic tetraparesis) or starting abruptly (some times unilateral), plus:	
	- Choreoathetosis, self-mutilation	Lesch-Nyhan disease (X-linked)
	- Acute-onset, pseudoencephalitis	Glutaric aciduria type I
	- Bilateral athetosis, hypertonicity	Cytochrome b5 reductase deficiency
	- Dystonia, stridor	Pelizaeus Merzbacher disease (X-linked)
	- Kernicterus syndrome	Criggler-Najjar syndrome
	- Low cerebral creatine	Creatine deficiency (GAMT)
	- Leigh syndrome	**PDH,** different mitochondrial genes, **thiamine transporter defect, biotinidase deficiency** Megdel syndrome
	Prominent chorea	**GA1, PA, MMA, homocystinurias,** GAMT, infantile NCL, FOLR mutations, NKH, sulfite oxidase/MOCO deficiency *PI4K2A* mutations (plus cutis laxa) L-2-hydroxyglutaric aciduria
Neurological regression	Progressive pyramidal hypertonia often painful, opisthotonos at advanced stage	Krabbe, Gaucher III, Niemann-Pick disease type C, Menkes diseases, untreated **MSUD** and **OAs**, GM3 synthetase deficiency, **arginase deficiency**
	Macrocephaly, startle response to sounds, cherry red spot, myoclonic jerks	Tay Sachs, Sandhoff, Canavan, Alexander diseases vacuolizing leucoencephalopathy
Epileptic Encephalopathy		Most IEM that produce neonatal seizures can also start during the first year of life. Consider also **GLUT-1 deficiency**, **Biotinidase deficiency,** Menkes disease, GPI-anchor biosynthesis pathway defects and other defects of complex lipid synthesis such as *FAH2* and *ELOVL4* mutations
	With prominent spasticity	NKH, SO, untreated **MSUD** and **OA** **MCD,** Menkes disease, GM3 synthetase deficiency
Ocular symptoms	Optic atrophy, incessant crying	Krabbe disease (infantile)
	Retinopathy	Respiratory chain defects, peroxisomal defects, CDG
Recurrent attacks of neurological crisis	Failure to thrive, hyperventilation attacks	Leigh syndrome (PC, PDH, respiratory chain defects), MAMEL syndrome, MEGDEL syndrome, **Thiamine transporter defect: (TBBGD)**
	Stroke-like episodes	**Urea cycle defects, MSUD, OA,** GA I CDG, Respiratory chain defects
	Thromboembolic accidents	**Homocystinurias,** CDG

Table 1.14 (continued)

Leading symptoms/signs	Other signs	Diagnosis (disorder/enzyme deficiency)
Without suggestive neurological signs		
Evidence of developmental arrest	May associate infantile spasms, hypsarrhythmia and autistic features	Untreated **PKU, biopterin defects,** Peroxisomal defects, Rett syndrome
Non specific symptoms, apparently non-progressive disorder	Frequent autistic feature Poor feeding, failure to thrive	**Hyperammonemia** (late-onset subacute) 4-OH-butyric, L2-OH-, D2-OH-glutaric acidurias
	Hypotonia, seizures	Mevalonic aciduria
With diverse neurological findings simulating cerebral palsy		
		Adenylosuccinase, pyrimidine defects 3-methylglutaconic type 1, Fumarase deficiency, other OA, Creatine deficiency **3-PGD**, 3-phosphoserine phosphatase **Homocystinurias**, Salla disease **Neurotransmitters defects, Cerebral folate deficiency due to diverse conditions (DHFR, FOLR1, autoantibodies against folate receptor)** Angelman syndrome, **GLUT-1**

3-PGD, 3-phosphoglycerate dehydrogenase; *ADC*, aromatic aminoacid decarboxylase; *CDG*, congenital disorders of glycosylation; *DHFR*, dihydrofolate reductase; *DTD*, dopamine transporter defect; *FOLR*, folate receptor; *GA*, glutaric aciduria; *GAMT*, guanidino acetate methyltransferase; *MAMEL*, methylmalonic aciduria, mitochondrial encephalopathy Leigh-like; *MMA*, methylmalonic; *MOCO*, molybdenum cofactor; *NCL*, neuronal ceroid lipofuscinosis; *PA*, propionic aciduria; *MSUD*, maple syrup urine disease; *OA*, organic acidurias; *PC*, pyruvate carboxylase; *PDH*, pyruvate dehydrogenase; *PGD*, phosphoglycerate deshydrogenase; *PNPO*, pyridox(am)ine phosphate oxidase; *SO*, sulfite oxidase; *TH*, tyrosine hydroxylase; **bold face**, treatable disorders

responsive dystonia due to **cyclohydrolase deficiency, tyrosine-hydroxylase deficiency and sepiapterin reductase deficiency**, as well as other less L-dopa responsive diseases (aromatic-l-amino-acid-decarboxylase deficiency and dopamine transporter defect), can masquerade as cerebral palsy. CSF studies should then be considered in patients with a cerebral palsy-like disorders and any other kind of undetermined neurological disturbances (▶ Chapter 29).

Late Infancy to Early Childhood (1–5 years)

In this period, diagnosis becomes easier. Seven general categories can be defined according to the accompanying signs and leading symptom: spastic or flaccid paraplegia, unsteady gait including ataxia and dyspraxia or myoclonia, epilepsy, arrest of development or regression, dystonia/abnormal movements, and behavioural disturbances.

Category 1: with visceral, craniovertebral, ocular, or other somatic abnormalities (Table 1.15) These symptoms associated with a slowing or regression of development, suggest MPS types I and II, mucolipidosis type III, oligosaccharidosis, multiple sulfatase deficiency, Niemann-Pick disease type C, Gaucher disease type III, and lactosyl ceramidosis, all disorders which are usually easy to recognize. Mucolipidosis type IV, which causes major visual impairment by the end of the first year of life, sometimes associated with dystonia, presents with characteristic cytoplasmic membranous bodies in cells. In Sanfilippo syndrome, coarse facies and bone changes may be very subtle or absent with only abundant and hirsute hair. Peroxisomal disorders may present at this age, with progressive mental deterioration, retinitis pigmentosa, and deafness, and in a very similar manner to Usher syndrome type II. Pyrroline-5-carboxylate-synthase deficiency presents with slowly progressive neurological and mental deterioration, severe hypotonia, joint laxity, and congenital cataracts (▶ Chapter 21). Sjogren Larsson syndrome and ELOVL4 deficiency present with ichthyosis and spastic paraplegia (▶ Chapter 40).

Category 2: with progressive paraplegia and spasticity (Table 1.15) Progressive paraplegia and spasticity are presenting signs in many IEM. Metachromatic leukodystrophy and infantile neuroaxonal dystrophy INAD (*PLA2G6* mutations) present between 12 and 24 months of age with flaccid paraparesis, hypotonia, and weakness (▶ Chapter 34). CSF protein content and nerve conduction velocity are disturbed in the former but normal in the latter (however axonal neuropathy can be present). Schindler disease is roughly similar to neuroaxonal dystrophy, though it is often associated with myoclonic jerks (▶ Chapter 38). A growing number of so called

Table 1.15 Prominent neurological involvement (1 to 5 years)

Symptoms	Diagnosis (disorder/enzyme deficiency)
With visceral, craniovertebral, or other somatic abnormalities	
- Coarse facies, skeletal changes, hirsutism, corneal opacities	MPS I, MPS II, MPS III, MLP III
- Coarse facies, subtle bone changes, lens/corneal opacities, hepatosplenomegaly, vacuolated lymphocytes	Mannosidosis (gingival hyperplasia) Fucosidosis (angiokeratoma) Aspartylglucosaminuria (joint laxity) Multiple sulfatase deficiency (ichthyosis)
- Hepatosplenomegaly, progressive dementia, myoclonic jerks	Niemann-Pick type C and related disorders (vertical supranuclear ophtalmoplegia)
- Splenomegaly + hepatomegaly, osseous lesions, (ataxia, myoclonus)	Gaucher disease type III (supranuclear ophtalmoplegia)
- Major visual impairment, blindness	Mucolipidosis type IV (corneal clouding)
- Retinitis pigmentosa, deafness	Peroxisomal defects, Usher syndrome type II
- Cataract, joint laxity, hypotonia	Pyrroline-5-carboxylase synthetase
With paraplegia (hypertonic or flaccid) due to spasticity, peripheral neuropathy or motor neuron involvement	
- Flaccid paraparesis (as initial sign)	
- With hyperproteinorrachia, progressive pyramidal signs	Metachromatic leukodystrophy (abnormal NCV)
- Normal proteinorrachia, optic atrophy, myoclonic jerks	Neuro-axonal dystrophy Schindler disease (normal NCV)
- »INAD« phenotype: hypotonia, flaccid paraparesis followed by spasticity, psychomotor deterioration, ataxia, axonal neuropathy	NBIA due to *PLA2G6* mutations
- Sensory and autonomic neuropathy, anhydrosis, cataract, psychomotor retardation	*SPTLC1, 2* mutations (complex lipid synthesis defects)
- With retinopathy, rhabdomyolysis, polyneuropathy	**LCHAD, TFP deficiencies**
- Progressive spasticity	
- With demyelinating neuropathy	**Metachromatic leukodystrophy** (initial hypotonia and hyporreflexia followed by pyramidal signs), Krabbe disease, peroxisomal disorders, consider different genetic leukodystrophies (Cockayne syndrome, HCC, HHHH syndromes)
- Spastic paraplegia with thin corpus callosum, white-matter abnormalities, and/or calcification of the basal ganglia	Complex lipid defects: SPG 28 (*DDHD1*), SPG 49 (*CYP2U1*) SPG 54 (*DDHD2*), SPG 35 (*FA2H*) SPG 46 (*GPA2*), SPG 26 (*B4GALNT1*). Consider different genetic leukodystrophies and genes involved in brain calcifications (Table 1.23)
- Spastic diplegia (scissoring or tiptoe gait) without relevant MRI findings	**Arginase deficiency** (high arginine, high orotic) **Cbl C deficiency** (subacute cord degeneration) **HHH** syndrome (recurrent attacks of hyperNH3) Costeff syndrome (*OPA3* mutation with 3-methylglutaconic aciduria) Untreated **dopamine synthesis defects** Untreated **biotinidase deficiency**
- With multysistem involvement, neurosensorial defects, cerebellar involvement, sensory motor neuropathy	Consider mitochondrial, peroxisomal and complex lipid defects (i.e. PEX genes, arylsulfatase 1 deficiency)
- With ichthyosis	Sjoegren-Larsson syndrome (*ALDH3A2*), *ELOVL4* mutations

Table 1.15 (continued)

Symptoms	Diagnosis (disorder/enzyme deficiency)
- Motor neurone disease: Hereditary spastic paraplegia with atrophy of distal leg and intrinsic hand muscles mimicking organophophorus intoxication	Neuropathy target esterase deficiency (*PNPLA6)*
- Mimicking spinal muscle atrophy	Peroxisomal and mitochondrial defects. A new phenotype of the classic Farber disease, associated with progressive myoclonic epilepsy (*ASAH1*)

HCC, hypomyelination with congenital cataracts; *HHHH*, hypomyelination, hypodontia, hypogonadotropic hypogonadism; *MLP*, mucolipidosis; *MPS*, mucopolysaccharidosis; *NBIA*, neuro-degeneration with brain iron accumulation; *NCV*, nerve conduction velocity; *LCHAD*, long-chain 3-hydroxyacyl-CoA dehydrogenase; *PEX*, peroxisome; *TFP*, trifunctional protein

hereditary spastic paraplegia (SPG) linked to mutations of genes implicated in phospholipids synthesis and remodeling very recently have been elucidated (▶ Chapter 34). **Arginase deficiency** is a rare disorder that presents early in infancy to childhood (2 months to 5 years) with progressive spastic diplegia, scissoring or tiptoe gait, and developmental arrest (▶ Chapter 19). A rapidly progressive flaccid paraparesis resembling subacute degeneration of the cord can be the presenting sign of inherited **Cbl-synthesis defects** (▶ Chapter 27). Spastic paraparesis is an almost constant finding in the **HHH syndrome** (▶ Chapter 21) and can be the leading symptom in **dopaminergic synthesis defects** (▶ Chapter 29) and **biotinidase deficiency**.

Category 3: with unsteady gait and uncoordinated movements (when standing, walking, sitting, reaching for objects, speaking, and swallowing) due to cerebellar ataxia, dyspraxia or myoclonia (Table 1.16) Several groups of disorders must be considered. A careful investigation of organic acid and amino acid metabolism is always mandatory, especially during episodes of metabolic stress.

- **Disorders without disturbances of classic IEM** markers (first line plasma and urine tests: organic acids, amino acids, lactic acid metabolism). Most cause progressive ataxias and can produce global neurological deterioration, such as the late-onset forms of GM-1 and GM-2 gangliosidosis, late infantile Krabbe disease, ataxia telangiectasia, and Niemann-Pick disease type C (▶ Chapter 38). Peroxisomal disorders and CDG present with signs that are sufficiently characteristic to warrant specific investigation. A severe early-onset encephalopathy with seizures and myoclonic jerks associated with hepatic disease is highly suggestive of Alpers syndrome due to mitochondrial disease (*POLG* mutations are the most common) (▶ Chapter 14). Diverse complex lipid biosynthesis and remodelling defects can cause an unsteady gait due to ataxia associated with movement disorders (NBIA syndromes) and progressive spasticity (▶ Section 1.5.2). Hypogonadotropic hypogonadism is a key sign of the neuropathy target enterase (NTE) spectrum that includes Boucher-Neuhäuser syndrome (with chorioretinal dystrophy) and Gordon Holmes syndrome (with peripheral neuropathy) (▶ Chapter 34).
- **Disorders with disturbances of OA and AA and/or other metabolic biomarkers** are numerous. **PDH** deficiency presents frequently with peripheral neuropathy, intermittent ataxia, dystonia and slight or moderate hyperlactatemia). Several mitochondrial disorders initially cause ataxia, intention tremor, dysarthria, epilepsy, myopathy, and (eventually) multiorgan failure. **Creatine deficiency** due to **guanidinoacetate-methyltransferase deficiency** can present in infancy, with an extrapyramidal disorder associated with epilepsy, neurological regression, and failure to thrive (▶ Chapter 15). **GLUT1 deficiency** can present with recurrent attacks of dystonia induced by exercise (▶ Chapter 10). **LCHAD deficiency**, L-2-hydroxyglutaric aciduria, 3-methylglutaconic acidurias, **MMA**, and **PA** significantly disturb organic acid excretion, although sometimes only slightly and intermittently (▶ Chapter 18). GA type I can also present with a permanent unsteady gait due to choreoathetosis and with dystonia developing abruptly after an acute episode resembling encephalitis and irreversible (▶ Chapter 22). Retinopathy and peripheral neuropathy are important signs to detect **vitamin E responsive ataxias.**

Category 4: with predominant epilepsy (Table 1.17) Predominant epilepsy and myoclonus with progressive deterioration result in ataxia and frequent falling and include two ceroid lipofuscinoses: Santavuori-Hagberg disease (CLN1) and Jansky-Bielchowski disease (CLN2), the latter being similar to Lennox-Gastaut syndrome (akinetic myoclonic petit mal). CLN14 (mutations in *KCTD7*) can also produce infantile refractory epilepsy. Late-onset forms of Niemann-Pick disease type C and Gaucher disease are easily suspected because of hepatosplenomegaly and supranuclear paralysis (▶ Chapter 38). Progressive myoclonic epilepsy is also a relevant feature of myoclonic-epilepsy ragged red fibre (MERRF) syndrome (▶ Chapter 14) and Schindler disease, which is similar to neuroaxonal dystrophy, sialidosis (macular cherry-red spot), and acid ceramidase deficiency due to *ASAH1* mutations (with spinal muscle atrophy) [37]. Some of the most representative

Table 1.16 Prominent neurological involvement (1 to 5 years) (follow)

With unsteady gait due to ataxia (cerebellar syndrome), dyspraxia or myoclonia	
Without disturbances of classic IEM markers (OA, AA, first line plasma and urine tests). Most are progressive ataxias.	
- Ataxia, choreoathetosis, oculocephalic asynergia	Ataxia telangiectasia
- With global deterioration: mental/speech/behaviour (psychosis)/neurosensorial	GM1 gangliosidosis (spastic quadriparesis, pseudobulbar signs) GM2 gangliosidosis (Tay-Sachs, Sandhoff) (late infantile form; psychotic behaviour, spinocerebellar degeneration) ; Krabbe disease (late infantile, peripheral neuropathy, vision loss) Niemann-Pick C (supranuclear gaze palsy may be not present at the first stages). Plasma oxysterols are normally elevated.
- Ataxia, muscular atrophy, peripheral neuropathy	Peroxisomal defects (may have normal plasma markers), CDG
- Seizures, myoclonic jerks, postictal coma, transient hemiplegia. Lactate in plasma and/or CSF may be high.	Alpers (hepatic signs, hyperlactatemia) and other mitochondrial diseases (in particular: MELAS, NARP, MERRF, MILS, KSS. **CoQ10 defects** (see ▶ Chapter 14)
- Spasticity, movement disorders and iron deposit in the brain MRI (not constant)	NBIA syndromes (▶ Section 1.5.2)
- With progressive spasticity as the most prominent feature	Consider HSP and genes involved in lipid synthesis and remodelling defects
- With hypogonadotropic hypogonadism	Boucher-Neuhäuser syndrome (chorioretinal dystrophy), Gordon Holmes syndrome (peripheral neuropathy, pons and pituitary may be atrophic). Both syndromes are due to neuropathy target esterase (NTE); (*PNPLA6* mutations)
With disturbances of OA, AA excretion or other metabolic biomarkers (sometines moderate or intermittent but may be progressive without treatment)	
- Progressive ataxia, intention tremor, cerebellar atrophy	L-2-OH-glutaric (spongiform encephalopathy) Combined degeneration of the spinal cord **Cobalamin defects** (CblC, CblE, CblF, CblG)
- Ataxia, peripheral neuropathy, dystonia	**PDH** (moderate hyperlactactemia), **biotinidase deficiency** (plasma biotinidase activity), NARP
- Ataxia, weakness, RP, myoclonic epilepsy	Respiratory chain, MERFF, methylglutaconic aciduria type 1,3
- Extrapyramidal signs	**Creatine deficiency (GAMT), GLUT1**
- Ataxia, peripheral neuropathy, RP	**LCHAD** (organic acids, acylcarnitine), CDG
- Acute attacks encephalitis-like, temporal lobe atrophy	**GA I** (dystonia, macrocephaly)
- Dystonia, athetosis, acute attacks	**MMA, PA, homocystinurias**
- Ataxia, dysarthria, optic atrophy, nystagmus	Ribose-5-phosphate isomerase (polyols)
- Retinopathy, neuropathy	**Vitamin E-responsive ataxias** (plasma vitamin E and lipoproteins), peroxisomal disorders, CDG, NARP
- Intellectual disability, behavioural problems, epilepsy	4-hydroxybutyric aciduria (SSDAH deficiency), **GLUT1** (hypoglucorrachia)
- Progressive deterioration, ophthalmoplegia	Niemann-Pick disease type C (oxysterols)
- Ataxia with oculomotor apraxia	Ataxia with oculomotor apaxia type 1 (*AOA1*) Low plasma albumin

BBGD, biotin-responsive basal ganglia disease; *CoA*, coenzyme A; *CSF*, cerebrospinal fluid; *CDG*, congenital disorder of glycosylation; *EEG*, electroencephalogram; *GAMT*, guanidino acetate methyltransferase; *HSP*, hereditary spastic paraplegia;*Ig*, immunoglobulin; *LCHAD*, long-chain 3-hydroxyacyl-CoA dehydrogenase; *INCL*, infantile-ceroid lipofuscinosis (*CLN1* mutations); *LINCL*, late infantile ceroid lipofuscinosis (*CLN2* mutations); *MMA*, *PA*, *GA*, methylmalonic, propionic, glutaric acidurias; *MERRF*, myoclonic epilepsy ragged red fibers; *MLP*, mucolipidosis; *MPS*, mucopolysaccharidosis; *NBIA*, neurodegeneration with brain iron accumulation; *PDH*, pyruvate dehydrogenase; *SPT*, serine palmitoyltransferase encoded by *SPTLC1*, *2*. **Bold face**, treatable disorders

IEM leading to refractory seizures as a major symptom without clear neurological deterioration are **creatine defects** (▶ Chapter 15), **GLUT1** deficiency (▶ Chapter 10), late onset **pyridoxine dependent** seizures (▶ Chapter 28) and the emergent group of GPI-anchor biosynthesis defects (▶ Chapter 41).

Category 5: isolated developmental arrest or regression (Table 1.17) Only a few disorders present between 1 and 5 years of age with an isolated developmental arrest or regression of cognitive and perceptual abilities without other significant neurological or extraneurological signs. Sanfilippo disease is one, although regression of high-level achievements, loss of speech, and agitation usually begin later than 5 years of age (▶ Chapter 38). Although non-metabolic, Rett syndrome is another such disease; it should be considered when a girl, without a family history, presents between 1 and 2 years of age with autistic behavior, developmental regression, typical stereotyped hand movements, and microcephaly.

Category 6: with dystonia/abnormal movements as the most prominent symptom (extrapyramidal syndrome) (Table 1.17) Extrapyramidal symptoms observed in IEM include dystonia, parkinsonism, chorea, and tremor, however dystonia is predominant in this age category (from 1-5 years of life) and until late infancy. Although usually associated with other neurological symptoms, some IEM can initially present as an isolated dystonia – e.g. **neurotransmitter defects** (in particular **Segawa disease**) (▶ Chapter 29), PKAN, Leigh syndrome, Lesch-Nyhan disease (▶ Chapter 35), **PDH deficiency** (▶ Chapter 11) and homocystinurias (▶ Chapter 19). However, in general, in intermediary and energy metabolism defects, dystonia tends to be abrupt, settles rapidly and is generalized and postural from the very first stages of the disease. Some illustrative examples are **GA1, PDH** deficiency, **thiamine transporter 2 deficiency due to *SLC19A3* mutations (biotin-thiamine basal ganglia responsive disorder: BTBGD)** and **homocystinurias**. **GLUT1** deficiency can cause paroxysmal exercise-induced dyskinesia and other paroxysmal complex movement disorders (▶ Chapter 10). Adenylate cyclase deficiency (*ADCY5*) can produce myokimia and paroxysmal chorea [38] (▶ Chapter 35). Most IEM with extrapyramidal symptoms exhibit abnormal brain MRI patterns, however brain image is usually normal in neurotransmitter defects, **GLUT1** deficiency and genetic primary dystonias.

Category 7: with intellectual disability and prominent behavioural disturbances (Table 1.17) Marked hyperactivity and agitation is very common in Sanfilippo disease (even before regression) (▶ Chapter 38), whereas autistic behaviour may be striking in creatine transporter defect, SSADH, untreated PKU, mild forms of **SLO (Smith-Lemli-Opitz)** (▶ Chapter 32) and a rare disease recently described due to inactivating mutations **in *BCKDK* (Branched Chain Ketoacid Dehydrogenase Kinase**) that is associated with low plasma branched chain amino acids.This disorder should be potentially treatable by BCAA supplementation (▶ Chapter 18).

Late Childhood to Adolescence (5-15 years)

It is important to recognize conditions in which cognitive function is primarily affected and those disorders with more extensive neurological involvement with normal or subnormal intellectual functioning. There are six clinical categories (Table 1.18).

Category 1: with predominant extrapyramidal signs (parkinsonian syndrome, dystonia, choreoathetosis) As already mentioned, dystonia is a major feature in many IEM. In fact, almost all neurometabolic disorders can cause dystonia at some stage, which is frequently a combined dystonia (with associated symptoms). At this period of life, IEM that produce dystonia as a major sign include those disorders that have been also included from 1 to 5 years of age (Table 1.18), however **Wilson disease** (▶ Chapter 37), **Segawa disease (dominant GTPCH)** (▶ Chapter 29) **GLUT1 deficiency** (▶ Chapter 10) and NBIA syndromes (▶ Chapter 34) are amongst the most relevant at this period of life. Pure dystonia is more common in primary DYTs (genetic dystonias), however some IEM can also initially present as pure dystonias such as NBIA syn-

Table 1.17 Prominent neurological involvement (1 to 5 years) (follow)

With seizures as the most prominent symptom	
Seizures and progressive deterioration	
- Rapid mental regression, myoclonic jerks, blindness	INCL (early-flattening EEG, CLN1, CLN14)
- Akinetic myoclonic petit mal, RP, typical EEG pattern	LINCL (misdiagnosed with Lennox-Gastaut)
- Rapid regression, myoclonic seizures, spasticity	Schindler disease (optic atrophy, severe osteoporosis)
- Myoclonic epilepsy, volitional and intentional myoclonias, muscular weakness	MERFF, Niemann-Pick disease type C, Gaucher disease type III (ophthalmoplegia, hepatosplenomegaly)
- Seizures and myoclonic jerks, uncoordinated movements	Alpers syndrome (hepatic symptoms, hyperlactatemia)
- Macular cherry-red spot and myoclonus	Sialidosis
- With spinal muscle atrophy	*ASAH1* mutations (new phenotype of Farber disease)

Table 1.17 (continued)

Without clear degeneration, often static, other neurological signs associated	
- Autistic signs, movement disorders	**Creatine defects** (GAMT, CTD)
- Ataxia, abnormal movements, worse before meals	**GLUT-1 deficiency** (low glucorrachia)
- Late onset pyridoxine dependent seizures	**Antiquitin defect** (elevated pipecolic acid (CSF, plasma, urine) and alpha-aminoadipic semialdehyde (urine).
- Intellectual disability, dysmorphia (not constant)	GPI-anchor biosynthesis pathway defects
Disorders with arrest or regression	
Autistic behaviour, regression of high-level achievements stereotyped movements of fingers microcephaly	Rett syndrome (girls), sporadic (acquired secondary epilepsy)
Regression of high-level achievements, loss of speech See causes of progressive deterioration associated with spasticity, ataxia and seizures in this table	Sanfilippo disease (hirsutism, agitation)
Disorders with Dystonia/ abnormal movements as the most prominent symptom (extrapyramidal syndrome)	
Dystonia associated with additional neurological signs:	
- Parkinsonism	**Neurotransmitter defects, Wilson disease** (very rare as neurological presentation at early infancy), mitochondrial disorders, *PANK2* and other NBIA genes, **manganese transporter defect,** consider early parkinsonism genes
- Myoclonus	D-2-hydroxyglutaric aciduria, mitochondrial and lysosomal diseases, ***DYT5* mutations**
- Developmental delay +/- spasticity	Lesch-Nyhan (self-mutilation), ADSL deficiency, untreated **homocystinurias,** mitochondrial and lysosomal disorders, **GAMT deficiency, manganese defects**
- Cerebellar ataxia	**GLUT-1, Vitamin E related defects,** SSADH deficiency, aprataxin deficiency, Niemann-Pick disease C, NBIA syndromes, mitochondrial and lysosomal disorders
- Myokimia, paroxysmal chorea	Adenylate cyclase deficiency (*ADCY5*)
Pure dystonia	**Neurotransmitter defects,** DYT genes Can present initially as pure dystonia: PKAN, Leigh syndrome, Lesch-Nyhan disease, **PDH, homocystinurias**
Abrupt dystonia (especially in catabolic states):	Glutaric aciduria, **thiamine transporter defect (TBBGD),** Mitochondrial disorders, **PDH deficiency, biotinidase deficiency, homocystinurias.** Consider *DYT12* (*ATP1A3*).
Normal brain MRI	**Neurotransmitter defects, GLUT1 deficiency,** genes related to primary dystonias
Disorders with behavioural disturbances	
Hyperactivity, agitation (usually before regression)	Sanfilippo disease (hirsutism, agitation)
Autism with comorbid intellectual disability that can be associated with epilepsy	**Branched chain ketoacid dehydrogenase kinase deficiency,** CTD, SSADH deficiency, untreated **PKU**, ADSL deficiency, mild forms of **SLO**

BBGD, biotin-responsive basal ganglia disease; *CoA*, coenzyme A; *CSF*, cerebrospinal fluid; *EEG*, electroencephalogram; *CTD*, creatinine transporter defect; *DYT*, dystonine; *GAMT*, guanidino acetate methyltransferase; *Ig*, immunoglobulin; *INCL*, infantile ceroid lipofuscinosis (CLN1 mutations); *LINCL*, late infantile ceroid lipofuscinosis (CLN2 mutations); *MERRF*, myoclonic epilepsy ragged red fibers; *MLP*, mucolipidosis; *MPS*, mucopolysaccharidosis; *NBIA*, neurodegeneration with brain iron accumulation; *PKAN*, panthothenate kinase associated neurodegeneration; *PDH*, pyruvate dehydrogenase; *SPT*, serine palmitoyltransferase encoded by *SPTLC1,2*; *SLO*, Smith Lemli Opitz syndrome; **Bold face**, treatable disorders

dromes, especially PKAN deficiency (▶ Chapter 37), PDH, Lesch-Nyhan syndrome, and juvenile metachromatic leukodystrophy. Usually, parkinsonism is more common in late infancy, adolescence and adulthood than in younger children. The complex dystonia-parkinsonism is in fact the most common presentation sign. As an example, NBIA cause progressive dystonia and bulbar symptoms in children, whereas parkinsonism usually appears later (late childhood, adolescence and adulthood). Although more common in older age groups, lysosomal disorders can also begin in childhood with Parkinsonism as the leading sign. Special consideration should be given to ceroid lipofuscinosis (CNL), GM1 gangliosidosis and Niemann-Pick disease type C (▶ Chapter 38). **Wilson disease**, **manganese transport defect** (▶ Chapter 37) and **cerebrotendinous xanthomatosis (CTX)** (▶ Chapter 33) tend to exhibit signs of parkinsonism later on in life but given therapeutic possibilities, they should also be considered at this period of life. **Neurotransmitter defects** (▶ Chapter 29) and **BTBGD due to thiamine transporter defect** (▶ Chapter 28) can also appear at late infancy and beyond the paediatric age, and they are treatable disorders, therefore should not be neglected. Mitochondrial disorders, especially *POLG* and different mitochondrial DNA mutations should be also considered since they can present as L-dopa or dopamine agonist responsive forms [39] (▶ Chapter 14). A phosphodiesterase deficiency linked to *PDE10A* mutations have been recently described in patients with childhood-onset chorea and characteristic brain MRI showing symmetrical striatal lesions [40].

Category 2: with severe neurological and mental deterioration and diffuse central nervous system involvement Category-2 patients have in common severe neurological dysfunction with pyramidal signs, incoordination, seizures, visual failure, impaired school performance, and dementia. In association with splenomegaly or hepatomegaly, these signs suggest Niemann-Pick disease type C or Gaucher disease type III. When visceral signs are absent, they may indicate juvenile metachromatic leukodystrophy, X-linked adrenoleukodystrophy (▶ Chapter 40), Krabbe disease, juvenile GM-1 and GM-2 gangliosidoses (▶ Chapter 38), or mitochondrial disorders. Peroxisomal biogenesis defects can also present in the second decade of life with peripheral neuropathy initially mimicking Charcot-Marie-Tooth type II disease, but which then evolves into a pyramidal syndrome, intellectual deterioration, dementia and, shortly thereafter, a neurovegetative state (▶ Chapter 40).

Category 3: with polymyoclonus and epilepsy Diverse types of ceroid lipofuscinosis have been described to produce progressive myoclonic epilepsy (◘ Table 1.18) although the most representative is Spielmeyer-Vogt or Batten disease due to *CLN3* mutations, which presents with loss of sight, retinitis, ataxia, and (at an advanced stage) extrapyramidal signs (▶ Chapter 38). After puberty (but also starting as early as 5 years of age) Lafora disease should also be considered (▶ Chapter 5). Gaucher disease type III, late onset GM-2 gangliosidosis, Niemann-Pick disease type C, sialidosis type I and mitochondrial disorders can also begin with polymyoclonus as an early major sign. Acid ceramidase deficiency due to *ASAH* mutations is a cause of progressive myoclonic epilepsy associated to spinal muscle atrophy [37]. Other genetic diseases (although not considered classic IEM) such as action myoclonus renal failure syndrome (AMRF), PRICKLE-1 related progressive myoclonus epilepsy with ataxia, and DRPLA (dentatorubral-pallidoluysian atrophy) should be also considered in the differential diagnosis of progressive myoclonic epilepsy.

Category 4: with predominant cerebellar ataxia Friedreich ataxia and other hereditary ataxias should be considered and are recognized on clinical and genetic grounds. Abetalipoproteinemia and ataxia telangiectasia are usually suspected because of the associated extraneurological signs. PBD, CDG, and **Refsum disease** (which can all present similarly to a peripheral neuropathy and retinitis pigmentosa) can be demonstrated by the analysis of plasma very-long-chain fatty acids, glycosylated transferrin profile, and plasma phytanic acid, respectively (▶ Chapter 40).Cerebellar ataxia in association with progressive mental deterioration, dementia, and epilepsy suggests Lafora disease (▶ Chapter 5), **CTX** (▶ Chapter 33), late-onset forms of gangliosidosis, Krabbe disease, Gaucher disease, Niemann-Pick disease type C, and metachromatic leukodystrophy (▶ Chapter 38). Mitochondrial disorders also can present with predominant ataxia and some forms of **CoQ10 synthesis defects** can respond to idebenone supplementation (▶ Chapter 14). Other forms of treatable ataxia such as **GLUT1 deficiency, vitamin E-responsive ataxias and Hartnup disease** (▶ Chapter 25) should also be considered. Ataxia and gait impairment may be present in IEM of complex lipid synthesis

◘ **Table 1.18** Prominent neurological involvement (5–15 years)

Symptoms	Diagnosis (disorder/enzyme deficiency)
With predominant extrapyramidal signs, parkinsonism, dystonia, choreoathetosis	
IEM starting with extrapyramidal signs in younger children can also appear at late infancy and adolescence	◘ Table 1.14
IEM that can present as pure dystonia (although other signs may associate later on)	**Segawa disease (GTP cyclo hydrolase)** and other **neurotransmitter defects, GLUT1 deficiency, classic homocystinuria, Wilson,** PDH, juvenile MLD, Lesch-Nyhan, PKAN

Table 1.18 (continued)

Symptoms	Diagnosis (disorder/enzyme deficiency)
Parkinsonism, dystonia-parkinsonism as initial sign - Consider first treatable disorders	
In general normal IQ when presenting at this age	**Neurotransmitter defects**
Dystonia, mood troubles, school failure	**Wilson disease**
Lens dislocation, marfanoid morphology	**Classic homocystinuria**
Reading/writing difficulties, alacrima, dysphagia due to achalasia	**Familial glucocorticoid deficiency** (with hypoglycaemia; *NNT* gene)
Cataracts, ataxia, peripheral neuropathy	**CTX**
Dysarthria, dysphagia, cogwheel ridigy, painful dystonias	**Thiamine transporter defect (TBBGD)**
Ophthalmoplegia, ataxia, visceromegaly	**Niemann-Pick disease type C**
Good response to L-dopa	**Neurotransmitter defects**
With dyskinesia, tremor (pill-rolling rest component), rigidity and severe bradykinesia, psychiatric symptoms	PLA2G6 defects
With markers of mitochondrial disease	POLG mutations
Mild response	CNL, **CTX**
- With cognitive decline, +/-cerebellar involvement, neurodegeneration	Niemann-Pick disease type C, gangliosidosis NBIA genes, manganese transport defect, neuroferritinopathy, **CTX,** CNL, mitochondrial disorders. Other than IEM: Consider spinocerebellar ataxias. *PARK2*.
- With prominent spasticity	Dystonia may be present in complex forms of HSP, especially SPG26 (*B4GALNT1*), SPG35 (*FA2H*) and SPG56 (*CYP2U1*). Lysosomal disorders at advanced stages of the disease. SPG11 and HSP genes in general.
- Choreoathetosis as prominent sign	**GAI, PA, MMA** **Galactosemia, homocystinuria** Non-ketotic hyperglycinemia, **pterin defects** Lesch-Nyhan disease **Niemann-Pick disease type C** *PI4K2A* (phosphatidylinositol 4-kinase type II-alpha) mutations (associated with cutis laxa) PKAN and other NBIA syndromes **Wilson disease** **GLUT1D** Phosphodiesterase deficiency (PDE10A mutations)
With diffuse central nervous system disorders, seizures, visual failure, dementia	
With hepatosplenomegaly	Niemann-Pick type C, Gaucher type III
Without visceral signs	Metachromatic leucodystrophy, X-ALD Peroxisomal biogenesis defects Krabbe disease, GM1 and GM2 gangliosidosis Leigh syndrome, mitochondrial disorders
With polymyoclonia and epilepsy (progressive myoclonic epilepsy)	
Intellectual deterioration, loss of sight, RP	JNCL (Batten, CLN3 mutations), CLN2, 4, 9, 11, 12, 13
Splenomegaly, osseous signs, dementia	Gaucher disease type III
Cerebellar ataxia, cherry red spot	Late GM2 gangliosidosis (Sandhoff, Tay-Sachs), sialidosis

Table 1.18 (continued)

Symptoms	Diagnosis (disorder/enzyme deficiency)
Hepatomegaly, splenomegaly, ophthalmoplegia,	Niemann-Pick disease type C
Deafness, cardiac problems, optic atrophy, short stature, lipomas, lactic acidosis	Mitochondrial disorders (MERFF and others)
After a period of normal development, visual hallucinations (occipital seizures)	Lafora disease
If there is associated renal failure and/or family members have renal failure	AMFR
Psychiatric symptoms, autosomal dominant	DRPLA
With ataxia	*PRICKLE-1*, mitochondrial disorders, Niemann-Pick disease type C, lysosomal disorders
With spinal muscle atrophy	Acid ceramidase deficiency (*ASAH1* mutations; Farber disease)
With predominant cerebellar ataxia	
Without significant mental deterioration	
- Dysarthria, pes cavus, cardiomyopathy	Friedreich ataxia
- Spinocerebellar degeneration	Other hereditary ataxias, peroxisomal defects
- Chronic diarrhea, low cholesterol, acanthocytosis	**Vitamin E related, abetalipoproteinemia**
- Retinitis pigmentosa, peripheral neuropathy	Refsum disease, peroxisomal defects, CDG, **vitamin E related ataxias**
- Oculocephalic asynergia, conjunctival telangiectasias	Ataxia telangiectasia
With deterioration and dementia	**CTX**, Lafora disease, GM1, GM2 gangliosidosis, Gaucher, Niemann-Pick disease type C, Krabbe disease, metachromatic leukodystrophy, mitochondrial disorders
With progressive spasticity and/or movement disorders	Consider genes related to NBIA syndromes and HSP
With predominant polyneuropathy	
Acute attacks	**Porphyrias, tyrosinemia type I**
Progressive	
- With demyelination (low NCV)	Metachromatic leucodystrophy, Krabbe disease β-mannosidase, Refsum disease, peroxisomal biogenesis MNGIE syndrome, X-ALD, PHARC syndrome (*ABHD12*)
- Predominantly axonal (normal NCV)	**LCHAD, trifunctional enzyme, PDH, homocysteine remethylation defects, CTX,** PBD, CDG, α-methyl-CoA racemase, **serine deficiency**, P5C synthetase, **OAT,** Leigh syndrome, respiratory chain defects, **neuropathy target esterase,** abetalipoproteinemia
With psychiatric symptoms as the only presenting sign	
Behaviour disturbances, personality and character changes, mental regression, dementia, schizophrenia before any significant neurological or extraneurologic sign	**OTC, homocystinurias** (CBS, MTHFR, CblC), Sanfilippo, metachromatic leucodystrophy, Krabbe, Niemann-Pick disease C, X-ALD, Leigh syndrome, JNCL (Batten), PKAN deficiency, **Wilson,** CDG, **CTX,** Huntington chorea (juvenile form), neuroferritinopathy

ALD adrenoleucodystrophy; *CBS*, cystathionine β-synthase; *JNCL*, juvenile neuronal ceroid lipofuscinosis; *MTHFR*, methylene tetrahydrofolate reductase; *OTC*, ornithinetranscarbamylase; *CTX*, cerebrotendinous xanthomatosis; *OAT*, ornithine amino transferase; *PBD*, peroxisome biogenesis disorders; *PDH*, pyruvate dehydrogenase; *PKAN*, panthotenate kinase associated neuro degeneration; *P5C*, pyrroline 5 carboxylase

and remodelling, in particular NBIA syndromes and HSP (hereditary spastic paraparesis) (▶ Chapter 34).

Category 5: with predominant polyneuropathy **Porphyrias** and **tyrosinemia type I** can present with an acute attack of polyneuropathy mimicking Guillain-Barre syndrome. Many other disorders can present with a late-onset progressive polyneuropathy that can mimic hereditary ataxia, such as Charcot-Marie-Tooth disease. These include lysosomal diseases (Krabbe disease, metachromatic leukodystrophy, β-mannosidase), PBD (peroxin 7, other PBD, Refsum disease with demyelination and reduced nerve conduction velocities, X-ALD, racemase deficiency), Refsum-like (PHARC syndrome) and Neuropathy target esterase *(PNPLA6)* (▶ Chapter 34), defects of energy metabolism (Leigh syndrome, mitochondrial disorders, histidyl-tRNA synthetase (*HARS* mutations) [41] PDH, **LCHAD** and **trifunctional-enzyme deficiencies**), **abetalipoproteinemia**, CDG syndrome and a variant form of Menkes disease presenting like an X-linked distal hereditary motor neuropathy, in older children or adults bearing missense mutations in *ATP7A* (▶ Chapter 37) (▶ Section 1.5.2 and ◘ Table 1.28).

Category 6: with behavioral disturbances as the presenting signs Some IEM can present between 5 and 15 years of age as psychiatric disorders. Behavioural disturbances (personality and character changes), loss of speech, scholastic failure, mental regression, dementia, psychosis, and schizophrenia-like syndrome are the most frequent symptoms. In addition, **OTC deficiency** can present with episodes of abnormal behavior and character change until hyperammonemia and coma reveal the true situation (see Recurrent Attacks of Coma above). **Homocystinuria** due to MTHFR deficiency has presented as isolated schizophrenia. Searching for these treatable disorders is mandatory including also **CTX** and **Wilson disease**.

▪ Onset in adulthood (>15 years to >70 years)

(▶ Chapter 2)

1.5.2 Specific Neurosensorial, Neurophysiological and Neuroradiological Signs and Symptoms (at any Age)

Neuroimaging, neurophysiology and ophtalmological investigations are helpful for elucidating neurologic and psychiatric syndromes. The most significant findings are presented in ◘ Table 1.19 to ◘ Table 1.29. Some are sufficiently distinctive to make a clinical diagnosis.

▪ Deafness

The hearing loss in metabolic disorders is mostly sensorineural, symmetrical and (at least initially) high frequency. Mitochondrial syndromic and non syndromic defects are the most frequent causes. Many genetic syndromes can mimic a metabolic disease; some are listed in ◘ Table 1.19.

◘ **Table 1.19** Sensorineural deafness

Detectable in neonatal to early infancy	Acyl-CoA oxidase deficiency Acetyl-CoA transporter (*SLC33A1* mutations) Adenylate kinase 2 (reticular dysgenesis) Alport syndrome (*COL4A* mutations) Cockayne syndrome Encephalopathy with hyperkinurininuria Galactose-4-epimerase deficiency Heimler syndrome (*PEX 1* and *7* mutations) MEDNIK syndrome **Riboflavin transporters (2 and 3) defects** Rhizomelic chondrodysplasia punctata Zellweger and variants
Detectable in late infancy to childhood	**Biotinidase deficiency (biotin responsive) (untreated or treated late)** Chanarin Dorfman syndrome Infantile Refsum disease (pseudo Usher syndrome) Kearns-Sayre syndrome MEDNIK syndrome Mucopolysaccharidosis type I, II and IV Mannosidosis (alpha) Mucolipidosis type II (I cell disease) **Megaloblastic anemia, diabetes and deafness(B1-responsive)** Mitochondrial encephalopathy (MELAS) Myoclonic epilepsy ragged red fibers (MERFF) PHARC syndrome Perrault syndrome PRPP synthetase overactivity **Riboflavin transporter defects** Sphingolipidose Wolframm syndrome
Detectable in late childhood to adolescence	Beta-mannosidosis Refsum disease (adult form) Usher syndrome type II MERFF, Kearns-Sayre syndrome **Riboflavin transporter defects**

▪ Head circumference, cephalhematomas, subdural hematomas (◘ Table 1.20)

Macrocephaly: Congenital macrocephaly may be an isolated early marker of **glutaric aciduria type 1** and a few other cerebral organic acidurias (▶ Chapter 22).

Microcephaly: There are many untreated IEM in which microcephaly results from a progressive non specific cerebral atrophy. A few disorders present with an antenatal (congenital) microcephaly, among them mild forms of **serine synthesis defects** may be treatable (▶ Chapter 24). Mutations in *MFSD2A*, required for omega-3 fatty acid transport in brain, have been recently shown to cause a lethal microcephaly syndrome [42]. Mutations in *PYCR2*, encoding pyrroline-5-carboxylate reductase 2, cause a unique syndrome of postnatal microcephaly with severe hypomyelination (▶ Chapter 21).

1

Table 1.20 Head circumference

Macrocephaly	Microcephaly
Alexander leukodystrophy Canavan disease (acetylaspartaturia) GM2 gangliosidosis (Sandhoff, Tay-Sachs) **Glutaric aciduria type I** Krabbe disease (infantile form) L-2-hydroxyglutaric aciduria Respiratory chain disorders Megalencephalic leukodystrophy with subcortical cysts	Congenital: - Infant born to untreated PKU mother - Amisch lethal microcephaly (mitochondrial TPP transporter) - Sulfite oxidase deficiency - **Serine synthesis defects improved by serine and brain serine transporter** including Neu Laxova syndrome - **BCDK (branched chain dehydrogenase kinase defect)** - MFSD2A defect (omega3 fatty acid transport defect) - PYCR2 (pyrroline-5-carboxylate reductase 2 deficiency) - Asparaginase synthetase deficiency - Dolichol kinase deficiency and different CDG syndromes Acquired: - **GLUT1 and PDH** - **Cerebral folate deficiency due to FOLR1 mutations and DHFR deficiency** - Mitochondrial encephalopathies (in particular, severe early onset mitochondrial DNA depletions) - Rett syndrome due to *MECP2* and Rett like mutations including *CDKL5* and *FOXG1* - DPM 13 (dolichol recycling defects) - Many untreated disorders in which microcephaly is a symptom of a non specific cerebral atrophy - Vici syndrome
Cephalhematomas	
Glutaric aciduria type 1 Menkes disease	

Neuroimaging signs

Morphological evaluation is best undertaken by MRI. Cranial computer tomography (CT scan) is still important when looking for calcifications or in an emergency. Proton MR spectroscopy is a tool for assessing brain metabolites but is diagnostic only in a few disorders, including cerebral creatine disorders (absence of creatine peak), Canavan disease (high peak of N-acetyl aspartate) and some complex lipids/fatty acid defects such as Sjogren-Larsson syndromes and ELOV4 in which there is an additional lipid peak [43] (▶ Chapter 40). Mutations in at least ten genes causing neurodegeneration with brain iron accumulation (NBIA) have been identified so far [44] [45].

Specific signs as listed in the following tables:

- Basal ganglia, brain stem hyperintensities: Table 1.21
- Brain deposits: Table 1.22
- White matter hyperintensities Table 1.23
- Brain dysplasia/malformation: Table 1.24
- Posterior fossa hypoplasia: Table 1.25

Neuro-ophtalmological signs

Abnormal fundoscopy findings (Table 1.26) *Retinal dystrophies* encompass retinitis pigmentosa, Leber congenital amaurosis (LCA), early onset retinal dystrophy and Stargardt disease. There are over four hundred known inherited diseases in which the retina, macula or choroids are substantially involved [46][47]. Most of metabolic causes involve complex molecules (mostly lipids) [48] and energetic process. LCA is a severe retinal dystrophy with infantile onset and is one of the most frequent cause of congenital blindness which is associated with several genes mutations. Mutations in *NMNAT* (coding for NAD synthetase) have been recently described and identify a new disease pathway for retinal degeneration [49].

Hereditary optic atrophy is common in neurodegenerative diseases due to IEM, especially white matter diseases and energy deficiencies, and deserves an extensive metabolic workup. Optic atrophy is a frequent early presenting sign of primary (Leber hereditary optic neuropathy, RCD, PDH deficiency, biotinidase deficiency, Costeff optic atrophy syndrome) or secondary (organic acidurias) mitochondrial dysfunction.

Table 1.21 Basal ganglia & brainstem hyperintensities

Leigh syndrome	Other type of hyperintensities	Hyperintensity of the Inferior olivary nucleus (brainstem)
Biotinidase deficiency **Mutations in SLC19A3 (thiamine-biotin responsive basal ganglia disease)** **CoQ10 deficiency** EPEMA syndrome (*ETHE1* mutations) *ECHS1* mutations (enoyl CoA hydratase) Fumarase deficiency Hydroxy-isobutyryl-CoA hydrolase LIPT1 (lipoyl transferase) SERAC1 (MEGDEL syndrome with five MRI disease stages, especial involvement of the putamen: »eye« on the dorsal putamen) Pyruvate carboxylase deficiency **Pyruvate dehydrogenase deficiency** Lipoilation defects >70 genes involved in oxidative phosphorylation (▶ Chapter 14) SUCLA2 (succinyl CoA synthetase) Sulfite oxidase deficiency 3-methylglutaconic aciduria 1 and 4 (MEGDEL: see above))	**Thiamine-biotin-responsive basal ganglia disease** **Cerebrotendinous xanthomatosis*** GM1 Gangliosidosis* (may produce T2 hypointensity especially in the pallidum and substantia nigra) Semialdehyde succinate dehydrogenase (pallidum) L-2-hydroxyglutaric aciduria **Methylmalonic aciduria** (pallidum) Mitochondrial cytopathies* MEDNIK **Pyruvate dehydrogenase deficiency*** **Wernicke encephalopathy*** (thalami, brain stem) **Wilson disease*** Methemoglobinemia type 2 (NAD cytochrome b5 deficiency)	*POLG* mutations **Wilson disease** **PDH** PMM2-CDG Ataxia telangiectasia Niemann Pick type C Leber hereditary optic neuropathy Dihydropyrimidine dehydrogenase deficiency Pontocerebellar hypoplasia (*TSEN, EXOSC3* mutations) Infantile neuroaxonal dystrophy Salla disease

Legend * observed in adulthood. *BG*, basal ganglia

Table 1.22 Basal ganglia & brain deposits

Calcifications on CT scan	Metals
Aicardi Goutières syndrome Biopterin metabolism defects (DHPR) Carbonic anhydrase deficiency (CAII gene) Cockayne syndrome Congenital lactic acidemias *COL4A1* mutations Cytochrome P450 hydroxylase (CYP2U1: SPG 56) **Folic acid metabolism defects** GM2 Gangliosidosis **Hypoparathyroidism** Krabbe disease Kearns-Sayre syndrome MELAS Other respiratory chain disorders 3-hydroxyisobutyric aciduria PANK and beta-propeller protein-associated neurodegeneration (both NBIA syndromes producing also calcifications) Primary familial brain calcifications (*SLC20A2, PDGFB, PDGFRB, XPR1* genes)*	Copper: **- Wilson disease*** - MEDNIK Manganese: **- Hypermanganesemia with cirrhosis** Iron: Neurodegeneration with brain iron accumulation (NBIA): - FTL Neuroferritinopathy*(pallidum,putamen,caudate) low ferritin - PANK2* (PKAN defect, HARP syndrome) tiger eye,RP - COASY (CoA synthetase) similar to PKAN - C2orf37/DCAF17 - *PLA2G6* mutations (pallidum>substantia nigra) cerebellar atrophy - *FA2H* mutations (pallidum>substantia nigra) - CPa **aceruleoplasminemia*** (diffuse hypointensity) low Cu and Cer - *C19orf12* mutations (pallidum>substantia nigra) optic atrophy - WDR45 (substantia nigra> pallidum) X linked dominant with MR - ATP13A2 (caudate,putamen)

Legend * observed in adulthood; *RP*, Retinitis pigmentosa; *MR*, mental retardation; *Cu*, copper; *CER*, ceruleoplasmin

Table 1.23 White matter hyperintensity

With increased head circumference

- Alexander disease (anterior)
- Canavan disease
- Glutaric aciduria type I (bitemporal atrophy)
- L-2-hydroxyglutaric aciduria
- Megalencephalic leukodystrophy with subcortical cysts (MLC1)
- Mucopolysaccharidosis (with vacuoles)
- Vacuolizing leukoencephalopathy

Without increased head circumference

Diffuse hypomyelination	Predominantly periventricular white matter (sparing U fibers)
Cerebral folate transport (*FOLR1* mutations) and folate metabolism defects in general	Aicardi Goutières syndrome (with calcifications)
Pelizaeus-Merzbacher disease (myelination arrest, PLP1)	CACH (vanishing white matter disease)
Ribose-5-phosphate isomerase*(arabitol, ribitol peaks)	**Cerebrotendinous xanthomatosis***
Untreated galactosemia	Cockayne (with calcifications)
Fucosidosis	**Homocysteine remethylation defects***
Sialidosis	**Glutaric aciduria type I***
GJA12 /GJA13 connexins (Pelizaeus-Merzbacher like)	Kearns-Sayre syndrome
Mitochondrial HSP 60 chaperronopathy	Menkes disease
SLC17A5 (Sialin)High sialic acid only in CSF	Metachromatic leucodystrophy*
Serine synthesis defects	Mitochondrial cytopathy
HABC (Hypomyelination with cerebellum atrophy and atrophy of the basal ganglia, *TUBB4A* gene)	MNGIE (with supratentorial cortical atrophy)
TACH (tremor ataxia, central hypomyelination)	Peroxisomal biogenesis defects*, PEX-7
4H syndrome (hypomyelination, hypogonadotropic hypogonadism, hypodontia; *POLR3A, B* genes)	**PKU (untreated, reversible)***
SPTAN1 (beta spectrin)	Polyglucosan body disease*
SLC 25 A12 (aspartate glutamate carrier)	X-ALD (posterior)
PYCR2 (pyrroline-5-carboxylate reductase 2 deficiency)	3-Methylglutaryl-CoA lyase defect*
Oculodentodigital dysplasia (GJA1)	Hypomyelination with congenital cataracts (FAM126A)
Trichothiodystrophy with photosensitivity (*ERCC2, 3, GTF2H5, MPLK1P* genes)	
RARS-associated hypomyelination	
Hypomyelination with brainstem and spinal cord involvement (*DARS*)	
Cockayne syndrome (*ERCC6,8*)	

Legend *observed in adulthood. *CPT*, carnitine palmitoyl transfease; *FAO*, fatty acid oxidation defects; *CTX*, cerebrotendinous xanthomatosis; *ALD*, adrenoleucodystrophy

	Without increased head circumference		
	Affecting U fibres	**Pyramidal tracts**	**With an extra lipid peak at 1 H-MRS**
	L-2-hydroxyglutaric Mitochondrial cytopathy **Glutaric** L-2-hydroxyglutaric aciduria Mitochondrial cytopathy **Glutaric aciduria type I*** Homocysteine remethylation defects* 3-methylglutaryl-CoA lyase deficiency* Polyglucosan body disease*	**Cerebrotendinous xanthomatosis*** Adrenomyeloneuropathy* Krabbe disease* Mitochondrial **cerebrotendinous xanthomatosis*** Adrenomyeloneuropathy* Krabbe disease* Mitochondrial cytopathies* Cavitating leukoencephalopathies Cystic leukoencephalopathy without megalencephaly (RNASET2-deficient leukoencephalopathy) COX deficiency due to mutations in *APOPT1* Lipoilation defects (especially *NDUFS1*) Pyruvate metabolism defects (pyruvate dehydrogenase deficiency, pyruvate carboxylase deficiency) Mitochondrial A83446 mutation Vanishing white matter disease (*EIF2B2*)	DDHD2, (SPG54 with thin corpus callosum) FALDH DDHD2, (SPG54 with thin corpus callosum) FALDH (Sjogren Larsson syndrome) CPT 2 and several other FAO defects X-ALD and several other peroxisomal defects Chanarin Dorfmann and several other complex lipid synthesis defects Gaucher and NP C disease CTX and Smith Lemli Opitz syndrome

Table 1.24 Brain dysplasia and malformations

Gyration abnormalities	Corpus callosum hypoplasia/agenesis
CEDNIK syndrome (snare protein mutation) O-glycosylation disorders: Muscle eye brain disease (POMGnT), Walker Warburg syndrome (POMT1), Fukuyama syndrome (fukutin), Congenital muscular dystrophy: DMC1C (fukutin related protein), DMC1D (protein large) Peroxisomal disorders (Zellweger and others) MFSD2A defect (omega3 fatty acid transport defect) FOXG1 defects Tubulin defects (*TUBA1A, TUBB2B, TUBB3*) Reelin gene (*RELN*) encoding an extracellular matrix associated glycoprotein Fumarase deficiency Different lyssencephaly genes (*L1S1, DCX, ARX, VLDLR*) Periventricular nodular heterotopia (bilateral due to *FLN1* mutations). Perisylvian polymicrogyria: *PIK3R2* gene (phosphoinositide 3 kinase)	With gyration abnormalities (see left column) ACTH deficiency Aicardi Goutières syndrome (with calcifications) Complex II mitochondrial cytopathies (with leucodystrophy) Non ketotic hyperglycinemia PDH defect (with basal ganglia abnormalities) 3-hydroxyisobutyric aciduria Phospholipids synthesis /remodelling defects associated with spastic paraplegia: SPG 28 (*DDHD1*), SPG 49 (*CYP2U1*),SPG 54 (*DDHD2*), SPG 35 (*FA2H*), SPG 46 (*GPA2*), *EPG5* mutations (Vici syndrome) Brain serine transporter defect Desmosterolosis, lathosterolosis In general, all amino acid synthesis defects may present corpus callosum hypoplasia Any disease with hypomyelination/white matter disturbances may have corpus callosum hypoplasia

Table 1.25 Posterior fossa (and olivo-ponto-cerebellar)

Hypoplasia	Progressive atrophy	Dentate nuclei of the cerebellum hyperintensities
CDG syndrome Mitochondrial cytopathies Peroxisomal disorders Congenital muscular dystrophies Joubert syndrome PCH (Pontocerebellar hypoplasias)	Mitochondrial cytopathies* Leigh syndrome GM2 gangliosidosis* Niemann-Pick disease type C* Cerebrotendinous xanthomatosis* Sialidosis type 1* Ceroid lipofuscinosis* L-2-hydroxyglutaric aciduria Mevalonic aciduria (mevalonate kinase) Adenylosuccinase deficiency Neuroaxonal dystrophy (infantile) and other phospholipid synthesis defects Schindler disease Smith-Lemli-Opitz Succinyl semialdehyde dehydrogenase deficiency 3-Methylglutaconic aciduria type 1 and CLPB mutations X-linked adrenoleukodystrophy* Spinocerebellar ataxias in general* Dentatorubralpallidoluysian atrophy* Progressive myoclonus epilepsies* Spinal and bulbar muscular atrophy*	Cerebrotendinous xanthomatosis* L-2-hydroxyglutaric aciduria Mitochondrial encephalopathy* Polyglucosan body disease* Semialdehyde succinate dehydrogenase deficiency* **Wilson disease*** NBIA (some late onset cases together with hypointensities) *

*observed in adulthood

Table 1.26 Abnormal fundoscopy findings

Cherry red spot	Retinitis pigmentosa and others	Optic atrophy (optic palor)
Niemann-Pick diseases type A, B Galactosialidosis (neuraminidase deficiency) Gangliosidosis GM1 (Landing) Gangliosidosis GM2 (Sandhoff, Tay Sachs) Nephrosialidosis Sialidosis type I Farber disease Cytochrome C oxidase deficiency	**Retinitis pigmentosa:** **Abetalipoproteinemia** **Vitamin E malabsorption (tocopherol carrier)** CDG Ceroid lipofuscinosis **CblC*** **Methylene tetrahydrofolate dehydrogenase defect (MTHFD1)** Panthothenate kinase deficiency Harp) Peroxisomal defects* **LCHAD deficiency** Respiratory chain disorders (Kearns Sayre, NARP, mtDNA deletions) * Mucopolysacchariddoses Dehydrodolichyl diphosphate synthase deficiency Fatty acid 2-hydroxylase (FA2H) deficiency **Refsum disease*** PHARC syndrome (*ABHD12*) **Others:** **Gyrate atrophy with OAT deficiency** **Aceruleoplasminemia*** Mucolipidosis type IV Heterozygous *ELOVL4* mutations (autosomal dominant juvenile form of macular degeneration Stargardt type 3) Sjögren Larsson syndrome (FALDH) macular dystrophy **Extinguished ERG with normal fundoscopy:** NAD synthase deficiency (NMNAT1) Leber congenital amaurosis	**Biotinidase deficiency** Canavan disease (early sign) **CblC*** Ceroid lipofuscinosis (CLN3*, CLN4*) Krabbe disease (infantile) LHON (Leber due to mitochondrial DNA deletions*) Leigh syndrome (all causes) Metachromatic leucodystrophy* Mitochondrial cytopathies* Infantile Neuroaxonal dystrophy (*PLA2G6*) Schindler disease Fatty acid 2-hydroxylase (*FA2H*) deficiency Pelizaeus-Merzbacher disease (presenting sign early in infancy) Peroxisomal biogenesis defects* **Pyruvate dehydrogenase deficiency*** Ribose-5-phosphate isomerase* Sulfite oxidase (infantile) X-ALD* 3-methylglutaconic aciduria type 3 (Costeff due to OPA mutations) Dolichol synthesis/recycling defects: SRD5A3-CDG (Ig) (with nystagmus colobomas,cataracts, glaucoma, micro-ophthalmia DPM1-CDG (CDG Ie) MPDU1-CDG (CDG IF) *SLC25A46* mutations Methylmalonic and propionic acidaemia

*observed in adulthood. *LHON,* Leber congenital optic atrophy

Ophthalmoplegia, abnormal eye movements (Table 1.27)

Table 1.27 Ophtalmoplegia, ptosis, eye movements, strabismus

Neonatal to early infancy (oculogyric crises)	Infancy to childhood	Adulthood
Aromatic amino acid decarboxylase deficiency **Tyrosine hydroxylase defect** **Biopterin synthesis defects** PMM2-CDG (with congenital strabismus) **Pyridox(am)ine-5-phosphate oxidase deficiency** Cogan syndrome (ocular contraversion) Chanarin-Dorfman syndrome(CGI58) (nystagmus and acquired strabismus)	**Gaucher disease type III (horizontal supranuclear paralysis)** **Niemann-Pick type C (vertical supranuclear paralysis)** Leigh syndrome (acute attacks of abnormal movements) **Pyruvate dehydrogenase (acute attacks of abnormal movements)** Respiratory chain (acute attacks of abnormal movements) Ataxia telangiectasia (ocular contraversion,telangiectasia) Fatty acid 2 hydroxylase (*FA2H*) deficiency (nystagmus and acquired strabismus)	Niemann-Pick disease type C **Gaucher disease type III (see above)** Mitochondrial cytopathies (mt DNA deletion) **Glutaric aciduria type I** GM2 gangliosidosis (abnormal eye movements) Non ketotic hyperglycinemia **Pyruvate dehydrogenase (abnormal movements)** Ataxia with oculomotor apraxia (*AOA1* and *2*)

Table 1.28 Polyneuropathy – Electroneuromyographic findings

Acute (recurrent attacks)	Demyelinating (low NVC)	Axonal (predominantly, normal NCV)	Miscellaneous
Porphyrias* Tyrosinemia type I* PDH deficiency*	**Refsum disease** (late childhood to adulthood) X-ALD (childhood to adulthood): leucodystrophy AMN (adulthood) Metachromatic leucodystrophy Multiple sulfatase defciency β-mannosidosis Farber lipogranulomatosis Krabbe (leucodystrophy) **Homocysteine remethylation defects (MTHFR, CblC)** MNGIE syndrome (leucodystrophy) **Tangier disease** **Cerebrotendinous xanthomatosis*** (with leucodystrophy) PHARC syndrome (ABHD 12) (presenting sign)	**Abetalipoproteinemia** (C) α-methylacylCoA racemase ° PMM2-CDG (childhood) GM2 gangliosidosis* **LCHAD, trifunctional** (C to Ado) PBD (late childhood to adult) Polyglucosan body disease* **PDH** (childhood to adulthood) **Vitamin E malabsorption** **CTX***(leucodystrophy) INAD, Schindler disease (early childhood) **OAT defect** (late complications) P5C synthetase defect (late C) **Porphyria*** Pyroglutamic aciduria (late complication) Respiratory chain defects (early C to Ado) Serine deficiency syndrome (Ado) Triose phosphate isomerase Presynaptic Choline Transporter* SLC5A7mutations [50] Neuropathy target esterase (C) SPTLC1/2* HSAN type1 AD	**Affecting small sensitive fibers and the autonomic nervous system**: - **Fabry disease*** (presenting sign) - GM2 gangliosidosis* - **Porphyria*** - Tangier disease* **Affecting anterior horn**: - GM2 gangliosidosis - Krabbe's disease - **Homocysteine remethylation defects (ClbC)** - Panthotenate kinase defect (Hallervorden-Spatz disease) (basal ganglia) - Polyglucosan body disease*

*observed in adulthood; *AD*, autosomal-dominant; *Ado*, adolescence; *ALD*, adrenoleucodystrophy; *AMN*, adrenomyeloneuropathy; *C*, childhood; *HSAN*, hereditary sensory autonomic neuropathy; *INAD*, infantile neuroaxonal dystrophy; *NCV*, nerve conduction velocity; *OAT*, ornithine aminotransferase; *PBD*, peroxisome biogenesis defect; *PDH*, pyruvate dehydrogenase; *P5C*, pyrroline 5 carboxylase; *SPTLC*, serine palmitoyl transferase

Neurophysiological signs (Table 1.28 and ► Section 1.5.1)

The diagnosis of peripheral neuropathies rely on clinical and electrophysiological criteria. The general classification depends on whether there is an involvement of large fibers (motor weakness, loss of deep reflexes, muscle atrophy, sensory ataxia), or small fibers (autonomic dysfunction, abnormal temperature, sensibility pinprick loss) and whether the neuropathy is predominantly demyelinating or axonal.

Two main groups of metabolic diseases give rise to peripheral neuropathies: lipid storage disorders and energy metabolism defects. In lipid storage disorders, both the peripheral and central myelin can be involved, leading to a low nerve conduction velocity (NCV) and leukoencephalopathy on brain MRI. In contrast, defects of energy metabolism are mostly responsible for axonal peripheral neuropathies with normal NCV and are usually associated with other signs of energy metabolism defects (optic atrophy and cerebellar ataxia in the case of respiratory chain disorders see Table 1.26). Many exceptions to this schematic view however exist. MNGIE syndrome (myoneurogastrointestinal neuropathy) caused by thymidine phosphorylase deficiency is typically responsible for a demyelinating polyneuropathy (► Chapter 35). Some lipid storage disorders such as cerebrotendinous xanthomatosis (► Chapter 32), adrenomyeloneuropathy and other peroxisomal diseases (► Chapter 40) may cause polyneuropathies that can be axonal, demyelinating or both.

Self mutilation and auto-aggression

Self mutilation and auto-aggression are mostly observed in Lesch Nyhan syndrome, untreated phenylketonuria, acute crises of tyrosinemia type I and 3-methylglutaconic aciduria.

1.5.3 Recommended Laboratory Tests in Neurological Syndromes

Table 1.29

Table 1.29 Main neurological syndromes from childhood to adolescence and recommended laboratory tests (focused on treatable disorders)

Predominant neurological syndrome	Laboratory tests (rational approach based on associated clinical signs and treatable disorders)	Treatable disorders
Isolated developmental delay/intellectual disability (ID)	- Basic laboratory tests (*): blood glucose, acid-base status, blood counts, liver function, creatine kinase, uric acid, thyroid function, alkaline phosphatase - Plasma: lactate, ammonium, amino acids, total homocysteine, folate, biotinidase activity - Urine: creatine metabolites, organic acids (including 4-hydroxybutyric acid), amino acids, glycosaminoglycanes (GAGs), purines, pyrimidines, - Consider maternal phenylalanine	Phenylketonuria (PKU), homocystinurias, urea cycle defects, amino acid synthesis defects, thyroid defects, biotinidase deficiency, Hartnup disease
With dysmorphic features	- Consider also: plasma sterols, peroxisomal studies (very long-chain fatty acids, phytanic acid, plasmalogens), transferrin isoelectric focusing for glycosylation studies (CDG), oligosaccharides in urine For the study of ID +/- dysmorphic features, genetic tests (cytogenetic studies, microarrays, NGS, and targeted studies) have the highest diagnostic yield.	Peroxisomal diseases (only partially by some supplements)
Behavioural and psychiatric manifestations including autistic signs	- Basic laboratory tests (*) - Plasma: ammonium, amino acids, homocysteine, total homocysteine, folate, sterols (including oxysterols), copper, ceruloplasmin - Urine: GAGs, organic acids (4-hydroxybutyric acid), amino acids, purines, creatine, creatinine and guanidinoacetate - Depending on additional clinical signs and brain MRI pattern: peroxisomal studies, lysosomal studies	PKU, urea cycle disorders, homocystinurias, folate metabolism defects, Wilson disease, BCKDH kinase deficiency, CTD, mild forms of SLO, Niemann-Pick disease type C, X-ALD (at some stages), Hartnup disease
Epilepsy	- Basic laboratory tests (*) adding calcium, magnesium - Plasma: lactate, ammonium, amino acids, total homocysteine, folate, biotinidase activity, copper and ceruloplasmin, VLCFA - Urine: organic acids, creatine, creatinine and guanidinoacetate, sulphite test, purines and pyrimidines, pipecolic acid and 5-AASA - CSF: glucose, lactate, amino acids, 5-methyltetrahydrofolate (5-MTHF), pterines, biogenic amines, GABA - Consider lysosomal studies and targeted tests if PME - Consider genetic tests for GPI-anchor biosynthesis pathway defects and other defects of complex lipid synthesis (FA2H, ELOVL4, GM3 synthethase)	GLUT-1, homocystinurias, IEM of folate metabolism, organic acidurias, biotinidase deficiency, creatine synthesis defects, serine biosynthesis defects, Menkes disease (only partially treatable), late onset forms of pyridoxine dependent epilepsy, pterin defects (DHPR), AADC deficiency, MoCo deficiency (cyclic pyranopterin monophosphate: treatment recently introduced)
Ataxia	- Basic laboratory tests (*) adding albumin, cholesterol, triglycerides, and alpha-foetoprotein - Plasma: lactate, pyruvate, ammonium, amino acids, biotinidase activity, vitamin E, sterols (including oxysterols), ceruloplasmin, peroxisomal studies (including phytanic acid), coenzyme Q10, transferrin electrophoresis - Urine: organic acids (including 4-hydroxybutyric and mevalonic acids), amino acids, purines - CSF: glucose, lactate, pyruvate - Consider lysosomal/mitochondrial/NBIA studies depending on the clinical and brain MRI signs - Consider lipidome studies (plasma, CSF) - Consider genetic panels of inherited ataxias and other NGS techniques	PDH deficiency (thiamine-responsive; ketogenic diet), biotinidase deficiency, GLUT-1, abetalipoproteinemia, CTX, Refsum disease, coenzyme Q10 deficiencies, Hartnup disease, Niemann-Pick disease type C

Table 1.29 (continued)

Predominant neurological syndrome	Laboratory tests (rational approach based on associated clinical signs and treatable disorders)	Treatable disorders
Dystonia-Parkinsonism	- Basic laboratory tests (*) - Plasma: lactate, pyruvate, ammonium, amino acids, biotinidase activity, sterols (including oxysterols), copper, ceruloplasmin, uric acid, manganese - Urine: organic acids, uric acid, creatine, creatinine and guanidinoacetate, purines, GAGs, oligosaccharides - CSF: glucose, lactate, pyruvate, amino acids, 5-methyltetrahydrofolate, pterines, biogenic amines, GABA - Consider lysosomal/mitochondrial/NBIA studies depending on the clinical and brain MRI signs - Consider genetic panels of inherited dystonias, parkinsonism, and other NGS techniques	Neurotransmitter defects, GLUT-1 deficiency, thiamine transport defects (TBBGD), PDH defects, organic acidurias, homocystinurias, IEM of folate metabolism, defects of creatine biosynthesis, Wilson disease, biotinidase deficiency, Niemann-Pick disease type C, CTX, manganese defects
Chorea	- Basic laboratory tests (*) - Plasma: lactate, pyruvate, ammonium, amino acids, total homocystinuria, folate, biotinidase activity, sterols (including oxysterols), copper, ceruloplasmin, uric acid, galactose 1 P, transferrin electrophoresis - Urine: organic acids, uric acid, creatine, creatinine and guanidinoacetate, purines, galactitol, sulphite test - CSF: glucose, lactate, pyruvate, amino acids, 5-methyltetrahydrofolate, pterines, biogenic amines, GABA - Consider NCL studies and GPI-anchor synthesis defect genetic tests - Consider genetic panels of inherited choreas, and other NGS techniques	Glutaric aciduria I and other classic organic acidurias (MMA, PA), GAMT, GLUT-1, homocystinurias, pterin and neurotransmitter defects, Niemann-Pick disease type C, Wilson disease, galactosemia, cerebral folate deficiency due to *FOLR* mutations, MoCo deficiency, NKH
Spasticity	- Basic laboratory tests (*) - Plasma: lactate, pyruvate, ammonium, amino acids, total homocystinuria, folate, biotinidase activity, vitamin E, triglycerides, cholesterol, sterols, peroxisomal studies - Urine: organic acids, amino acids, GAGs, oligosaccharides, sialic acid - CSF: biogenic amines, pterines and 5-MTHF - Consider lysosomal/mitochondrial/ NBIA studies depending on clinical and MRI findings - Consider genes related to HSP and plasma, CSF lipidome	HHH, arginase deficiency, ornithine amino transferase deficiency, homocysteine remethylation defects, biotinidase deficiency, cerebral folate deficiencies, dopamine synthesis defects (atypical TH), CTX, vitamin E deficiency
Peripheral Neuropathy	- Basic laboratory tests (*) - Plasma: lactate, pyruvate, ammonium, amino acids, folate, vitamin E, triglycerides, cholesterol, acylcarnitines, sterols, peroxisomal studies, transferrin electrophoresis - Urine: amino acids, GAGs, oligosaccharides, thymidine, porphyrins - Consider lysosomal/mitochondrial/ NBIA studies depending on clinical and MRI findings	Refsum disease, X-ALD (treatable at some stages), homocysteine remethylation defects, CTX, abetalipoproteinemia, LCHAD, trifunctional protein, PDH, vitamin E malabsorbtion, ornithine amino transferase, serine deficiency

(*) These basic laboratory tests should be considered as a routine screening in every neurological syndrome.
AADC, amino acid decarboxylase; *5-AASA*, 5-aminoadipic semialdehyde; *BCKDH*, branched chain ketoacid dehydrogenase; *CTX*, cerebrotendinous xanthomatosis; *DHPR*, dihydropteridine reductase; *HHH*, hyperammonemia, hyperornithinemia, homocitrullinuria; *FOLR*, folate receptor; *GA1*, glutaric aciduria type 1; *GAG*, glycosamineglycan; *GAMT*, guanidinoacetate methyltransferase; *GTPCH*, GTP cyclohydrolase I; *LCHAD*, long-chain 3-hydroxyacyl-CoA dehydrogenase; *HSP*, hereditary spastic paraparesis; *MMA*, methylmalonic aciduria; *MoCo*, molybdenum cofactor deficiency; *NGS*, next generation sequencing; *NKH*, nonketotic hyperglycinemia; *PA*, propionic acidemia; *PDH*, pyruvate dehydrogenase deficiency; *PME*, progressive myoclonus epilepsy; *X-ADL*, x-linked adrenoleukodystrophy; *TH*, tyrosine hydroxylase

1.6 Specific Organ Signs and Symptoms

IEM can involve any organ /system in any scenarios at any age. Some of these phenotypes are rare and very distinctive (e.g., lens dislocation and thromboembolic accidents in homocystinuria) whereas others are common and non-specific (e.g., hepatomegaly, seizures, mental retardation). The most important are listed below in tables classified by alphabetical order. The following diagnostic checklist is primarily based upon the authors'personal experience and, of course, is not exhaustive. It should be progressively updated according to the personal experiences of all readers.

1.6.1 Cardiology

All pertinent information is presented in ▶ Section 1.3.1, ▶ Section 1.4.1 and ◘ Table 1.11 and ▶ overview (cardiomyopathies).

1.6.2 Dermatology

- **Hair signs (◘ Table 1.30)**

◘ **Table 1.30** Hair Signs

Alopecia	Brittle Hair	Pili Torti, Hirsutism	Trichorrhesis Nodosa
Age at onset: neonatal to infancy			
Acrodermatitis enteropathica **Biotin-responsive MCD** **Calciferol metabolism defects** RFT1-CDG (In) **Congenital** erythropoietic porphyria Conradi-Hunermann syndrome Ehlers-Danlos type IV **Essential fatty acid deficiency** **Hepatoerythropoietic porphyria** Menkes disease (X-linked) **Methylmalonic and propionic acidurias** Netherton syndrome Zinc deficiency	**Argininosuccinic aciduria** **Citrullinemia** Menkes syndrome Pollitt's syndrome Trichothiodystrophy Mucopolysaccharidosis	**Pili Torti** Menkes disease Netherton syndrome **Hirsutism** MPS III Oliver-McFarlane syndrome (Trichomegaly,bushy eyebrows, sparse scalp hair)	**Argininemia** **Argininosuccinic aciduria** **Lysinuric protein intolerance** Menkes disease Netherton syndrome
Age at onset: Adulthood			
Porphyria cutanea tarda Steinert disease Woodhouse Sakati syndrome			

- **Hyperkeratosis/Ichthyosis (◘ Table 1.31)**

The classification of inherited ichthyoses is clinically based and distinguishes between syndromic and non syndromic ichthyosis forms [51]. Bullous ichthyosis/epidermolytic hyperkeratosis was redefined as keratinopathic ichthyosis.Collodion babies present with a tight shiny cast that cracks after some time, resulting in irregularly branched fissures. Autosomal recessive congenital ichthyosis (ARCI) refers to harlequin ichthyosis, lamellar ichthyosis, and congenital ichthyosiform erythroderma. About 30 inherited disorders of complex lipids synthesis, remodeling, catabolism, and transport presenting with ichthyosis have been described so far [48], the vast majority of which present as neuro-cutaneous syndromes, chondrodysplasia punctata or multiple congenital anomalies, CDG or Neu Laxova syndrome (▶ Chapter 24). Among the neuro-cutaneous syndromes comprising spastic paraparesis, **Sjögren-Larsson syndrome** presents at birth with a very severe pruriginous ichthyosis that responds dramatically to Zileuton (▶ Chapter 40). **Sterol methyl-C4 oxidase**, a sterol metabolism disorder, is another treatable ichthyosis with a spectacular improvement on statin and cholesterol supplement (▶ Chapter 32). Ichthyosis and keratodermia are also cardinal signs of CEDNIK [4] and MEDNIK syndromes (▶ Chapter 37).

Table 1.31 Hyperkeratosis, Ichthyosis

Hyperkeratosis	
CEDNIK (neuro-cutaneous syndrome: keratosis on palms and soles) MEDNIK syndrome: keratodermia (see below) Ichthyosis (see below) **Tyrosinemia type II (keratosis on palms and soles)**	
Angiokeratosis	
Aspartylglucosaminuria β-mannosidosis **Fabry disease** (presenting sign) Fucosidosis Galactosialidosis Kawasaki disease Schindler disease (adult form)	
Ichthyosis (with Congenital Erythrodermia)	
Lysosomal diseases	X-linked steroid sulfatase (non pruritic)
	Austin disease: multiple sulfatase deficiency
	Gaucher disease type II (collodion baby)
Complex phospho lipids and fatty acid synthesis/remodeling defects	Early ARCI:Phospholipase A1 deficiency *(PNPLA1)*
	Late ARCI: Epidermal lipase N deficiency (*LIPN)*
	Chanarin Dorfmann syndrome (*ABDH5)*
	Sjogren Larsson syndrome (FADH) (pruritic)
	Elongase 4 deficiency (*ELOVL4*)
	Serine synthesis defects
Cholesterol synthesis defect	Conradi Hunermann syndrome (X-Linked) Δ8Δ7-sterol isomerase (transient ichthyosis)
	CHILD syndrome (3-beta-hydroxysteroid C-4 dehydrogenase) unilateral
	Sterol-C4 methyl oxidase deficiency (spares palms and soles)
Peroxisomal disorders	**Adult Refsum disease**
	Chondrodysplasia punctata (CDP) type I, II, III (plasmalogens synthesis defect)
Dolichol synthesis and recycling defects	MPDU1-CDG (1f): Mannose-P-Dolichol Utilization Defect 1)
	SRD5A3-CDG (1q): Steroid 5-a-Reductase 3 with eye findings
	DK1- CDG (1m): Dolichol Kinase with loss of hair eyebrows and eyelashes
	CEDNIK syndrome (*SNAP29* coding for a SNARE protein) Cerebral Dysgenesis, Neuropathy, Ichthyosis, palmoplantar Keratodermia
	MEDNIK syndrome (*AP1S1* mutations) (Mental Retardation, Enteropathy, Deafness, Neuropathy, Ichthyosis, Keratodermia (with low copper and ceruleoplasmin)

■ Vesiculobullous lesions /skin rashes/photosensitivity (■ Table 1.32)

■ Table 1.32 Vesiculous Bullous Lesions, Photosensitivity

Photosensitivity & Skin Rashes	Vesiculo Bullous Lesions	Acrocyanosis
Age at onset: neonatal to childhood		
- Biotinidase deficiency **- Congenital erythropoietic porphyria** - Ethylmalonic encephalopathy (EPEMA syndrome) **- Erythrohepatic porphyria** **- Erythropoietic protoporphyria** **- Hartnup disease** - Mevalonic aciduria (with fever and arthralgia) - Respiratory chain disorders - *SECISPB2* mutations (seleno protein defect) **- Zinc deficiency**	**- Acrodermatitis enteropathica** **- Biotinidase deficiency** **- Holocarboxylase synthetase deficiency** **- LPI (lupus like skin lesions)** **- Lipin 2 deficiency (Majeed syndrome)** **- Methylmalonic, propionic acidemias** (isoleucine deficiency) **- Zinc deficiency**	- EPEMA syndrome (ETHE1 mutations) (orthostatic) - Aicardi Goutières syndrome (Chilblains)
Age at onset: adulthood		
- Hereditary coproporphyria **- Porphyria variegata** **- Porphyria cutanea tarda**		

LPI, lysinuric protein intolerance

■ Cutis laxa/Nodules/Xanthomas/ Miscellaneous (■ Table 1.33)

Ehlers-Danlos syndrome (EDS) are collagenopathies that comprise a clinically and genetically heterogeneous group of heritable connective tissue disorders. Its principal clinical features reflect varying degrees of connective tissue fragility, affecting mainly the skin, ligaments, blood vessels, and internal organs. There are 16 EDS variants described so far, which include defects in noncollagenous proteins including genes involved in glycosaminoglycans (GAG) synthesis (*B4GALT7* and *B3GALT6* encoding galactosyltransferase I, and II respectively and *CHST14*, encoding dermatan 4-O-sulfotransferase). Deficiency of galactosyltransferase I and II affects the initial steps in the formation of the GAG chains [52].

Cutis laxa syndrome forms a group of diseases, mostly elastinopathies characterized by wrinkled, redundant, inelastic and sagging skin due to defective synthesis of elastic fibers and other proteins of the extracellular matrix. Syndromic forms of cutis laxa are caused by diverse genetic defects, mostly coding for structural extracellular matrix proteins [53]. A number of metabolic disorders have been also found to be associated with inherited cutis laxa among them copper metabolism defects like Menkes disease (▶ Chapter 37), Glut 10 (▶ Chapter 10), combined disorder of N- and O- linked glycosylation (mutations in *ATP6V0A2),*COG7-CDG and other CDG defects (▶ Chapter 41), and proline synthesis defects (▶ Chapter 21), all disorders associated with neurologic disease.

1.6.3 Endocrinology (■ Table 1.34)

IEM may be associated with endocrine dysfunction, the most frequent being disorders of carbohydrate metabolism (diabetes and hyperinsulinism) (▶ Chapter 9). Diabetes may occur with iron overload, mitochondriopathies, and thiamine sensitive disorders. The clinical spectrum of some forms of IEM changes from hypoglycemia in childhood to diabetes in adulthood [54]. Mitochondriopathies can be associated with all types of endocrine disorders, the most frequent being diabetes and dysthyroidism. Hypothyroidism is encountered in mitochondriopathies, cystinosis and primary hyperoxaluria. The Allan-Herndon-Dudley syndrome, an X-linked mental retardation syndrome due to the monocarboxylate transporter 8 gene, involves the transport of tri-iodothyronine into neurones and disturbs blood levels of thyroid hormone [55]. Long term consequences of IEM on fertility,reproduction and bone metabolism are still poorly understood and should be prospectively investigated [56]. Hypogonadism is almost constant in women with classic galactosemia, frequent in CDG syndromes, cystinosis, and iron overload and in some complex lipids disorders like in *PNPLA 6* mutations spectrum (▶ Chapter 34).

Table 1.33 Cutis laxa and Laxity,Nodules, Xanthoma, and Miscellaneous

Cutis laxa, Skin laxity	Xanthoma	Nodules, Lipodystrophy and lipomatosis	Miscellaneous
- **Copper defects** Menkes disease, occipital horn syndrome - **Proline synthesis defects (de Barsy syndrome)** P5C-synthetase, P5-phosphate reductase - **CDG syndromes** *ATP6V0A2,* COG7-, GALNT1-CDG - **Phospholipids synthesis defects** *PTDSS1* (Lenz-Majewski), *PIK2A* - **GLUT 10** (arterial tortuosity) - **TALDO** (transient)	- Apo CII defect (eruptive) - Apolipoprotein A1 defect (planar) - **Autosomal dominant hypercholesterolemia** - Autosomal recessive hypercholesterolemia - Dysbetalipoproteinemia (hyperlipoproteinemia type III) - Cerebrotendinous xanthomatosis - Hepatic lipase - Lipoprotein lipase (eruptive) - Sitosterolemia (childhood)	**Nodules** - PMM2-CDG syndrome - Farber lipo granulomatosis - *PSMB8* (mutations in Proteasome) **Lipodystrophy and lipomatosis** - Triglycerides synthesis defects - Perilipin deficiency - AGPAT II and SEIPIN mutations - Phospholipids synthesis defects - *PCYT1A* mutations (with spondylometaphyseal dysplasia) - PIK3CA-related overgrowth spectrum - Mitochondrial defect: MERFF syndrome: multiple lipomas	**Telangiectasias, Purpuras, Petechiae** **- Ethylmalonic aciduria (EPEMA)** - Prolidase deficiency **Ulceration (Skin Ulcers)** - Prolidase deficiency - HSAN type 1 (▶ Chapter 38) **Inflammatory dermatosis** - Sweet syndrome, Majeed syndrome (*LIPIN2)* - Aplasia cutis congenita: *EOGT-CDG* - Progressive reticular hyper- and hypopigmentation: POGLUT1-CDG - Salt and peper syndrome: ST3GAL5-CDG
Laxity, dysmorphic scarring, easy bruising Ehlers-Danlos syndrome (16 types) of which *B4GALT7B-3GALT6* and *CHST14*			

1.6.4 Gastroenterology and Nutritional Findings

Gastrointestinal and nutritional findings occur in a wide variety of IEM. Unfortunately, their cause often remains unrecognized, thus delaying the correct diagnosis. Persistent anorexia, feeding difficulties, chronic vomiting, failure to thrive, frequent infections, osteopenia, and generalized hypotonia in association with chronic diarrhea may be the presenting symptoms and signs in a number of IEM in infancy. They are easily misdiagnosed as cow's milk protein intolerance, celiac disease, chronic ear, nose & throat infections, late-onset chronic pyloric stenosis etc. Congenital immunodeficiencies are also frequently considered, although only a few present early in infancy with this clinical picture.There are two groups of IEM presenting with chronic diarrhea and failure to thrive:

- Disorders of the intestinal mucosa or the exocrine function of the pancreas with almost exclusive intestinal effects, for example **congenital chloride diarrhoea, glucose-galactose malabsorption, lactase and sucrase-isomaltase deficiencies, abetalipoproteinemia type II (Anderson disease), enterokinase deficiency, acrodermatitis enteropathica and selective intestinal malabsorption of folate and vitamin B12**, the latter also causing systemic disease. A new congenital diarrhea disorder linked to mutations in *DGAT1* involved in triglycerides synthesis has been recently described (▶ Chapter 34).
- Systemic disorders which also give rise to GI and nutritional abnormalities.

In clinical practice, these groups are sometimes very difficult to distinguish, because a number of specific intestinal disorders can give rise to various systemic clinical abnormalities and vice versa.There are also several metabolic phenocopies linked to chronic deficient intake in one specific indispensable nutrient (mostly vitamins). This is summarized in Table 1.35.

Table 1.34 Endocrine Abnormalities

Pancreas	Thyroid / Parathyroid and Growth hormone	Adrenal/Sex glands
Diabetes (and pseudodiabetes) - Abnormal proinsulin cleavage - **Aceruleoplasminemia** - **Diabetes, deafness and TRMA syndrome** - Diabetes type II: FAO? - Kir 6.2, Glucokinase mutations - **Hemochromatosis** (adult) - Mitochondrial pyruvate carrier defect (MCT1) - **Organic acidurias (MMA, PA, IVA, ketolytic defects)** - Respiratory chain defects - Untreated cystinosis - Wolfram syndrome - Woodhouse Sakati syndrome (C2orf37)(diabetes, hypogonadism, alopecia, deafness, mental retardation and extrapyramidal syndrome)	**Hyperthyroidism** - **Glutaric aciduria** (glutarylCoA oxidase deficiency?)	**Hypogonadism ,Sterility** - PMM2-CDG - **Galactosemia** - PLA2G6 mutation spectrum (Gordon Holmes syndrome) - Kalman syndrome Idiopathic Hypogonadotropic Hypogonadism - Perrault syndrome (several mitochondrial genes: C10orf2, CLPP, HARS2, LARS2, HSD17B4) DBifunctional protein - X-linked ALD - **Fabry disease** - **Cystinosis (males)** - Alstrom disease - **Hemochromatosis** - Endosomal ferrireductase defect(STEAP 3) - Selenoprotein defect - C2orf37 mutations - 4H syndrome (Table 1.23)
	Hypothyroidism - Allan-Herndon-Dudley syndrome (monocarboxylate transporter 8) - Respiratory chain defect - **Cystinosis** - **Fabry disease** - Selenoprotein defect	
	Hypoparathyroidism - **LCHAD deficiency** - Respiratory chain defect - **Trifunctional enzyme deficiency**	
	Growth Hormone Deficiency - Respiratory chain defects	
Hyperinsulinism - SUR1 and KIR6.2 mutations - Glucokinase overactivity - **GDH overactivity** - **SCHAD deficiency** - Monocarboxylic transporter overactivity		**Sexual Ambiguity** - Congenital adrenal Hyper- and Hypoplasia - Disorders of adrenal steroid metabolism
		Salt-Losing Syndrome - **Disorders of adrenal steroid metabolism** - **FAO** (CPT II) - Respiratory chain (mit DNA deletions

Abdominal pain (recurrent):

See acute symptoms section ▶ Section 1.4.1 and Table 1.10

Acute pancreatitis

- Hyperlipoproteinemia type I and IV,
- **Lysinuric protein intolerance,**
- **Organic acidurias** (MMA, PA, IVA, MSUD),
- Respiratory chain disorders (Pearson, MELAS).

Chronic diarrhea, failure to thrive, osteoporosis (Table 1.35)

Table 1.35 Chronic diarrhea, poor feeding, vomiting, failure to thrive

Leading symptoms	Other signs	Age of onset	Diagnosis (disorder/enzyme deficiency)
Severe watery diarrhea, attacks of Dehydration	Nonacidic diarrhea, hypochloremic alkalosis	Congenital to infancy	**Congenital chloride diarrhea**
	Acidic diarrhea, reducing substances in stools	Neonatal	**Glucose galactose malabsorption** **Lactase deficiency**
	Acidic diarrhea, reducing substances in stools after weaning	Neonatal to infancy	**Sucrase isomaltase deficiency**
	Skin lesions, alopecia	Neonatal or post weaning	**Acrodermatitis Enteropathica**
Protein losing enteropathy	Non bloody, watery diarrhea Cholangitis crisis	Neonatal	AcylCoA:diacylglycerol acyltransferase 1 deficiency (*DGAT*)
		Infancy	**MPI-CDG (Ib),** ALG8- CDG (Ih), ALG6-CDG (Ic)
	Hypoglycemia		PMM2-CDG (1a)
Fat-soluble vitamins malabsorption, severe hypocholesterolemia, osteopenia, steatorrhea	Cholestatic jaundice	Neonatal to infancy	**Bile acid synthesis defects**
			Infantile Refsum
	Ichthyosis, keratodermia, deafness, MR		MEDNIK
	Hepatomegaly, hypotonia, retinitis pigmentosa, deafness	Infancy	Infantile Refsum
			PMM2-CDG (1a)
	Abdominal distension, ataxia, acanthocytosis, peripheral neuropathy, retinitis pigmentosa	Infancy	**Abetalipoproteinemia I and II** (no acanthocytes, no neurological sign in type II)
	Pancreatic insufficiency, neutropenia, pancytopenia	Early in infancy	Pearson syndrome
			Schwachman syndrome
Severe failure to thrive, anorexia, poor feeding, with predominant hepatosplenomegaly	Severe hypoglycemia, inflammatory bowel disease, neutropenia,	Neonatal to early infancy	**Glycogenosis type Ib** (no splenomegaly)
	Hypotonia, vacuolated lymphocytes,adrenal gland calcifications	Neonatal	**Wolman disease**
	Recurrent infections, inflammatory bowel disease,	Infancy	**Chronic granulomatosis** (X-linked)
	Megaloblastic anemia, neuropathy, homocystinuria, MMA	1-5 years	**Intrinsic factor deficiency**
	Leuconeutropenia, osteopenia, hyperammonemia, interstitial pneumonia,	Infancy	Lysinuric protein intolerance
	Recurrent fever, inflammatory bowel syndrome, hyper-IgD	Infancy	Mevalonate kinase
Severe failure to thrive, anorexia, poor feeding, with megaloblastic anemia	Oral lesion, neuropathy, infections, pancytopenia, homocystinuria, MMA	1-2 years	**TC II deficiency**
			Intrinsic factor deficiency
	Stomatitis, peripheral neuropathy, infections, intracranial calcifications	Infancy	**Congenital folate malabsorption**
	Severe pancytopenia, abnormal marrow precursors, lactic acidosis	Neonatal	Pearson syndrome

Table 1.35 (continued)

Leading symptoms	Other signs	Age of onset	Diagnosis (disorder/enzyme deficiency)
Severe failure to thrive, anorexia, poor feeding, no significant hepato-splenomegaly, no megaloblastic anemia	Severe hypoproteinemia, putrefaction diarrhea	Infancy	**Enterokinase**
	Diarrhea after weaning, cutaneous lesion (periorificial), low plasma zinc	Infancy	**Acrodermatitis, Enteropathica**
	Ketoacidotic attacks, vomiting	Infancy	Organic acidurias (**MMA**, **PA**)
			Mitochondrial DNA deletions
	Vomiting, lethargy, hypotonia, hyperammonemia	Infancy	**Urea cycle defects** (mainly OTC)
	Frequent infections, lymphopenia,	Infancy	**Adenosine deaminase defect**
	Developmental delay, relapsing petechiae, orthostatic acrocyanosis	Infancy	EPEMA syndrome
	Skin laxity, pili torti, hypothermia, hypotonia, seizures, facial dysmorphism		Menkes disease

MMA, methylmalonic acidemia; *PA*, propionic acidemia; *CDG*, congenital disorder of glycosylation; *OTC*, ornithine transcarbamylase; *MR*, mental retardation. **Bold face**, treatable disorders

Hypocholesterolemia

- Abetalipoproteinemia type I and II,
- PMM2-CDG (1a),
- Infantile Refsum disease (peroxisome biogenesis defect),
- Mevalonic aciduria,
- Smith-Lemli-Opitz syndrome,
- **Tangier disease** (alpha-lipoprotein deficiency).

HELLP Syndrome (baby born to mothers with)

- Carnitine palmityl transferase I deficiency,
- LCHAD deficiency and other fatty acid β-oxidation disorders,
- Respiratory chain defects.

Intestinal obstruction

- MNGIE syndrome is a mitochondrial cytopathy due to mutations in thymidine phosphorylase and other mitochondrial genes (▶ Chapter 14)

1.6.5 Haematology

Red blood cell disturbances

Many IEM can cause anemia (Table 1.36). Over 95% of macrocytic anaemias are secondary to acquired deficiencies of folate or vitamin B12, but many IEM of vitamin B12 and folate metabolism also present with macrocytic anaemia (with the notable exception of MTHFR deficiency) (▶ Chapter 27) and one thiamine transporter deficiency (▶ Chapter 28). Haemolytic anaemias are due to deficiencies of glycolytic and pentose phosphate shuttle enzymes (some of which are associated with abnormal neurological signs) (▶ Chapter 7), abnormal erythrocyte nucleotide metabolism (▶ Chapter 35), porphyrias (▶ Chapter 36), disorders of lipid metabolism or hypersplenism. Sideroblastic anaemias are observed in mitochondrial disorders such as Pearson syndrome or mitochondrial tyrosyl-tRNA synthetase deficiency presenting with MLASA: myopathy, lactic acidosis, and sideroblastic anaemia syndrome (▶ Chapter 14). The **pyridoxine-responsive anaemia** (or X-linked sideroblastic anaemia) caused by a defect in the erythroid-specific form of 5-aminolevulinate synthase presents in the 2nd decade of life; 90% of patients are B_6 responsive (▶ Chapter 28).

Table 1.36 Red blood cells disturbances

Acanthocytosis Polycythemia	Anemias : Macrocytic	Anemias : Non Macrocytic, Hemolytic,Congenital dyserythropoietic or Due to Combined Mechanisms
Acanthocytosis - Abetalipoproteinemia - Panthothenate kinase deficiency - Inborn errors of cobalamin **(Cbl C)** - Wolman disease **Polycythemia** - Inherited manganism	**Cobalamin metabolism defects** - Imerslund-Gräsbeck disease - Intrinsic factor deficiency - TC II deficiency - Cbl C, Cbl D,Cbl E,Cbl F, Cbl G deficiencies - **Methionine synthase deficiency** **Folate metabolism defects** - Dihydrofolate reductase deficiency - Glutamate formimino transferase deficiency - **Congenital folate malabsorption** - **MTHDF1-deficiency** **Others** - **Hereditary orotic aciduria** - Mevalonic aciduria - Pearson syndrome (due to mitochondrial DNA deletion) (dyserythropoiesis) - Respiratory chain disorders - **Thiamine responsive megaloblastic anemia**	- Abetalipoproteinemia - Adenylate kinase deficiency (reticular dysgenesis) - Adenosine triphosphatase deficiency - **Carnitine transport defect** - **Congenital erythropoietic porphyria** - **Erythropoietic protoporphyria** - Di-metal transporter 1 deficiency (*SLC11A2)* - Endosomal ferri reductase (STEAP 3) - G**alactosemia** - **Glycolytic and pentose-P pathway deficiencies** - Hemochromatosis - IRIDA (*TMPRSS6* coding for Matriptase*)* - Lecithin cholesterol acyltransferase deficiency - Majeed syndrome *(LIPIN2)* (dyserythropoietic) - Mevalonic aciduria - Mitochondrial tyrosyl-tRNA synthetase deficiency (MLASA) - Pyroglutamic aciduria - Pyrimidine 5-nucleotidase deficiency - SEC23B-CDG (congenital dyserythropoietic anemia II) - Severe liver failure (all causes) - Sitosterolemia (with stomatocytes) - Transaldolase deficiency - **Wilson disease** - Wolman disease - **X-linked sideroblastic anemia (B6 responsive**)

White blood cells disturbances (Table 1.37)

Table 1.37 White blood cells

Pancytopenia - Thrombocytopenia - Leucopenia	Vacuolated Lymphocytes	Miscellaneous
- Aspartylglucosaminuria - Barth syndrome (neutropenia) - SLC35A-CDG (IIf) - Dursun syndrome (neutropenia) - **Gaucher disease type I** and III - **Glycogenosis type Ib** (neutropenia) - **Cobalamin metabolism defects** - **Folate metabolism defects** - Johansson-Blizzard syndrome - **Lysinuric protein intolerance** - **Organic acidurias** (methylmalonic, propionic, isovaleric in acute attacks) - All conditions with major splenomegaly - Pearson syndrome - Respiratory chain disorders - Adenylate kinase 2 deficiency (reticular dysgenesis with deafness) - Schwachman syndrome - Transaldolase deficiency	- Aspartylglucosaminuria - Multiple sulfatase deficiency - Ceroid lipofuscinosis - Chanarin Dorfman syndrome - I-cell disease - GM1-gangliosidosis - Mucopolysaccharidosis - Neutral lipid storage (Jordan anomaliy) - Niemann-Pick type Ia - Pompe disease - Sialidosis - Wolman disease	**Hyperleucocytosis (>100.000):** - Leucocyte adhesion deficiency syndrome (SLC35C1-CDG (IIc): GDP fucose transporter 1) **Hemophagocytosis**: - **Cobalamin C** - Gaucher disease - **Lysinuric protein intolerance** - Niemann-Pick disease - **Propionic acidemia** - **Methylmalonic aciduria**

1.6.6 Hepatology

Cholestatic Jaundice and cirrhosis (Table 1.38)

Table 1.38 Cholestatic jaundice and cirrhosis

Cholestatic Jaundice	Cirrhosis
- α-1-antitrypsin deficiency - Arginase deficiency - Byler disease - CDG - **Cerebrotendinous xanthomatosis** - Cholesterol synthesis defects (Smith-Lemli-Opitz syndrome) - **Citrin deficiency** - COG 6 and 7-CDG - Cystic fibrosis - **Galactosemia** - **Inborn errors of bile acid metabolism** - **LCHAD deficiency** - Methylacyl-CoA racemase deficiency - Mevalonic aciduria - **MEDNIK syndrome** - MEGDHEL syndrome - N-Glycanase deficiency - **Niemann-Pick disease type C** - Peroxisomal disorders - Transaldolase deficiency - **Tyrosinemia type I**	- Alpers progressive infantile poliodystrophy - α-1-antitrypsin deficiency - **Arginase deficiency** - Argininosuccinate lyase deficiency - CDG syndromes several types - Cholesterol ester storage disease - Cystic fibrosis - **MPI-CDG (Ib)** - **Galactosemia** - **Gaucher disease** - Glycogenosis type IV - Glycerol-3-phosphate dehydrogenase 1 deficiency - Hemochromatosis - **Hereditary fructose intolerance** - Hypermanganesemia with dystonia - **LCHAD deficiency** - Niemann-Pick disease - Peroxisomal disorders - S-adenosine homocysteine hydrolase deficiency - Transaldolase deficiency - **Tyrosinemia type I** - **Wilson disease** - Wolman disease

Liver Failure (Ascites, Edema) see ►Section 1.3.1, ►Section 1.4.1, and Table 1.6, Table 1.12)

Acute liver failure is defined as the rapid development of severe impairment of hepatic synthetic function including hypoalbuminemia (responsible for ascites and oedema) and the development of coagulopathy (prolonged blood prothrombin time and/or a prolonged blood activated partial thromboplastin time). When acute neurologic symptoms are present it may mimick a Reye like episodes (► Section 1.4.1). Less severe liver dysfunction includes abnormal biochemical markers of liver function (mostly transaminases: alanine ALT and aspartate AST aminotranferases) but without clinical symptoms (no ascites, no hemorragic syndrome). In the recently described *TMEM 199* mutations the adolescent individuals presented with a mild phenotype of hepatic steatosis, elevated aminotransferases and alkaline phosphatase, hypercholesterolemia, low serum ceruloplasmin and abnormal N- and mucin-type O-glycosylation (► Chapter 41).

Hepatomegaly and Hepatosplenomegaly without prominent hepatic dysfunction (► Section 1.3.1)

There are four mechanisms by which IEM can lead to hepatomegaly in pediatrics:

I. Storage (glycogen, neutral lipids, complex lipids),
II. Cholestasis,
III. Fibrosis/cirrhosis, and
IV. Inflammatory and immune processes.

A few clinical criteria allow an initial diagnostic approach:

I. Consistency of the liver (rock hard: cirrhosis; firm to hard: fibrosis and cholestasis; soft to normal: storage with or without splenomegaly)
II. Ultrasound findings (nodules, others.)
III. Clinical context: coarse facies and dysostosis, neurological deterioration, failure to thrive and gastrointestinal signs, inflammatory, immunologic or hematologic signs, and hypoglycemia.

A firm or rock-hard consistency may indicate tyrosinemia type I, galactosemia, GSD type IV, severe neonatal hemochromatosis, α_1-antitrypsin deficiency, Wilson disease, cystic fibrosis, Niemann-Pick and Gaucher disease.

When the liver consistency is normal or soft and there is associated splenomegaly (HSM), a LSD should be considered; coarse facies, bone changes, joint stiffness, ocular symptoms, vacuolated lymphocytes, and neurologic deterioration are strongly suggestive of the mucolipidoses and MPS. Failure to thrive, anorexia, poor feeding, severe diarrhea, hypotonia, hypothermia, and frequent infections are presenting signs in Niemann-Pick disease type A, Farber disease, Gaucher disease

type II, and Wolman disease, and also in chronic granulomatous disease, intrinsic factor deficiency, GSD type Ib, and lysinuric protein intolerance (LPI). HSM can be the only presenting sign in Gaucher disease type I and in Niemann-Pick disease type B (with asymptomatic interstitial pneumonia in the latter). Familial lipoprotein lipase presents with HSM, abdominal pain, xanthomas, acute pancreatitis and massive hypertriglyceridemia. In late infancy or childhood, HSM associated with myoclonic jerks, opthalmoplegia, and neurologic deterioration strongly suggest the late-onset forms of Niemann-Pick disease type C or subacute neuronopathic Gaucher disease type III. *CCDC 115* mutations may present with a storage-disease-like phenotype involving hepatosplenomegaly which regresses with age (▶ Chapter 41).

When hepatomegaly is not associated with splenomegaly, three clinical circumstances should be considered. Situations with fasting hypoglycemia suggest GSD type I or type III (in which the liver can extend down to the iliac crest) or Fanconi-Bickel syndrome (in which glycogenosis is associated with tubulopathy) (▶ Chapter 10); these patients have a doll-like appearance and short stature. FBPase deficiency is considered when hypoglycemia is associated with recurrent attacks of lactic acidosis triggered by fasting or by intercurrent infections. In argininosuccinic aciduria there can be hepatomegaly and failure to thrive that can mimic hepatic GSD (▶ Chapter 19).

Isolated hepatomegaly with a protuberant abdomen is a presenting sign of GSD type VI and IX (phosphorylase and phosphorylase b kinase deficiency) but may be also the only presenting sign in GSD type III. It is also observed in the rare entities, cholesteryl ester storage disease, Tangier disease, neutral lipid storage disorders (liver steatosis with myopathy) due to phospholipase (*PNPLA2*) deficiency, and Chanarin Dorfman syndrome (*ABHD5* mutations). Cytoplasmic glycerol 3 phosphate dehydrogenase 1 deficiency, presenting with isolated soft asymptomatic hepatomegaly and transient hypertriglyceridemia in infancy, has been recently described (▶ Chapter 34).

1.6.7 Immunology (see also ▶ Section 1.6 Neutropenia)

Combined immunodeficiencies (CID, SCID) involvingT/B cells, phagocytes deficiencies involving polymorphonuclear, monocytes or mastocytes, diseases of immune dysregulation and auto-inflammatory disorders are the main immunologic manifestations of IEM.

Some disorders are restricted to the immune system while some other are associated with extraimmune manifestations like deafness, anemia, dermatologic, osseous, or neurologic signs that may be preponderant.

Inflammatory syndrome, recurrent fever

- Hyper-IgD syndrome and mevalonate kinase deficiency (▶ Chapter 32):episodic fever and generalized inflammation, lymphadenopathy, hepatosplenomegaly,abdominal pain, arthralgia,myalgia, skin rash,
- Aicardi Goutières syndrome (altered cytokine expression) [57][58]),
- Majeed syndrome (*LPIN2* mutations) (▶ Chapter 34): Recurrent osteomyelitis and cutaneous inflammation with congenital dyserythropoietic anaemia,
- Fabry disease: bouts of fever (▶ Chapter 38),
- *PSMB8* mutations in proteasome: Autoinflammation,recurrent fever, nodular erythema, muscular weakness and progressive lipodystrophy [59],
- *HOIL1/LUBAC* mutations (involved inlinear ubiquitination): Autoinflammation, immunodeficiency, and amylopectinosis [60],
- *RBCK1* (E3 ubiquitin ligase) Autoinflammation with recurrent episodes of sepsis,
- COG7-CDG (malignant hyperthermia).

Macrophage activating syndrome, hemophagocytosis

- **Gaucher disease,**
- **Lysinuric protein intolerance**,
- Niemann-Pick disease type A and B,
- **Propionic acidemia.**

Severe combined immune deficiency (SCID)

As a predominant presenting sign (▶ Chapter 35)

- **Adenosine deaminase 1 deficiency** (with costochondral abnormalities),
- **Purine nucleoside phosphorylase** (with hypouricemia and developmental delay),
- **Adenylate kinase 2** (reticular dysgenesis with deafness),
- Cytidine deaminase deficiency (autosomal recessive type II hyper-IgM syndrome),
- Transferrin receptor 1 deficiency.

As as associated finding:

- **Hereditary orotic aciduria** (with megaloblastic anemia) (▶ Chapter 35),
- VICI syndrome,
- **Folate and B12 disorders** (▶ Chapter 27):

Hereditary folate malabsorption: The severity of immunodeficiency ranges from hypogammaglobulinemia to full-blown SCID phenotype.

Transcobalamin II deficiency (severe neutropenia, lymphopenia, hypogammaglobulinaemia ~SCID + failure to produce specific antibodies).

Methylene tetrahydrofolate dehydrogenase deficiency (MTHFD1), with atypical haemolytic uremic syndrome).

- Deletions of the *PLCG2* encoding phospholipase Cγ(2), an enzyme expressed in B cells, natural killer cells, and mast cells present with cold urticaria, immunodeficiency and autoimmunity [61] (▶ Chapter 34).

1.6.8 Myology

Many IEM can present with severe hypotonia, muscular weakness, and poor muscle mass. These include most of the late-onset forms of UCD and many OA. Severe neonatal generalized hypotonia and progressive myopathy with or without an associated nonobstructive idiopathic cardiomyopathy, can be the specific presenting findings in a number of inherited energy deficiencies; the most frequent conditions are mitochondrial RCD and other congenital hyperlactatemias, FAO defects, PBD, muscular GSD, alpha-glucosidase deficiency, and some other LSD (▶ Section 1.3.1). Hypotonia, generalized weakness, reduced muscle mass and developmental delay are also the presenting features of the Allan-Herndon-Dudley syndrome [55]. Several defects of cytoplasmic triglycerides and phospholipids synthesis present with congenital progressive myopathy (▶ Chapter 34). Choline kinase beta deficiency displays a congenital muscular dystrophy, characterized by early-onset muscle wasting, mental retardation and abnormal mitochondrial morphology (▶ Chapter 34). Severe neonatal hypotonia with elevated CK and brain dysfunction are major findings in most of the dolichol synthesis and recycling defects. All these disorders can be screened by routine characterization of transferrin glycosylation by isoelectric focusing (▶ Chapter 41). A congenital myasthenic syndrome pyridostigmine responsive can be a presenting sign in ALG2, ALG14, DPAGT1, GFPT1, and GMPPB-CDGs that bridges myasthenic disorders with dystroglycanopathies [62] (▶ Chapter 41). It has been also recently shown that *ISPD* mutations (coding for isoprenoid synthase containing domain) are a common cause of congenital and limb girdle muscular dystrophy [63].

Exercise Intolerance, Myoglobinuria, Cramps, Muscle Pain, elevated CK

See acute symptoms ▶ Section 1.4.1.

Myopathy (progressive)

There are many metabolic myopathies but only a few have an effective treatment.

- Adenylate deaminase deficiency,
- **Carnitine transport defect and fatty acid oxidation disorders**,
- **ETF, ETF dehydrogenase, FAD synthase and mitochondrial FAD transporter deficiencies**,
- Glycogenosis type II (acid maltase deficiency), Danon disease (LAMP-2),
- Glycogenosis type III,IV,0b (muscle type), *AMPK* mutations,
- Phosphoglucomutase deficiency,
- Allan-Herndon-Dudley syndrome (monocarboxylate transporter 8 deficiency),
- Choline kinase deficiency,
- Neutral Lipid Storage Diseases: ATGL and CGI-58 Deficiencies (Chanarin Dorfman syndrome),
- CDG syndromes: **DPAGT1- CDG**, ALG14 –CDG and ALG 2-CDG (myasthenic syndrome),
- Dolichol synthesis defects,
- *RBCK1* mutations (E3 ubiquitin ligase),
- Respiratory chain disorders (Kearns-Sayre, MLASA syndrome and others),
- *ISPD* mutations (isoprenoid synthase containing domain): limb girdle muscular dystrophy [63],
- Vici syndrome.

1.6.9 Nephrology (◘ Table 1.39)

Nephrolithiasis/nephrocalcinosis, polycystic kidneys, tubulopathy, abnormal urine color/odor) are the main renal manifestations of IEM.

Atypical Hemolytic Uremic Syndrome (HUS), nephrotic syndrome and tubulointerstitial nephropathy may also be presenting signs. *DGKE* mutations (coding for diacylglycerol kinase ε) responsible for HUS with nephrotic syndrome has been recently described and provides an interesting new mechanism of atypical HUS (▶ Chapter 34).

1.6.10 Neurology and Psychiatry

See also ▶ Section 1.4.1.

1.6.11 Ophthalmologic Signs

See also neuroophtalmologic signs, ▶ Section 1.5.

Cataracts

Metabolic causes of cataracts are presented according to age of onset in ◘ Table 1.40.

Corneal clouding

◘ Table 1.41

Ectopia Lentis (Dislocation of the Lens)

- **Classical homocystinuria**,
- Sulfite oxidase deficiency,
- Marfan syndrome,
- Marchesani syndrome.

Keratitis with corneal opacities

These are presenting signs of two treatable disorders:

- **Tyrosinemia type II**,
- **Fabry disease** (X-linked).

Miscellaneous

- Microcornea: Ehlers Danlos type IV
- Macular colobomata: **Familial hypomagnesemia** (▶ Chapter 37)
- Conjunctivitis, blepharitis: **Acrodermatitis enteropathica, cystinosis, tyrosinemia type II, PA, MCD**
- Alacrimia: N-glycanase 1 deficiency (▶ Chapter 41)

1

Table 1.39 Nephrology

Nephrolithiasis (stone composition)/Nephrocalcinosis	Polycystic kidneys	Tubulopathy	Urines (color,odor)	Miscellaneous
- APRT deficiency (2-8 dihydroxy adenine) - **Cystinuria** (cystine) - Hereditary hyperparathyroidism (calcium) - Hereditary renal hypouricemia (uric acid) - **Hyperoxaluria type I and II** (oxalic acid) - Lesh-Nyhan (uric acid) - Molybdenum cofactor deficiency (xanthine) - PRPP synthase superactivity (uric acid) - Renal tubular acidosis type I - **Xanthine oxidase deficiency** (xanthine) - Familial juvenile hyperuricemic nephropathy (uromodulin) - 5 Oxoprolinuria	- CDG syndromes - CPT II deficiency - Glutaric aciduria type II - Zellweger syndrome	**Fanconi syndrome**: - **Fructose intolerance** - **Galactosemia** - Respiratory chain disorders (complex III, IV, mitDNA deletion/depletion) - **Tyrosinemia type I** - Bickel Fanconi syndrome: Glut II - Lowe syndrome (X-linked *OCRL*1) - **Cystinosis** - **Wilson disease**	**Abnormal Color** - Alkaptonuria (black) - Indicanuria (blue) - Myoglobinuria (red) - **Porphyria** (red)	**HUS**: **Folate and cobalamin defects** (Cbl C, Cbl G, MTHFD1 - DGKE (▶ Chapter 34)
		Renal tubular acidosis: - **Renal tubular acidosis type I and II** - Pyruvate carboxylase deficiency - **Methylmalonic aciduria** - **Glycogenosis type I** - **CPT I deficiency** - Dent disease (*CLCN5* mutations) - Carbonic anhydrase II (proximal)	**Abnormal Odor** - Dimethylglycine dehydrogenase (fish) - 3-CH3-crotonylglycinuria (cat) - **Glutaric aciduria type II** (sweaty feet) - **Isovaleric acidemia** (sweaty feet) - **MSUD** (maple syrup) - **PKU** (musty odor) - **Trimethylaminuria** (fish) - **Tyrosinemia type I** (boiled cabbage)	**Nephrotic Syndrome**: - Respiratory chain disorders (coenzyme Q synthesis defects - DGKE
		Hypochloraemic alkalosis - Bartter and Gitelman syndromes - Congenital chloride diarrhea - Hupra syndrome (▶ Chapter 14)		**Nephropathy (tubulointerstitial)**: - **Glycogenosis type I** - MMA - Respiratory chain disorders (pseudo Senior-Loken syndrome)

HUS, Hemolytic uremic syndrome

1.6.12 Orthopedy (Table 1.42)

Over 20 IEM affecting the synthesis or remodeling of phosphatidylcholine, phosphatidylserine, phosphatidylinositol, plasmalogens or cholesterol present with major bone and cartilage involvement. Schematically, 3 clinical entities may be recognized: congenital bone dysplasia, overgrowth disorders and inflammatory presentations [48].

Congenital bone dysplasia

Beside rhizomelic chondrodysplasia punctate and defects of cholesterol (▶ Chapter 32) and plasmalogen biosynthesis (▶ Chapter 40), responsible for many bone dysplasia and malformative syndromes, several entities affecting phospholipids metabolism have been recently described (▶ Chapter 34). They involve mostly the synthesis, transport or activating pathways of phosphoserine and phosphatidyl inositides: (i) Lenz-Majewski syndrome,(ii) spondylometaphyseal dysplasia with cone-rod dystrophy, (iii) Yunis-Varon syndrome (iv) Opsismodysplasia, and v) the Schneckenbecken dysplasia, a severe spondylodysplastic disorders caused by mutations in *SLC35D1* or in the gene encoding inositol polyphosphate phosphatase-like 1 (*INPPL1*) [64].

Segmental overgrowth disorders with congenital lipomatosis

The molecular etiology of somatic overgrowth syndromes has been recently clarified and allowed the clinical delineation and

Table 1.40 Cataracts

Detectable at birth (congenital)	Detectable in the newborn period (<1 months)	Detectable in infancy (<1 years)	Detectable in childhood (1 to 15 years)	Detectable in adulthood (>15 years)
-Conradi Hunermann syndrome - Cockayne syndrome - Lowe syndrome (*OCRL1* X-linked mutation) - Peroxisomal biogenesis defects (Zellweger and variants) - Phosphoglycerate dehydrogenase deficiency - RCDP - Smith Lemli Opitz syndrome (rare) - Sorbitol dehydrogenase deficiency -Sengers syndrome (AGK) (may be isolated) - Vici syndrome	- **Galactosemias** - Marginal maternal galactokinase deficiency - Peripheral epimerase deficiency (homozygotes and heterozygotes) - Sjogren-Larsson syndrome (may be isolated) - Glucocerebrosidase II deficiency - Acetyl CoA carrier defect *(SLC33A1)* - B4GALNT1 (rare)	- Alpha-mannosidosis - ELOVL4 - Galactitol or sorbitol accumulation of unknown origin - **Galactokinase deficiency** - Hypoglycemia (various origins) - P5C synthetase deficiency - Respiratory chain disorders - Sialidosis - Steroid 5-α-Reductase 3 *(SRD5A3)* deficiency - Chanarin-Dorfman syndrome *(CGI-58)* - Sterol-C4-methyl oxidase deficiency	- Chanarin Dorfman syndrome - **Diabetes mellitus** - Dominant cataract with high serum ferritin - **Hypoparathyroidism** - **Lysinuric protein intolerance** - Mevalonic aciduria - Neutral lipid storage disorders - PHARC syndrome (*ABHD12*) - Pseudo-hypoparathyroidism - Sjögren-Larsson syndrome - **Wilson disease**	- Carriers for Lowe syndrome - **Cerebrotendinous xanthomatosis** - **Fabry disease** - G6PD deficiency - **Heterozygotes for GALT and galactokinase deficiency** - **Homocystinurias** - **Lactose malabsorbers** - Mevalonate kinase defect - Mitochondrial cytopathies - **OAT deficiency** - PEX 7 mutations - Refsum disease - Steinert dystrophy (cataract can be presenting sign) - **Tangier disease**

G6PD, glucose-6-phosphate dehydrogenase; *GALT*, galactose uridyl transferase; *RCDP*, rhizomelic chondrodysplasia punctata; *OAT*, ornithine aminotransferase deficiency

Table 1.41 Corneal clouding

Visible in early infancy (3 to 12 months)	Visible in late infancy to early childhood (1 to 6 years)	Visible in late childhood, adolescence to adulthood:
- **Tyrosinemia type II** (presenting sign) - **Cystinosis** (presenting sign) - Hurler, Scheie (MPS I) syndromes - I-cell disease (mucolipidosis type II) - Maroteaux-Lamy (MPS VI) - Steroid sulfatase deficiency	- Mucolipidosis type IV (presenting sign) - Alpha-mannosidosis defect (late-onset form) - Lecithin cholesterol acyl-transferase deficiency - Morquio disease (MPS IV) - Pyroglutamic aciduria (presenting sign) - **Tangier disease**	- **Fabry disease** (X-linked) - Galactosialidosis (juvenile form) - **Wilson disease** (green Kaiser Fleischer ring) also observed in cholestatic liver disease

MPS, mucopolysaccharidosis

natural history of the PIK3CA-related overgrowth spectrum [65][66] (▶ Chapter 34).

Inflammatory presentations

Recurrent aseptic necrosis and medullary infarction of long bones in an inflammatory context are a frequent diagnostic finding in Majeed syndrome (▶ Chapter 34) and Gaucher disease. These symptoms represent a major clinical concern.

The most frequent causes of osteopenia, bone infarction, punctate epiphyseal calcifications and exostosis and hyperostosis are listed in Table 1.42.

Table 1.42 Orthopedy

Osteopenia Bone infarction	Punctate Epiphyseal Calcifications Chondrodysplasia punctata	Exostosis and Hyperostosis
Osteopenia - **Cerebrotendinous xanthomatosis** - CDG - **Glycogenosis type I** - **Galactosemia** (long term) - **Gaucher type 1** - **Homocystinurias** - I-cell disease (mucolipidosis type II) - Infantile Refsum disease - **LPI** - **All organic acidurias** (chronic forms)	- Beta-glucuronidase deficiency - X-linked dominant chondrodysplasia punctate (Conradi-Hunermann syndrome) - Child syndrome - X-linked steroid sulfatase deficiency - Peroxysomal RCDP types I,II,III (with plasmalogen deficiency) - Familial resistance to thyroid hormone - Peroxisomal disorders (Zellweger and variants) - Spondylo enchondromatosis - Warfarin embryopathy	**Exostosis** - O-glycosylation defects (EXT1-EXT2) **Hyperostosis** - Hyperphosphatemic hyperostosis syndrome (GALNT3-CDG) - Osteopetrosis type 2 (carbonic anhydrase 2) - Lenz Majewski syndrome: Sclerosing bone dysplasia (*PTDSS1)* (▶ Chapter 34)
Bone infarction - **Gaucher disease type I** - Majeed syndrome (*LPIN2*)		

RCDP, rhizomelic chondrodysplasia punctata; *LPI*, lysinuric protein intolerance

1.6.13 Pneumology

- **Hyperventilation Attacks**
  - **Hyperammonemias**
  - Joubert syndrome
  - Leigh syndrome in acute attacks (due to many inborn errors)
  - **Metabolic acidosis**
  - Rett syndrome (girls only)

- **Interstitial pneumopathy is a frequent complication and may occasionally be the presenting sign in the following disorders:**
  - **Gaucher disease**
  - **Lysinuric protein intolerance**
  - Niemann-Pick disease type B
  - **CblC deficiency**
  - *MARS* mutations (methionyl t-RNA synthetase) [67]

- **Stridor**
  - **Biotinidase deficiency**
  - Hypocalcemia
  - Hypomagnesemia
  - **MADD** (riboflavin responsive)
  - Pelizaeus-Merzbacher
  - Farber disease (hoarseness)

- **Pulmonary hypertension**
  - Dursun syndrome (*G6PC3* mutations) with neutropenia
  - **Glycogenosis type I**
  - Non ketotic hyperglycinemia
  - HUPRA syndrome (with hyperuricemia see ▶ Section 1.4.2)
  - Gaucher disease
  - Mitochondrial disorders: *NFU1, LIPT1, TMEM70 mutations* (▶ Chapter 14)

1.6.14 Psychiatry

(See ▶ Section 1.4.1 and **Table 1.8**, **Table 1.17**, **Table 1.18**)

1.6.15 Rheumatology

- **Arthritis - Joint Contractures - Bone Necrosis**
  - Aicardi Goutières syndrome (chronic progressive deforming arthropathy with chilblains and contractures) [57]
  - Alkaptonuria
  - ***ANKH* mutations** (progressive ankylosis with deafness, mental retardation and hypophosphataemia)
  - Familial gout
  - Farber disease
  - **Gaucher disease type I**
  - **Homocystinuria**
  - I-Cell disease, mucolipidosis type III
  - Lesch-Nyhan syndrome
  - Mevalonic aciduria (recurrent crisis of arthralgia)
  - Mucopolysaccharidosis type I S
  - *NT5E* mutations and arterial and joint calcifications
  - PRPP synthetase, HGPRT defects
  - Uromoduline mutation (familial hyperuricaemic nephropathy)
  - Majeed syndrome *(LPIN2)*

- **Pain in extremities**

Bone Crisis: See ▶ Section 1.4.1.

1.6.16 Stomatology

- **Glossitis, stomatitis, mouth ulcers**
  - **CblF deficiency**
  - **Folate malabsorption**
  - **Intrinsic factor deficiency**
  - **Transcobalamin II**
  - Aicardi Goutières syndrome
- **Macroglossia**
  - Beckwith-Wiedemann syndrome
  - Congenital muscular dystrophies (DMC1C)
  - Complex IV deficiency
  - Pompe disease
  - Hurler syndrome (MPS 1S)
  - GM1 gangliosidosis
- **Hypodontia**
  - Leucoencephalopathy with ataxia
  - 4H syndrome (Table 1.23)
  - Heimler syndrome (*PEX 1,6* mutations): enamel hypoplasia (▶ Chapter 40).

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Inborn Errors of Metabolism in Adults: A Diagnostic Approach to Neurological and Psychiatric Presentations

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Late-onset forms of IEM presenting initially in adulthood are often unrecognised, so that their exact prevalence is unknown [1]. Most often they have psychiatric or neurological manifestations, including atypical psychosis or depression, unexplained coma, peripheral neuropathy, cerebellar ataxia, spastic paraparesis, dementia, movement disorders and epilepsy [2][3][4][5].

Physicians caring for adult patients with IEM are also involved in the management of those with early onset forms who reach adulthood. The transfer of such patients from paediatric to adult care raises a number of medical, dietetic and social concerns.

A further important issue is the diagnosis of adult patients who had their first clinical signs in childhood but for whom the diagnosis was missed, either because IEM were not considered or because the disease or its mild clinical form had not been described at that time.

2.1 Differences Between Paediatric and Adult Phenotypes

Adults' physicians who want to specialise in IEM are faced with the fact that, with the exception of several review articles, most if not all existing books and diagnostic algorithms refer to paediatric forms of these diseases. Late-onset forms of IEM tend to display attenuated phenotypes, which in some instances are associated with one or more clinical manifestations that differ from the classic clinical picture described in children. Table 2.1 gives some examples of differences between childhood and adult onset presentations. Although the limited information available about adult forms of IEM makes the specialty new and quite exploratory, the diagnostic approach in adults is facilitated by the fact that the nervous system is already mature. Therefore, clinical presentations are more homogeneous than in children, in whom clinical signs usually differ depending on their stage of maturation (▶ Chapter 1).

2.2 General Approach to IEM in Adulthood

As stated above, adult-onset presentations of IEM are essentially neurological or psychiatric. The typical situation is that of a patient with an unexplained and bizarre neurological or psychiatric problem in whom the usual aetiologies have been excluded by appropriate tests. The diagnostic approach in such a situation is always based on the two questions of when to suspect an IEM and, when an IEM is suspected, what type of metabolic investigations must be performed [6].

Some general clinical features are highly suggestive of an IEM: when clinical signs or symptoms are fluctuating, especially when triggered by fasting, exercise, fever, catabolic circumstances or post-partum; when clinical signs suggest a diffuse disease including neurological signs plus systemic signs (eye or skin problems, organomegaly etc.) or involvement of different parts of the nervous system (optic nerves and cerebellum; leukoencephalopathy and polyneuropathy).

In addition, some clinical signs are highly suggestive of a particular IEM or of a particular group of IEM. Some of these 'red flags' are listed in Table 2.2.

Unfortunately, in many circumstances, highly specific signs or symptoms are lacking and the presentation is that of a less specific neurological or psychiatric disorder (epilepsy, cognitive decline, psychiatric signs). In such situations, the diagnostic approach is based on the type of clinical signs, their clinical course (acute, acute-relapsing, with diurnal variations, progressive, static), brain MRI findings, eye findings and electroneuromyography. Some matching between clinical, imaging, ophthalmological and electrophysiological findings and IEM is shown in the text and tables below.

Metabolic diseases involving the nervous system can be divided into five main categories, all of which display some similarities in clinical presentation, diagnostic methods and treatment strategies.

2.2.1 Disorders of Energy Metabolism

Disorders of energy metabolism include respiratory chain disorders (that can be primary or secondary, as can occur in organic acidurias), pyruvate dehydrogenase deficiency, Krebs cycle deficiencies, GLUT1 deficiency, β-oxidation defects and disorders involving co-factors such as ETF deficiency, vitamin E deficiency, biotinidase deficiency, biotin-responsive basal ganglia disease, creatine deficiency syndromes and coenzyme Q synthesis defects. Acute manifestations are often triggered by infections and encompass Leigh syndrome, acute optic neuropathy, acute cerebellar ataxia, pseudo-strokes or status epilepticus. Chronic presentations often involve muscles, cerebellum, basal ganglia (parkinsonism, dystonia), cortex (epilepsy, myoclonus) or the peripheral nervous system (axonal polyneuropathy). In adults, the brain white matter is less involved and spastic paraparesis is uncommon.

2.2.2 Disorders of Lipid Metabolism

Disorders of lipid metabolism include sphingolipidoses (Krabbe disease, metachromatic leukodystrophy, Niemann Pick A and B, Fabry disease and Gaucher disease), peroxisomal disorders (adrenomyeloneuropathy, Refsum disease, disorders of pristanic acid metabolism, peroxisome biogenesis disorders), sterols disorders (cerebrotendinous xanthomatosis, Niemann-Pick C, spastic paraplegia type 5 and Tangier disease) and the newly described group of metabolic diseases affecting the synthesis and remodelling of phospholipids (mutations in *PLA2G6*, *DDHD1*, *DDHD2*, *NTE*, *CYP2U1*, *ABHD12*) and sphingolipids (mutations in *FA2H*, *GBA2*, *B4GALNT1*) [7][8] (▶ Chapter 34, ▶ Chapter 38, ▶ Chapter 40). Given the great proportion of lipids in the nervous system, these diseases can produce almost all kinds of symptoms but spastic paraparesis is very common. Leukodystrophy and demyelinating polyneuropathy are hallmarks of disorders interfering with myelin formation or maintenance. A past history

Table 2.1 Phenotypic differences between childhood-onset and adult-onset forms of inborn errors of metabolism

Disease	Classic presentation in childhood	Adult-onset forms
AGAT deficiency	Psychomotor delay, severe language impairment, failure to thrive and autistic-type behaviour	Mild mental retardation with myopathy
AMACR deficiency	Neonatal cholestasis, mental retardation, retinitis pigmentosa	Recurrent encephalopathy, epilepsy, psychiatric disorders, polyneuropathy
α-Mannosidosis	Mental retardation, deafness, upper airways infections, dysmorphic features	Episodes of psychosis, confusion, cerebellar ataxia, posterior leukoencephalopathy
Biotinidase deficiency	Muscular hypotonia, lethargy, grand mal and myoclonic seizures, ataxia, stridor, skin lesions	Bilateral optic atrophy, spastic paraparesis, motor neuropathy
Cerebral glucose transporter (GLUT1) deficiency	Epilepsy, psychomotor delay, dystonia, ataxia, acquired microcephaly	Isolated seizures, exercise-induced dystonia, lethargy triggered by fasting, dystonic tremor
Cobalamin C disease	Progressive encephalopathy, abnormal movements, epilepsy, comas, multisystem pathology (renal failure, hepatic dysfunction, cardiomyopathy), retinopathy, macrocytosis	Psychiatric problems, confusion, subacute myelopathy, peripheral neuropathy, thromboembolic events. MRI: normal or leukoencephalopathy, macrocytic anaemia is rare
Coenzyme Q10 deficiency	Leigh syndrome, myoglobinuria, encephalopathy	Cerebellar ataxia, myopathy
Cerebrotendinous xanthomatosis	Mental retardation, chronic diarrhoea, epilepsy, juvenile cataract, neonatal cholestasis	Tendon xanthomas, cerebellar ataxia, spastic paraparesis, dementia, psychiatric signs
Cystathionine β-synthase deficiency	Mental retardation, marfanoid habitus, epilepsy, autism, lens dislocation, scoliosis	Strokes (internal carotid dissection), deep vein thrombosis, psychiatric disorders
Fatty acid β-oxidation defects	Non-ketotic hypoglycaemia, cardiomyopathy, liver disease, rhabdomyolysis, peripheral neuropathy, retinitis pigmentosa (LCHAD)	Encephalopathy (MCAD), rhabdomyolysis, proximal myopathy
Fabry disease	Crises of acroparaesthesia	Strokes, vertigo, cardiomyopathy, hearing loss, proteinuria
GAMT deficiency	Epilepsy, movement disorders, mental retardation, behavioural problems	Isolated myopathy
Glutaric aciduria type 1	Encephalopathy or movement disorders with bilateral lesions of basal ganglia, dystonia predominates	Leukoencephalopathy with subependymal nodules, spastic paraparesis, cephalalgia, dysexecutive syndrome, peripheral neuropathy
Glycogenosis type IV (glycogen branching enzyme deficiency)	Neuromuscular form, combined hepatic and myopathic form	Polyglucosan body disease: spastic paraparesis, peripheral neuropathy, leukoencephalopathy with spinal cord atrophy
GM1 gangliosidosis	Dysmorphic features, organomegaly, macular cherry red spot, progressive spasticity, seizures, decerebrate posturing	Generalised dystonia, parkinsonism, dysarthria, kyphoscoliosis, vertebral and hip dysplasia. MRI: high signal of posterior putamen
GM2 gangliosidosis	Motor weakness, visual loss, progressive spasticity, macular cherry red spot, epilepsy	Psychosis, lower motor neuron disease, cerebellar ataxia, dystonia, sensory neuronopathy
Krabbe disease	Progressive encephalopathy, hyperaesthesia, tonic spasms, signs of peripheral neuropathy, blindness, loss of bulbar function, seizures	Spastic paraparesis with or without peripheral neuropathy, specific leukoencephalopathy involving cortico-spinal tracts
Lesch-Nyhan syndrome	Severe generalised dystonia, cognitive disability and self-injurious behaviour	Isolated dystonia, mild cognitive or behavioural problems
L-2-Hydroxyglutaric aciduria	Seizures, progressive ataxia, spasticity, mental retardation, progressive macrocephaly, leukoencephalopathy with cerebellar atrophy	Epilepsy, progressive dystonia and parkinsonism, leukoencephalopathy involving the subcortical white matter, malignant brain tumours
MERRF	Myoclonic epilepsy, generalised epilepsy, cerebellar ataxia	Cerebellar ataxia, hearing loss, peripheral neuropathy, lipomatosis

Table 2.1 (continued)

Disease	Classic presentation in childhood	Adult-onset forms
Metachromatic leukodystrophy	Progressive gait problems, hypotonia, peripheral neuropathy, spasticity in all four limbs, optic atrophy, cerebellar ataxia	Psychiatric form: »psychosis-like features (mimics schizophrenia), cognitive decline« Motor form: »spastic paraparesis, cerebellar ataxia, dystonia, demyelinating polyneuropathy«
3-Methylglutaconyl-CoA hydratase deficiency	Mental retardation or motor delay, movement disorders, febrile seizures	Ataxia, dementia, optic atrophy, spasticity, leukoencephalopathy
Maple syrup urine disease	Coma, failure to thrive	Episodes of vomiting, lethargy, ataxia triggered by fever
MTHFR deficiency	Progressive encephalopathy with apnoea, epilepsy, microcephaly	Psychiatric disorders, spastic paraparesis, thromboembolic events, polyneuropathy
Neurotransmitter defects (dopamine synthesis)	Seizures, mental retardation, oculogyric crises, abnormal movements	Focal or generalised dopa-responsive dystonia or parkinsonism
Niemann-Pick disease type C	Liver disease, hypotonia, psychomotor delay, epilepsy, spasticity, ataxia, cataplexy, vertical supranuclear gaze palsy	Psychosis, cognitive decline, cerebellar ataxia, vertical supranuclear gaze palsy, dystonia, isolated splenomegaly
Non ketotic hyperglycinemia	Epilepsy with suppression bursts, encephalopathy	Paroxysmal choreic movement disorders, confusion triggered by fever, mental retardation with aggressive behaviour
PDH deficiency	Lactic acidosis, corpus callosum agenesis, Leigh syndrome, polyneuropathy	Episodic ataxia triggered by fever, optic neuropathy, MRI can be normal
Peroxisome biogenesis defects	Mental retardation, liver disease, deafness, cerebral malformations, dysmorphic features (high forehead, epicanthic folds), skeletal abnormalities, retinopathy, cataracts, seizures	Various combinations of peripheral neuropathy, cerebellar ataxia, deafness, retinitis pigmentosa, leukoencephalopathy
Phenylketonuria (untreated)	Mental retardation, autistic behaviour, seizures, movement disorders	Spastic paraparesis, optic atrophy, dementia, parkinsonism
***POLG* mutations**	Severe encephalopathy with intractable epilepsy and hepatic failure	Ptosis, ophtalmoplegia, sensory neuronopathy, cerebellar ataxia
Thiamine transporter (*SLC19A3*) mutations	Biotin responsive basal ganglia disease (encephalopathy, coma, epilepsy, generalised dystonia)	Wernicke-like encephalopathy
Serine deficiency	Mental retardation, epilepsy, microcephaly	Polyneuropathy
Sialidosis type 1	Dysmorphic features, mental retardation, progressive encephalopathy	Action and stimulus-sensitive myoclonus, cerebellar ataxia
SSADH deficiency	Epileptic encephalopathy	Behavioural/psychiatric disorders, isolated seizures
Urea cycle disorders	Coma, failure to thrive	Nausea, vomiting, cephalalgia, confusion, psychiatric disorders, ataxia, stroke-like episodes, coma
Wilson disease	Hepatic failure	Psychiatric signs, tremor, parkinsonism, dystonia, dysarthria

Treatable disorders are shown in **boldface type**

Table 2.2 Examples of syndromes or signs with very high diagnostic value (see also ▶ Chapter 1)

Syndromes	Metabolic pathways involved
Neurological	
Recurrent coma of unknown cause	Urea cycle disorders (mainly)
Dopa-responsive dystonia	Monoamine synthesis defects
Acute or subacute myelopathy	Homocysteine remethylation defects
Exercise-induced paroxysmal dyskinesia	GLUT1 deficiency
Brain MRI	
Abnormally high signal of basal ganglia on T_2-weighted sequences (Leigh syndrome)	Energy metabolism defects (pyruvate dehydrogenase, respiratory chain, coenzyme Q10)
Abnormally low signal of basal ganglia on T_2-weighted sequences	Neurodegeneration with brain iron accumulation
Abnormally high signal of basal ganglia on T_1-weighted sequences	Disorders of manganese metabolism, porto-systemic shunts
Stroke-like episodes	Energy metabolism defects (mitochondrial DNA mutations, *POLG* mutations)
Ophthalmological	
Supranuclear gaze palsy	Lysosomal diseases (Gaucher, Niemann Pick C)
Bilateral optic neuropathy	Energy metabolism defects (pyruvate dehydrogenase, respiratory chain, organic acidurias), *PLA2G6* mutations
Macular cherry red spot	Sialidosis
Cataract	Cerebrotendinous xanthomatosis, *GBA2* mutations
Retinitis pigmentosa	Energy metabolism defects (respiratory chain), peroxisomal disorders, Sjögren Larsson syndrome, *NTE* mutations, *DDHD1* mutations
Cutaneous	
Progressive dysmorphia	Lysosomal diseases
Angiokeratoma	Lysosomal diseases
Xanthomata (Achilles tendons)	Cerebrotendinous xanthomatosis
Ichthyosis	Sjögren Larsson syndrome, Refsum disease, *ELOVL4* mutations
Visceral	
Splenomegaly	Lysosomal diseases, Tangier disease
Venous and arterial thrombosis	Hyperhomocystinemia
Gout, nephrolithiasis, tophi	Purine salvage (Lesch-Nyhan syndrome)
Past history of neonatal cholestasis	Sterols metabolism (Niemann-Pick C, hereditary spastic paresis type SPG5, cerebrotendinous xanthomatosis, alpha-methyl-acyl-CoA racemase deficiency), citrin deficiency

of prolonged neonatal jaundice is suggestive of disorders of sterols metabolism (▶ Chapter 1 ▶ Section 1.3.1). Splenomegaly is highly suggestive of Niemann-Pick B and C, Gaucher disease and Tangier disease.

2.2.3 Intoxication Syndromes

These include porphyrias, urea cycle defects, organic acidurias, aminoacidopathies and homocysteine remethylation defects. The occurrence of acute symptoms that accompany the metabolic crisis can be very characteristic. However, in mild adult forms, symptoms can be progressive giving rise to leukoencephalopathies, epilepsy, psychiatric disorders or spastic paraparesis.

2.2.4 Disorders of Neurotransmitter Metabolism

Disorders of neurotransmitter metabolism are mostly represented by defects in the synthesis of serotonin and dopamine. Clinical signs are related to dopamine deficiency (dystonia, parkinsonism, oculogyric crisis), noradrenergic deficiency (ptosis, myosis, hypotension) or serotonin deficiency (sleep disturbance, dysthermia, behavioural disturbance). Dopa-responsive dystonia or parkinsonism is highly suggestive. Diurnal fluctuations of symptoms are also characteristic, with improvement in the morning and worsening during the day. Diagnosis of these disorders relies on analysis of neurotransmitter metabolism in the CSF.

2.2.5 Metal Storage Disorders

Metal storage disorders include Wilson disease (interfering with copper metabolism), the group of NBIA (neurodegeneration with brain iron accumulation such as neuroferritinopathy, aceruloplasminaemia, *PANK2*-associated neurodegeneration and *PLA2G6* mutations) interfering, even if only partially, with iron metabolism and a recently identified disorder of manganese metabolism. The hallmark of these diseases is the metal deposits that occur in the basal ganglia and that are visible on brain MRI (▶ Chapter 1 ▶ Section 1.5.2). The main presentations are movement disorders because of the primary involvement of the basal ganglia. Treatments, when they exist, are mainly based on metal chelators.

2.3 Specific Approaches to Neurometabolic Presentations in Adults

The clinical diagnostic strategies are illustrated in the Sections below, starting from the main neurological and psychiatric syndromes seen in adults with IEM. For each syndrome, the signs (clinical or radiological) indicative of an IEM and the approach leading to the specific metabolic investigations are discussed (see also ▶ Chapter 1 ▶ Section 1.5.1).

Table 2.3 Diagnostic approach to metabolic causes of encephalopathy, strokes or pseudo-strokes (see also ▶ Chapter 1 ▶ Section 1.4.1)

Diseases	Encephalopathy/coma	Strokes or pseudostrokes
Energy metabolism disorders		
Respiratory chain disorders (MELAS and others)	+	+
Thiamine transporter (*SLC19A3*) mutations, PDH deficiency	+	
MCAD deficiency	+	
Intoxication syndromes		
Urea cycle disorders	+	+
Homocysteine RD	+	+
CBS deficiency		+
Acute intermittent porphyrias	+	
Lysinuric protein intolerance	+	
MSUD	+	
Non ketotic hyperglycinemia	+	
Lipid metabolism/storage		
AMACR deficiency	+	+
Fabry disease		+
Pompe disease		+

AMACR, α-methyl-acyl-CoA racemase; *CBS*, cystathionine-β-synthase; *MCAD*, medium-chain acyl-CoA dehydrogenase; *MELAS*, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; *MSUD*, maple syrup urine disease; *RD*, remethylation defects.
Treatable disorders are shown in **boldface type**

2.3.1 Encephalopathies/Comas

In a patient with an unexplained encephalopathy or coma, certain features are highly suggestive of an IEM, firstly when the encephalopathy is triggered by an external factor (surgery, fasting, exercise, high protein intake, new medication) and secondly when specific brain lesions are present on brain MRI [5] (▶ see also Chapter 1 ▶ Section 1.4.1).

Two main groups of IEM are responsible for encephalopathies in adults: intoxication syndromes and energy metabolism defects (Table 2.3). In the first group MRI is usually normal or shows nonspecific features (brain oedema, generalised leukoencephalopathy), whereas in the second group MRI is often abnormal, showing bilateral lesions of basal ganglia (Leigh syndrome) or stroke-like lesions.

In addition, some clinical signs suggest specific diagnoses. Encephalopathies in the context of urea cycle disorders, organic aciduria and aminoacidopathies are usually associated with gastrointestinal symptoms such as nausea or vomiting. Porphyria crises are associated with abdominal pain, acute neuropathy or hyponatraemia. Homocysteine remethylation defects cause acute or subacute myelopathy and are often preceded by psychiatric symptoms lasting for months or years.

Fatty acid oxidation disorders usually cause muscular symptoms; however, patients with MCAD deficiency can

present with isolated encephalopathies starting in adolescence or adulthood with normal MRI.

Lastly, α-methyl-acyl-CoA racemase (AMACR) deficiency can cause a very severe relapsing encephalopathy. Patients with this disease often have characteristic MRI findings including abnormal signals of the thalami and brain stem, with cortical lesions mimicking infectious encephalitis or pseudostrokes (▶ Chapter 40).

2.3.2 Strokes and Pseudostrokes

Some IEM cause ischaemic strokes in adulthood (see also ▶ Chapter 1 ▶ Section 1.4.1). This is the case in Fabry disease and homocystinurias. In the former, strokes typically involve small arteries of the vertebrobasilar system, leading to acute deafness, vertigo, diplopia, hemiplegia. In homocystinurias (cystathionine β-synthase deficiency) or homocysteine remethylation defects, thrombosis or dissection or large vessels (carotid arteries) is observed. Ischaemic brain lesions have also been reported in patients with Pompe disease. In addition, acute focal neurological signs mimicking strokes (pseudo-strokes) are very evocative of mitochondrial diseases, especially mitochondrial DNA mutations (MELAS, MERRF, NARP) and can also be seen in patients with urea cycle disorders and AMACR deficiency. These pseudo-strokes differ from real strokes in that they do not correspond to usual arterial territories and are often associated with signs of encephalopathy, including cephalalgia, confusion and epileptic seizures. A good way to distinguish pseudostrokes from true ischaemic strokes is diffusion imaging: the diffusion coefficient is typically normal or increased in the former and decreased in the latter.

2.3.3 Movement Disorders

In patients with movement disorders, an IEM should be suspected in several situations: (1) when a patient displays several types of abnormal movements (example dystonia + parkinsonism); (2) when movement disorders are associated with other neurological signs (epilepsy, dementia, etc.); (3) when dystonia involves the orofacial region; (4) when bilateral lesions of the basal ganglia are observed on brain MRI; and (5) when paroxysmal movement disorders are triggered by fasting and exercise [9] (◘ Table 2.1 and ◘ Table 2.2).

Generally, a particular movement disorder can be seen in many different IEM, and conversely, a given IEM can present with different abnormal movements. As a consequence, the classic phenomenological diagnostic approach to movement disorders (i.e. dystonia, parkinsonism, chorea, myoclonus) is less applicable to the diagnosis of IEM. As in the case of acute encephalopathies, brain MRI can help the diagnostic approach. A T2 hypersignal of the basal ganglia suggests an energy metabolism disorder (see above) whereas a T2 hyposignal of the pallidum suggests NBIA [10] (◘ Table 2.5). Diffuse T2-hypersignal involving thalami, brain stem and cerebellar peduncles are suggestive of Wilson disease.

When MRI is normal, the diagnostic approach can be based on the course of the disease. Dystonia or parkinsonism with diurnal fluctuations suggests a neurotransmitter metabolism defect. Paroxysmal dystonia triggered by exercise is highly suggestive of GLUT1 deficiency but can also be observed in PDH deficiency. In addition, paroxysmal dyskinesias (not triggered by exercise) have been observed in several IEM, including Wilson disease and neurotransmitter metabolism defects.

2.3.4 Peripheral Neuropathies

Peripheral neuropathies in the context of IEM are often labelled 'Charcot-Marie-Tooth disease'. These types of neuropathy are characterised by long-standing chronic, predominantly motor, distal and symmetrical polyneuropathy with claw toes, diffuse, and severe homogeneous electrical abnormalities. IEM should be suspected in such patients if the neuropathy is associated with other incongruous neurological signs (leukoencephalopathy, ataxia, pyramidal signs, psychiatric or visual signs) or with systemic disease (skin problems, xanthomas, splenomegaly, cataract). In some cases, peripheral neuropathies may be acute or relapsing, and may involve multiple nerves (mononeuropathy, multiplex), motor neurons or dorsal root ganglia [11] (◘ Table 2.6).

Two main groups of metabolic diseases give rise to peripheral neuropathies: lipid storage disorders and energy metabolism defects. In lipid storage disorders, both the peripheral and central myelin can be involved, leading to a leukoencephalopathy seen on brain MRI. In contrast, defects of energy metabolism are mostly responsible for axonal peripheral neuropathies and are usually associated with other signs of energy metabolism defects (cerebellar ataxia in the case of respiratory chain disorders). Many exceptions to this schematic view exist, however. MNGIE (mitochondrial neurogastrointestinal encephalomyopathy) syndrome caused by thymidine phosphorylase deficiency (▶ Chapter 35) is typically responsible for a demyelinating polyneuropathy. Some lipid storage disorders, such as cerebrotendinous xanthomatosis (▶ Chapter 33), adrenomyeloneuropathy and other peroxisomal diseases, may cause polyneuropathies that can be axonal, demyelinating or both. Metabolic neuropathies may also present as autosomal dominant diseases such as the hereditary sensory and autonomic neuropathy type 1 (HSAN1) related to mutations in the gene encoding serine palmitoyltransferase and leading to toxic accumulation of abnormal sphingolipids [12] (▶ Chapter 38) and mutations in the α-N-acetyl-glucosaminidase (*NAGLU*) gene associated with a late-onset painful sensory neuropathy [13]. Acute polyneuropathies mimicking Guillain-Barré syndrome can be observed in acute attacks of porphyria and in pyruvate dehydrogenase deficiency, acute exacerbations of Refsum disease or untreated tyrosinaemia type 1. Painful peripheral neuropathy involving small fibres is reminiscent of Fabry disease, Tangier disease, GM2 gangliosidosis and porphyria. Motor neuron involvement mimicking spinal muscular atrophy is characteristic of late-onset Tay-

Table 2.4 Diagnostic approach to metabolic causes of movement disorders

Disease	Parkinsonism	Dystonia	Chorea	Myoclonus	Paroxysmal dystonia
Energy metabolism disorders					
Respiratory chain disorders	+	+	+	+	
Adenylate cyclase (*ADCY5*)		+	+	+	+
PDH deficiency	+	+	+		+
GLUT1 deficiency		+	+		+
BBGD (*SLC19A3*)		+			
Vitamin E deficiency		+			
Lipid metabolism disorders					
Cerebrotendinous xanthomatosis	+		+		
Niemann-Pick type C	+	+	+	+	
GM1 gangliosidosis	+	+			
GM2 gangliosidosis	+	+	+		
Gaucher disease	+	+		+	
CYP2U1 mutations (SPG56)		+			
FA2H mutations (SPG35)		+			
B4GALNT1 mutations (SPG26)		+			
Intoxication syndromes					
Phenylketonuria	+				
Homocystinuria	+	+	+		
L-2-Hydroxyglutaric aciduria	+	+			
Neurotransmitter metabolism defects					
GTP cyclohydrolase-1 deficiency	+	+			+
Tyrosine hydroxylase deficiency	+	+			
Dopamine transporter deficiency	+	+			
PTP synthase deficiency		+			+
Sepiapterin reductase deficiency	+	+			
Non-ketotic hyperglycinaemia			+		
Metal storage disorders					
Wilson disease	+	+	+		+
Aceruloplasminaemia	+	+	+		
Panthotenate kinase deficiency	+	+	+		
Neuroferritinopathy	+	+	+		
Manganese metabolism disorder (*SLC30A10*)	+	+			
PLA2G6 mutations	+	+			
Others					
Ceroid-lipofuscinosis	+	+		+	
Lesch-Nyhan disease		+	+		
Sialidosis				+	

BBGD, biotin-responsive basal ganglia disease; *PDH*, pyruvate dehydrogenase; *PTP*, 6-pyruvoyl-tetrahydropterin. Treatable disorders are shown in **boldface type**

Table 2.5 Diagnostic approach to metabolic causes of basal ganglia lesions on brain MRI

Diseases	Pallidum	Thalamus	Putamen	Brain stem nuclei	Dentate nuclei
Energy metabolism disorders					
Respiratory chain disorders	+	+	+	+	+
BBGD (*SLC19A3*)			+	+	
PDH deficiency	+	+	+	+	+
Co-enzyme Q10 deficiency			+	+	
Mitochondrial thiamine pyrophosphate transporter (*SLC25A19*)			+		
CoA synthase deficiency (COASY)	+	+	+		
Lipid storage disorders					
Cerebrotendinous xanthomatosis	+				+
AMACR deficiency		+		+	
GM1 gangliosidosis			+		
Fabry disease		+			
Intoxication syndromes					
Methylmalonic/propionic aciduria	+				
SSADH deficiency	+				+
Urea cycle disorders	+				+
Glutaric aciduria type 1			+		
Metal storage disorders					
Wilson disease	+	+	+	+	+
Aceruloplasminaemia	+	+	+	+	+
Neuroferritinopathy	+		+	+	+
PANK2 mutations	+				
PLA2G6 mutations	+			+	
FA2H mutations (SPG35)	+				
Manganese metabolism disorder (*SLC30A10*)	+		+		+

AMACR, α-methylacyl coenzyme A racemase; *BBGD*, biotin-responsive basal ganglia disease; *SSADH*, succinic semialdehyde dehydrogenase deficiency. Treatable disorders are shown in **boldface type**

Sachs disease. Lastly, involvement of dorsal root ganglia is highly suggestive of *POLG* mutations (mtDNA polymerase γ). In summary, the type of metabolic investigations is mainly based on the type of the peripheral neuropathy (demyelinating versus axonal), its topography and course and on brain MRI results.

2.3.5 Leukoencephalopathies

The first step in the diagnostic approach of leukoencephalopathies is to search for acquired, potentially treatable causes. These causes are numerous and include inflammatory, infectious, metabolic, neoplastic, paraneoplastic, toxic or vascular diseases. In metabolic leukoencephalopathies, lesions are usually bilateral and symmetrical involving specific white matter tracts (pyramidal tracts, cerebellar peduncles, U-fibres, etc.). Furthermore, the existence of an associated polyneuropathy is highly suggestive of an IEM [14][15][16].

Table 2.6 Diagnostic approach to metabolic causes of peripheral nervous system

Diseases	Demyelinating	Axonal	Motor neurone involvement	Small fibres	Dorsal root ganglion	Acute	Mononeuropathy multiplex
Energy metabolism disorders							
Respiratory chain disorders		+			+		
MNGIE	+						
PDH deficiency		+				+	
Vitamin E deficiency		+			+		
β-Oxidation defects (LCHAD, TFP)		+			+		
Biotinidase deficiency			+			+	
Lipid storage/oligosaccharidoses							
Cerebrotendinous xanthomatosis	+	+					
GM2 gangliosidosis			+	+			
Fabry disease				+			
Metachromatic leukodystrophy	+						
Krabbe disease	+	+					
Adrenoleukodystrophy/adrenomyeloneuropathy	+	+					
Refsum disease	+					+	+
ABHD12 mutations (PHARC)	+						
AMACR deficiency	+	+					
Peroxisome biogenesis defects	+	+					
Tangier disease				+		+	+
β-Mannosidosis	+						
Serine palmitoyltransferase mutations (HSAN1)		+	+	+			
PLA2G6 mutations		+					
CYP2U1 mutations (SPG56)		+					
GBA2 mutations (SPG46)		+					
B4GALNT1 mutations (SPG26)		+					
NTE mutations (SPG39)		+					
Presynaptic choline transporter (*SLC5A7*)		+					
Others							
Serine deficiency		+					
Homocysteine RD	+	+			+		
APBD		+	+				
Acute porphyrias		+		+		+	+
CDG syndromes		+					
Tyrosinaemia type 1						+	

AMACR, α-methylacyl coenzyme A racemase; *APBD*, adult polyglucosan body disease; *CDG*, congenital disorders of glycosylation; *HSAN1*, dominant hereditary sensory and autonomic neuropathy; *LCHAD*, long-chain 3-hydroxyl-CoA dehydrogenase; *PDH*, pyruvate dehydrogenase; *MNGIE*, mitochondrial neurogastro intestinal encephalomyopathy; *RD*, remethylation defects; *TFP*, trifunctional protein.
Treatable disorders are shown in **boldface type**

Table 2.7 Diagnostic approach to metabolic causes of leukoencephalopathies

Diseases	Periventricular	Pyramidal tracts	Cerebellum	Spinal cord	Juxtacortical	Brain stem	Corpus callosum
Lipid storage							
Metachromatic leukodystrophy	+						+
Adrenoleukodystrophy		+					+
Krabbe disease		+					+
Cerebrotendinous xanthomatosis	+	+	+			+	+
Refsum disease	+						
AMACR deficiency						+	
Peroxisome biogenesis disorder	+	+				+	
α-Mannosidosis	+						
Niemann-Pick C	+						
Intoxication syndromes							
Homocysteine RD	+			+	+		
Phenylketonuria	+						+
Glutaric aciduria type 1	+				+		
L-2-Hydroxyglutaric aciduria					+		
3-methylglutaconyl-CoA hydratase deficiency					+		
Disorders of energy metabolism							
Respiratory chain	+	+	+		+	+	+
APBD	+	+	+	+		+	
MNGIE	+				+		
DARS2 mutations	+	+	+	+		+	
Others							
Wilson disease			+			+	

AMACR, α-methylacyl coenzyme A racemase; *APBD*, adult polyglucosan body disease; *MNGIE*, mitochondrial neurogastrointestinal encephalomyopathy.
Treatable disorders are shown in **boldface type**

The diagnostic approach to genetic leukoencephalopathies should be guided by the clinical examination, the MRI aspect and electroneuromyographic studies (see also ▶ Chapter 1 ▶ Section 1.5.2). Some IEM are responsible for a specific pattern of leukoencephalopathy (Table 2.7). In general, two main groups of IEM are responsible for leukoencephalopathies: lipid storage disorders and aminoacidopathies/organic acidurias. In the first group, brain abnormalities are usually restricted to specific tracts within the deep brain white matter and the cortico-spinal tracts, cerebellar peduncles, corpus callosum and optic radiations, while U-fibres (juxtacortical fibres) are relatively spared. Furthermore, involvement of the peripheral nerves (polyneuropathy) is usually present (see paragraph »Polyneuropathies«). In contrast, aminoacidopathies and organic acidurias involve the peripheral nerves only exceptionally (one exception being homocysteine remethylation defects), and brain abnormalities usually extend to U-fibres.

2.3.6 Epilepsy

Although epilepsy is a frequent presentation of IEM in neonates and children, several IEM may also manifest in adults with onset of epileptic seizures, but these are usually observed as part of a larger clinical spectrum.

In a patient with epilepsy, several clinical, radiological or electrophysiological features suggest an IEM: (1) the form of epilepsy does not match with any classic epileptic syndrome, i.e. atypical electroclinical presentation, mixture of generalised and partial epileptic manifestations (e.g. association of myoclonus and partial seizures); (2) progressive myoclonic epilepsy; (3) association with other neurological impairments (cerebellar, pyramidal, etc.), with unexplained mental retardation, or with other organ disorders; (4) seizures that are related to the times of eating (fasting, protein rich meal); (5) inefficacy of or worsening with classic antiepileptic drugs; (6) unexplained status epilepticus; (7) abnormalities on proton magnetic resonance spectroscopy, e.g. creatine deficiency or increased lactate; (8) slowing of the background activity on the EEG, photo-paroxysmal responses during the photic intermittent stimulation at low frequencies (1-6 Hz) [17].

The three main groups of IEM presenting with epilepsy in adults are disorders of energy metabolism, intoxications and LSD (Table 2.8). Myoclonic epilepsy suggests lysosomal disorders or certain respiratory chain disorders (MERRF syndrome). Partial motor or occipital seizures are frequent in respiratory chain disorders together with slow waves predominating in posterior brain regions. Tonic-clonic seizures are often observed in intoxications but are not really specific.

2.3.7 Psychiatric Disorders

IEM frequently present with psychiatric diseases in adolescents or adults. Retrospective analysis of patients with various IEM shows that psychiatric signs may remain isolated for years before more specific organic involvement becomes obvious. Since psychiatrists' awareness of these rare disorders is low, IEM presenting with a pure psychiatric illness are often missed. In most cases, treatments are more effective at the 'psychiatric stage' of the disease, before the development of irreversible neurological lesions.

The diagnosis is especially difficult when psychiatric signs are initially isolated, without a family history or clinical somatic involvement. In addition, it is sometimes difficult, in a patient with physical signs, to determine whether psychiatric problems are due to the same disease or not. Furthermore, physical signs may not be evident after a simple clinical examination (as examples, leukodystrophies may be missed if a brain MRI is not performed, peripheral neuropathy, cataract or xanthomas may not be symptomatic, organomegaly is often missed clinically in an adult). It is therefore important to determine which psychiatric symptomatology points to an IEM and should lead to further investigations (Table 2.9). Diseases can be schematically classified into three groups [18][19].

Group 1 includes diseases with acute and recurrent attacks of confusion and behavioural changes, which are usually associated with physical signs (gastrointestinal signs, cephalalgia, dysautonomia, pyramidal signs, alteration of consciousness). This group corresponds mainly to intoxications (urea cycle defects, homocysteine remethylation defects and porphyrias) but also energy defects (mitochondrial diseases).

Table 2.8 Diagnostic approach to metabolic causes of epilepsy

Diseases	Generalised or focal epilepsy	Progressive myoclonic epilepsy
Energy metabolism disorders		
Respiratory chain disorders (MERRF, MELAS, NARP, *POLG* and others)	+	+
GAMT deficiency	+	
GLUT1 deficiency	+	+
***SLC19A3* mutations**	+	
Lipid metabolism/storage		
Cerebrotendinous xanthomatosis	+	
Niemann-Pick C	+	+
Gaucher type 3	+	+
Ceroid lipofuscinosis	+	+
LIMP2 deficiency	+	+
Sialidosis	+	+
Lafora disease	+	+
Intoxication syndromes		
Homocysteine RD	+	
L-2-Hydroxyglutaric aciduria	+	
SSADH deficiency	+	
Acute intermittent porphyrias	+	
Others		
Hyperinsulinism-hyperammonaemia	+	+

GAMT, guanidinoacetate N methyl transferase; *LIMP2*, lysosomal integral membrane protein type 2; *MELAS*, mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke; *MERRF*, myoclonic epilepsy with ragged red fibers; *NARP*, neuropathy, ataxia, and retinitis pigmentosa; *RD*, remethylation defects; SSADH, succinate semialdehyde dehydrogenase. Treatable disorders are shown in **boldface type**

Therefore, plasma ammonia, homocysteine and lactate should be measured in unexplained acute psychiatric presentations.

Group 2 is made up of diseases with isolated psychiatric signs arising in adolescence or adulthood in a previously non symptomatic patient. This group includes hyperhomocystinaemia (homocysteine remethylation defects and cystathionine β-synthase deficiency) and lipid metabolism disorders (metachromatic leukodystrophy, GM2 gangliosidosis, Nie-

Table 2.9 Diagnostic approach to metabolic causes of psychiatric disorders

Diseases	Adult-onset psychiatric disorders without mental retardation	Behavioural/psychiatric disorders with mental retardation
Energy metabolism disorders		
Respiratory chain disorders	+	+
Creatine transporter deficiency		+
Intoxication syndromes		
Urea cycle disorders	+	+
Homocysteine remethylation defects	+	+
CBS deficiency	+	+
Acute intermittent porphyrias	+	
Non ketotic hyperglycinemia		+
SSADH deficiency		+
Phenylketonuria	+	+
Lipid storage/oligosaccharidoses/MPS		
Niemann-Pick C	+	
GM2 gangliosidosis	+	
Metachromatic leukodystrophy	+	
Adrenoleukodystrophy	+	
Cerebrotendinous xanthomatosis	+	+
β-Mannosidosis		+
α-Mannosidosis	+	+
Ceroid lipofuscinoses	+	+
MPS type III (San Filippo syndrome)		+
AMACR deficiency	+	
Metal storage disorders		
Wilson disease	+	
Aceruloplasminaemia	+	
Neuroferritinopathy	+	
PANK2 mutations	+	+

AMACR, α-methyl-acyl-CoA racemase; *CBS*, cystathionine-β-synthase; *MPS*, mucopolysaccharidoses; *SSADH*, sucinate semialdehyde dehydrogenase.
Treatable disorders are shown in **boldface type**

mann-Pick type C disease, adrenoleukodystrophy and cerebrotendinous xanthomatosis). Patients in this group may initially present with recurrent psychotic attacks, chronic delusion or disorganised behaviour, which may mimic schizophrenia. It also includes behavioural and personality changes. The diagnosis is particularly difficult in this group given the relative non-specificity of psychiatric signs, especially when they remain isolated for years or decades. However, catatonias, visual hallucinations, fluctuating symptoms, mental confusion, resistance or even deterioration with treatments and associated cognitive decline constitute atypical features that suggest an IEM.

Group 3 includes patients with mild mental retardation from childhood and disorders of behaviour or personality without a definite psychiatric syndrome. This group includes chronic intoxications (homocystinurias, non ketotic hyper-

glycinaemia, succinic semialdehyde dehydrogenase deficiency), some neurotransmitter metabolism defects, (monoamine oxidase A deficiency and probably disorders of serotonin synthesis), and some miscellaneous diseases (creatine transporter deficiency, α- and β-mannosidosis, MPS III).

Given the important number of IEM presenting with chronic psychiatric symptoms, minimal investigations include brain MRI, ophthalmological examination, abdominal ultrasonography, electromyogram as well as plasma biomarkers such as homocysteine, cholestanol and oxysterols (cholestane-3β,5α,6β-triol and 7-ketocholesterol).

2.3.8 Spastic Paraparesis

Spastic paraparesis is a general term describing progressive stiffness and weakness in the lower limbs caused by pyramidal tract degeneration. This clinical situation is frequently encountered in adult neurology. The diagnostic strategy (Table 2.10) is usually limited to searching for acquired causes (spinal cord compression, inflammatory, metabolic, infectious diseases) and the so-called hereditary spastic paraplegias (HSP). To date, more than 70 forms of HSP have been identified, with various modes of inheritance [20]. HSP are clinically classified as »uncomplicated« (or »pure«) when symptoms are limited to spastic paraparesis and as »complicated« (or »syndromic«) when accompanied by other neurological or systemic signs.

However, although poorly recognised by neurologists, spastic paraparesis is also one of the many presentations of IEM in children and adults [21]. Pyramidal signs are usually included in a diffuse neurological or systemic clinical picture, but in some cases spastic paraparesis remains the only symptom for years. In a patient with spastic paraparesis some signs are suggestive of an IEM: (1) when a polyneuropathy is present on EMG; (2) when a leukoencephalopathy is present on MRI; (3) when the course is acute or subacute, with sensory ataxia suggesting subacute degeneration of the spinal cord.

Two groups of IEM give rise to spastic paraparesis: (1) disorders of lipid metabolism and (2) intoxication syndromes, including homocysteine remethylation defects. In the first group, polyneuropathy and leukoencephalopathy are often present. It should be noted that dopamine synthesis defects (guanosine-5'-triphosphate [GTP] cyclohydrolase and tyrosine hydroxylase deficiencies) can produce dystonia mimicking spastic paraparesis in the lower limbs. In such cases, treatment with levodopa is highly effective in alleviating the symptoms.The first metabolic autosomal dominant form of HSP has also been recently identified in patients with mutations in *ALDH18A1* encoding delta-1-pyrroline-5-carboxylate synthetase (P5CS) who present with hypocitrullinaemia [22]. The exploration of spastic paraparesis should therefore comprise the measurement of plasma ammonia, amino acids, homocysteine, vitamin B12, folate, very long chain fatty acids, pristanic and phytanic acids, oxysterols (27- and 25-hydroxycholesterol) and cholestanol.

Table 2.10 Diagnostic approach to metabolic causes of acute myelopathy or spastic paraparesis

Diseases	Chronic	Acute
Lipid metabolism		
Cerebrotendinous xanthomatosis	+	
CYP7B1 mutations (SPG5)	+	
Adrenoleukodystrophy	+	
Krabbe disease	+	
Peroxisomal biogenesis disorder	+	
DDHD1 mutations (SPG28)	+	
DDHD2 mutations (SPG54)	+	
CYP2U1 mutations (SPG56)	+	
NTE mutations (SPG39)	+	
FA2H mutations (SPG35)	+	
GBA2 mutations (SPG46)	+	
B4GALNT1 mutations (SPG26)	+	
PGAP1 mutations (GPI-Anchor synthesis pathway)	+	
Intoxication syndromes		
Phenylketonuria	+	
Arginase deficiency	+	+
Triple H syndrome	+	
ALDH18A1 mutations	+	
Homocysteine RD	+	+
L-2-Hydroxyglutaric aciduria	+	
Neurotransmitter metabolism defects		
GTP cyclohydrolase 1 deficiency	+	
Tyrosine hydroxylase deficiency	+	
Others		
Biotinidase deficiency		+
APBD	+	

APBD, adult polyglucosan body disease; *RD*, remethylation defects. Treatable disorders are shown in **boldface type**

2.3.9 Cerebellar Ataxia

Except for focal cerebellar lesions, the many causes of cerebellar ataxia include inflammatory diseases, paraneoplastic disorders, acquired metabolic disorders, alcohol intoxication, multiple system atrophy, and genetic diseases (Friedreich ataxia, dominant spinocerebellar ataxias etc.). Cerebellar ataxia in the context of IEM may be

Table 2.11 Diagnostic approach to metabolic causes of cerebellar ataxia

Diseases	Chronic cerebellar ataxia	Spinocerebellar ataxia	Episodic or acute ataxia	Myoclonic ataxia
Energy metabolism disorders				
Respiratory chain disorders	+	+	+	+
PDH deficiency	+		+	
Vitamin E deficiency	+	+		
Co-enzyme Q10 deficiency	+			
***SLC19A3* mutations (Wernicke-like)**			+	
Lipid metabolism/oligosacccharidoses				
Cerebrotendinous xanthomatosis	+	+		
Niemann-Pick type C	+			+
GM2 gangliosidosis	+			
Gaucher type 3	+			+
Adrenomyeloneuropathy		+		
Refsum disease	+			
NTE mutations (SPG39)	+	+		
Acyl-CoA oxidase deficiency	+			
PEX10 mutations	+			
α-Mannosidosis	+			
Sialidosis				+
Abetalipoproteinaemia	+	+		
Intoxication syndromes				
Urea cycle disorders			+	
Urocanase deficiency			+	
Mevalonate kinase deficiency	+			
Others				
Hartnup disease			+	

PDH, pyruvate dehydrogenase. Treatable disorders are shown in **boldface type**

acute, triggered by fever (PDH deficiency, respiratory chain disorders or *SLC19A3* mutations) [23], or chronic (Table 2.11). Chronic cerebellar ataxia is rarely pure. In practice, several situations are usually encountered.

- Associated polyneuropathy: the association of cerebellar ataxia with an axonal polyneuropathy suggests a disorder of energy metabolism (*POLG* mutations, NARP syndrome, PDH deficiency, etc.) or a peroxisomal disease (PEX10). Association with a demyelinating polyneuropathy suggests a neurolipidosis such as Refsum disease or cerebrotendinous xanthomatosis.
- Association with a pyramidal syndrome and a proprioceptive ataxia (so-called spinocerebellar ataxia) suggests lipid metabolism disorders (cerebrotendinous xanthomatosis or adrenomyeloneuropathy).
- Isolated cerebellar ataxia can be the only presenting sign of GM2 gangliosidosis or of Niemann-Pick disease type C.

2.3.10 Myopathy

Metabolism within muscles is very different from that of the nervous system (Table 2.12). Except for mitochondrial disorders that can affect both the muscle and the nervous system, diseases affecting the muscle usually spare the nervous system. Hallmarks of metabolic myopathies are exercise intolerance

Table 2.12 Diagnostic approach to metabolic causes of myopathies

Diseases	Permanent weakness	Exercise intolerance and/or myglobinuria	Cardiomyopathy
Glycogen storage disorders			
McArdle disease (GSD-V)		+	
Pompe disease (GSD-II)	+		+
Debranching enzyme (GSD-III)	+		
Branching enzyme (GSD-IV)	+		
Glycolysis defects		+	+
Respiratory chain disorders			
MELAS	+	+	+
MERRF	+	+	+
MNGIE	+		
PEO-Kearns Sayre	+	+	+
POLG mutations	+		
Cytochrome B deficiency		+	
Fatty acid oxidation defects			
VLCAD deficiency	+		+
ETF and ETFDH deficiencies	+	+	
TFP deficiency		+	
CPT2 deficiency		+	
Primary carnitine deficiency	+		+
Other lipids metabolism			
Lipin 1 deficiency (*LPIN1*)		+	
Barth syndrome (*TAZ*)	+		+
CHKB mutations	+		+
PNPLA2 mutations (neutral lipid storage disorder)	+		+
Others			
Cystinosis	+		
AGAT deficiency	+		
GAMT deficiency	+		
AMACR deficiency		+	

AGAT, I-Arginine glycine amidinotransferase; *AMACR*, α-methyl-acyl-CoA racemase; *CPT2*, carnitine palmitoyltransferase 2; *ETF*, electron transfer flavoprotein; *ETFDH*, electron transfer flavoprotein dehydrogenase; *GAMT*, guanidinoacetate N-methyltransferase; *MELAS*, mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke; *MERRF*, myoclonic epilepsy with ragged red fibers; *MNGIE*, mitochondrial neurogastrointestinal encephalomyopathy; *PEO*, progressive external ophthalmoplegia; *TFP*, trifunctional protein; *VLCAD*, very long-chain acyl-CoA dehydrogenase.
Treatable disorders are shown in **boldface type**

(exertional cramps or fatigue) and recurrent rhabdomyolysis [24][25] (see also ▶ Chapter 1 ▶ Sections 1.4.1 and 1.6.8). However, presentations may be less specific, with progressive proximal myopathy or cardiomyopathy. Muscle histology may be suggestive of a metabolic disorder when it shows ragged red fibres, lipid droplets or high glycogen content with PAS staining. The four main groups of metabolic diseases affecting muscle are (1) mitochondrial disorders, (2) fatty acids β-oxidation defects (FAOD), (3) glycogen storage disorders (GSD), and (4) complex lipid synthesis disorders.

Mitochondrial diseases may show a wide range of manifestations including exercise intolerance with premature fatigue or myalgia out of proportion to weakness. These symptoms are frequently associated with progressive external ophthalmoplegia, which is highly suggestive of *POLG* mutations or other mtDNA deletion syndromes.

FAOD may manifest with rhabdomyolysis triggered by prolonged exercise or prolonged fasting, but progressive proximal weakness with lipid storage is also a common presentation in adults. Lipin deficiency 1 has been observed only in few adults so far (▶ Chapter 34).

Clinical presentations of muscle glycogenoses are various, ranging from exercise intolerance to isolated progressive muscle weakness. Patients with McArdle disease typically exhibit premature fatigue and contractures, frequently accompanied by muscle breakdown. A sign considered pathognomonic of this disease is the »second wind phenomenon«, which is a marked improvement in exercise tolerance about 10 minutes into aerobic exercise involving large muscle masses (jogging or cycling).

Adipocyte triglyceride lipase (ATGL) deficiency (*PNPLA2* mutations) gives neutral lipid storage with myopathy typically presenting in young adults with weakness and fatty infiltration of muscle or with cardiomyopathy. Weakness can be proximal, distal or generalized. The disease is progressive: some patients are athletic in childhood. Jordan anomaly on a blood smear is highly diagnostic (▶ Chapter 34).

2.3.11 Others

■ Table 2.13 and ■ Table 2.14 summarise other important signs, including sensorial disorders and miscellaneous presentations that can be helpful in determining which investigations should be undertaken.

■ **Table 2.13** Diagnostic approach to metabolic causes of deafness/visual problems

Diseases	Deafness	Corneal deposits	Retinitis	Macula cherry red spot	Optic nerve disorders	Cataract	Gaze palsies
Energy metabolism disorders							
Respiratory chain disorders	+		+		+	+	+
β-Oxidation defects (LCHAD, TFP)			+				
Vitamin E deficiency			+				
Biotinidase deficiency	+				+		
PDH deficiency					+		
Intoxication syndromes							
Homocysteine RD			+		+		
CBS deficiency						+	
Phenylketonuria					+		
Galactokinase deficiency						+	
Lipid storage							
Niemann-Pick C	+						+
Metachromatic leukodystrophy					+		
Adrenoleukodystrophy	+				+		
Gaucher disease							+
Fabry disease	+	+				+	
Cerebrotendinous xanthomatosis						+	
GBA2 mutations (SPG46)						+	
Sjögren-Larsson disease			+			+	
ELOVL4 mutations			+				

Table 2.13 (continued)

Diseases	Deafness	Corneal deposits	Retinitis	Macula cherry red spot	Optic nerve disorders	Cataract	Gaze palsies
DDHD1 mutations (SPG28)			+				
NTE mutations (SPG39)			+				
ABDH12 mutations (PHARC)	+		+			+	
FA2H mutations (SPG35)					+		
Acyl-CoA oxidase deficiency			+			+	
AMACR deficiency			+				
Refsum disease	+		+			+	
Peroxisome biogenesis disorder	+		+		+	+	
Mucopolysaccharidosis	+	+	+			+	
Fish eye syndrome		+					
Cystinosis		+					
α-Mannosidosis	+						
β-Mannosidosis	+						
Sialidosis type 1				+			
Metal storage disorders							
Wilson disease		+				+	
Aceruloplasminaemia			+				
PANK2 mutations			+				
PLA2G6 mutations					+		
Others							
CDG syndrome (PMM2-CDG)			+				
Ceroid lipofuscinoses			+		+		

AMACR, α-methylacyl-CoA racemase; *CBS*, cystathionine β-synthase; *CDG*, congenital disorders of glycosylation; *LCHAD*, long-chain L-3 hydroxyacyl-CoA dehydrogenase; *PDH*, pyruvate dehydrogenase; *RD*, remethylation defects; *TFP*, trifunctional protein.
Treatable disorders are shown in **boldface type**

Table 2.14 Diagnostic approach to metabolic causes of miscellaneous presentations

Diseases	Dysautonomia	Gliomas	Aseptic meningitides	Pseudotumor cerebri	Abdominal pain	Ichthyosis
Energy metabolism disorders						
Respiratory chain disorders	+		+			
MNGIE	+				+	
APBD	+					
Succinate dehydrogenase		+				
L-2-Hydroxyglutatic aciduria		+				
Intoxication syndromes						
Acute porphyrias	+				+	
Galactokinase deficiency				+		

Table 2.14 (continued)

Diseases	Dysautonomia	Gliomas	Aseptic meningitides	Pseudotumor cerebri	Abdominal pain	Ichthyosis
Lipid storage						
Fabry disease			+		+	
Sjogren-Larsson						+
ELOVL4 mutations						+
GM2 gangliosidosis	+					
Cystinosis	+			+		
Others						
CDG syndrome (ALG6-CDG)				+		

APBD, adult polyglucosan body disease; *CDG*, congenital disorders of glycosylation; *MNGIE*, mitochondrial neurogastrointestinal encephalomyopathy.
Treatable disorders are shown in **boldface type**

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Diagnostic Procedures

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3.1 Introduction

Unlike most other genetic disorders, IEMs are usually diagnosed from biochemical analyses prior to molecular testing. Basal metabolic investigations remain the gold standard for many clinical presentations (hypoglycaemia, liver disease, epilepsy, neurodevelopmental delay, movement disorders, neuro-sensorial deficit, peripheral neuropathy, etc.). If an IEM is suspected, then blood, urine and cerebrospinal fluid should be collected for the appropriate investigations (▶ Chapter 1, ▶ Fig. 1.1). If no material is available or if the results are ambiguous, a provocative test that challenges a metabolic pathway may provide clues to a diagnosis and indicate which specific enzymatic or genetic analysis should be undertaken. Functional tests are dynamic investigations based on the measurement of intermediary metabolites in body fluids. They are most useful in disorders that give rise to toxicity or energy deficiency. The best functional test is elicited by nature itself during episodes that cause metabolic stress, including acute infection, inadvertent fasting, or consumption of a nutrient that induces a metabolic intolerance.

With its unprecedented throughput, scalability, and speed, next-generation sequencing changes profoundly our diagnostic approaches in rare diseases, including IEM. Dedicated gene panels, but also whole exome sequencing, are now becoming first line investigations in some diagnostic centres. However, due to very complex dataset resulting from such large testing – including variants in several candidate genes – the strength of metabolic investigations relies on their ability to functionally validate genetic variants. Metabolic biomarkers are also invaluable for the therapeutic follow-up of many inborn errors of metabolism.

3.2 Basal Metabolic Investigation

3.2.1 Amino and organic acids

The initial evaluation of amino acid disorders usually requires contemporaneous analysis of amino acids in blood and urine. For acute disorders, samples should be taken as early as possible after the patient's arrival. For chronic disorders, a fasting blood sample (in the morning or at least 4 hours after last meal) and 24 hour urine collection are usually preferred. Samples collected under different conditions (e.g. post-prandial blood) or using other fluids (mainly CSF) may also be useful (e.g. encephalopathy).

Similarly, for the initial investigation of organic acid disorders, a urine sample should be collected as soon as possible after the onset of acute symptoms for analysis or for storage at -20°C if the analysis cannot be performed immediately. In non acute situations, a 24-h or a 12-h overnight collection is usually the first investigation.

For these analyses, strict conditions for collection, handling and storage of the samples are necessary to prevent artefactual changes. For example, in a plasma sample that is badly preserved, cystine and homocystine will bind to protein, leading to a falsely low level, and hydrolysis of asparagine and glutamine will result in falsely high concentrations of aspartic and glutamic acids and low concentrations of glutamine. Specific total homocysteine measurement for detecting a slight increase in homocystine is better than an amino acid chromatography which detects only free homocystine.

The concentration of each metabolite should be considered not only in absolute terms but also compared with the laboratory's age-related reference values and relative to other constituents. In many cases, the final interpretation needs knowledge of the clinical and nutritional status, and the conditions of sampling. This is best achieved by close co-operation between clinician and biochemist.

Some clues for the interpretation of the main variations in amino acids are given in ◘ Table 3.1. The main abnormal organic acids that may be found in IEM are given in ◘ Table 3.2. Abnormal acylcarnitine profiles are discussed in ▶ Chapters 12 and ▶ Chapter 18.

3.2.2 Metabolic Profile over the Course of the Day

▪ Indications

This assessment may be performed when an initial or recurrent clinical presentation is associated with a disturbance in intermediary metabolism such as hypoglycaemia, hyperlactataemia, hyperketosis or hypoketosis. In these situations, it should always be undertaken before any provocative test that might lead to metabolic decompensation. The metabolic profile is also used for monitoring treatment in many disorders.

▪ Procedure

Blood samples from an indwelling venous catheter (kept patent with a saline infusion) are taken before and after meals, and once during the night, as outlined in ◘ Table 3.3. To allow a reliable interpretation of the results, the correct method of sampling and processing of blood and urine is specified in ◘ Table 3.3. It is important to record the conditions under which sampling is undertaken, for example local or general anoxia may influence the results for lactate, lactate/pyruvate ratio and ammonia measurements.

Continuous glucose monitoring over a period of 2–3 days during normal activities, using a highly portable subcutaneous probe and recording device, is commonly used in the assessment of individuals with known glycogen storage disease, but is also useful in the investigation of patients who have symptoms at home that may or may not be related to hypoglycaemia [1].

▪ Interpretation

This investigation may show abnormalities in the metabolic and endocrine profiles throughout the day or specifically only during either the fasting or fed states. The data must be compared to age related reference values [2][3]. All physiological (food refusal) or pathological conditions (malnutrition, cardiac, renal or liver failure) that may influence the results need to be taken in account.

Table 3.1 Interpretation of main plasma amino acids variations

Plasma Amino Acid	Variation	Other Plasma Amino Acids	Investigations in other fluids	Diagnoses
Alanine	↑	See Gln, Pro, Gly		Hyperlactacidemia
Arginine	↑	Gln ± ↑, Cit ± ↓, Orn ↓	U: ± ↑	Arginase deficiency
	↓	Gln ± ↑, Pro ↓, Cit ↓, Orn± ↓		P5CS deficiency
		Orn ↓, Lys ↓	U: ↑++, Orn ↑, Lys ↑ UOA: Orotic ↑	LPI
Argininosuccinic acid	±↑	Gln ± ↑, Cit ± ↑	U: ASA ↑	ASLD late-onset form
	↑	Gln ↑, Cit ↑	U: ASA ↑++	ASLD neonatal form
Asparagine	↓	All normal	CSF ↓	Asn synthetase deficiency
Branched chain AA	↑	no Alle, other AA ± ↓		Starvation
		no Alle, other AA ± ↑		Fed state
		Alle +++, Ala ↓	U: ↑	MSUD
		Alle± ↑, Ala ↑, Gln ↑	UOA: Lac ↑, 2KG ↑	E3 deficiency
	↓	All normal	CSF ↓	BCKAD kinase deficiency
		Met ↑, Tyr ↑		Hepatic failure
		Cit ↑, Cys2 ↑, 3Mhis ↑		Renal failure
Citrulline	↑ or ↓	See Gln		UCDs
	↓	Gln ± ↑, Pro ↓, Arg ↓, Orn± ↓		P5CS deficiency
	↓	All normal		ATP synthase deficiency (NARP)
	↑	Cys2 ↑, 3MHis ↑, BCAA ↓ Alone ± ↑		Renal failure Heterozygote ASS
Cys2	↓+++	SulfoCys ↑	U:SulfoCys ↑, Tau ↑	Sulfite oxidase deficiency
	↑	Cit ↑, BCAA ↓, 3MHis ↑		Renal failure
	N	All normal	U:Cys ↑, Lys ↑, Orn ↑, Arg ↑	Cystinuria
Glutamine	↑	Cit ↓, Orn ↓,Arg ↓	UOA: Orotic ↑ (OTC,OAT)	Mitochondrial UCD, Neonatal OAT deficiency
		Cit ↓ or N, Ala ↑, Pro ↑	P: Lac ↑ UOA : Orotic N, 3-OHP ↑, PG ↑, MC ↑, 3-MCG ↑	CA-VA deficiency
		Cit ↑+++	U: Cit ↑+++	ASSD
		Cit ↑, ASA ↑	U: Cit ↑, ASA ↑	ASLD
		Cit N, Arg ↑	U: Arg N or ↑	ARGD
		Cit ↓, Orn ↑, HomoCit ↑	U: Orn N or ↑, Homocit ↑	HHH
		Cit ± ↑, Orn ↓, Lys ↓,Arg ↓	U: Cit ↑, Orn ↑, Lys ↑, Arg↑	LPI
	N or ↓	Cit ± ↑, Ala ↑, Lys ↑		PC deficiency
		Cit ↑,Thr ↑,Orn ↑, Lys ↑, Arg ↑		Citrin deficiency
		Cit N, Gly↑, Ala↑, Lys↑	U: Abnormal organic acids	Organic acidurias
	↓ +++		CSF: ↓ +++	Glutamine synthesis deficiency

Table 3.1 (continued)

Plasma Amino Acid	Variation	Other Plasma Amino Acids	Investigations in other fluids	Diagnoses
Glycine	↑	Alone	CSF: ↑, U ↑	NKH
		Ala ↑	CSF: N, U ↑	Valproate
		Ala ↑±, 2-aminoadipate	CSF: ↑, U ↑ P: Lac ↑ UOA: Lac ↑, 2KG ↑, 2KA ↑	Lipoic acid synthesis deficiency (LIAS, BOLA3, GLRX5, NFU1)
		Gln N, Ala ↑, Lys ↑	CSF: N, U ↑, UOA	Organic acidurias
		Thr ↑ ±	CSF: Gly ↑ ±, Thr ↑ ±	PNPO deficiency
Histidine	↑	Alone	U: ± ↑	Histidase deficiency
Homocystine	± ↑	All normal		Secondary to Q12, folate deficiency
	↑	See Methionine		
Lysine	↑ ++	Alone		Saccharopine dehydrogenase deficiency
	↑	Gln ↑, NH3 ↑		Urea cycle disorders
		Gln ↓, NH3 ↑, Ala ↑,Cit ↑±		PC deficiency
	↓	Gln ↑, Cit ± ↑, Orn ↓, Arg ↓	U: Cit ↑, Orn ↑, Lys ↑, Arg ↑ UOA: Orotic ↑	LPI
Methionine	↓	HCy2 ↑	U: HCy2 ↑ alone	MTHFR deficiency
			UOA: MMA ↑	Cobalamin metabolism
	↑	Alone		MATD,SAHHD,ADKD, GNMTD
		HCy2 ↑, Cys2 ↓, Disulfide	U: HCy2 ↑	CBS deficiency
		Tyr ↑, BCAA ↓		Hepatic failure
Ornithine	↑	Alone	U: ± ↑	OAT deficiency
		Gln ↑, Cit ↓, HomoCit ± ↑	U: Orn ± ↑, HomoCit ↑	HHH syndrome
	↓	Gln± ↑, Pro ↓, Cit ↓, Arg ↓		P5CS deficiency
		Arg ↓, Lys ↓	U: Cit ↑, Orn ↑, Lys ↑, Arg ↑ UOA: Orotic ↑	LPI
Phenylalanine	↑ +++	Tyr ↓		PKU
	↑ ±	Tyr ± ↓		Biopterin synthesis disorders
Pipecolic	↑	All normal	CSF: Pip ± ↑, P: VLCFA CSF: Pip ± ↑, P and U: αAASA	Peroxisomal diseases αAASADH deficiency
Proline	↑	Alone	U: N or ↑	Hyperprolinemia I
		Alone	U: N or ↑, P5C ↑	Hyperprolinemia II
		Alone		SLC25A22 deficiency
		Ala ↑		Hyperlactacidemia
	↓	Cit ↓, Orn ↓, Arg ↓		P5CS deficiency
Serine	↓	All others N	CSF: ↓	Serine synthesis deficiency
Sulfocysteine	↑	Cys2 low Cys2 very low	U: SulfoCys 0, all AA normal U: SulfoCys ↑, Tau ↑	Anticoagulant Sulfite oxidase deficiency

Table 3.1 (continued)

Plasma Amino Acid	Variation	Other Plasma Amino Acids	Investigations in other fluids	Diagnoses
Threonine	↑	See Glycine		PNPO deficiency
Tyrosine	↑ +++	Alone	U: ↑ alone	Tyrosinemia type II
	↑	Met ↑, BCAA ↓	UOA: succinylacetone 0	Hepatic failure
			UOA: succinylacetone +	Tyrosinemia type I

Def, deficiency; ±, sligthly modified
AA, amino acids; *αAASA*, alpha-amino adipic semi aldehyde; *Ala*, alanine; *2-aminoAd*, 2-aminoadipate; *Alle*, alloisoleucine; *Arg*, arginine; *ASA*, argininosuccinic acid; *Asn*, asparagine; *BCAA*, branched chain amino acids (valine, isoleucine, alloisoleucine, leucine); *BCKAD*, branched-chain ketoacid dehydrogenase; *CA-VA*, carbonic anhydrase VA; *Cit*, citrulline; *Cys2*, cystine; *Disulfide*, disulfide cysteine-homocysteine; *Gln*, glutamine; *Gly*, glycine; *Hcy2*, homocystine; *HomoCit*, homocitrulline; *2KA*, 2 ketoadipate; *2KG*, 2-ketoglutarate; *Lac*, lactate; *Lys*, lysine; *MC*, methylcitrate; *Met*, methionine; *3-MCG*, 3-methylcrotonylglycine; *3MHis*, 3-methylhistidine; *MMA*, methyl-malonic acid; *NH3*, ammonia; *3-OHP*, 3-hydroxy-propionic; *OAT*, ornithine aminotransferase; *Orn*, ornithine; *P5C*, Δ1-pyrroline-5-carbox-ylate; *PG*, propionylglycine; *Pip*, pipecolic; *PNPO*, pyridox(am)ine-5-phosphate oxidase; *Pro*, proline; *SulfoCys*, sulfocysteine; *Tau*, taurine; *Thr*, threonine; *Tyr*, tyrosine; *UOA*, urine organic acids; *VLCFA*, very long chain fatty acids; *αAASADH*, alpha-aminoadipic semialdehyde dehydrogenase; *ADKD*, adenosine kinase deficiency; *ARGD*, arginase deficiency; *ASLD*, argininosuccinic lyase deficiency; *ASSD*, argin-inosuccinic synthetase deficiency; *BCKDD*, branched-chain ketoacid dehydrogenase kinase deficiency; *CA-VA*, carbonic anhydrase; *CBS*, cystathionine-β-synthase; *GNMTD*, glycine N-methyltransferase deficiency; *HHH*, triple H syndrome (Hyperammonemia, Hyperornith-inemia, Homocitrullinuria); *LIAS*, Lipoic acid synthetase; *LPI*, lysinuric protein intolerance; *MATD*, methionine s-adenosyltransferase deficiency; *MSUD*, maple syrup urine disease; *MTHFR*, methylene tetrahydrofolate reductase; *NARP*, neuropathy, ataxia, and retinitis pigmentosa; *NKH*, non ketotic hyperglycinemia; *OAT*, ornithine aminotransferase; *PC*, pyruvate carboxylase; *P5CS*, Δ1-pyrroline-5-car-boxylate synthase; *PKU*, phenylketonuria; *PNPO*, pyridox(am)ine 5′-phosphate oxidase; *SAHHD*, S-adenosylhomocysteine hydrolase deficiency; *SLC25A22*, potential transporter of P5C, glutamate-γ-semialdehyde or glutamate; *UCD*, urea cycle disorder

Table 3.2 Interpretation of urine organic acids analysis

Principal OA	Other OAs	Causes of variation	Other investigations
Adipic	Very high isolated	Non metabolic (plastifier?)	
	See 3-OH- butyric and EMA lines. Remark:		
	- if Adipic > Sebacic	Ketosis, beta oxidation disorder	
	- if Adipic < Sebacic	MCT supplementation	
Dicarboxylic acids (DCA)	See Adipic, 3-OH-n-butyric and EMA lines		
3,6-Epoxyoctanedioic	Other epoxy (C10, C12, C14), 2-OH-sebacic, DCA with Ad>Sub	Peroxisomal diseases	
	Idem but Ad<Sub	MCT supplementation	
Ethylmalonic	>20 µmol/mmol C alone	SCAD deficiency	Acylcarnitines
	>20 µmol/mmolC ± IBG, 2MBG, IVG	Valproate, RC, GAII, ETHE1	
	>100 µmol/mmolC + IBG, nBG, 2MBG, IVG, HG, SG, 2OHG, DCA, Glut	Glutaric aciduria type II	
	>100 µmol/mmolC + nBG	SCAD deficiency	
Fumaric	High ± succinate, malate	Fumarase deficiency	
	± High with other KC derivatives + lactate	Respiratory hain disorders	
Glutaric	3-OH-glutaric	Glutaric aciduria type I	Acylcarnitines
	EMA, 2-CH3succinic, IBG, nBG, 2MBG, IVG, HG, SG, DCA, 2-OHG	Glutaric aciduria type II	

Table 3.2 (continued)

Principal OA	Other OAs	Causes of variation	Other investigations
Glyceric	Glyceric	Glycerate kinase deficiency	
	L isomer	D-glycerate dehydrogenase or glyoxylate reductase def (hyperoxaluria type II)	
Glycolic	Oxalic	Type I oxalosis	
	4HB	SSADH deficiency	
	Lactic, ethyleneglycol	Ethyleneglycol intoxication	
Hexanoylglycine	High ± and SG ±	Mild or asymptomatic MCAD deficiency	Acylcarnitines
	High + SG + DCA	MCAD deficiency	
	High + SG + DCA + EMA + Glut + IBG + EMBG + IVG + nBG	Glutaric aciduria type II	
Homogentisic	Alone	Alcaptonuria	
3-Hydroxy-n-butyric	High ++, AcAc, DCA	Ketosis (starvation, diabetes)	AACp
	High ±, DCA, 3HDC	Hepatic failure	AACp
	Low, DCA, 3HDC, ± acylglycines	Fatty acid oxidation defects	Redox + acylcarnitines
4-Hydroxy-butyric	Alone	Drug addiction	
	4,5 diOH-hexanoic lactone and acid, 3,4-diOH-butyric, 2,4-diOH-butyric, glycolic	Succinic semialdehyde dehydrogenase deficiency	
3-Hydroxy-dicarboxylic acids (3HDC)	See 3-Hydroxy-n-butyric line		
2-Hydroxy-glutaric	Very high	D or L-2-OH glutaric aciduria	
	High ± acylglycines	Glutaric aciduria type II	Acylcarnitines
	Moderately high	Respiratory chain disorders	
3-Hydroxy-glutaric	Glutaric normal or high	Glutaric aciduria type I	
	3-OH-butyric elevated	Ketosis	
3-Hydroxy-isobutyric	2-Ethylhydracrylic	3-OH-isobutyric dehydrogenase def	
2-Hydroxy-isovaleric	2-OH-3-CH3Val, 2-OH-isocaproic, 2-oxo-isovaleric, 2-oxo-3-CH3Val, 2-oxo-isocaproic, AcLeu, AcIle	Maple syrup urine disease	AACp
3-Hydroxy-isovaleric	Slightly elevated	Valproate treatment	
	See: - 3-Hydroxy-propionic - Isovalerylglycine - 3-Methyl-crotonylglycine - 3-Methyglutaconic - 3-Methyl-3-OH-glutaric		
3-Hydroxy-propionic	Alone	Bacterial infections	AACu
	PG, TG, MC, (2M3KB, 2M3HB, 3HIV)	Propionic acidemia	
	PG, TG, MC, 3MCG, (2M3KB, 2M3HB, 3HIV)	Biotinidase or holocarboxylase synthetase deficiency	
	Lactic, PG, MC, 3MCG	CA-VA deficiency	Ammonia plasma AACp Redox
	MMA, PG, TG, MC, (2M3KB, 2M3HB, 3HIV)	Methylmalonic aciduria (different causes)	AACp+u

Table 3.2 (continued)

Principal OA	Other OAs	Causes of variation	Other investigations
Isovalerylglycine	3-OH-isovaleric	Isovaleric acidaemia	Acylcarnitines
	Other acylglycines, glutaric, EMA	Glutaric aciduria tpye II, *ETHE1* mutations	
2-ketoglutaric	Alone	2-KGD deficiency TPK1 deficiency SLC25A19 transporter deficiency E3 deficiency DOOR syndrome	Redox
	Lactic, BCKA, BCHA	E3 deficiency	Redox AACp
	Lactic, KC der	Respiratory chain disorders	Redox
	Lactic, Glut, TG, 2OHG,3OHG, 2-oxoAD, 2OHAd	Lipoic acid synthesis deficiency	AACp Redox
Lactic	Alone	Bacterial infections	AACp+u
	2HIB, 2HB, Pyr, KC derivatives	Respiratory chain disorders	Redox AACp
	KC derivates + 3MG	Pearson, respiratory chain disorders	CAAp+u
	High KB, low or very low KC derivatives	PC deficiency	AACp Redox
	3-OHProp, PG, MC, 3MCG, 3HIV	CA-VA deficiency	Ammonia plasma AACp Redox
	Glut, TG, 2OHG,3OHG, TG, 2-oxoAD, 2-OH-adipic, 2KG	Lipoic acid synthesis deficiency	AACp Redox
	Other specific organic acids	Organic acidurias	
Malonic	Alone	Malonyl-CoA decarboxylase deficiency	
	+ Methylmalonic	ACSF3 def	
3-Methyl-crotonyl-glycine	3-OH-isovaleric	3-CH3-crotonyl-CoA carboxylase deficiency	Acylcarnitines
3-Methyl-glutaconic	Very High + 3-CH3-glutaric	3-CH3-glutaconyl-CoA hydratase deficiency	
	3-CH3-glutaric ±	Costeff syndrome *CLPB* mutations MEGDEL (*SERAC* mutations) *POLG* mutations Barth syndrome ATP synthase (*TMEM70* mutations)	
	3-CH3-glutaric, lactate, KC derivatives	Respiratory chain disorders, Pearson	
	3-CH3-glutaric, 3-OH-3-CH3-glutaric, 3HIV	HMG-CoA lyase deficiency	
2-Methyl-3-hydroxy-butyric	3-OHProp, PG, TG, MC	Propionate metabolism defects	
	3-OH-nBut, AcAc, 2-CH3-3-oxo-but, TG	β-ketothiolase deficiency	
3-Hydroxy-3-methyl-glutaric	3HIV, 3MG, 3-CH3-glutaric	HMG-CoA lyase deficiency	
Methylmalonic	15 to 250 µmol/mmol crea, isolated	SUCLA2/SUCLG1 Methylmalonyl-CoA racemase deficiency	
	15 to 250 µmol/mmol crea + 3HIB, 3-OHProp	Methylmalonic semialdehyde dehydro-genase deficiency	

Table 3.2 (continued)

Principal OA	Other OAs	Causes of variation	Other investigations
Methylmalonic	High (> 250) with same OA as propionic acidemia (not always)	Methylmalonic acidurias: - methylmalonyl-CoA mutase deficiency - CblA, CblB - IF, IGS, TCII, CblC, D, F, J CblX (*HCFC1* deficiency) nutritional B12 deficiency	AACp (Met↓, Hcy+)
Mevalonolactone	Mevalonic	Mevalonate kinase deficiency	
N-acetylaspartate	Alone	Canavan disease or aspartoacylase deficiency	
Orotic		Urea cycle disorders	AACp
		UMP synthase deficiency (hereditary orotic aciduria)	
Phenyllactic	Phenylacetic, mandelic, phenylpyruvic, 4-OH-phenylacetic, 4-OH-phenyllactic, 4-OH-phenylpyruvic	Phenylketonuria	AACp
	Phenylpyruvic, 4-OH-phenylacetic, 4-OH-phenyllactic, 4-OH-phenylpyruvic, N-AcTyr	Hepatic insufficiency	AACp
Pyroglutamic (Oxoproline)	Alone, very high	Glutathione synthetase or oxoprolinase deficiency	
	± High	Secondary: amino acid infusion, UCD, paracetamol intoxication	
Suberylglycine	See Hexanoylglycine		
Succinylacetone	Several peaks, succinylacetoacetic, 4-OH-phenyllactic, 4-OH-phenylpyruvic, N-AcTyr	Fumarylacetoacetate lyase def (Tyrosinemia type I)	AACp: not specific
Uracil	PyroGlu, Orotate	Urea cycle disorders	AACp
	Thymine	Dihydropyrimidine dehydrogenase def	
Vanillactic	Vanilpyruvic	Transitory in newborns	
		Dopa treatment	
		Aromatic amino acids decarboxylase deficiency	Neurotransmitters in CSF

AcAc, acetoacetic; *AcIle*, acetylisoleucine; *AcLeu*, acetylleucine; *Ad*, adipic; β-AIB, β-aminoisobutyric; β-Ala, β-alanine; *BCHA*, branched-chain 2-hydroxy acids; *BCKA*, branched-chain keto acids; *DCA*, dicarboxylic acids (adipic, suberic, sebacic, dodecanedioic, tetradecanedioic); *EMA*, ethylmalonic acid; *Glut*, glutaric; *2HB*, 2-hydroxy-n-butyric; *2HIB*, 2-hydroxy-isobutyric; *3HDC*, 3-hydroxydicarboxylic acids (3-OH-adipic, 3-OH-suberic, 3-OH-sebacic, 3-OH-dodecanedioic, 3-OH-tetradecanedioic); *3HIB*, 3-hydroxy-isobutyric; *3HIV*, 3-hydroxy-isovaleric; *4HB*, 4-hydroxy-butyric; *HG*, hexanoylglycine; *HMG*, 3-hydroxy-3-methyl-glutaric; *IBG*, isobutyrylglycine; *IVG*, isovalerylglycine; *KB*, ketone bodies (3-hydroxy-n-butyrate; + acetoacetate); *KC der*, krebs cycle derivatives; *2KG*, 2-ketoglutaric; *2MBG*, 2-methylbutyrylglycine; *MC*, methylcitrate; *3MCG*, 3-methyl-crotonylglycine; *3MG*, 3-methylglutaconic; *2M3HB*, 2-metyl-3-hydroxy-butyric; *2M3KB*, 2-methyl-3-ketobutyric; *MMA*, methylmalonic acid; *N-AcTyr*, N-acetyltyrosine; *nBG*, n-butyrylglycine; *2-OHAd*, 2-hydroxy-adipic; *2-OH-3CH3Val*, 2-hydroxy-3-methylvaleric; *2OHG*, 2-hydroxyglutaric; *3OHG*, 3-hydroxyglutaric; *2-OH-isocaproic*, 2-hydroxy-isocaproic; *3-OH-n-But*, 3-hydroxy-n-butyric; *3-OHProp*, 3-hydroxy-propionic; *2-oxoAd*, 2-oxo-adipic; *PG*, propionylglycine; *Pyr*, pyruvate; *PyroGlu*, pyroglutamic (or oxoproline); *Seb*, sebacic; *SG*, suberylglycine; *TG*, tiglylglycine

β-ox def, fatty acids β-oxidation defects; *CA*, carbonic anhydrase; *Cb*, cobalamin variant; *def*, deficiency; *DOOR syndrome*, deafness, onychodystrophy, osteodystrophy, mental redardation; *E3*, common protein of 2-ketoacid dehydrogenase complexes; *ETHE1*, ethylmalonic encephalopathy; *GA*, glutaric aciduria; *IF*, intrinsic factor; *IGS*, Imerslund-Gräsbeck syndrome (cubilin/amnionless deficiency); *2KGD*, 2-ketoglutarate dehydrogenase; *MCAD*, medium-chain acyl-CoA dehydrogenase; *MCT*, medium chain triglycerides; *MMSA*, methylmalonic semialdehyde; *MSUD*, maple syrup urine disease; *PC*, pyruvate carboxylase; *Redox*, simultaneous measurement of plasma lactate, pyruvate, 3-OH-butyrate and aceto-acetate; *RC*, respiratory chain; *SCAD*, short-chain acyl-CoA dehydrogenase; *SLC25A19* transporter (thiamine carrier) (Amisch lethal microcephaly); *SSADH*, succinic semialdehyde dehydrogenase; *SUCLA*, succinyl-CoA synthetase; *SUCLG*, succinyl-CoA ligase; *TC II*, transcobalamin II; *TPK1*, thiamine pyrophosphate kinase 1; *UCD*, urea cycle deficiency; *UMP*, uridyl-monophosphate

AAC, aminoacid chromatography (*p*, plasma; *u*, urine); *CSF*, cerebrospinal fluid

Table 3.3 Assessment of intermediary metabolism over the course of the day. The protocol of investigation is adapted to the clinical situation for each patient

Parameters in blood	Breakfast		Lunch		Dinner		Night
	Before	1 h after	Before	1 h after	Before	1 h after	04 h
Glucose[1]	X	X	X	X	X	X	X
Acid-base	X	X					
Lactate[2]	X	X	X	X	X	X	X
Pyruvate[2]	X	X	X	X	X	X	X
Free fatty acids	X	X	X	X	X	X	X
Ketone bodies	X	X	X	X	X	X	X
Ammonia	X	X	X	X	X	X	X
Amino acids	X						
Carnitine	X						
Acylcarnitines	X						
Hormones[3]	X	X	X	X	X	X	X
Urine 24 h collection[4]	Amino acids, organic acids, ketone bodies, urea, creatinine						

[1] Glucose should be determined immediately.
[2] Immediate deproteinization (with perchloric acid) at the bedside is the only way of ensuring that the results for calculating redox potential ratios can be accurately interpreted.
[3] Hormones (insulinemia, cortisol, growth hormone) are useful in the investigation of hypoglycaemia.
[4] Urine samples are collected both overnight and during the day and should be frozen immediately.

1. In glycogenosis (GSD) type I and in disorders of gluconeogenesis, blood glucose and lactate move in opposite directions, with hypoglycaemia and hyperlactataemia more pronounced in the fasted than in the fed state. In GSD type III, VI and IX, glucose and lactate change in parallel, with a moderate increase of glucose and lactate in the post-prandial state. Fasting hypoglycaemia and ketosis with postprandial hyperlactataemia and postprandial hyperglycemia is usual in glycogen synthase deficiency. Repeated assays are required for glucose and insulin in primary hyperinsulinism, as hyperinsulinemia is frequently erratic and difficult to prove. An insulin level >3 µU/ml with a glucose concentration lower than 2.8 mmol/l should be considered abnormal.
2. In patients with pyruvate dehydrogenase (PDH) deficiency, plasma lactate, in association with pyruvate, may be persistently raised, but usually decreases during fasting. Lactate may be normal, moderately raised or very high in mitochondrial respiratory chain (RC) disorders [2]. It may be difficult to distinguish a moderate elevation of lactate from a falsely raised level due to difficult sampling. However, the presence of a lactaturia with an elevation of alanine in blood is very suggestive of a true hyperlactatemia (the upper threshold for lactate reabsorbtion is at approx 4mmol/l). Lactate measurement in cerebrospinal fluid (CSF) may also be of help in patients with neurological disorders.
3. Measurements of ketone bodies are useful for the diagnosis of hyperketotic states, i.e. ketolysis defects or some RC disorders. The simultaneous measurement of blood glucose, free fatty acids and ketone bodies is necessary for the diagnostic and therapeutic evaluation of hypoketotic states, i.e disorders of fatty acid oxidation (FAO) or ketogenesis (▶ Chapters 13 and 14); data must be interpreted with regard to age and length of fasting (▶ Fasting Test [below] and also ▶ Fig. 1.3).
4. The lactate/pyruvate ratio (L/P), normally around 10:1, and the 3-hydroxy-butyrate/acetoacetate ratio (3OHB/AcAc), normally >1 after an overnight fast and <1 in the fed state, reflect the redox states of the cytoplasm and the mitochondrion, respectively, and may provide additional information [3] as follows:
   - L/P increased, 3OHB/AcAc normal or decreased: pyruvate carboxylase (PC) deficiency or 3-ketoglutarate dehydrogenase deficiency.
   - L/P and 3OHB/AcAc both increased with persistent hyperlactatemia: RC disorders.
   - L/P normal or low and 3OHB/AcAc normal, with varying hyperlactatemia: PDH deficiency, pyruvate carrier defect.

Table 3.4 Main metabolic abnormalities in lactic acidosis due to inborn errors of metabolism (from [2])

	Disorders of gluconeogenesis (G6Pase, FBPase deficiencies)	Glycogen Storage Disorders type III, VI	PDH deficiency	PC deficiency	KGDH deficiency	Fumarase deficiency	E3 deficiency	Respiratory chain defects
Hyperlactatemia	Maximum during fasting and when hypoglycaemic	Only in fed state	Permanent, maximum in fed state; can be moderate	Permanent	Permanent	Moderate	Permanent	Permanent, maximum in fed state very variable
L/P ratio	<15	<15	<10	>30	15-30	<15	15-30	>20
Ketone bodies	↑ at fast or N	only at fast	absent	+ (postprandial)	+	N	N	↑ + or N
3OHB/AcAc	N	N	N	↓↓	N or ↓	N	N	↑
Glucose	↓ at fast	↓ at fast	N	N or ↓	N	N	N	N or ↓
Ammonia	N	N	N	↑	N or ↑	N	N	N
Alanine	↑ at fast	N	↑ postprandial	N or ↓	N	N	↑	↑
Glutamine	N	N	N	↓	↑	N	↑	↑
Proline	N	N	N or ↑	↑	N	N	↑	↑
BCAA	N	N	N	↓	N	N	↑	N
Citrulline	N	N	N	↑	N	N	N	N or ↓
Organic acids in urine	Lactate	Lactate	lactate, pyruvate	lactate KB	αKG, lactate fumarate	Fumarate	branched-chain keto acids	N or lactate ± Krebs intermediates, methylglutaconic acid

It should be noted that all metabolic abnormalities are highly variable and that many patients affected with respiratory chain defects have no hyperlactatemia.
BCAA, branched chain amino acids; *FBPase*, fructose-1,6-bisphosphatase; *G6Pase*, glucose-6-phosphatase; *KB*, ketone bodies; *αKG*, α-ketoglutarate; *KGDH*, α-ketoglutarate dehydrogenase; *3OHB/AcAc*, 3-hydroxybutyrate/acetoacetate; *PC*, pyruvate carboxylase; *PDH*, pyruvate dehydrogenase; *N*, normal; ↑ increased; ↓ decreased.

The usual metabolic abnormalities observed in lactic acidosis due to inborn errors of metabolism are summarized in Table 3.4 (► also Table 1.3).

3.3 Metabolomic Approaches: the Example of *In Vitro* ^{1}H-NMR Spectroscopy of Body Fluids

Inborn errors of intermediary metabolism can generally be detected by the analysis of amino acids, organic acids, acylcarnitines and purines/pyrimidines. Other biochemical investigations are needed for the diagnosis of metabolic diseases involving organelles such as lysosomes or peroxisomes. However, these techniques can fail to reveal abnormal metabolic profiles in many patients in whom an IEM is suspected, emphasising the need for additional investigatory tools. Metabolomics is a rapidly developing field of -omics science in which the metabolome, i.e. the complete collection of metabolites present in an organism or in its cells, tissues or body fluids, is characterized using state of the art analytical tools, including nuclear magnetic resonance spectroscopy and high resolution mass spectrometry.

Proton nuclear magnetic resonance spectroscopy (^{1}H-NMRS) of body fluids shows the majority of proton-containing compounds, therefore offering an overall view of metabolism. The technique is of special interest because it requires no derivatisation or extraction, can simultaneously detect different compounds, and offers structural information on the metabolites present in body fluids. In vitro ^{1}H-NMRS can be used for the detection of known inborn error of metabolism [4][5][6][7][8][9], but also of new metabolic diseases. In the last decade, in vitro ^{1}H-NMRS has contributed to the identification of several metabolic diseases, some of which are amenable

to therapeutic intervention, for example involving the metabolism of glycine [10], pentose phosphates [11], pyrimidines [12], N-acetylaspartylglutamic acid [13] and N-acetylneuraminic acid [14]. Altogether, more than 100 resonances can be assigned in serum and CSF spectra, and even more than 200 in urine spectra [15]. In addition to the many metabolites that are also detected by metabolic screening techniques, such as amino acids and organic acids, ^{1}H-NMRS can detect other metabolites that would not be detected by standard methods. These include, for example, (1) betaine, choline, dimethylglycine and trimethylamine N-oxide in urine and (2) N-acetylneuraminic acid, N-acetylaspartylglutamic acid, citric acid, creatine, creatinine and myoinositol in CSF [15]. The detection limit of ^{1}H-NMRS is in the low micromolar range in the less crowded regions of the spectrum. It must be noted that urine NMR spectra are very complex and that information on the medication and on any special dietary regimens or habits is critical for a proper interpretation of the spectrum. Patients with complex and undiagnosed diseases despite extensive metabolic and genetic screening are therefore candidates for additional investigations by in vitro ^{1}H-NMRS. This is particularly relevant in the context of abnormal in vivo MRI spectra, as illustrated by IEM affecting creatine, polyols or lipid metabolism [9][16][17]. In addition, ^{1}H-NMRS can be very helpful in extending the spectrum of known diseases [18] and/or providing biomarkers that are relevant to certain subgroups of neurometabolic diseases [19]. However, novel statistical methods are needed before implementing metabolomics approaches in a clinical setting and statistical health monitoring is a promising one [20]. Furthermore, the combination of metabolomic approaches and whole exome sequencing techniques holds promises for the elucidation of new metabolic diseases. Due to the abundance of lipids in the brain – about 40% of dry mass, lipidomics stands as a key exploratory method in the field of neurometabolic diseases and is best addressed by mass spectrometry techniques rather than ^{1}H-NMRS [21][22].

3.4 Functional Tests

When performing a functional test, it is important to adhere to a strictly defined protocol in order to attain the maximum amount of interpretable diagnostic information and to minimise the risk of metabolic complications. Some provocative tests are now used infrequently, since simpler direct assays of metabolites and DNA have reduced their diagnostic value. Some have fallen into total disuse and are not considered here. These include the galactose and fructose loading tests, the glucagon test for the differentiation of glycogen storage diseases, the fat loading tests for the differentiation of fatty acids oxidation (FAO) defects and the phenylpropionate loading test for diagnosis of medium-chain acyl-coenzyme A dehydrogenase deficiency.

3.4.1 Fasting Test

Indications

This test [23] has been used for the clarification of hypoglycaemia observed in disorders of gluconeogenesis, fatty acid oxidation and ketogenesis, ketolysis and in some endocrinopathies. However, as it can be a highly dangerous procedure, its indications are now restricted to unexplained hypoglycaemia when basal metabolic investigations (organic acids analysis, acylcarnitines profile, enzymatic or molecular studies) have ruled out a FAO disorder or adrenal failure, or as a means to assess fasting tolerance during the treatment of certain disorders.

Procedures

The fasting test should only be performed in a specialized metabolic unit and under close medical supervision. The results of the basal investigations should be known before the test is planned. If permanent abnormalities exist, the diagnostic workup should be adjusted accordingly. During the three days before the test the patient should be adequately fed and the energy intake appropriate for his age. No intercurrent infection or metabolic incident should have occurred during the preceding week.

Fasting tolerance differs considerably depending on the age of the patient and on the disorder. The recommended period of fasting is as follows: 12 h for children less than 6 months of age, 20 h for those 6–12 months, and 24 h from age one year onwards. The test should be planned to ensure that the final and most important period (during which complications may arise) takes place during the daytime, when the best facilities for close supervision are available.

An indwelling venous catheter with a saline drip is inserted at zero time. The patient is encouraged to drink plain water during fasting. Table 3.5 gives the time schedule for the laboratory investigations. The main metabolic monitors for continuing the test safely are glucose and HCO_3^- concentrations in blood. Blood for a complete metabolic and endocrine profile is collected at the start of the test and twice before the end. If glucose drops below 3.2 mmol/l, glucose and HCO_3^- should then be determined at 30-min intervals. If glucose drops below 2.6 mmol/l and/or HCO_3^- drops below 15 mmol/l, or if neurological symptoms develop, the test should be stopped immediately. At that time, blood is taken for the complete metabolic and endocrine profile. The urine is collected and kept on ice for each 8 h period of the fast and for a further 4 h period after the end. From each 8 h or 4 h collection, a sample of 10 ml should be frozen at –70°C for the determination of lactate, ketone bodies, amino acids and organic acids.

Interpretation

The interpretation of this investigation is difficult and the results must be compared with the normal values for the particular age (Table 3.6).

Blood measurements: the tentative diagnoses are as follows:

- Hyperinsulinaemia: glucose <2.8 mmol/l, insulin >3 mU/l, and FFA <0.6 mmol/l, simultaneously. Ketone bodies (KB) remain very low during the fast.

Table 3.5 Fasting-test flow sheet. The duration of the test is adapted to the age of the patient or is determined by the length of time for the onset of spontaneous symptoms (▶ text). A complete sample is taken at the end of the fast if the test is stopped before 24 h

Time (h)	0	8	12	16	20	24
Blood						
Glucose	+	+	+	+	+	+
HCO_3^-	+	+	+	+	+	+
Lactate	+	+	+	+	+	+
3-Hydroxybutyrate	+	+	+		+	+
FFA	+	+	+	+	+	+
Carnitine	+		+		+	+
Acylcarnitines	+	+	+	+	+	+
Amino acids	+		+		+	+
Insulin	+		+		+	+
Cortisol	+					+
ACTH	+					+
Growth hormone	+					+
Urine	0–8 +			16–24 +		
Organic acids						

ACTH, adrenocorticotrophic hormone; *FFA*, free fatty-acids

- Fatty-acid oxidation and ketogenesis defects: glucose <2.8 mmol/l, increased FFA and low KB with FFA/KB ratio >2 (normal <1) and glucose in mmol/l × total KB in mmol/l <4.
- Gluconeogenesis defect: glucose <2.8 mmol/l and lactate >3.0 mmol/l simultaneously.
- Ketolysis defect: ketone bodies are already high in the basal state and increase dramatically during the fast, with possible acidosis. Glucose × total KB >10.
- PDH defect: high lactate (L) and pyruvate (P) with normal L/P ratio, L and P decrease during the fast. The fasting test is usually not useful for this disorder.
- Defects of the citric-acid cycle and the respiratory chain: variable levels of lactate and KB. The fasting test is usually not informative for these disorders.
- Adrenal-cortex insufficiency: glucose <2.8 mmol/l and cortisol <250 nmol/l simultaneously. Adrenocorticotrophin hormone (ACTH) deficiency: ACTH <80 pg/l. The fasting test is dangerous and contraindicated if this disorder is suspected.
- Human growth hormone (hGH) deficiency: glucose <2.8 mmol/l and hGH <10 ng/ml simultaneously. The fasting test is usually not useful for this disorder.
- Urine measurements: the best approach is to compare the results of the last period with those of the first.

Complications

Hypoglycaemia, metabolic acidosis, cardiac dysrythmia, cardiomyopathy, organ failure may occur. Fluids and medication must be immediately available in the patient's room.

Table 3.6 Metabolic profiles during fasting tests in children of different ages (from [3]). Normal blood values of hormones at the end of the fast or when the patient is hypoglycaemic, irrespective of age, are: insulin <3 mU/l at a glucose level of <2.8 mmol/l; cortisol >120 ng/ml; adrenocorticotrophic hormone (ACTH) <80 pg/ml; growth hormone >10 ng/ml

	Less than 12 months	1–7 years		7–15 years	
	20 h	20 h	24 h	20 h	24 h
Glucose (mM)	3.5–4.6	2.8–4.3	2.8–3.8	3.8–4.9	3.0–4.3
Lactate (mM)	0.9–1.8	0.5–1.7	0.7–1.6	0.6–0.9	0.4–0.9
FFA (mM)	0.6–1.3	0.9–2.6	1.1–2.8	0.6-1.3	1.0-1.8
KB (mM)	0.6–3.2	1.2–3.7	2.2–5.8	0.1–1.3	0.7–3.7
3OH-B (mM)	0.5–2.3	0.8–2.6	1.7–3.2	<0.1–0.8	0.5–1.3
3OH-B/AcAc	1.9–3.1	2.7–3.3	2.7–3.5	1.3–2.8	1.6–3.1
FFA/KB	0.3–1.4	0.4–1.5	0.4–0.9	0.7–4.6	0.5–2.0
Carnitine (free; µM)	15–26	16–27	11–18	24–46	18–30

AcAc, acetoacetate; *FFA*, free fatty-acids; *3OH-B*, 3-hydroxybutyrate; *KB*, ketone bodies

3.4.2 Oral Glucose Loading Test

Indications

This test is used for elucidation of hypoglycaemia or moderate/intermittent hyperlactatemia of unknown origin.

Procedures

It should follow a period of fasting of 3 to 8 h, depending on the age and the patient's usual interval between meals. In the case of previously recorded hypoglycaemia, the test is started at a plasma glucose concentration between 3.3 mmol/l and 2.8 mmol/l. An indwelling venous catheter is inserted 30 min before the expected start of the test and kept patent with a saline drip. A glucose load (2 g/kg with a maximum of 50 g), as a 10% solution in water, is administered orally or through a nasogastric tube over 5–10 min. The blood is sampled from the indwelling venous catheter twice at zero time (just before glucose administration) and every 30 min thereafter until 3–4 h.

All blood samples are assayed for glucose, lactate, pyruvate, 3OHB and AcAc. A urine sample collected just before the test and a second aliquot from a sample collected during the 8 h after glucose administration are tested for lactate, ketone bodies and organic acids.

Interpretation

- Glucose: a short-lived increase followed by a precipitous decrease may be observed in some cases of hyperinsulinism.
- Lactate: a marked decrease from an elevated fasting level occurs in disorders of gluconeogenesis and glucose-6-phosphatase deficiency (GSD type I) [24]. An exaggerated increase from a normal fasting level occurs in other GSDs including glycogen-synthase deficiency. Lactate remains increased or increases even further after glucose administration in PDH deficiency and RC disorders [3] [25]. Any increase in lactate must be compared to control values. The L/P ratio, normally around 10:1, is usually increased in PC deficiency and in mitochondrial disorders and remains normal or low in PDH deficiency and in mitochondrial pyruvate carrier defect.
- Ketone bodies: Ketone bodies may increase paradoxically in PC deficiency (with a low 3OHB/AcAc ratio) and in RC disorders (with a high 3OHB/AcAc ratio). Fasting ketone bodies are very low in hyperinsulinism.

Complications

The test should be stopped if plasma glucose drops below 2.6 mmol/l. The complete metabolic profile should be taken at that time.

In patients with PDH deficiency, a glucose load may precipitate lactic acidosis.

3.4.3 Glucagon Test

Indications

The glucagon test has now been abandoned for the investigation of conditions associated with fasting hypoglycemia where it can be dangerous. However, it remains very useful in patients with spontaneous and repeated hypoglycemias in the fed state, where hyperinsulinism is suspected. For these patients, it is a rapid diagnostic method and also a useful therapeutic test.

Procedure

Intra-muscular injection of glucagon (0.5 mg in newborn and infant, 1 mg in children) with measurements of blood glucose before and after 10, 20 and 30 minutes.

Interpretation

An increase in blood glucose > 50% of the basal value is considered significant and suggestive of an hyperinsulinism.

Complications

The test is contraindicated in the fasted state where it can be dangerous and may precipitate catabolic decompensation.

3.4.4 Protein and Allopurinol Loading Test

Indications

These tests have been used for the detection of late-onset forms of ornithine transcarbamylase deficiency (OTC). They may be indicated in a patient with intermittent clinical signs suggestive of OTC deficiency when no samples have been taken during the acute episode and when basal investigations (ammonia levels, plasma amino acids, urinary orotic acid and mutation analysis) are normal. They may also be used to detect heterozygotes in a family of an affected patient [26][27]. This latter indication has now been superseded by molecular analysis.

Procedures

Three procedures have been used:

1. Acute protein-loading (1 g/kg) test.
2. Allopurinol loading test: 100 mg in children <6 years, 200 mg in children aged 6 to 12 years, 300 mg in patients >12 years.
3. Combined protein + allopurinol loading test.

The patient should avoid caffeine, tea, chocolate or cola during the day before the test. After an overnight fast, an oral protein load (1 g/kg) is given. An indwelling venous catheter is inserted for the collection of blood samples at zero time and 1, 2 and 4 h after the protein load. Ammonia and amino acids are measured. If allopurinol is added, urine is collected in 5 fractions: before the test, 0–6, 6–12, 12–18 and 18–24 h after the challenge. Quantification of orotic acid and orotidine is performed on an aliquot from each collection using a specific method (HPLC).

A fourth procedure may be used in patients where there is a high suspicion of intermittent hyperammonaemia if these simple tests are negative. A protein-load is performed with a high protein diet: 5 g/kg/day for 5 days. This test has significant risks and should only be performed in a metabolic unit where blood ammonia can be measured rapidly (after each meal) and emergency procedures started if the level increases. The test must be stopped as soon as the blood ammonia increases or if even minor clinical symptoms occur.

Interpretation

These tests are positive if the protein load leads to an increase in blood ammonia or if the protein and/or allopurinol load induces a high excretion of orotic acid. False positive results can occur due to a small increase in orotic acid excretion in normal subjects following allopurinol ingestion. More importantly, false negative tests are not uncommon in patients with partial OTC deficiency [28].

3.4.5 Exercise Test

Indications

The exercise test is used to identify patients suspected of having a metabolic myopathy. Several methods exist:

- A non ischemic forearm-exercise test [29][30].
- Bicycle ergometer test [31].
- Treadmill test.

The best exercise test for the widest age range is the treadmill test.

Exercise testing may help in the diagnosis of glycogen storage disorders [32] affecting muscle (▶ Chapter 5) and for AMP deaminase deficiency. In mitochondrial myopathies, an exercise test is neither sufficiently specific nor sensitive for diagnosis [33]. This test may also be suitable for assessing the effects of therapeutics or exercise training in glycogen storage disorders [34] and mitochondrial myopathies.

Procedures

The forearm and the bicycle test are only applicable in adults and older children who are able to adhere to the protocols. The treadmill test has the advantage that it can be used from the age at which the child is able to walk. All exercise tests should be carried out at a submaximal workload. This is a safeguard to prevent severe complications, such as rhabdomyolysis, myoglobinuric anuria and metabolic acidosis.

The original forearm test, and its semi-ischemic modification have been abandoned and replaced by a more accurate non ischemic test [29][30].

In the bicycle ergometer test, the duration of the exercise and a submaximal workload associated with a pulse rate below 150 beats/min for adults or between 150 and 180 beats/min for children are adapted to the patient's condition [31].

In the treadmill test, the speed of the belt and its angle of inclination can be manipulated to a walking velocity of 3–5 km/h and a pulse rate of 150–180 beats/min. Exhaustion arises rapidly in those with myopathies due to defects of glycolysis and in defects of the citric-acid cycle and the respiratory chain. It occurs later in patients with defects of fatty-acid oxidation (after the exhaustion of energy from glycogen via aerobic and anaerobic glycolysis). The interpretation of the results of each exercise test should take this time sequence into consideration. In plasma and urine, the parameters to be compared before, during and after exercise are the following:

- Plasma: lactate, pyruvate, throughout the study. Acylcarnitines, ammonia, creatine kinase (CK) and potassium (K+) at the start and the end of the test.
- Urine: lactate, organic acids.

Interpretation

Lactate normally rises during muscle contraction reflecting a disturbed equilibrium between its production from glycolysis and its expenditure in the citric-acid cycle. No increase in lactate reflects deficient glycolysis which can be caused by phosphorylase deficiency and other more rare muscle glycolysis defects (▶ Chapter 6). Abnormally high elevations of lactate can be found with mitochondriopathies and muscle AMP deaminase deficiency.

Ammonia normally rises owing to deamination of adenosine monophosphate (AMP) during muscle contraction. There is no increase in ammonia in muscle AMP deaminase deficiency (▶ Chapter 35). The exercise test may reveal specific acylcarnitine accumulation in fatty acid oxidation disorders. An elevation of CK and K^+ reflects abnormal myolysis.

3.5 Next Generation Sequencing and Gene panels

The important cost reduction and the possibility to considerably accelerate diagnosis make next generation sequencing (NGS) more and more attractive among the complex journey of patients with rare diseases, such as inborn errors of metabolism. Some centres have even implemented these techniques as first-line biological investigations as soon as a genetic disease is suspected in a patient. Other centres have elected to pursue performing metabolic investigations before targeting one candidate gene or several candidate genes, often grouped in the so-called gene panels.

Gene panels allow analysing simultaneously several genes that belong to a common clinical or radiological or biochemical pattern, while ensuring an optimal sequencing coverage in order to reduce the number of false negative and false positive analyses. Gene panels have been successfully developed for patients with neurodevelopmental delay, neuro-sensorial deficit, peripheral neuropathy and leukodystrophy. More specifically in the field of IEM, gene panels are also being developed for major biochemical entries such as hyperhomocystinemia, hyperammonemia as well as peroxisome biogenesis disorders and lysosomal disorders. Although gene panels allow the analysis of a more restrictive number of genes than whole exome approaches, they do not preclude the use of stan-

dard genetic validation approaches, especially the analysis of variants frequency in the general population, the analysis of variants segregation in the family and functional analyses when possible. Furthermore, the possibility for a given gene to be associated with both dominant (heterozygous mutation) and recessive (homozygous or compound heterozygous mutations) inheritance, as reported in a growing number of metabolic disorders [35][36][37] further complicates the interpretation of NGS datasets. However, such global approaches may help further understanding the additional pathological effects of variants in distinct genes that encode proteins belonging to the same pathway. For whole exome sequencing, it is important to keep in mind that the chances to identify disease genes significantly increase in either of the 3 situations: family consanguinity, affected siblings or trio analyses (the patient and his/her parents) in the case of a sporadic patient.

Altogether, instead of replacing metabolic investigations, the development of NGS is increasingly likely to require the use of functional tools in order to validate genetic variants before they can be implicated in a patient's disease. Moreover, some biochemical investigations should remain first-line tools for certain clinical presentations as they are cheap to perform and very sensitive to diagnosis. We recommend, for example, to measure (i) plasma very long chain fatty acids (X-adrenoleukodystrophy), 25- and 27-hydroxycholesterol (SPG5), amino acids chromatography (arginase deficiency) and homocysteine (MTHFR deficiency) in any patient with spastic paraplegia; (ii) plasma cholestanol (cerebrotendinous xanthomatosis) and 7-ketocholesterol and cholestane-3β,5α,6β-triol (Niemann-Pick Type C disease) in any patient with atypical psychotic symptoms. Of importance, the interpretation of datasets obtained by NGS requires the access to advanced bioinformatics pipelines in order to sort out the complexity of the genetic information of a given patient.

3.6 Postmortem Protocol

Since the first description of a post-mortem protocol by Kronick [38], some refinements have become available to enhance the diagnostic value of the original recommendations [39][40]. In the protocol given below, the time schedule for proper preservation of specimens determines the sequence of the diagnostic procedures.

3.6.1 Cells and Tissues for Enzyme Assays

Liver (minimum 10–20 mg wet weight) and muscle (minimum 20–50 mg wet weight) biopsies are taken by needle puncture or, preferably, by open incision. The tissues are immediately frozen in small plastic cups in liquid nitrogen, followed by storage at –80°C. Part of the liver biopsy should be fixed for histological and electron-microscopic investigation prior to freezing (▶ »Autopsy« below). A total of 20 ml of blood is collected by peripheral or intracardiac puncture in a heparin-coated syringe; 10 ml is transferred to the laboratory for isolation of erythrocytes or white blood cells, and the biochemist is notified. At least 10 ml is conserved for chromosome analysis and DNA extraction.

3.6.2 Cells and Tissues for Chromosome and DNA Investigations

Of the 10 ml of fresh heparinized blood collected, 1–2 ml is reserved for chromosome analysis; the remaining 8–9 ml can be used for DNA extraction. Additionally, blood spots dried on filter paper (as in the Guthrie test) are useful for many investigations and should always be collected. These samples as well as paraffin-embedded tissues can also be used for DNA analysis.

3.6.3 Skin Fibroblasts

At least two biopsies (diameter 3 mm) are taken under sterile conditions as early as possible; one from the forearm, one from the upper leg (fascia lata ▶ above). Although a delay decreases the chance of successful fibroblast cultivation, fibroblasts may often be cultivated even from biopsies taken many hours after death. A biopsy may also be taken from the pericardium in case of delayed autopsy. These samples are conserved in culture medium or, if not immediately available, on sterile gauze wetted in sterile saline and sealed in a sterile tube for one night at room temperature.

3.6.4 Body Fluids for Chemical Investigations

Plasma from the centrifuged blood sample, urine (~10 ml), and cerebrospinal fluid (~4 ml) are immediately frozen at –20°C (◘ Table 3.7). If no urine can be obtained by suprapubic puncture or catheterization, the bladder may be filled with 20 ml of saline solution and diluted urine may be harvested. Alternatively, vitreous humor can also be collected (by intraocular puncture) and frozen. This liquid is comparable to blood plasma with respects to its solubility for organic acids. Bile, readily available at autopsy, has been found to be useful material for the post-mortem assay of acylcarnitines [41].

Many biochemical parameters are impossible to interpret post-mortem due to rapid tissue lysis. These include lactate, ammonia, carnitine (total and free), and amino acids, all of which rapidly increase without any specific significance. In contrast, the acylcarnitine-ester profile, determined from dried blood spots or from bile, may be highly diagnostic for many disorders of fatty-acid oxidation and for organic aciduria.

Table 3.7 Collection, processing and storage of blood, urine, and cerebrospinal fluid (CSF) for metabolic and endocrine investigation. The volumes of blood, urine, and CSF are subject to local practice, which must be taken into account

Blood	Urine	Cerebrospinal fluid
Hematology: 0.5 ml in EDTA tube	pH, amino acids, organic acids, ketone bodies, lactate, reducing substances: 5 ml (at least), freeze at –20°C	Cells, protein, glucose: 0.5 ml in plastic tube
Blood gases: 0.5 ml on heparin-coated syringe (eject air bubble, cap syringe immediately)		Lactate/pyruvate: 1 ml, add to 0.5 ml perchloric acid (18% v/v, keep on ice), centrifuge under refrigeration, store supernatant at –20°C
Electrolytes, urea, creatinine, urate, total protein, liver function tests: 1–2 ml (centrifuge after clotting)		Amino acids: 0.5 ml in plastic tube
Glucose: 0.3 ml fluoride-heparin tube (dry heparin and fluoride salts, no solution)		Culture: 1 ml in sterile tube
Lactate/pyruvate and 3OHB/AcAc: 1 ml blood (no forcing), mix immediately with 0.5 ml perchloric acid (18% v/v, keep on ice, centrifuge under refrigeration, store supernatant at –20°C)		
Ammonia: 0.5 ml in heparin-coated syringe on ice (eject air bubble, cap syringe immediately)		
Amino acids: 1–2 ml in EDTA or heparin tube		
Carnitine: 1–2 ml in EDTA tube on ice, centrifuge under refrigeration, store at –20°C		
Free fatty acids: 0.3 ml in fluoride-heparin tube (dry heparin and fluoride salts, no solution)		
Insulin: 1 ml in EDTA tube on ice, centrifuge under refrigeration, store at –20°C		
Cortisol and ACTH: 1 ml in plastic, heparin-coated syringe (keep on ice, centrifuge under refrigeration in plastic tube, store at –20°C)		
Growth hormone: 1 ml (centrifuge under refrigeration after clotting, store at –20°C)		
Glucagon: 3 ml in heparin tube (centrifuge under refrigeration, store in plastic vial at –20°C)		

AcAc, acetoacetate; *ACTH*, adrenocorticotrophic hormone; *EDTA*, ethylenediaminetetraacetic acid; *3OHB*, 3-hydroxybutyrate

3.6.5 Autopsy

The autopsy is important, particularly in undiagnosed patients and foetus, where it may give important clues to the underlying disorder. It should be as complete as soon as possible and include the cranium, provided that the parents have given permission [42]. The pathologist freezes fresh samples of liver, spleen, muscle, heart, kidney and brain and conserves important tissues for histology and electron microscopy in buffered formaldehyde (4%) and Karnofski fixative, respectively.

If a complete autopsy is refused, it is important to obtain permission to take photographs, X-rays, blood, urine and CSF samples, skin biopsies, and to do needle biopsies of liver and muscle. A kit containing all the material necessary for collecting and conserving specimens is highly recommended as a means of ensuring that the post-mortem protocol is completed as fully and as quickly as possible.

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Emergency Treatments

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4.1 Introduction

As soon as the diagnosis of a metabolic disorder is suspected, a plan for its emergency management should be made. As stated in ▶ Chapter 1, both the presentation and the management depend mainly on the pathophysiology involved. This chapter focuses on the main clinical presentations in neonates, children and adults with those inborn errors of metabolism for which emergency treatment may be life saving and outlines the first steps of such treatment up to when the exact diagnosis is known. The subsequent management of patients is addressed in the specific chapters.

In neonates, the main clinical presentations are as follows:

1. Neurological deterioration (metabolic encephalopathy): This is the most common presentation and the main causes are maple syrup urine disease (MSUD), branched-chain organic acidurias (BCOAs) and urea cycle defects (UCDs). Treatment must be started immediately to avoid severe cerebral sequelae.
2. Liver failure: Galactosaemia, and tyrosinaemia type I (though the latter is rare before 3 weeks of life) are amenable to specific emergency treatment.
3. Hypoglycaemia: Blood glucose levels must be corrected immediately. The three groups of disorders usually implicated are hyperinsulinism, glycogen storage diseases (GSD) and mitochondrial fatty acid oxidation (FAO) defects.
4. Cardiac failure: In neonates treatable disorders include FAO defects, infantile Pompe disease and TMEM70 deficiency.
5. Primary hyperlactataemia: This is associated with a general lack of cellular energy and may be due to various enzymatic defects. Some patients may benefit from high-dose vitamin treatment.
6. Intractable seizures: Vitamin responsiveness (pyridoxine, pyridoxal phosphate, folinic acid, biotin) must be assessed systematically.

In older children all these clinical situations can also arise whereas, in adults, emergencies mainly comprise encephalopathy and rhabdomyolysis. In particular, any type of coma or acute psychiatric symptoms can be the presenting sign of a metabolic disorder. In addition, recurrent attacks of unexplained dehydration, abdominal pain, muscle pain and myolysis or peripheral neuropathy can also be indications of a metabolic disease.

Such situations require careful and urgent biochemical investigation. Emergency treatment should be started concurrently and subsequently modified when necessary. Good collaboration with the metabolic laboratory is essential. The results of all laboratory investigations relevant to the diagnosis of metabolic disorders and for which specific emergency therapy exists should be available within 24 h.

4.2 General Principles

4.2.1 Supportive Care

Many patients, especially newborn infants, will require ventilatory and circulatory support. Most will need rehydration and correction of electrolyte, calcium and phosphate imbalance, but such treatments, despite their importance, should not delay the start of specific therapeutic measures. Patients with a metabolic crisis frequently suffer from septicaemia, which can result in persistent catabolism and lead to therapeutic failure. Consequently, infections must be prevented: patients must be thoroughly investigated for infection, and any infection must be actively treated.

4.2.2 Nutrition

Whatever the disease, nutrition is extremely important and both the method of administration and the composition of feeds must be rapidly determined. Briefly, four types of diet can be considered: normal, low-protein, carbohydrate-restricted, and high-glucose, with or without lipid restriction. To promote anabolism, the age-related recommended daily energy should be provided [1][2][3]. In some situations, the anabolic effect of insulin may reduce energy requirements [4].

The mode of administration will depend on the disorder and the clinical status. Oral (enteral) nutrition is preferable if the condition and clinical status allow it. Continuous enteral tube feeding can be temporarily useful in many patients whose initial condition is poor. Total parenteral nutrition (TPN) is the method of choice in those cases where effective enteral nutrition is precluded (e.g. by intestinal intolerance, high energy or high glucose requirements or invasive techniques required for toxin removal).

4.2.3 Specific Therapies

Specific therapies can be used for some disorders. These mainly comprise substrates that enable the excretion of ammonia by alternate pathways (▶ Chapter 19), carnitine and vitamin supplementation and administration of additional specific drugs [5] (▶ Appendix: ▶ Chapter 43). All intensive care units should ensure that these are readily available.

4.2.4 Extracorporeal Procedures for Toxin Removal

For those disorders associated with acute metabolic toxicity, such as UCDs and BCOAs, extracorporeal procedures to remove toxins are necessary when less invasive methods are insufficient. Of the available techniques continuous veno-venous haemodiafiltration (CVVHDF) and haemodialysis (HD) are more efficient than peritoneal dialysis (PD) [6]. However,

the choice of the technique is highly influenced by local facilities and experience.

4.3 Emergency Management of Particular Clinical Presentations

As previously stated, the management depends on the pathophysiology and the main clinical presentation.

4.3.1 Neurological Deterioration

The most common treatable disorders causing an acute toxic encephalopathy are MSUD, BCOAs and UCDs, and these should always be considered, particularly in a newborn infant who presents with a sepsis-like illness following an asymptomatic period. In caring for neonates with BCOAs and UCDs there are three main risks: overhydration, cerebral oedema and acute protein malnutrition [7][8][9]. The management of children and adults (late-onset coma) is essentially similar to that of neonates [8][9]. In adults, clinical symptoms may often precede and/or predominate over biochemical abnormalities – the recommendations for treatment are therefore different than for children.

▪ Supportive Care

▪▪ Neurological Deterioration with Ketoacidosis

This is the usual presentation in infants with BCOAs including MSUD. There is, however, no significant metabolic acidosis in MSUD. In general, in addition to disease-specific therapies, patients require supportive care, procedures for the removal of toxins, and high-energy protein-free nutrition.

From a practical point of view, two situations should be considered:

- Some patients may not initially appear seriously unwell and may have only a mild acidosis (pH >7.20, HCO_3 >15 mmol/L), mild to moderate dehydration (<10% of birth weight) and normal or moderately raised blood ammonia (<400 µmol/L). Blood glucose, lactate, calcium and cell count are normal. This is frequently the early presentation in MSUD and in methylmalonic, propionic and isovaleric acidurias when recognised early or diagnosed by newborn screening.
- In other patients, the situation appears more severe. This is especially the case for patients with organic acidurias whose diagnosis has been delayed for a few days. They present with severe ketoacidosis (pH <7.10, HCO_3 <10 mmol/L), are seriously dehydrated (by >10% of birth weight), and may have overt hyperammonaemia (>400 µmol/L), mild hyperlactaemia (<5 mmol/L), hypo- or hyperglycaemia, hypocalcaemia, leukopenia and thrombocytopenia.

▪▪ Neurological Deterioration with Hyperammonaemia

This is most commonly due to primary disorders of the urea cycle. Affected neonates have acute neurological deterioration with vasomotor instability, apnoeas and fits. Biochemically, they exhibit respiratory alkalosis, with plasma ammonia levels above 400 µmol/L, and often very much higher. All other routine laboratory tests are normal and, in particular, ketonuria is not usually present. As a general rule, the treatment is similar to that in the previous group. However, newborn infants with UCDs have in most cases a poor outlook, and even among those who receive the most aggressive treatment the majority of survivors will be handicapped [6]. Infants treated prospectively do better, but there may still be significant complications [10][11]. Thus, the wisdom of starting treatment should be carefully considered. Some children with organic acidurias diagnosed late may have a similar presentation with severe hyperammonaemia without ketoacidosis [12]. The need for urgent management, and unfortunately the poor prognosis, are the same as for UCDs [6][13].

▪▪ Mildly Affected Infants

These neonates should be hydrated over a 24-h period, while a procedure for toxin removal is prepared. Hydration can be performed using a standard 5-10% glucose solution containing 75 mmol/l of Na+ (4.5 g/l of NaCl) and 20 mmol/l of K+ (1.5 g/l of KCl). High-calorie, protein-free nutrition should be started in parallel, using carbohydrates and lipids to provide 100 kcal/kg/day. Initially, for the 24- to 36-h period needed to test gastric tolerance, parenteral and enteral nutrition are used together. The requirement for toxin removal is dependent on the diagnosis, the levels of metabolites and the short-term clinical and biochemical course. In order to prevent acute protein malnutrition, the protein-free diet must not be used for more than 2 days. Once the levels of toxic metabolites have decreased, natural proteins are introduced using measured amounts of infant formula (▶ Section »Enteral Nutrition«).

▪▪ Severely Affected Infants

Neonates with severe ketoacidosis present with intracellular dehydration that is often underestimated. In this situation, aggressive rehydration with hypotonic fluids and alkalisation may cause or exacerbate pre-existing cerebral oedema. Therefore, rehydration should be planned over a 48-h period, with an infusion of less than 150 ml/kg/24 h that contains an average concentration of 75 to 150 mmol/l of Na+ (4.5 to 9 g/l of NaCl), 30–40 mmol/l of K+ (2–3 g/l of KCl) and 5% glucose. Acidosis can be partially corrected with i.v. bicarbonate, especially if it does not improve with the first measures applied for toxin removal. However, aggressive therapy with repeated boluses of i.v. bicarbonate may induce hypernatraemia, cerebral oedema, and even cerebral haemorrhage [14][15][16]. In order to compensate for bicarbonate consumption, sodium bicarbonate may be substituted for one-quarter to one-half of the sodium requirements during the first 6–12 h of rehydration. To prevent precipitation with calcium, the bicarbonate solution should be connected to the infusion line with a Y-connector. These supportive measures are applied in parallel with a procedure for toxin removal that, in addition to the dialysis of the toxic organic acids, can compensate for some of the fluid and electrolytic imbalance and allow for nutritional support.

Presentation and Management in Adults

Behavioural changes (irritability, anxiety) and confusion are the most common presenting symptoms in adults with hyperammonaemia. During the first 48 hours, hydration and parenteral nutrition using a central i.v. line is the method of choice while starting the administration of nitrogen scavengers. Total daily energy intake shall exceed 2500 kcal in women and 3000 kcal in men. Depending on the severity of neurological symptoms (coma) and the ammonia levels (>400 µmol/l), haemodialysis is indicated and should be promptly initiated. However, haemodialysis should only be performed during short intervals to lower the risk of central pontine myelinolysis as the adult brain is very sensitive to osmolarity changes. After 48 hours, proteins should be reintroduced, ideally using enteral feeding.

Nutrition

Parenteral Feeding

Total parenteral nutrition (TPN) is the method of choice in infants with severe illness who are at high risk of gastric intolerance. The amino acid-free TPN solution is suitable for the first 48 h; protein must then be added using a commercially available amino acid solution. Initially, amino acids are introduced in amounts sufficient to meet the minimal daily requirements, and then titrated according to biochemical monitoring. The method is safe if the amino acid solution is evenly distributed over the whole day [17][18][19]. The minimal isoleucine requirement in neonates is at least equal to that of valine. However, many i.v. amino acid solutions provide less of the former than the latter. Consequently, when the TPN solution only provides the minimal requirement for L-valine, additional oral supplementation of L-isoleucine (25–100 mg/day) is often necessary. Vitamins, mineral and micronutrients must always be provided to prevent deficiencies.

Enteral Feeding

As soon as the enteral feed is available the switch from parenteral nutrition is scheduled over a 4- to 5-day period, using continuous nasogastric tube feeding [3][20]. To ensure gastric tolerance, small volumes, e.g. 60 ml/day, are given initially and then increased every 24 h until the full fluid requirement is met. As enteral feeds are increased, the parenteral infusion rate is decreased reciprocally. Ondansetron (0.15 mg/kg in 15 min i.v., up to three times daily) may be tried if there is persistent vomiting. In terms of the formulation of feeds, the first step is to progressively increase the amount of protein given to reach the desired daily requirements using human milk or infant formula.Urinary urea excretion assessment is a simple and useful tool for guiding the reintroduction of natural proteins [21]. Next, calories are slowly added using either glucose polymer and lipids or a commercially available protein-free powder. Minerals, vitamins and micronutrients are also given. Addition of an amino acid mixture, if necessary, is the final step, since it increases the osmolarity of the solution and can induce diarrhoea. However, in MSUD, a branched-chain-free amino acid mixture is always required. During this process, the volume of water is increased to cover the requirement for age and weight.

In mild decompensation, enteral nutrition may be sufficient to result in a rapid clinical and biochemical recovery [20]. In this situation the composition of the enteral formula is initially based on a glucose-lipid mixture. However, to prevent acute protein malnutrition, a protein-free diet should not be used for more than 2 days. In infants, the diet should provide 130–150 kcal/kg/day. Micronutrients, osmolarity, and renal solute load must be assessed to make it possible to provide the recommended dietary allowance (RDA) and prevent diarrhoea and dehydration. Depending on the disorder, an appropriate amino acid mixture can be added to cover the protein requirement. The latter is an absolute requirement in MSUD [3][17][19][22]. Once the toxic metabolites have normalised, natural proteins are introduced using measured amounts of infant formula. Attention must be paid to both the total protein and essential amino acid requirements. For patients with an inborn error blocking an amino acid catabolic pathway, intake of natural protein and essential amino acids must provide the minimal safe requirements (protein accretion + non urinary losses), which are 50–60% below the normal requirements (protein accretion + non urinary losses + urinary losses) and consequently less than the RDA [23]. These minimal requirements represent the basis for initiation of a protein-controlled diet. Next, the natural protein and amino acid intakes are adjusted for growth and according to the specific biochemical control. The final step is transition to appropriate long-term dietary treatment.

Specific Therapies

Enhancing Anabolism: Insulin

Owing to its anabolic effect insulin is used to suppress severe catabolism; however, this will only be achieved if dehydration and acidosis are also corrected. Infusion of insulin in high doses (0.05–0.2 IU/kg/h) used in association with large amounts of glucose provided by TPN may be useful [4][24][25]. The dose of insulin must be adjusted frequently to control glycaemia. Sustained normalisation of the blood glucose level, which is an indirect marker of effective anabolism, allows for insulin withdrawal. Human growth hormone has been useful in promoting anabolism in a variety of organic acidurias, but is unlikely to be effective in the acute situation.

Alternative Pathways

Neurological damage is primarily related to the duration and the severity of hyperammonaemia; consequently ammonia must be removed as rapidly as possible [13]. In acute situations, L-arginine is an essential amino acid in all disorders of the urea cycle (except arginase deficiency) and is administered together with sodium benzoate and/or sodium phenylbutyrate, the latter providing alternative pathways for nitrogen excretion by conjugation with glycine and glutamine, respectively [26]. There has been some debate as to whether sodium benzoate or sodium phenylbutyrate should be used for detoxification of ammonia before the diagnosis is known in organic acidurias, as there is the theoretical risk of additional intramitochondrial coenzyme A depletion [27][28]. However,

sodium benzoate is now regularly used, without apparent adverse effects [5][13][29]. Sodium phenylbutyrate is given as ammonia scavenger because, following its conversion to phenylacetate, it binds to glutamine to form phenylacetyl-glutamine, which is rapidly excreted. Its use is not recommended in organic acidurias in which glutamine levels are normal or low [30][31][32]. Enteral sodium phenylbutyrate is used to provide a source of phenylacetate. The i.v. combination of both sodium benzoate and sodium phenylbutyrate may also be used, exclusively by a central line. However, the use of these drugs must be limited before a precise diagnosis indicating hyperammonaemia is obtained, since glutamine is elevated only in urea cycle defects and is in the low-normal range in organic acidurias. In N-acetylglutamate synthetase deficiency, N-carbamoylglutamate has become available as the treatment of choice. It may also be efficacious in hyperammonaemia attributable to N-acetylglutamate synthetase inhibition by acyl-coenzyme A in organic acidurias [33][34][35].

L-Carnitine is given to compensate for secondary carnitine deficiency caused by urinary excretion of carnitine-bound organic acids [36][37]. As a rule, L-carnitine supplementation is never contraindicated in these disorders. Only if a long-chain FAO defect is suspected should the administration of carnitine be avoided, at least as a bolus, because of the acute accumulation of toxic long-chain acylcarnitines and the potential risk of cardiac arrhythmia (▶ Chapter 12).

Vitamin Therapy

Megadoses of specific vitamins should be systematically tested in each case of a potentially vitamin-dependent disorder. Vitamin responsiveness is more likely in late-onset forms than in those presenting in the newborn period. As the response may be masked by the simultaneous use of other therapies, the trial should be repeated later in a stable metabolic period and the results compared with those of in vitro studies.

Biotin is essential in the treatment of both holocarboxylase synthetase and biotinidase deficiency. Hydroxocobalamin should be tried in all cases of methylmalonic aciduria, riboflavin in glutaric aciduria types I and II and thiamine in MSUD. In any severe metabolic decompensation accompanied by insufficient nutritional intake and severe lactic acidaemia a trial with thiamine should also be performed [38][39].

Additional Drugs

In methylmalonic aciduria, forced diuresis and alkalinisation of urine with sodium bicarbonate help to eliminate methylmalonic acid because of its high urinary clearance. In isovaleric aciduria, glycine can be used in combination with carnitine to promote the excretion of glycine conjugates and is particularly useful for long-term treatment. In the emergency treatment, carnitine alone is adequate and essential to correct secondary carnitine deficiency [36][40]. N-carbamoylglutamate can be used to treat some urea cycle disorders (i.e. N-acetylglutamate synthase deficiency or CPS deficiency) and it has been shown to be helpful as an adjunctive medication in the treatment of hyperammonemic crises secondary to organic acidurias.

Extracorporeal Toxin-removal Procedures

In some cases, the situation deteriorates so rapidly that extracorporeal toxin-removal becomes necessary. Such treatment should be considered if the ammonia concentration exceeds 300 to 400 μmol/l and/or if ammonia levels do not decrease adequately within the first 4–6 h with conservative treatment. This is often the case in multiorgan failure, as alternative pathway therapy requires intact hepatic and renal function for the formation and excretion of conjugates. In all cases of neonatal hyperammonaemic coma, the dialysis team should be informed immediately. MSUD may require extracorporeal detoxification if leucine levels exceed 20 mg/dl (1500 μmol/l).

The choice of the technique is highly influenced by local facilities and experience. Haemodialysis (HD) [6][41][42][43], continuous veno-venous haemofiltration (CVVHF) [44][45][46] and hemodiafiltration (CVVHDF) have been shown to be more effective than peritoneal dialysis (PD). Extracorporeal membrane oxygenation has been used in driving HD and haemofiltration (HF) [47]. A delayed extracorporeal treatment was not superior to PD in improving the short-term outcome in a large series of hyperammonemic neonates [48]. Therefore, the main determinant of neonatal outcome is an early initiation of medical and dialysis treatment according to local facilities, regardless of the dialysis modality. If such management is unavailable locally, the patient should be transferred to a specialist centre. The advantages and disadvantages of the respective techniques in the emergency treatment of various acute metabolic disorders are as follows:

Peritoneal Dialysis

Manual PD requires minimal technical expertise, can be rapidly initiated in any paediatric intensive care unit and can be effective in newborns [49][50]. The main cause of failure is poor splanchnic blood flow secondary to shock and septicaemia. It appears that PD is far less effective in older children owing to a smaller peritoneal area relative to body weight.

Continuous Haemofiltration

CVVHF consists in a low-resistance extracorporeal circuit connected to a small-fibre haemofilter that is permeable to water and non-protein-bound small solutes [51]. The ultrafiltrate of plasma is concurrently replaced by an electrolyte and TPN solution. CVVHDF increases solute removal by the addition of diffusive transport from a dialysis solution flowing upstream through the ultrafiltrate compartment of the haemofilter [52]. The advantages of CVVHF and CVVHDF are logistical simplicity, good tolerance in neonates or infants who present with haemodynamic instability, multiorgan failure and a hypercatabolic state, and the ability to use a large volume of TPN without the risk of overhydration. Nevertheless, these procedures should not be applied except in a paediatric intensive care unit by staff trained in the techniques of extracorporeal circulation [6][42][44][45][46][53][54].

Haemodialysis

HD is a very effective and rapid method for removing small solutes [6][43][55]. However, multiple dialysis sessions are

most often necessary, owing to a rebound in the circulation of toxic metabolites. In addition, clearance is hampered by vascular instability [6][41][52].

Assessment of Biochemical Progress

In order to evaluate the efficiency of toxin removal it is necessary to undertake regular biochemical monitoring in blood, urine and dialysate or ultrafiltrate within set timed periods. Blood glucose, plasma electrolytes and calcium should be corrected when necessary. Regular blood cell counts are also important since, in organic acidurias, neutropenia and thrombocytopenia may be present or may develop after the initiation of therapy and may require specific treatment. Urinary urea excretion and plasma uric acid concentration provide readily available information on catabolism. Repeated assessments for septicaemia must be undertaken and treatment initiated as soon as there is any suspicion of infection.

4.3.2 Liver Failure

Liver failure is a predominant finding in children with galactosaemia, hereditary fructose intolerance (HFI) and tyrosinaemia type I and requires urgent and specific treatment. Neonatal and late-onset forms of these disorders may present with acute deterioration, vomiting, seizures, dehydration, hypoglycaemia, liver failure and tubulopathy. A number of abnormalities are associated with advanced liver disease, including mellituria, hyperammonaemia, hyperlactataemia, hypoglycaemia, hypertyrosinaemia and hypermethionaemia. Tyrosinaemia type I rarely presents before the 3rd week of life. Galactosaemia usually presents in the newborn period, but HFI should not become manifest until after weaning, since fructose is not normally part of infant formulas. As soon as these disorders are considered, galactose, fructose and protein must be excluded from the diet (with normal intake of all other nutrients) pending confirmation of the diagnosis. When galactosaemia or HFI is confirmed, protein can be reintroduced (▶ Chapters 7 and 9). When tyrosinaemia type I is confirmed, treatment with NTBC, along with a low-phenylalanine and low-tyrosine diet must be started urgently, in order to prevent production of toxic metabolites and to promote rapid recovery from acute liver failure [56][57] (▶ Chapter 17).

Of note, patients with urea cycle defects may also present with acute liver failure. The following observations should prompt hepatologists to consider a primary UCD and search for hyperammonaemia: the severity of the neurological associated symptoms and/or the discrepancy between severe liver dysfunction and relatively mild cytolysis (▶ Chapter 19). Liver failure may also be observed in mitochondrial FAO defects (▶ Chapter 12), mitochondrial respiratory chain disorders (▶ Chapter 14), transaldolase deficiency (▶ Chapter 7), Wolman disease (▶ Chapter 31) and Wilson disease (▶ Chapter 37).

4.3.3 Neonatal Hypoglycaemia

Whatever the cause of hypoglycaemia, blood glucose levels must be corrected immediately with a glucose bolus (0.5–1 g/kg) followed by a continuous infusion. However, because abnormal metabolites may quickly become normal with therapy, adequate samples for metabolic studies (acylcarnitines, glucose, insulin, free fatty acids, ammonia and ketone bodies) should be obtained first. Glucose should then be started via a peripheral i.v. line, initially at 150 ml/kg/day of a 10% solution (~10 mg/kg/min). Observation of the patient's glucose requirement to maintain normoglycaemia is useful for both diagnosis and management. A glucose supply at a rate equivalent to hepatic glucose production (7–8 mg/kg/min in the newborn) is usually sufficient for disorders such as GSD I and disorders of gluconeogenesis. Patients with congenital hyperinsulinism will require much higher rates (10–20 mg/kg/min) (▶ Chapter 9).

Glycogen Storage Disease Type I and Fructose-1,6-Bisphosphatase Deficiency

In these disorders, fasting hypoglycaemia is associated with hyperlactataemia and metabolic acidosis. In Fructose-1,6-bisphosphatase deficiency, there may be glyceroluria which strongly indicates a defect of gluconeogenesis [58]. As soon as the blood glucose values have returned to normal, continuous enteral feeding is substituted for the glucose infusion. At first a milk-based formula containing maltodextrin as the source of carbohydrate is used. Giving a normal energy intake for age in which 50–60% of the energy is supplied by carbohydrates, this allows for a glucose infusion of 10–12 mg/kg/min. This diet can subsequently be changed according to the final diagnosis (▶ Chapters 5 and 8).

Neonatal Hyperinsulinism

This disorder presents with recurrent hypoglycaemia without ketoacidosis. The newborn requires a continuous supply of glucose that exceeds the capacities of the peripheral i.v. route and continuous enteral feeding. Consequently, central venous catheterisation is unavoidable. In cases of persistent hypoglycaemia, treatment with glucagon and/or diazoxide can be started. The emergency treatment of neonatal hyperinsulinism is discussed in ▶ Chapter 9.

Fatty Acid Oxidation Defects

FAO defects cause severe energy deprivation and can be suspected in both newborns and children who present with fasting hypoglycaemia and/or acute deterioration associated with lethargy, hepatomegaly and liver failure, cardiac dysrhythmia, and high blood creatine-kinase, lactate and uric acid levels. These are serious disorders that may require resuscitation. In order to suppress lipolysis it is at first necessary to give an i.v. solution providing 10–12 mg/kg/min of glucose (120–150 ml/kg/day of a 12–15% glucose solution), preferably in combination with insulin. The initial diet should be fat free. Medium-chain triglycerides (2–3 g/kg/day) can be of advantage in long-chain FAO defects as a fuel for the compro-

mised energy metabolism especially in the heart. However, supplementation should be postponed until the exact site of the defect is known. Hypocarnitinaemia is usually present. The efficacy and safety of carnitine supplementation is still controversial, except in carnitine transporter defect, where it is life saving (▶ Chapter 2).

4.3.4 Cardiac Failure

One of the treatable disorders that lead to presentation with cardiac failure in the neonatal period is the group of mitochondrial FAO defects associated with cardiomyopathy or conduction abnormalities. In addition to the usual cardiac drugs and symptomatic treatment of cardiac failure, specific emergency treatments are as discussed above (▶ Chapter 12). Infantile Pompe disease should also be considered in the setting of severe neonatal hypotonia with cardiac failure and short PR interval on the ECG (▶ Chapter 5).

4.3.5 Primary Hyperlactataemia

Whatever the enzyme defect, most newborns with primary hyperlactataemia present with acute metabolic acidosis with increased anion gap (with or without ketosis) and dehydration requiring supportive care similar to that described for the branched-chain organic acidurias. Usually, this treatment is sufficient to reduce the lactate to a level that does not cause severe metabolic acidosis. In some cases, sustained hyperlactataemia is due to a high-glucose infusion and can be corrected by using a 5% or even a 2.5% i.v. glucose solution. Thus, none of these patients require any procedures for toxin removal except in TMEM70 deficiency (▶ see below).

Few strategies are of proven efficacy in congenital lactic acidaemia. A trial should be performed with thiamine (cofactor for the pyruvate dehydrogenase [PDH] complex), riboflavin (cofactor for complex I) and biotin (cofactor for pyruvate carboxylase). Secondary carnitine deficiency is treated with L-carnitine. It is essential to correct metabolic acidosis with sodium bicarbonate or, if the sodium level exceeds 160 mmol/L, with trometamol. Dichloroacetate (50 mg/kg/day in one or two divided doses), an inhibitor of PDH kinase, can be an effective mean for lowering lactate accumulation, and hence correction of acidosis, in both PDH and respiratory chain disorders [59][60]. However, it has little effect on the clinical status except for reducing tachypnoea. In one patient with the French phenotype of pyruvate carboxylase deficiency, the early administration of triheptanoin allowed survival for several months [61] (▶ Chapters 12, 15, 17). However, the child collapsed during his next decompensation. The only congenital lactic acidosis in which extracorporeal depuration should be considered for the treatment of severe hyperammonaemia and lactic acidosis is TMEM70 deficiency, a disorder presenting in the neonatal period or early infancy with poor feeding, hypotonia, lethargy, respiratory and heart failure, cardiomyopathy, with or without pulmonary hypertension accompanied by severe lactic acidosis, 3-methylglutaconic aciduria and hyperammonaemia [62].

4.3.6 Intractable Seizures

When seizures are the preponderant or presenting sign, pyridoxine, pyridoxal phosphate [63], biotin and folinic acid [64] must be systematically tested. Familial hypomagnesaemia with secondary hypocalcaemia should be considered, and if present treated with enteral magnesium supplementation. Disorders of methyl group transfer (including methylenetetrahydrofolate reductase deficiency and disorders of cobalamin metabolism) may require treatment with hydroxocobalamin, folinic acid, pyridoxine, betaine and/or methionine, depending on the underlying enzymatic defect. GLUT1 deficiency can be efficiently treated with a ketogenic diet (▶ Chapter 11).

In suspected metabolic disorders those drugs that may inhibit mitochondrial function should be used only in acute emergencies where no other effective treatment is available. These include the antiepileptic drugs sodium valproate and chloral hydrate.

4.4 Final Considerations

Once the patient is discharged from hospital precautions must be taken to avoid further episodes of decompensation. Parents must be aware of possible causes and be taught to recognise the early signs and when to initiate the first steps of the emergency treatment at home [65]. Every patient should be supplied with an emergency card detailing their particular management scheme to be followed both at home and in the primary care hospital. If there are recurrent episodes of decompensation, insertion of a gastrostomy and/or a portacath system should be considered.

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Section II
Disorders of Carbohydrate Metabolism

The Glycogen Storage Diseases and Related Disorders

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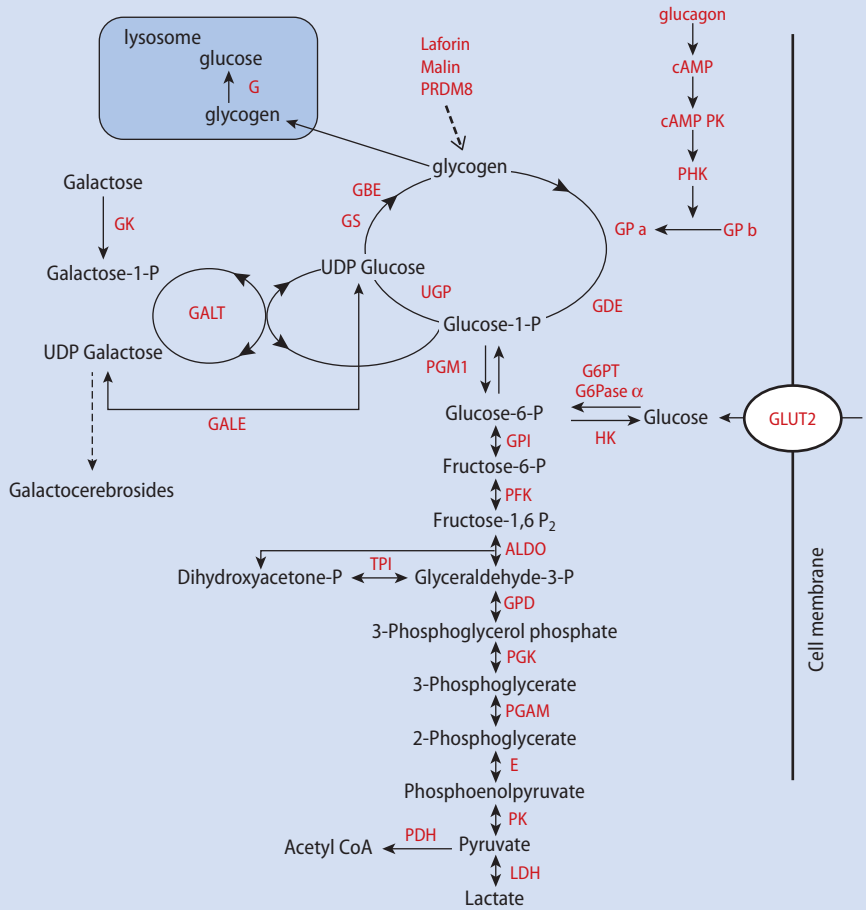
Glycogen Metabolism

Glucose is a key fuel for energy production and an absolute requirement for the central nervous system. In view of the importance of glucose homeostasis to prevent cerebral energy deficiency evolution has ensured that the mechanisms for the maintenance of a satisfactory blood glucose concentration are as robust as possible. In the immediate post prandial state carbohydrate absorbed from the gut can be released into the circulation. Glucose sensors in the pancreatic beta cells trigger a release of insulin in response to an increasing blood sugar. Insulin increases glucose uptake into cells where it can be catabolised by aerobic and anaerobic metabolism to produce ATP (◘ Fig. 5.1) or be stored as glycogen primarily in in the liver but also in muscle. Glycogen is a polysaccharide polymer consisting of glucose molecules in straight chains but with numerous branch points and a molecular weight of between 1 and 20 million. It is structurally similar to animal starch (amylopectin) but has more branch points and a higher MW. At the centre of each glycogen macromolecule is a protein known as glycogenin. Glycogenin 1 is present in skeletal and heart muscle and glycogenin 2 in liver. Glycogen can form up to 8% of the wet weight of the liver after a meal. In muscle there is approximately 2% by weight and much smaller amounts are also present in the kidneys, in glial cells in the nervous system, and in leucocytes. Only liver glycogen is available for glucose release into the circulation.

Glycogen synthesis

Glycogen synthesis is an energy requiring process. The initial step involves the auto-glucosylation of apo-glycogenin at



◘ **Fig. 5.1 Glycogen, Glucose and Galactose metabolism.** *ALDO*, Aldolase A; *cAMP PK*, Cyclic AMP protein kinase; *E*, Enolase; *G*, lysosomal a-1,4-glucosidase; *G6Pase-α*, glucose 6 phosphatase-*α*; *G6PT*, glucose-6-phosphate transporter; *GALE*, Galactose epimerase; *GALT*, Galactose 1 phosphate uridyl transferase; *GBE*, glycogen brancher; *GDE*, glycogen debrancher; *GK*, Galactokinase; *GLUT2*, Solute Carrier Family 2; *GP*, glycogen phosphorylase; *GPD*, Glyceraldehyde phosphate dehydrogenase; *GPI*, Glucose phosphate isomerise; *GS*, glycogen synthase; *LDH*, Lactate dehydrogenase; *PDH*, Pyruvate dehydrogenase; *PFK*, muscle phosphofructokinase; *PGK*, Phosphoglycerate kinase; *PGAM*, Phosphoglyceratemutase; *PGM1*, Phosphoglucomutase; *PHK*, Phosphorylase kinase; *PK*, Pyruvate kinase; *TPI*, Triosephosphate isomerise; *UGP*, UDP-glucose/galactose pyrophosphorylase

tyrosine residue 194 by UDPglucose. The same catalytic site of the glycogenin molecule adds a number of glucose residues with an alpha 1,4 linkage. Further elongation of the chain requires the enzymes glycogen synthase for alpha 1,4 linkage and glycogen branching enzyme for alpha 1,6 branch points. The final macromolecule, consisting of up to 30,000 glucose molecules, forms a granule in the cytoplasm.

Glycogenolysis
In the post absorptive state with decreasing glucose concentrations there is a fall in the insulin/glucagon ratio, activation of adenylate cyclase and an increase in cAMP. The initial changes in the cAMP results in a cascade effect to stimulate glycogenolysis. This amplification of this stimulatory process using a number of enzymes (cyclic AMP – dependent protein kinase, glycogen phosphorylase kinase and glycogen phosphorylase) enables very large quantities of glucose to be released from hepatic glycogen rapidly. In addition to glucagon the 'stress' hormones (adrenaline, cortisol and growth hormone) inhibit glycogenesis and stimulate glycogenolysis. The effect of growth hormone on glycogenolysis is limited to muscle. Thyroxine does not have a direct effect on glycogen metabolism but increases sympathetic activity.

Disorders of glycogen metabolism primarily involve liver and/or muscle although there are rare neurological phenotypes associated with some enzyme deficiencies (▶ Glycogen Metabolism; ◘ Table 5.1). Most are referred to by a roman numeral or by the specific enzyme that is deficient. The use of eponyms is now largely historical. The hepatic glycogenosis generally cause hepatomegaly (apart from GSD 0a) and fasting hypoglycaemia whereas the muscle disorders are associated with skeletal and/or cardiomyopathy. The clinical phenotypes are extremely heterogeneous.

5.1 Hepatic Glycogenoses

5.1.1 Liver Glycogen Storage Disease Type 0 (GSD 0a)

▪ Clinical Presentation

Individuals with GSD 0a present with symptomatic fasting ketotic hypoglycaemia or are found incidentally with post prandial hyperglycaemia and glycosuria. Symptoms generally become apparent in late infancy after weaning or in childhood. Physical examination is normal although some children may have poor growth, short stature and osteopenia; unlike other GSDs there is no hepatomegaly. For review see [1].

▪ Metabolic Derangement

Deficiency of hepatic glycogen synthase (GS2) results in very low levels of glycogen in liver and consequently limited post prandial and fasting hepatic glucose release. Overnight fasting or fasting related to intercurrent infections are associated with ketosis. Hyperglycaemia and moderate hyperlactataemia occurs post-prandially due to reduced uptake of glucose by the liver. Hyperlipidaemia and raised liver transaminases may be found.

▪ Genetics

GSD 0a is an autosomal recessive disorder caused by mutations in *GYS2* which codes for the liver isoform of glycogen synthase. It has 70% homology with *GYS1* which codes for the muscle isoform. A number of mutations have been described. One (R246X) is most common in patients of Italian descent. The disorder appears rare but is likely to be underdiagnosed.

▪ Diagnostic Tests

GSD 0a should be considered in patients with ketotic hypoglycaemia, particularly if they are also found to have postprandial hyperglycaemia or glycosuria. GS2 can be assayed in liver tissue but the diagnosis is now best confirmed by mutation analysis.

▪ Treatment and Prognosis

Treatment consists of preventing fasting hypoglycaemia, hyerketosis and hyperlactataemia. This is achieved by regular meals using a diet with increased protein to stimulate gluconeogenesis and complex carbohydrates to provide slow release of glucose. Daytime snacks may be required between meals. Uncooked cornstarch (1.5 to 2 gm/kg) may be necessary before bedtime to prevent morning hypoglycaemia and also regularly during intercurrent infections. The prognosis for GSD 0a is good. Treatment allows for normal growth and the disorder is not associated with long term hepatic or other complications. Significant hypoglycaemia is less frequent in older children and adults but continued dietary treatment may be required to prevent fasting ketosis.

5.1.2 Glycogen Storage Disease Type I (GSD I)

▪ Clinical presentation

There are two genetically distinct forms of GSD I: GSD Ia caused by deficiency in glucose 6 phosphatase-α and GSD Ib by deficiency in glucose 6 phosphate (G6P) transporter [2]. Both disorders cause severe fasting hypoglycaemia, lactic acidosis and hepatomegaly. In GSD Ib there is additionally immunological disease due to neutrophil dysfunction.

Patients with GSD I generally appear normal at birth although hepatomegaly and hypoglycaemia can be present in the newborn period. From early infancy there is poor growth, abdominal distension, episodes of tachypnoea, and irritability. Some children become extremely unwell with severe acidosis. Those with GSD Ib may present with frequent and often severe bacterial infections.

Table 5.1 Phenotypes associated with enzyme deficiencies

Number	Enzyme deficiency	Gene(s)	Eponym	Main phenotype(s)
0a	Liver glycogen synthase	*GYS2*		Ketotic hypoglycaemia
0b	Muscle glycogen synthase	*GYS1*		Cardiac & skeletal myopathy
Ia	Glucose-6-phosphatase α	*GSPC*	von Gierke	Hypoglycaemia, hepatomegaly, lactic acidosis
Ib	Glucose-6-phosphate transporter	*SLC37A4*		As Ia + neutrophil dysfunction, infections/colitis
II	Lysosomal α-1,4-glucosidase	*GAA*	Pompe	Cardiac & skeletal myopathy
III	Glycogen debrancher	*AGL*	Cori	Hypoglycaemia, hepatomegaly
IV	Glycogen brancher	*GBE1*	Andersen	Cirrhosis; Myopathy/cardiomyopathy; Adult polyglucosan body disease
V	Muscle glycogen phosphorylase	*PYGM*	McArdle	Skeletal myopathy
VI	Liver glycogen phosphorylase	*PYGL*	Hers	Hepatomegaly, hypoglycaemia, growth delay
VII	Muscle phosphofructokinase	*PFKM*	Tarui	Skeletal myopathy
IXa	Phosphorylase kinase (various subunits Table 5.2)	*PHKA2*		Hepatomegaly, hypoglycaemia, growth delay
IXb		*PHKB*		
IXc		*PHKG2*		
IXd		*PHKA1*		Skeletal myopathy
X	Muscle phosphoglycerate mutase	*PGAM2*		Skeletal myopathy
XI	GLUT2	*SLC2A2*	Fanconi-Bickel	Hepatomegaly, hypoglycaemia, renal tubular disease, growth delay
XII	Aldolase A	*ALDOA*		Skeletal myopathy and anaemia
XIII	β-Enolase	*ENO3*		Skeletal myopathy
XIV	Phosphoglucomutase	*PGM1*		Myopathy; CDG1T
XV	Muscle glycogenin deficiency	*GYG1*		Cardiac & skeletal myopathy
	AMP-activated protein kinase	*PRKAG2*		Fatal congenital cardiomyopathy; WPW syndrome with or without cardiomyopathy
	Lysosomal-associated membrane protein 2 (LAMP2)	*LAMP2*	Danon	Cardiomyopathy, skeletal myopathy, mental retardation, maculopathy
	Lafora Body Disease Laforin deficiency Malin deficiency	*EPM2A &* *EPM2B*		Epilepsy, dementia

On examination infants are generally miserable with a 'dolls like' face, a large abdomen and thin arms and legs. The liver is large and easily palpable. On investigations there is fasting hypoglycaemia and lactic acidosis. Fasting tolerance is often very short with blood sugar falling after only 2 to 3 hours. Other abnormalities include hyperlipidaemia and hyperuricaemia. Liver functions test are usually unremarkable without the severe transaminitis seen in GSDIII. In GSD Ib neutropenia may or may not be evident in infancy.

Long term complications are frequent in GSD I and include liver tumours, renal disease, osteoporosis, anaemia, and gastrointestinal disease. They and their treatment are considered in more detail below.

Metabolic Derangement

GSD I is both a disorder of glycogen metabolism and of gluconeogenesis. The failure of glucose dephosphorylation to release free glucose from G6P severely inhibits hepatic glycogen breakdown resulting in excessive glycogen storage and hepatomegly. Hyperlactataemia occurs as a consequence of the disruption to gluconeogenesis and increases with fasting. Since lactate may be used as alternative energy substrate by the

brain, patients are, to some extent, protected from the adverse effects of hypoglycaemia on the CNS.

Secondary abnormalities include hyperlipidaemia, particularly hypertriglyceridaemia and hyperuricaemia. Both occur as a consequence of increased levels of G6P with increased *de novo* lipogenesis and increased flux through the pentose phosphate pathway to form uric acid from ribose-5-phosphate. Additionally, there appears also to be a reduction in lipid clearance [3].

G6P translocase is necessary for normal neutrophil function. Individuals with GSD Ib have both abnormally functioning neutrophils and low neutrophil numbers, the latter caused by increased neutrophil apoptosis (for discussion of the molecular mechanisms involved see [4]). Neutropenia, however, is not always evident in infants. Bacterial infections are common and may be severe.

Genetics

GSD Ia is caused by homozygous or compound heterozygous mutations in *G6PC* and GSD Ib by those in *SLC37A4*. Both disorders are panethnic. GSD I has a frequency of approximately 1 in 100,000 with GSD Ia accounting for 80% of cases. Numerous mutations, mostly missense, have been described in both disorders. Despite some being associated with a complete loss of activity and others with varying degrees of residual function no genotype-phenotype correlation has been established.

Diagnostic tests

The diagnosis of GSD I and its differentiation into 1a and 1b was previously confirmed by assay of glucose 6 phosphatase-α activity in intact (fresh) and disrupted hepatocytes. Absent activity in intact cells, but normal activity in disrupted cells is found in GSD Ib whereas in GSD Ia activity is absent in both preparations. However, the diagnosis is now made on the basis of mutation analysis thus avoiding the need for liver biopsy. If a liver biopsy is performed the histology shows excess storage of cytoplasmic glycogen with large lipid vacuoles but no fibrosis. Prenatal diagnosis can be performed by mutation analysis.

Treatment, Complications and Prognosis

Prior to effective dietary treatment most patients with GSD I died during childhood. Now children can be expected to survive into adulthood. Cognitive development is normal providing there have not been episodes of severe hypoglycaemic encephalopathy. There remain, however, a number of long term complications that significantly impinge on their health and quality of life.

The aim of treatment is to correct the metabolic abnormalities as far as is possible and to prevent or manage any complications. Consensus guidelines for the management of GSD I have been published in 2002 [5] and in 2014 [6].

Dietary treatment

(For a practical review see [7].)

The major requirement is to maintain blood sugar within the normal range by frequent feeds. Carbohydrate should make up 60–70% of total calories, fat 20–25% and protein 10–15%. The glucose requirement is based on normal, age related, hepatic glucose production (8–9 mg/kg/min in infants, 5–7 mg/kg/min in children and 2–4 mg/kg/min in adolescents and adults) but must be adjusted according to biochemical control. In the most severely affected patients feeding is required every 60 to 90 minutes. Such a regimen is extremely onerous, particularly at night. Continuous pumped overnight feeds given via a nasogastric tube or gastrostomy are often employed. However, there are risks with such treatment. Nasogastric tubes can become displaced leading a disruption to the supply of feeds or to inhalation pneumonia. Since patients on treatment have reduced hyperlactataemia a disruption in the supply of glucose can cause severe symptomatic hypoglycaemia in the absence of lactate as alternative fuel. Gastrostomies are generally contraindicated in GSD Ib as, except in the case of very mild disease, invariably become chronically infected. Uncooked cornstarch prolongs carbohydrate absorption from the gut and can increase the period of normoglycaemia between feeds. Commercial extended release cornstarch preparations which prolong the period of normoglycaemia are available [8][9]. It is poorly tolerated in infants but can be introduced gradually in children over the age of 6 months to 2 years. It has been advocated as an alternative to continuous overnight feeding with better blood sugar control [10] although other factors need also be considered [11]. The recommended dose is 1.6 g/kg/3 to 4 hourly in young children and 1.7–2.5 g/kg 4–5 hourly thereafter. In adults where glucose requirements are less, a pasta meal at bedtime may be sufficient [12].

In the absence of glucose 6 phosphatase-α neither fructose nor galactose can be converted to glucose and consequently can lead to a worsening of hyperlactataemia. It is proposed that both sucrose and lactose be restricted in GSD I but there is no agreement on how strict this should be.

Assessing the efficacy of dietary treatment to prevent hypoglycaemia is best achieved by the use of a portable continuous glucose monitor over several days [13].

Hepatic tumors

Benign hepatocellular adenoma (HCA) occur commonly in older patients with GSD I and are evident on liver ultrasound, CT or MR imaging. The adenomas are generally asymptomatic but if large can cause abdominal pain, with bleeding or rupture. Increased production of tumour derived hepcidin can cause a severe refractory anaemia. Adenomas may regress with improved metabolic control [14]. Malignant tumours arising from adenomas are uncommon but have occurred in a number of adult patients. Patients with adenomas need to be monitored for any evidence of malignant change. Consensus recommendations for the surveillance of hepatic adenomas after the onset of puberty in GSD have been published [6]. In summary these are as follows:

- Blood tests, 6 monthly to include liver transaminases, albumin, bilirubin, serum creatinine, international normalized ratio (prothrombin time/partial thromboplastin time), α-fetoprotein (AFP) and chorionic embryonic

antigen (CEA) levels. However, AFP and CEA can be normal in HCC in GSD I and are not a reliable marker of malignant transformation.
- Abdominal ultrasound at baseline and then every 12–24 months in children.
- Abdominal computed tomography/magnetic resonance imaging with contrast in older patients or in paediatric patients once adenomas are detected on ultrasound. A rapid increase in size or number of adenomas or an increase in their vascularity of adenomas may be an indication of malignant change.

Treatment of adenomas that are considered worrying include percutaneous ethanol injections, radiofrequency ablation, and partial liver resection. Liver transplant should be considered for patients with multiple adenomas that are growing and in hepatocellular carcinoma (HCC) where there is no evidence of metastatic spread.

Although the aetiology of adenomatous formation and malignant change in GSD I are not fully understood a number of molecular and cellular abnormalities which may be implicated have been demonstrated in HCA from patients, including alterations in chromosome 6, a lack of HNF1A activation, a reduction in IGF2R and LATS1 and deregulation of microRNAs [15][16][17].

Gastrointestinal Disease

Inflammatory bowel disease occurs frequently in patients with GSD Ib with histology similar to Crohns disease and is thought to be related to neutrophil dysfunction. The enterocolitis may improve on treatment with 5-aminosalicylic acid and granulocyte colony stimulating factor (GCSF). Adalimumab, a monoclonal antibody, has been reported of benefit in one patient unresponsive to aminosalicylate, GCSF, and antibiotic therapy [18]. Although clinical symptoms of colitis appear to be infrequent in GSD Ia, a higher incidence of abnormal serological markers for IBD has been reported [19]. Pancreatitis has been reported in several patients with GSD I. It is assumed that the increase risk is related to hypertriglyceridaemia.

Renal Disease

The kidneys may be large on imaging due to glycogen deposition. Renal disease starts in childhood and effects both proximal and distal renal tubular and glomerular function. In older patients glomerular hyperfiltration, microalbuminuria, proteinuria, nephrocalcinosis and nephrolithiasis can occur, the latter associated with reduced citrate and increased calcium excretion that predisposes to calcium oxalate precipitation. End stage renal failure can occur in the most severely affected patients requiring renal replacement therapy or transplantation. Renal function should be regularly monitored from childhood. The progression of renal disease may be delayed by good metabolic control, oral citrate supplements in those with low urinary citrate, thiazide diuretics if there is hypercalciuria, and an angiotensin-converting enzyme (ACE) inhibitor or an angiotensin receptor blocker (ARB) medication started at the onset of glomerular hyperfiltration [6]. Hyperlipidaemia has been reported to be correlated with the severity of renal disease and severe hyperlipidaemia to effect the efficacy of ACE inhibitors [20]. Hyperuricaemia may increase the risk of developing renal disease and developing gout. Plasma uric acid should be monitored regularly and hyperuricaemia treated with allopurinol. Renal failure is associated with decreased erythropoietin (EPO) production by the kidney and hence anaemia. Treatment with EPO is advised in children if the haemoglobin falls to less that 100 g/l and in adults to prevent transfusion dependency.

Haematological Disease

Anaemia Anaemia may result from iron deficiency, colitis in GSD Ib, associated with large adenomas or from chronic renal disease. Treatment is dependent on the cause and is discussed in the relevant sections.

Coagulopathy Clotting abnormalities in GSD I may manifest as frequent nose bleeds, menorrhagia or increased bruising. The aetiology is not fully understood but is attributed to abnormal platelet function. In some patients there is reduced or dysfunctional von Willebrand factor antigen. The coagulopathy may improve with improved metabolic control otherwise conventional treatment for platelet & factor VIII abnormalities is indicated. This is of particular importance before any surgical procedure.

Neutrophil dysfunction Infections are a serious, potentially life threatening complication in GSD Ib. Mouth ulcers, chronic and severe periodontits and inflammatory bowel disease are common. Treatment with GCSF improves both neutrophil numbers and function and reduces the frequency and severity of infections and improves the colitis in most patients. Treatment is well tolerated in the short term but patients on treatment develop splenomegaly. Acute myeloid leukaemia has been reported after continuous treatment over many years. Published guidelines recommend using GCSF in GSD Ib only if any of the following occur: a neutrophil count below 0.2×10^9/l, a single life-threatening infection requiring intravenous antibiotics, serious enterocolitis documented by abnormal colonoscopy and biopsies or severe diarrhoea requiring hospitalization [21]. Further guidelines recommend using the lowest effective dose, starting at 0.5–1.0 μg/kg given daily or every other day and increasing at 2 weekly intervals until the neutrophil count has increased to 0.5 to 1.0×10^9/l [6]. A prospective trial of vitamin E supplementation in GSD Ib has been reported to improve the neutrophil count and reduce the frequency of infections thus allowing the dose GCSF to be reduced with a reduction of GCSF related side effects [22].

Cardiovascular Disease

Systemic hypertension, secondary to renal disease may affect older children and adults and should be managed conventionally. There remains debate as to whether hyperlipidaemia in GSD I may cause premature atherosclerosis. Lipid levels are lowered with improved metabolic but there are no specific

published guidelines as to the use of statins or other pharmacological treatments. Pulmonary arterial hypertension has been described in a few patients with GSD I, the majority of whom had other concomitant risk factors.

Bone disease

Low levels of vitamin D, which are common in GSD I, should be routinely monitored and treated [23]. Osteopenia and osteoporosis are common in adults. Monitoring of bone density every 2 years is recommended.

Other Treatments

Liver transplant Liver transplant is not solely indicated to treat or prevent HCC in GSD I but has the potential to significantly improve the quality of life for patients and families. Fasting tolerance and other metabolic disturbances become normal following successful transplant and most patients show catch up growth. Of those post transplant complications that were unrelated to the procedure, the most frequent have been renal disease in GSD Ia and a persistent neutropenia in GSD Ib. It is not known whether renal disease might be a progression of pre-existing damage and/or caused by immunosuppressive treatment or ongoing disease still effecting the kidney (a kidney-specific G6PC knockout mouse, has been shown to develop nephropathy despite normal liver enzyme activity [24]). In GSD Ib neutropenia has been found to improve in some but not all patents. Complications associated with the transplantation procedure itself have been relatively common occurring in 18 of the 58 patients reviewed by Boers et al [25]. Potentially, to avoid such complications, cell-based therapies, such as hepatocyte transplantations and liver stem cell transplantations may have role in the future if these can be shown to be efficacious.

Contraception and Pregnancy

Progesterone only oral contraception is recommended since those containing oestrogen increase the risk of hepatic adenomas. Intrauterine devices (IUDs) should be avoided in GSD Ib in view of the risk of infection.

A number of successful pregnancies have been reported both in women with GSD Ia and GSD Ib [26][27]. Despite a high frequency of polycystic ovaries fertility appears to be normal [28]. An increase in glucose requirements has been observed during the first trimester, worsening of renal function during the pregnancy, and in 3 out of 15 patients with GSD Ia, lactic acidosis during delivery [26]. ACE inhibitors must be stopped during pregnancies in women with GSD I.

5.1.3 Glycogen Storage Disease Type III (GSD III)

GSD III is caused by deficiency in glycogen debrancher enzyme (GDE). The disorders is both a hepatic glycogenosis and (in most cases) also a myopathic disorder.

Clinical presentation

The clinical presentation is generally not as severe as in GSD I. Children present in the first year with poor growth, delayed motor milestones and abdominal distension.

Fasting hypoglycaemia occurs but the fasting tolerance is usually longer than in GSD I. Unlike GSD I, fasting ketosis is prominent. Since gluconeogenesis is normal there is no fasting hyperlactataemia although there is a moderate post prandial increase in lactate. The majority of patients have a myopathy. The liver is often very large. On initial investigation, in addition to low glucose levels and ketosis with fasting, there is hyperlipdaemia and markedly raised liver transaminases. Creatine kinase levels are raised in the myopathic form (but not always in infancy). Uric acid levels are not raised. If a liver biopsy is undertaken it shows the accumulation of cytoplasmic glycogen in non membrane bound vacuoles and peri-portal fibrosis.

The skeletal myopathy may worsen with age. Left ventricular hypertrophy is common but symptomatic cardiomyopathy is rare. Unlike GSD I, renal disease is not a long term complication. Hepatic adenomas may occur although these are smaller and less frequent than in GSD I. Liver fibrosis may develop into cirrhosis and there is an increased risk of malignant change. Insulin resistance with maturity onset diabetes may occur in some older patients.

Metabolic Derrangement

GDE has both glucosidase and transferase activity; it cleaves α 1,4 glucose linkages of the terminal glucose molecules and then breaks α 1,6 linkage to remove branch point. Most individuals with GSD III have a defect in both liver and muscle (IIIa) but about 15% have only liver involvement (IIIb) and do not develop a myopathy. Very rarely there is a loss of only one of the two activities; glucosidase deficiency in GSD IIIc and transferase deficiency in GSD IIId.

GDE deficiency leads to the accumulation of abnormal glycogen (limit dextran). Since some, albeit limited, glucose release is possible from glycogen and there is no defect in gluconeogenesis, hypoglycaemia is usually less severe than in GSD I and lactic acidosis does not develop. Raised liver transaminases indicate chronic hepatocellular damage (even though the increase in aspartate aminotransferase may also in part be related to myopathy). Hyperlipidaemia is common in children but less severe than in GSD I. Hypertriglyceridaemia had been found to improve with age [29].

Genetics

GSD III is an autosomal recessive disorder caused by mutations in *AGL*. Mutations, mostly nonsense, associated with GSD IIIa, occur throughout *AGL* whereas two specific mutations in exon 3 are associated with the GSD IIIb [30].

Diagnostic Tests

The diagnosis can be made by the assay of DBE activity in leucocytes or by mutation analysis. Prenatal diagnosis is possible by mutation analysis.

Treatment and Prognosis

Expert derived detailed guidelines for the clinical management of GSD III have been published [31]. The aim of treatment is to maintain normoglycaemia, reduce the hyperlipidaemia and ketosis and ensure adequate growth. This is achieved by regular meals and the use of uncooked cornstarch. Overnight continuous feeding is less commonly needed in GSD III than in GSD I. It is possible that the use of intensive high carbohydrate diets worsens skeletal myopathy and increases the risk of cardiomyopathy by encouraging excessive tissue glycogen deposition. Reducing carbohydrate as a proportion of total calories by increasing protein and fat and the use of oral D,L-3-hydroxybutyrate have been advocated (for review see [32]). Bone health may be affected; it is important to ensure that the diet contains sufficient calcium and vitamin D.

The long term outcome for individuals with GSD III is generally good with survival into adulthood. Growth may continue into the third decade so that a satisfactory final height is reached. The hepatomegaly may lessen or resolve after puberty. Although polycystic ovaries are common, fertility appears normal and successful pregnancies reported [33]. However, life threatening complications can occur in older patients including worsening of skeletal and or cardiomyopathy (with a risk of sudden death from arrhythmia), end stage liver failure from cirrhosis and hepatic malignancy. As a consequence, it is important that patients remain under follow up throughout life.

5.1.4 Glycogen Storage Disease Type IV (GSD IV)

Glycogen disease type IV is caused by deficiency in glycogen brancher enzyme (GBE).

Clinical Presentation

There are a number of phenotypes associated with GBE deficiency ranging from death in utero to adult presentation. The following clinical disorders are described although these subtypes may overlap:

Liver Disease

- Progressive liver disease in infancy. This presents within the first few months of life with failure to thrive and hepatomegaly. Cirrhosis develops with eventual end stage liver disease and portal hypertension. Death is usual by 5 years of age.
- Non-progressive liver disease in childhood. Patients present with hepatomegaly and liver dysfunction, hypotonia and myopathy. However, the liver disease does not progress and survival is into adulthood.

Neuromuscular disease

- Congenital onset – including fetal loss in pregnancy, fetal akinesia deformation sequence (FADS) with athrogryposis, hydrops and perinatal death, or a severe congenital myopathy similar to spinal muscular atrophy but which may also be associated with cardiomyopathy.
- Juvenile onset – with a myopathy and/or cardiomyopathy [34].
- Adult onset – adult polyglucosan body disease (APBD) or more rarely, myopathy. APBD is described in ▶ Section 5.3.2

Metabolic Derrangement

GBE is responsible for transferring short glucosyl chains to form branch points with an alpha 1,6 linkage. Deficiency of GBE results in an abnormal poorly soluble glycogen with fewer branch points (polyglucosan) that leads to tissue damage. This abnormal glycogen accumulates to a varying degree in various organs, including liver, muscle, heart, nervous system and skin. The different clinical manifestations are likely to be related to the degree of enzyme deficiency with an almost complete absence of GBE in the congenital forms of the disease but significant residual activity in APBD. Liver transaminases are raised in those with hepatic involvement. Fasting hypoglycaemia is uncommon except in end stage liver failure. Liver and muscle histology show swollen hepatocytes that contain periodic acid-Schiff (PAS)-positive and diastase resistance inclusions and evidence of interstitial fibrosis.

Genetics

GBE deficiency is a rare autosomal recessive disorder caused by mutations in *GBE1* (for review see [35]). A common mutation found in the the Ashkenazi Jewish population is associated with APBD. There appears to be some degree of genotype-phenotype correlation; none of the mutations found in patients with the congenital form have been found in adult onset patients. More severe disease is most often associated with two null mutations or large deletions rather than missense mutations.

Diagnosis

Enzyme analysis can be undertaken in liver tissue, cultured skin fibroblast, peripheral lymphocytes and muscle. The diagnosis is confirmed by *GBE1* mutation analysis.

Prenatal diagnosis is possible by enzyme analysis in cultured amniocytes or chorionic villi cells and by *GBE1* mutation analysis [36].

Treatment and outcome

Liver transplant is the only treatment for the progressive liver form [37]. Although following transplant most children have done well with no progressive skeletal myopathy or cardiomyopathy, a few have had a fatal progression of the disease affecting other organs, particularly the heart [38]. Heart transplant may be considered in those with heart failure caused by cardiomyopathy. There is no specific treatment for the other forms of the disease.

5.1.5 Glycogen Storage Disease Type VI (GSD VI)

GSD VI is caused by deficiency in hepatic glycogen phosphorylase.

Clinical Presentation

GSD VI is generally a relatively mild disorder often diagnosed following an incidental finding of hepatomegaly. However, it may also present with symptomatic ketotic hypoglycaemia and growth retardation. During adolescence symptoms and signs normally resolve and most adults are asymptomatic. Liver adenoma, liver fibrosis, mild cardiomyopathy are rare complications [39]. Hepatocellular carcinoma has been reported in a single patient [40].

Metabolic Derrangement

Hepatic glycogen phosphorylase catalyses the release and phosphorylation of terminal glucosyl units from glycogen forming glucose-1-phosphate. Ketosis with or without hypoglycaemia may occur with fasting [41]. Although plasma lipids may be raised, uric acid and creatine kinase are normal. In those with more severe variants recurrent hypoglycaemia and post prandial lactic acidosis can occur.

Genetics

GSD VI is an autosomal recessive disorder caused by mutations in *PGYL* which codes for the liver isoform of glycogen phosphorylase. A high frequency of missense mutations have been reported [42].

Diagnosis

The diagnosis can be confirmed by mutation analysis or by finding enzyme deficiency in hepatic tissue, erythrocytes, and leukocytes. However, enzyme activity may not always be reduced in blood and even in liver tissue may be difficult to interpret due to residual activity and the effect of other factors. For example, deficiency of glycogen phosphorylase kinase will cause low activity of glycogen phosphorylase.

Treatment and Outcome

Treatment for asymptomatic children may not be required but those with growth failure or fasting ketosis may benefit from regular meals and snacks and uncooked cornstarch (1.5–2 g/kg). The outcome for individuals with GSD VI is generally excellent with catch up growth occurring for those with short stature in childhood.

5.1.6 Glycogen storage disease type IX (GSD IX)

GSD IX is caused by deficiency in hepatic glycogen phosphorylase kinase (PHK) [43]. The enzyme is a multi-enzyme complex consisting of four homo-tetramers, each made up of an α, β, γ and δ subunit. The γ subunit is catalytic and the other subunits regulatory. There are tissue specific isoforms of the α and γ subunits. The δ subunit, calmodulin, is ubiquitous. The various subtypes of GSD IX are listed in Table 5.2.

Clinical presentation

Most patients with GSD IX, have a relatively benign disorder, with hepatomegaly often detected incidentally but there may be short stature, fasting hypoglycaemia and ketosis, with raised liver transaminases, cholesterol and triglycerides. Blood lactate and uric acid are normal. There is usually resolution of signs and symptoms by adulthood. GSD IXc can be more severe with an increased risk of hepatic fibrosis and cirrhosis.

Metabolic derangement

PHK phosphorylates glycogen phosphatase leading to a conformational change to form the active ›a‹ form. Decreased PHK activity results in more glycogen phosphatase remaining in the inactive ›b‹ form with a subsequent reduction in glucose-1-phosphate release from glycogen.

Genetics

The most common form, GSD IXa is an X-linked recessive disorder. The other, rarer variants are autosomal recessive. The various sub-units and their respective genes involved are listed in Table 5.2.

Diagnosis

The diagnosis should be considered in children with unexplained hepatomegaly and in those with ketotic hypoglycaemia. PHK can be measured in liver, erythrocytes and leukocytes. However, in view of variable tissue expression enzyme assays may be difficult to interpret. Diagnosis is best achieved by mutation analysis using a DNA panel.

Treatment and outcome

Asymptomatic patients may not need treatment. For those with growth failure or symptomatic hypoglycaemia frequent meals and uncooked cornstarch may be used. Protein can be increased to 15 to 20% of calories to provide a gluconeogenesis substrate. The outcome for most patients is good with resolution of hepatomegaly and catch up growth by adulthood. A patient with IXb has been reported with mild cardiomyopathy [39]. Patients with GSD IXc may have a more severe disease with an increased risk of hepatic fibrosis and cirrhosis [44].

5.1.7 Fanconi-Bickel Syndrome

Fanconi-Bickel syndrome, a hepatic glycogen storage disease associated with renal tubular disease, is caused by deficiency in Solute Carrier Family 2 protein (GLUT2) due to mutations in *SLC2A2*. The disorder is discussed in ▶ Chapter 10.

Table 5.2 PHK deficiency

	Gene	Location	Subunit	Inheritance	Tissue involved
GSD IXa	*PHKA2*	Xp22.13	α_2	XLR	GSD IXa1 (XLG1): liver & blood GSDI Xa2 (XLG2): liver*
GSD IXb	*PHKB*	16q12.1	β	AR	liver & muscle
GSD IXc	*PHKG2*	16p11.2	γTL (Liver/testis isoform)	AR	liver
GSD IXd	*PHKA1*	Xq13.1	α_1	XLR	muscle**

*Mutations causing GSD IXa2 are missense, small in-frame deletions, or insertions with some residual enzyme activity in erythrocytes [42].
**GSD IXd is a cause of, cramps and exercise intolerance, middle age onset and raised CK, with normal red cell & liver PHK activity. There is a variable lactate response with the non-ischaemic exercise test.
No disorder has yet been described caused by mutations in *PHKAG1* (at 7p11.2) which codes for the muscle isoform of the γ subunit.
A fatal autosomal dominant congenital heart glycogenosis is now known not to be caused by PHK deficiency but by mutations in *PRKAG2* which codes for the γ_2 subunit of AMP-activated protein kinase [45] (see below ► Section 5.2.6).
For review see [43]

5.2 Muscle and Cardiac Glycogenoses

At rest, muscle predominantly utilizes fatty acids. During submaximal exercise, it additionally uses energy from blood glucose, mostly derived from liver glycogen. In contrast, during very intense exercise, the main source of energy is anaerobic glycolysis following breakdown of muscle glycogen. When the latter is exhausted, fatigue ensues. Enzyme defects within the pathway affect muscle function.

5.2.1 Glycogen Storage Disease Type V (Myophosphorylase Deficiency, McArdle Disease)

Clinical Presentation

GSD V, the most common muscle glycogenosis is characterized by exercise intolerance with myalgia and stiffness of exercising muscles, which are relieved by rest. Onset of the disease occurs during childhood, but diagnosis is frequently missed at an early age because affected children are often considered to be just lazy. Two types of effort are more likely to cause symptoms: brief intense isometric exercise, such as lifting heavy weights, or less intense but sustained dynamic exercise, such as running or climbing a hill. Moderate exercise, such as walking on level ground, is usually well tolerated. All patients experience a constant phenomenon, named the »second wind«: if they rest briefly after the onset of exercise-induced myalgia, they are then able to continue to exercise with a lower level of pain and fatigue. This phenomenon is considered to be related to the ability to metabolize free glucose that is mobilized in the bloodstream. Myoglobinuria is the major complication, and occurs in about half of the patients. Creatine kinase (CK) can increase to more than 100,000–1,000,000 UI/l during episodes of rhabdomyolysis, leading to a risk of developing acute renal failure. With carnitine palmitoyl transferase II (CPT II) deficiency, GSD V is the second most common disorder leading to episodes of recurrent myoglobinuria [46], although lipin1 deficiency is now also recognised as a relatively frequent cause in children (► Chapter 34). Clinical examination is usually normal between crises, but proximal muscle weakness and wasting occur in approximately 35% of the patients over 40 years of age [47]. Two patterns of muscle weakness may be observed: (1) proximal and symmetrical, or (2) scapulo-humeral and asymmetrical. Resting serum CK is consistently elevated in McArdle patients. Electromyography (EMG) can be normal or show nonspecific myopathic features at rest, but documents electrical silence in contracted muscles.

Metabolic Derangement

There are three isoforms of glycogen phosphorylase: brain/heart, liver and muscle, all encoded by different genes. GSD V is caused by deficient myophosphorylase activity.

Genetics

GSD V is an autosomal recessive disorder caused by mutations in *PYGM*. The number of known pathogenic mutations has rapidly increased to over 100 [48]. By far the most common mutation in Caucasians is the p.R50X mutation, which accounts for 81% of the alleles in British patients [49] and 63% of alleles in US patients (50).

No genotype-phenotype correlations have been detected. In addition, an angiotensin-converting enzyme (ACE) insertion/deletion polymorphism might play a significant role as a phenotype modulator in individuals with GSD V [51].

Diagnosis

The ischaemic forearm exercise test (IFET) was first used by McArdle to describe the absence of elevation of lactate during

exercise, but its main drawbacks are muscle pain with possible rhabdomyolysis (▶ Chapter 3). Consequently the ischaemic test should be abandoned and replaced by the standardized non-ischaemic FET, which has a sensitivity of 100% in McArdle's disease [52][53]. Ammonia levels should be also assessed in parallel with lactate, as an abnormal increase in ammonia is always observed in GSD V. This measurement of ammonia also allows discrimination of patients with disorders of glycogenolysis from those with nonorganic muscle symptoms, because in the latter the lack of an increase in both lactate and ammonia indicates insufficient effort due to lack of cooperation. Alternative diagnostic tests include (1) a cycling test at a moderate and constant workload, during which patients with GSD V show a consistent decrease in heart rate between the 7th and the 15th minutes of exercise, indicating the second wind phenomenon [54] or (2) ^{31}P-magnetic resonance spectroscopy to demonstrate abnormal alkalinisation after exercise [55]. Muscle biopsy shows vacuoles and subsarcolemmal accumulation of glycogen that is normally digested by diastase. Negative staining using a specific myophosphorylase confirms the diagnosis, but muscle biopsy should always be performed several weeks after an episode of rhabdomyolysis, as the histochemical abnormalities may be overshadowed by the intensity of the necrotic process. However, muscle biopsy should be avoided when the diagnostic is suspected, as sequencing of *PYGM* gene is now available.

Treatment

There is no pharmacological treatment, but exercise intolerance may be alleviated by aerobic conditioning programs [56] or by ingestion of oral sucrose (37 g), which may have a prophylactic effect when taken 5 to 15 minutes before planned activity [57]. This effect is explained by the fact that sucrose is rapidly split into glucose and fructose; both bypass the metabolic block in GSD V and hence contribute to glycolysis [58]. It has been reported that work capacity and exercise tolerance are improved after a carbohydrate-rich diet, an effect that needs to be explored in larger controlled trials [59]. Patients should also avoid strenuous efforts and leisure activities that put them at risk, such as swimming far from the shore and mountaineering.

5.2.2 Disorders of Glycolysis

Several other enzyme deficiencies affecting the glycolytic pathway have been reported and are described fully in ▶ Chapter 6 (◘ Table 5.1). They all present with exercise intolerance and possibly also with episodes of rhabdomyolysis similar to those in GSD V. Additional clinical, biological and morphological features may allow these very rare disorders to be distinguished from GSD V.

When a disorder of glycolysis is suspected, the first step in the evaluation of patient should be a forearm exercise test for measurements of lactate and ammonia levels. Absent or blunted lactate production with an abnormal rise of ammonia levels is a characteristic feature, which should always be followed by search for *PYGM* mutations, before performing a muscle biopsy. ^{31}P-NMR spectroscopy, which is available only in highly specialized centers, allows detection of an abnormal increase in the phosphomonoester peak in PFK and PGK deficiencies, a useful criterion for distinguishing these enzyme deficiencies [56].

Muscle histology shows inconstant subsarcolemmal vacuoles and glycogen accumulation on PAS staining. This glycogen is normally digested by diastase, except in PFK deficiency, which can also lead to accumulation of abnormally branched glycogen (polyglucosan) with a hyaline aspect on standard haematein-eosin stain and resistance to diastase digestion. Specific anomalies such as tubular aggregates may be observed in PGAM deficiency. A specific histochemical reaction is also available for PFK and may help to confirm the diagnosis of GSD VII. The diagnosis is made from the biochemical analysis of enzyme deficiencies either on muscle biopsy for all enzymes, or in erythrocytes for PFK, PGK and aldolase A and from molecular analysis.

5.2.3 Glycogen Storage Disease Type II (Pompe Disease)

GSD II, also named Pompe disease, acid α-glucosidase deficiency or acid maltase deficiency, is caused by deficiency of the lysosomal enzyme acid α-glucosidase. It is the second most common cause of muscle glycogenosis after GSD V.

Clinical Presentation

Pompe disease presents as a spectrum, with infantile, juvenile and adult forms named according to the age at onset, rate of progression and extent of organ involvement [60].

The classic infantile form usually presents within the first months of life with hypotonia (floppy infant syndrome) and hypertrophic cardiomyopathy, which can be detected on chest X-ray and electrocardiogram. Additional clinical features can be dysphagia, smooth muscle dysfunction, enlargement of the tongue and liver and failure to achieve major motor milestones. Most untreated infantile onset patients die from cardiopulmonary failure or aspiration pneumonia prior to one year of age [61]. The juvenile forms are characterized by predominant skeletal muscle dysfunction, with motor and respiratory problems, but rarely cardiac involvement. Calf hypertrophy can be present, mimicking Duchenne muscular dystrophy in boys. Myopathy and respiratory insufficiency deteriorate gradually, and patients may become dependent on a ventilator or wheelchair.

The adult form develops in the 3rd or 4th decade and affects the trunk and proximal limb muscles, mimicking inherited limb-girdle muscle dystrophies [62]. Involvement of the diaphragm is frequent, and acute respiratory failure may be the initial symptom in some patients. Therefore, the presence of a severe respiratory insufficiency in a patient with moderate limb-girdle muscle weakness is a major clue in the diagnosis of adult-onset Pompe disease. By contrast with the infantile form, the heart is generally not affected. The major cause of

death in adults is respiratory insufficiency. Pulmonary function tests should be undertaken annually and respiratory support started when necessary, as in some patients this can prolong life for decades. Rarer causes of death are strokes related to intracranial aneurysm or arteriopathy due to accumulation of glycogen in vascular smooth-muscle cells [63][64].

Metabolic Derangement

The enzyme defect results in the accumulation of glycogen within the lysosomes, with different critical thresholds depending on the organ, explaining why the heart is unaffected in adults who have significant residual enzyme activity. Intermediary metabolism is unaffected. Autophagy probably also has a major role in the pathogenic process, with recent work showing an autophagic build-up due to dysfunction of endocytic and autophagic pathways in the muscle fibres [65]. A failure to digest glycogen could result in local starvation, inducing autophagy with a pathological cycle due to lysosomal dysfunction.

Genetics

Over 200 mutations have been reported in *GAA* encoding acid α-glucosidase, about 75% of these being pathogenic mutations (www.pompecenter.nl). There is some degree of genotype-phenotype correlation with severe mutations (such as del exon18) associated with the infantile form and ›leaky‹ mutations associated with the adult variant. The most common mutation in adults and children with a slowly progressive course is c.-32-13T>G (approximately 80% of Caucasian patients).

Diagnosis

The diagnosis always relies on demonstrating acid α-glucosidase deficiency; infants with the classic infantile form have less than 1% residual activity, whereas children and adults have residual activity of no more than 30% of normal values. Sensitivity and specificity of enzymatic assays performed in various tissues may be altered by interference with neutral α-glucosidase activities, and skin fibroblasts are the best tissue for diagnosis owing to lower biochemical interferences. Screening methods for acid α-glucosidase deficiency using assays in dried blood spots have been developed [66] and could be suitable for neonatal screening. Enzymatic prenatal diagnosis is also possible on chorionic villi, but DNA analysis is a far better procedure in this context if mutations have already been detected in the parents or a previously affected child.

Muscle biopsy shows a severe vacuolar myopathy with accumulation of both lysosomal and free glycogen in the infantile form, but this procedure is not recommended in babies because of the anaesthetic risks. Conversely, the diagnosis is frequently established in adults from the result of a muscle biopsy performed in the context of diagnostic work-up of a muscle dystrophy. A vacuolar myopathy with PAS-positive material is present in approximately two thirds of adults, but in one third of cases the muscle biopsy may be normal or show nonspecific changes, potentially leading to a mistaken diagnosis [67]. Electromyography may also help in establishing the diagnosis in the myopathic forms of the disease, showing pseudo-myotonic discharges, more frequently in paraspinal muscles, in addition to the myopathic features.

Treatment

Palliative therapy relies on prevention of cardiorespiratory failure, with the possibility of long-lasting survival in adults with ventilatory support. A major step towards treatment of Pompe disease has been achieved with the large-scale production of recombinant acid α-glucosidase (rhGAA), initially in milk from transgenic rabbits and subsequently from CHO cells (alglucosidase alfa). Alglucosidase alfa (Myozyme or Lumizyme) has been commercially approved since 2006, and two large studies in infants showed major beneficial effects on cardiomyopathy and muscle weakness, with increased survival [68]. Doses of 20 mg/kg by infusion every other week are recommended though higher does are now currently being considered [69]. However, only about one third of children on enzyme replacement therapy (ERT) gain normal motor function status and become ventilator free [70][71] although the outcome appears better for those started on treatment following identification by newborn screening [72]. Several factors may limit the efficacy of ERT in children, including the the severity and extent of muscle damage at the start of treatment and the appearance of high levels of IgG antibodies to rhGAA [73]. In adults, a double-blind placebo-controlled trial showed improvement of the walking distance and stabilisation of vital capacity after 18 months of treatment [74]. Long-term follow-up data in adults confirms a tendency to stabilisation of muscle function and respiratory involvement [75]. Further data are currently being collected across the entire spectrum of Pompe disease in order to expand understanding of the effects of ERT and to formulate guidelines for treatment.

5.2.4 Danon Disease (LAMP-2 Deficiency)

Danon disease is a rare X-linked disorder caused by a primary deficiency of lysosomal-associated membrane protein 2 (LAMP2).

Clinical Presentation

The disease presents after the first decade, and the characteristic clinical features in male patients include cardiomyopathy in all cases and mild skeletal myopathy and mental retardation in approximately 70%. Eye fundus examination may detect either retinopathy or maculopathy, and these visual abnormalities may be important clues to this diagnosis in patients with unexplained hypertrophic cardiomyopathy [76]. Hemizygous females can also be affected, with a later age at onset and either hypertrophic or dilated cardiomyopathy.

Metabolic Derangement

This disease has been classified with glycogenoses because of the appearance on muscle biopsy, in most cases, of small cytoplasmic vacuoles containing autophagic material and glycogen in muscle fibres [77].

Genetics

Danon disease is caused by mutations in *LAMP2* on Xq24.

Diagnosis

The diagnosis may be confirmed with muscle biopsy, by the absence of LAMP-2 staining on immunohistochemistry and detection of mutations in *LAMP2* [78].

Treatment

No specific therapy is available, but cardiac transplantation should be considered (79).

5.2.5 Glycogen Depletion Syndromes: Muscle Glycogen Synthase Deficiency (Muscle GSD Type 0, GSD 0b) and Glycogenin 1 Deficiency

Two muscular glycogenoses that are due to deficiencies of enzymes involved in the initial steps of glycogen synthesis, glycogenin and glycogen synthase, have recently been identified [80][81].

Clinical Presentation

Both disorders present with myopathy and cardiomyopathy.

Metabolic Derangement

In both diseases the major pathological hallmark is a profound depletion of glycogen in muscle on PAS staining, associated with a marked predominance of oxidative (type 1) muscle fibres and mitochondrial proliferation. However, there is an unexplained difference between them in the cardiac pathology, with an absence of glycogen in cardiomyocytes in GSD 0b, whilst PAS-positive material lacking the normal ultrastructural appearance of glycogen was present in the patient with glycogenin 1 deficiency.

Genetics

Glycogenin 1 Deficiency Glycogenin is an autoglycosylated glycosyltransferase that catalyses the formation of a short glucose polymer of approximately ten glucose residues. There are two glycogenin isoforms: glycogenin-1, encoded by *GYG1*, is the muscle isoform, but is also expressed in other tissues to a minor degree; glycogenin-2, encoded by *GYG2*, is the liver isoform and is also expressed in cardiac muscle and other tissues, but not in skeletal muscle. Recessively inherited mutations of *GYG1*, leading to inactivation of autoglycosylation of glycogenin-1, have been detected in a young patient with exercise intolerance, muscle weakness and cardiac arrhythmia associated with hypertrophic cardiomyopathy [80]. Different *GYG1* mutations have also been found in a number of patients with a polyglucosan skeletal myopathy without cardiomyopathy (see below).

Muscle Glycogen Synthase Deficiency (Muscle GSD Type 0, GSD 0b) Muscle glycogen synthase is ubiquitously expressed and encoded by *GYS1*, whereas *GYS2*, encoding for hepatic glycogen synthase, is only expressed in the liver. A recessively inherited stop mutation in *GYS1* has been reported in three siblings with muscle fatigability and hypertrophic cardiomyopathy. Epilepsy was observed in the oldest child, who died of cardiac arrest at the age of 10 years. Glucose tolerance was investigated in the two younger siblings and was found to be normal [81].

Treatment

No specific treatment has been reported apart from selective β_1-receptor blockade for cardiac protection.

Glycogen Storage Disease Type III (Debranching Enzyme Deficiency)

(▶ Section 5.1.3).

5.2.6 Muscle and Cardiac Glycogenosis with Polyglucosan Bodies Due to *RBCK1* and *GYG1* Mutations

Two new forms of glycogenosis have been recently described, characterised by accumulation of polyglucosan in skeletal muscle or heart [82][83].

Clinical Presentation

Patients with *RBCK1* mutations present progressive muscle weakness and may also develop a rapidly progressive cardiomyopathy which may require cardiac transplantation. Patients with polyglucosan accumulation in skeletal muscel due to *GYG1* mutations, in contrast to the patient with glycogen depletion in skeletal muscle and *GYG1* mutations, described above, have not developed cardiomyopathy.

Genetics

Glycogenin 1 deficiency *GYG1* mutations which have been identified in 7 patients with polyglucosan body myopathy are missense, nonsense, or frameshift pathogenic variants, distributed all over the gene. There was either reduced or complete absence of glycogenin-1 protein, in accordance with the deleterious effects of the variants. The most frequent variant was c.14313G>C, identified in patients from different ethnic backgrounds. This common splice site variant caused a complete or nearly complete alternative splicing, with profound reduction of wild-type glycogenin-1 [83].

RBCK1 mutations

All patients are homozygous or compound heterozygous for missense or truncating mutations in this gene which encodes an E3 ubiquitin ligase. How RBCK1 is involved in glycogen metabolism remains unknown. Furthermore, children with loss-of-function mutations in the same gene had a distinct phenotype characterised by failure to thrive, autoinflammation, and recurrent episodes of sepsis [84].

5.2.7 AMP-activated Protein Kinase (AMPK) Deficiency

Clinical Presentation

Symptoms start typically in late adolescence with ventricular pre-excitation (Wolff-Parkinson-White syndrome) predisposing to supraventricular arrhythmias. There is a progressive mild to severe cardiac hypertrophy and an increased risk of sudden cardiac death. The disorder is usually described as familial hypertrophic cardiomyopathy with Wolff-Parkinson-White syndrome. Although glycogen storage typically affects only the heart, a skeletal muscle involvement with myalgias or muscle weakness may also occur in some patients, and a skeletal muscle glycogenoses has been reported in a patient with exercise intolerance, high CK levels and a forearm exercise test showing a blunted lactate increase [85].

Metabolic Derangement

AMPK is a cellular energy sensor that is activated by exercise in muscle and an increase in the AMP/ATP ratio, stimulating fatty acid oxidation, glycolysis and glucose oxidation. This enzyme forms a heterotrimeric complex comprising a catalytic subunit (α) and two regulatory subunits (β and γ). Three isoforms of the gamma subunits are known (γ1, γ2 and γ3) with different tissue expression, and each contains four repeats of a structural module known as a cystathionine β–synthase (CBS) domain [86]. Pathological examinations of the hearts from affected patients revealed vacuoles containing polysaccharide.

Genetics

The *PRKAG2* gene coding for the γ-subunit of AMPK is located on chromosome 7q36. Mutations in the γ2-subunit of AMPK are transmitted as an autosomal dominant trait with full penetrance [87]. Interestingly, molecular analysis performed in babies who had died of cardiac congenital glycogenoses, which had been attributed to a heart-specific variant of phosphorylase b-kinase deficiency, revealed a recurrent activating mutation in *PRKAG2*. Therefore, it appears that the low PHK activities that were determined in the hearts of these patients were either artefacts or secondary to a down-regulation induced by AMPK dysfunction or cardiac glycogen deposition [45].

Diagnosis

The diagnosis, if clinically suspected, is based on ECG, echocardiography and molecular genetics. The differential diagnosis includes Pompe, Danon (LAMP2) and Fabry diseases.

Treatment

Treatment requires a pacemaker/defibrillator and heart transplant.

5.3 Brain Glycogenoses

In the brain branching enzyme, glycogen synthase, debranching enzyme and phosphorylase are present in both astrocytes and neurons. In neurons, however, there is no glycogen synthesis, since glycogen synthase is directed toward glycogen degradation in the proteasome system by the laforin-malin complex. In astrocytes glycogen is degraded to supply energy during brief episodes of hypoglycaemia and hypoxia. Glycogenolysis in astrocytes produces lactate, which is exported by a monocarboxylic transporter to neurons, where it is oxidised in the mitochondria [88]. Brain GSDs present with adult neurodegeneration/epilepsy syndromes associated with accumulation of polyglucosan bodies. Polyglucosan deposition in the nervous system is characteristic of Lafora disease and adult polyglucosan body disease, but can also occur in normal ageing.

5.3.1 Lafora Disease (Neuronal Laforin/Malin Defects)

Clinical Presentation

Lafora disease is an autosomal recessive form of myoclonic epilepsy that typically manifests during adolescence and is characterised by tonic-clonic, myoclonic and absence seizures, or focal seizures frequently associated with visual symptoms. As the disease progresses, affected individuals develop a rapidly progressive dementia with visual loss, apraxia and aphasia, leading to a vegetative state and death within a decade of disease onset.

Metabolic Derangement

The hallmark of Lafora disease is the presence of large inclusions (Lafora bodies) composed of abnormal glycogen molecules in the axons and dendrites of neurons, especially in the cerebral cortex, substantia nigra, thalamus, globus pallidus and dentate nucleus. The abnormal glycogen has an elevated phosphate content and reduced branching. Polyglucosan bodies are also seen in muscle, liver, heart, skin and retina, showing that Lafora disease is a generalised glycogenoses. The mechanisms by which accumulation of abnormally branched glycogen triggers neuronal apoptosis are undetermined [88] [89].

Genetics

Lafora disease has been found associated with mutations in two genes: Epilepsy, Progressive Myoclonus 2a (*EPM2A*) and Epilepsy, Progressive Myoclonus 2b (*EPM2B*). *EPM2A* is mutated in about 50% of individuals and encodes laforin; *EPM2B* is mutated in 30-40% and encodes malin. These two mutations share an identical phenotype, as these two proteins operate through a common physiological pathway. An early onset form of the disorder has been described in a single family with mutations in *PRDM8*. The PRDM8 protein is of unknown function but has been shown to interact with laforin and malin [90].

Diagnosis

A skin biopsy will reveal the pathognomonic Lafora bodies in most patients. Mutation analysis will confirm the diagnosis.

Treatment

No treatment is available.

5.3.2 Adult Polyglucosan Body Disease

Clinical Presentation

Adult polyglucosan body disease (APBD) is the adult-onset form of branching enzyme deficiency, and this rare disorder is characterised by progressive central and peripheral nervous system involvement. The first symptoms of APBD usually occur between the fourth and fifth decade of life, with early onset bladder dysfunction followed by progressive spastic paraplegia, and to a lesser extent, peripheral neuropathy and cognitive impairment occurring in about 50% of cases in the later stages of the disease. An earlier relapsing, remitting course is also described [91]. Several clinical variants, mimicking spinocerebellar ataxia, extrapyramidal disorders, or motor neuron disease, have been described [92][93][94]. Brain MRI most often show atrophy of the cervical spine and white matter abnormalities on T2 sequences predominantly in the periventricular regions, pyramidal tracts and the medial lemniscus of the pons and medulla [95].

Genetics

Most of the patients with APBD are of Ashkenazi Jewish ancestry, with a prevalent mutation in *GBE1* (p.Tyr329Ser) in this population. However, in a significant number of patients, only one heterozygous mutation has been found despite similar residual enzymatic activity than patients with two identified mutations. In fewer cases, no mutation has been found in *GBE1*, suggesting a genetic heterogeneity [95].

Diagnosis

Although the presence of polyglucosan bodies in skin, muscle or nerve tissues can further orientate the diagnosis, APBD is primarily confirmed by a reduction of GBE enzymatic activity to 10-20% normal in patients' leukocytes or fibroblasts.

Treatment

No treatment is currently available, but a clinical trial with triheptanoin is ongoing.

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Disorders of Galactose Metabolism

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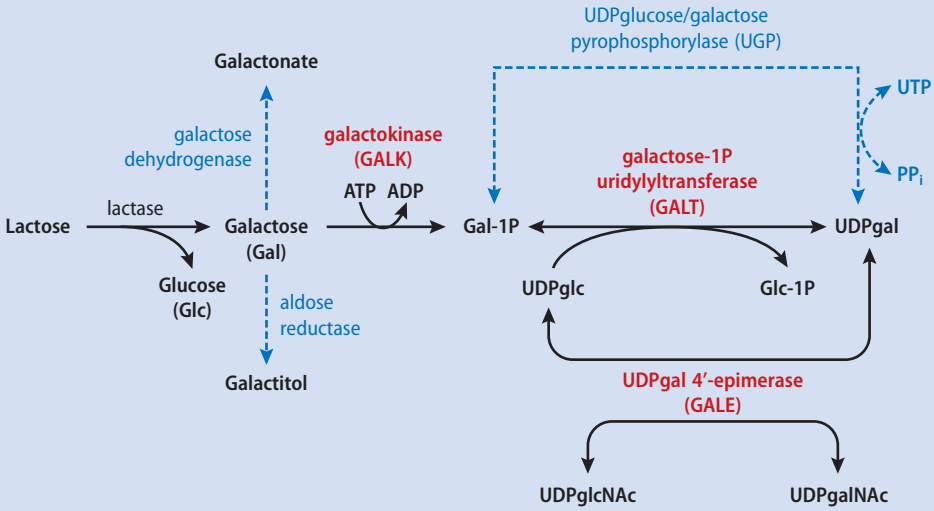
Galactose Metabolism

In nearly all mammals, glucose and uridine diphosphogalactose (UDPgal) are used in mammary tissue to form the disaccharide lactose, which is the principal carbohydrate in milk. Although other foods may contain free or bound galactose, dairy products are, by far, the largest exogenous source of galactose in man. Endogenous galactose production is also significant.

Ingested lactose is hydrolysed by lactase, liberating galactose and glucose (◘ Fig. 6.1). Galactose is then absorbed using a sodium/glucose-galactose cotransporter (SGLT1). Although the enzymes involved in galactose metabolism are widespread, the liver is the major organ responsible for galactose metabolism. The integration of galactose within hepatic carbohydrate metabolism is shown in ◘ Fig. 6.2. Galactose is phosphorylated to form galactose-1-phosphate (Gal-1P) by galactokinase (GALK, EC 2.7.1.6). Galactose-1-P-uridylyltransferase (GALT, EC 2.7.7.12) converts uridine diphosphoglucose (UDPglc) and Gal-1P into UDPgal and glucose-1-phosphate (Glc-1P). Glc-1P is metabolised into glucose-6-P, from which glucose is formed by glucose 6 phosphatase and pyruvate and lactate by glycolysis (▶ Chapter 5, ◘ Fig. 5.1). Galactose can also be reduced by aldose reductase to form galactitol, or oxidized by galactose dehydrogenase to form galactonate. UDPglc (or UDP-N-acetylglucosamine, UDPglcNAc) can be interconverted with UDPgal (or UDP-N-acetylgalactosamine, UDPgalNAc) by UDP-galactose 4′-epimerase (GALE, EC 5.1.3.2). The utilisation of UDPgal in the synthesis of glycoconjugates, including glycoproteins, glycolipids and glycosaminoglycans, and their subsequent degradation (◘ Fig. 6.2) may constitute the pathways of de novo and salvage production of endogenous galactose. All four substrates of GALE: UDPgal, UDPglc, UDPgalNAc, and UDPglcNAc, are used for glycoconjugate synthesis. UDPglc is also the key substrate in all tissues for glycogen production, and as mentioned above, UDPgal is also used in mammary tissue for lactose synthesis. The UDPglucose pyrophosphorylase (UGP) enzyme (◘ Fig. 6.1) that is primarily responsible for synthesis of UDPglc from Glc-1P and UTP can also catalyse, albeit in a limited way, the synthesis of UDPgal from Gal-1P and UTP, and therefore may also contribute to metabolism of galactose.



◘ **Fig. 6.1 Major reactions of galactose metabolism.** The three enzymes of the Leloir Pathway (GALK, GALT, and GALE) are presented in red font. Enzymes that provide alternative or bypass routes of galactose metabolism are presented in blue font. The four UDP sugars listed are all key substrates for glycosylation. Inherited deficiencies of GALK, GALT, or GALE result in the primary disorders of galactosemia metabolism described here

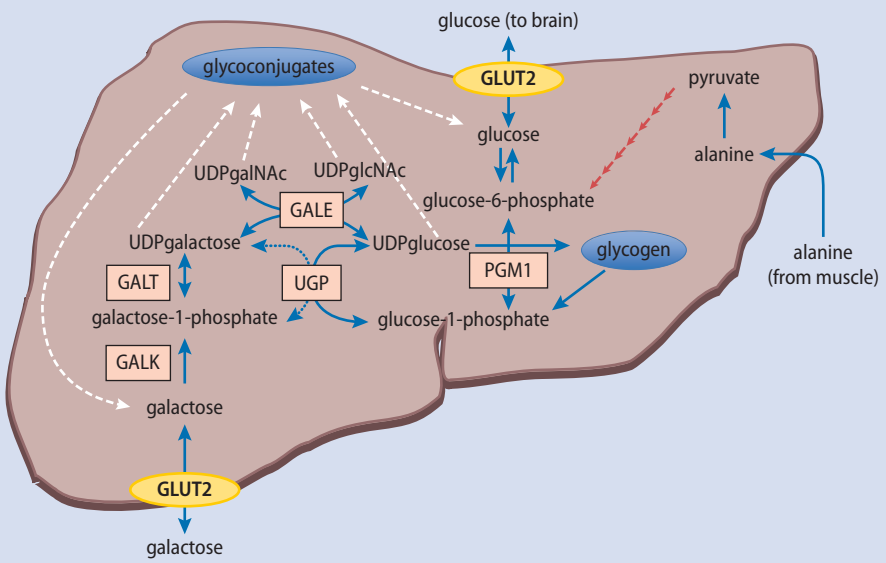


Fig. 6.2 Hepatic carbohydrate metabolism. Red arrows indicate the various reactions in the gluconeogenesis pathway. White dashed lines indicate known or putative steps or reactions in the pathway for glycoconjugate synthesis and catabolism. The fine blue dotted line indicates that galactose-1-phosphate is a poor substrate for the UGP enzyme compared to glucose-1-phosphate

Three inborn errors of galactose metabolism are known (► Galactose Metabolism). The clinically best recognized is classic galactosemia caused by a complete or profound deficiency of galactose-1-phosphate uridylyltransferase (GALT). Classic galactosemia can be life threatening in infancy with multiorgan involvement; long-term developmental and other complications are also common. Partial GALT deficiency ranges from having serious consequences in the newborn period to being generally mild or benign. Uridine diphosphate galactose 4′-epimerase (GALE) deficiency exists as a continuum. The very rare profound, but not complete, GALE deficiency clinically resembles classic galactosemia, at least in the neonatal period. Partial GALE deficiency, which is much more common, at least in some populations, appears to be mild or benign. Galactokinase (GALK) deficiency is extremely rare in many populations but more common in others and can lead to the formation of nuclear cataracts and possibly also long-term developmental deficits, often without provoking acute symptoms of intolerance. Deficiency of the sodium-dependent monosaccharide carrier SGLT1 causes congenital glucose/galactose malabsorption (► Chapter 10). Deficiency of the glucose and galactose transporter, GLUT2, leads to Fanconi-Bickel syndrome (► Chapter 10), a congenital disorder characterized by renal tubular dysfunction and glycogen storage that can be misdiagnosed as galactosemia due to hypergalactosaemia in the neonatal period. Other secondary causes of impaired liver handling of galactose in the neonatal period are congenital portosystemic shunting and multiple hepatic arteriovenous malformations. Whenever an infant is suspected of having a disorder of galactose metabolism, it is imperative that all milk feeding is ceased immediately and replaced with soy or elemental formula to minimize the risk of acute disease or death while the diagnosis is pursued.

6.1 Galactose-1-Phosphate Uridylyltransferase (GALT) Deficiency

6.1.1 Clinical Presentation of GALT Deficiency

A diagnosis of classic galactosemia is most often made following the onset of clinical illness in infancy or from a positive newborn screening (NBS) result [1][2]. Infants with classic galactosemia may appear normal at birth, but within a few days of starting breast or formula milk feeds develop a life-threatening illness with hepatic, renal and cerebral involvement. Early signs include vomiting, diarrhea, poor feeding, jaundice, excessive weight loss, and lethargy. Other findings may include liver enlargement, excessive bruising or bleeding, nuclear cataracts detectable by slit lamp examination, and a full fontanelle. Death from septicaemia, particularly with *E. coli*, may occur. The severity of acute illness may wane when milk feeds are temporarily withdrawn and replaced by intravenous glucose nutrition. Occasionally chil-

dren may first present with a more chronic illness characterised by failure to thrive and developmental delay.

Blood tests may show liver disease evident by unconjugated or mixed hyperbilirubinaemia, abnormal clotting, raised liver transaminases and an increase in certain amino acids, particularly phenylalanine, tyrosine and methionine (which may result in an abnormal newborn screening test for PKU or homocystinuria). Urine studies may show renal tubular disease manifest by metabolic acidosis, galactosuria, glycosuria and aminoaciduria. The absence of reducing substances or galactose in urine can be misleading, especially if the sample was collected only a few hours after a milk feed. Even if present, they may be masked by the glycosuria and aminoaciduria resulting from renal tubular dysfunction. Hypoglycaemia can occur but is rare. Vitreous hemorrhage has been detected in a minority of affected infants [1]. Partial GALT deficiency with up to 10% residual activity may cause acute illness if untreated; infants with 25% or more GALT activity are generally, but not always, mild to asymptomatic.

6.1.2 Metabolic Derangement in GALT Deficiency

Infants with profound GALT deficiency accumulate galactose, Gal-1P, galactitol and galactonate in blood and tissues, especially following dietary exposure to high levels of lactose or galactose. Disturbances in the glycosylation of glycoproteins also occur prior to dietary galactose restriction, as demonstrated by abnormalities in both N- and O-linked glycans [3]. Cataract formation results from galactitol accumulation in the ocular lens. Metabolites responsible for the hepatic, renal and cerebral pathogenesis remain unknown, but might include Gal-1P and perhaps galactitol.

Although the amounts of galactose and galactose metabolites detected in blood and urine decrease significantly following dietary galactose restriction they often remain elevated. This may reflect endogenous galactose production and/or cryptic dietary sources. Endogenous galactose production occurs *in utero* and throughout life. It is age related, with higher levels detected in children than in adults [4][5]. Studies of adults on restrictive diets showed that endogenous galactose production exceeded the estimated 20–40 mg/day of dietary galactose intake [6].

6.1.3 Genetics of GALT Deficiency

Galactosemia is considered an autosomal recessive disorder with a recurrence risk of 1:4, though one case of *de novo* mutation has been reported [7]. The birth incidence is 1/50,000 in the United States [8]. In Ireland it is about 1/16,476 [9]. In Asian populations it is extremely rare. The human *GALT* gene is encoded on chromosome 9. More than 335 mutations or polymorphisms have been described (http://www.arup.utah.edu/database/GALT/GALT_welcome.php [10].) Q188R (c.563A>G) is the most prevalent mutation in northern European populations; it is particularly common in Ireland and Great Britain where it accounts for over 70% of mutant alleles. S135L (c.404C>T) is common among patients with African ancestry. Though genotype-phenotype matching is complicated by allelic heterogeneity, it appears that *GALT* genotypes associated with even trace residual GALT activity, including S135L and others, are associated with milder clinical outcomes [11][12].

Many alleles of *GALT* associated with substantial residual activity have been reported. The Duarte variant galactosemia (D2) allele is the best known; it includes an N314D(c.940A>G) polymorphism that exists, in *cis*, with 3 intronic changes and a small deletion in the promoter region of the gene (c.-119_-116del) that is believed to be causal [13]. Patients who inherit a Duarte allele in *trans* with a »classic« *GALT* mutation, such as Q188R, have what is called Duarte variant galactosemia (D/G). D/G is associated with approximately 25% residual GALT activity and is generally considered benign, at least in infancy. Whether these patients are at risk for subtle developmental deficits later in childhood remains unclear [13]. D/G is identified by newborn screening in some populations at up to 10 times the prevalence of classic galactosaemia [13].

6.1.4 Diagnostic Tests for GALT Deficiency

Newborn screening (NBS) for galactosaemia is undertaken in many countries by measurement of GALT enzyme activity, with or without blood total galactose measurement, using dried blood spots usually collected within 48 hours after birth [8]. Because the GALT activity assay (Beutler test) is coupled, infants with G6PDH deficiency may show apparently diminished activity. Some programmes quantify Gal-1P in addition to total galactose. Depending on the screening approach and cut-offs used, false positive and even some false negative results can occur [14]. If the NBS turn-around time is long, infants with classic galactosemia may already be ill before the NBS result is received.

The diagnosis of GALT deficiency galactosaemia is confirmed or refuted by a quantitative assay of GALT activity in freshly drawn erythrocytes. All assays of red cell GALT activity can give a false-negative result if the patient received a blood transfusion within 2–3 months prior to the blood draw. In this situation, more informative tests will include assay of urinary galactitol, red cell Gal-1P, or mutation analysis in *GALT*. Cultured skin fibroblasts can also be used for study. If taken post-mortem, liver or kidney cortex may provide diagnostic enzyme information, but these specimens must be adequately collected and stored.

Infants with D/G or another partial GALT deficiency are detected at high rates by some but not all NBS programs [8]. Follow-up assessment of an infant with suspected partial GALT deficiency involves quantitation of urine galactitol and/or erythrocyte Gal-1P, repeat quantitative investigation of GALT enzyme activity, and *GALT* sequencing, if available. Galactose tolerance tests present a potential risk to the child and should not be used to evaluate the need for treatment of partial GALT deficiency.

Prenatal Diagnosis

GALT deficiency is inherited as an autosomal recessive trait. If familial *GALT* mutations are known, informative prenatal diagnosis can be performed by genotyping DNA from a chorionic villous (CVS) biopsy or amniotic fluid cells. Full *GALT* sequencing may be informative even in the absence of known familial mutations. Historically, prenatal testing has also been achieved by measuring GALT activity in primary or cultured CVS cells, or in cultured amniotic fluid cells, or by measuring galactitol in amniotic fluid; however, genetic testing of a fetal DNA source may be preferable [1].

6.1.5 Treatment and Prognosis for GALT Deficiency

Prenatal and Newborn Considerations

Due to family history some infants are diagnosed with classic galactosemia prenatally. Though some of these mothers have been advised to restrict their own dietary lactose or galactose intake during pregnancy, this does not seem to prevent the accumulation of Gal-1P and galactitol in the fetus or amniotic fluid, presumably as the result of endogenous synthesis. Furthermore, the outcomes for infants whose mothers restricted milk intake in pregnancy were no better than for those whose mothers did not [3].

Newborns suspected of classic galactosemia must be treated immediately by exclusion of all lactose from the diet, including both breast milk and milk-based formula. Most newborns suspected of having classic galactosemia are switched to a soya-based formula with no apparent adverse effects [15], although an elemental formula may be used if soya presents a problem. In the presence of significant liver disease, a medium-chain triglyceride containing casein hydrolysate preparation may be preferable. Infants who are seriously ill at diagnosis may require considerable supportive care, including the management of a coagulopathy and septicaemia. When a lactose-free diet is instituted early enough, signs disappear promptly, jaundice resolves within days, cataracts may clear, liver and kidney functions return to normal and liver cirrhosis may be prevented [1].

Infants and Young Children

Dietary restriction of high lactose or galactose foods becomes more complex as the infant with classic galactosemia transitions to solid foods; calcium (+ vitamin D) supplementation may also be necessary. Parents may require assistance in learning to scrutinize food labels to look for hidden sources of galactose or lactose from milk powder or solids, hydrolysed whey (a sweetener), drugs in tablet form, toothpaste, baking additives, fillers, sausages, etc. Further, not all dairy products are problematic; for example, some hard cheeses contain very little, if any, galactose because milk sugars were cleared by the fermenting microorganisms [16].

Galactose is present at high levels in milk but also at low levels in a great number of vegetables, fruits, legumes (beans, peas, lentils etc.) and other foods [17]. When compared with endogenous production, however, it is unlikely that absorption of galactose from cryptic dietary sources has a significant impact on the expanded whole-body pool. Further, there is no evidence that their restriction leads to any improvement in long-term outcome [16].

Older Children and Adults

Current recommendations are that patients with classic galactosemia should continue dietary restriction of galactose for life; however, it remains unclear how rigorous that restriction should be for older children and adults. Anecdotal reports suggest that children and/or adults may experience elevated red blood cell Gal-1P and suffer cataracts and liver or brain disease after ingesting high levels of lactose [18] (G.T. Berry, unpublished observations). However, at least two cases of classic galactosaemia have been reported in which stopping dietary restriction of galactose after early childhood resulted in outcomes that were no worse than those of patients who continued treatment [19][20]. Several small studies have also demonstrated that defined quantities of galactose appear to be well tolerated by adolescents and adults with classic galactosaemia [21][22], although individual differences among patients may render some more sensitive to exposure than others. Finally, the failure to understand long-term disease mechanisms, the lack of true disease-related biomarkers, and the marked variability in clinical outcomes among patients has led to a continued diversity in degrees of dietary galactose restriction, especially among older children and adults [23][24].

Biochemical Monitoring

Erythrocyte Gal-1P concentration is the most common biochemical marker used to monitor treatment. The level can be very high at diagnosis if the infant is drinking milk, and then falls gradually over weeks to months after the initiation of dietary galactose restriction. Even with good dietary compliance, the Gal-1P concentration may plateau above normal. The usefulness of monitoring Gal-1P is open to question, however, as the levels have not been shown to correlate with outcome [1][3]. Other galactose metabolites, including red cell or urine galactitol and red cell galactonate, are also consistently increased in patients and have been suggested as alternative or additional markers [25]. Abnormal glycans may also be informative, at least in infants [1]. However, there are currently no data available to demonstrate the superiority of any of these other markers over Gal-1-P for biochemical monitoring.

Long-term Outcome and Complications

Despite the rapid clinical response to lactose exclusion in newborns with classic galactosaemia, long-term complications are common, and appear to be largely independent of the severity of initial illness or the strictness of dietary compliance [1][3][26]. Decreased bone density is common and early identification by DXA scan may allow intervention to help reduce the risk of osteoporosis later in life [1]. Mild growth retardation, delayed speech development, verbal dyspraxia, difficulties in spatial orientation and visual perception, and intellectual defi-

cits have been variably described as complications of treated galactosaemia [1][3]. Reduced leptin levels [27][28], tremor, ataxia, dystonia and choreic movements [28][29], and introverted personality and/or anxiety/depression [28][30] have also been reported. The quality of life in treated patients has been unfavourably compared with that of patients with PKU [31][32].

By mid-childhood or later, patients vary markedly in terms of the number of long-term sequelae present and the severity of those sequelae. Contrary to some early reports, IQ is no longer thought to decrease with age [1][3]. A minority of patients also develop severe neurological disease with cerebellar dysfunction, and brain MRI [33] and FDG-PET scans [34] may reveal abnormalities, though results have been highly variable.

Fertility and Pregnancy

Premature ovarian insufficiency (POI) occurs in the large majority of girls and women with classic galactosaemia, but not Duarte variant galactosemia [35]. Galactosemia-associated POI presents clinically with delayed puberty and menarche, primary or secondary amenorrhoea, or oligomenorrhoea [1][36]. Male gonadal function appears largely unaffected [37]. The cause of ovarian dysfunction in classic galactosemia remains unclear, but is often signalled early in infancy or childhood by hypergonadotropism with a perturbation in granulosa cell function, as evidenced by reduced circulating levels of anti-Müllerian hormone (AMH) [12][38]. Indeed, by AMH levels, a vast majority of girls with classic galactosemia show evidence of severely diminished ovarian reserve by 2–6 months of age [12], suggesting the initial damage may have occured very early in development, perhaps *in utero*.

The hormonal intervention required to help some girls achieve or complete puberty, and women avoid the negative general health consequences of early menopause, can be complicated by the fact that seemingly all oral hormone drug tablets contain lactose as an »inactive« filler. However, some women with classic galactosemia have received these pills for many years with no obvious negative side effects (G.T. Berry, personal observation). It is important to stress that the hormonal treatments that enable girls and women with classic galactosemia to achieve or complete puberty and maintain good bone health, etc., are not known to have any impact on fertility [36][39]. Although uncommon, a small number of women with classic galactosaemia, including those with no detectable residual GALT activity in blood, have experienced one or more successful pregnancies and deliveries. Some of these women subsequently developed secondary amenorrhoea. Galactose metabolite concentrations in blood do not appear to increase significantly during pregnancy, or following 1 week postpartum even in those who choose to breast feed [40]. Infants born to mothers with classic galactosaemia appear normal and healthy.

Management of Partial GALT Deficiency Attributable to Duarte galactosemia (D/G)

At present there is no uniform standard of care for infants with Duarte (D2) galactosaemia (D/G) [13]. Some centres advise lactose restriction in infancy or until erythrocyte Gal-1P levels normalise and remain normal following a lactose challenge or a normal lactose-containing diet, often tested at 1 year of age. Other centres advise no follow-up testing or intervention. Whether D/G galactosaemia is a benign condition remains unclear and a point of some controversy [13].

Heterozygotes for Classic Galactosaemia

Heterozygotes for GALT deficiency are predicted to occur at a frequency of about 1/112 individuals, and have not been shown to be at increased risk of premature menopause, presenile cataracts or other disease manifestations [41].

6.2 Uridine Diphosphate Galactose 4′-Epimerase (GALE) Deficiency

6.2.1 Clinical Presentation of GALE Deficiency

GALE deficiency ranges from an apparently benign »peripheral« condition associated with GALE deficiency restricted to circulating red and white blood cells to a severe »generalized« disorder resulting from widespread GALE impairment that presents with life-threatening illness in the newborn period [42]. Of note, unlike GALT deficiency, even the most severely affected patients with GALE deficiency exhibit some residual GALE activity, at least in some tissues. The severe or generalized form of GALE deficiency is extremely rare, with a total of 6 patients from three families reported [43][44]. Those affected infants exposed to milk showed clinical presentation similar to classic galactosemia; they were rapidly switched to low-galactose formula. One child died from unexplained liver failure at 4 months of age. Despite continued dietary restriction, some, but not all, affected children showed learning difficulties, sensorineural hearing loss, and other long-term complications; however, POI has not been reported. Patients with an intermediate form of GALE deficiency have also been described [45], with clinical findings ranging from transient illness with seizures, to vomiting and hypoglycaemia that resolved upon dietary restriction of galactose, to juvenile cataracts and developmental delay [42].

6.2.2 Metabolic Derangement in GALE Deficiency

Patients with GALE deficiency exposed to milk accumulate galactose, galactitol, Gal-1P, and UDPgal in blood (Fig. 6.1). As in GALT deficiency, patients with severe GALE deficiency exposed to high levels of dietary lactose may also show abnormal glycosylation of proteins in blood [43]; this may be a secondary biochemical complication not primarily related to the genetic defect. Of note, red cell GALE activity does not correlate well with that seen in other tissues, such as lymphoblasts, and is poor at differentiating between peripheral and generalised forms of the disease [42].

6.2.3 Genetics of GALE Deficiency

GALE deficiency is inherited as an autosomal recessive trait; the recurrence risk for couples with an affected child is therefore 1:4. Due to incomplete ascertainment, the true population incidence is not known, and may vary significantly between groups [42]. Human GALE is encoded on chromosome 1. A number of mutations have been identified and characterised. The c.280G>A (V94M) mutation has been found in the homozygous state in the majority of patients with the severe phenotype [46], whereas other mutations are associated with the intermediate or asymptomatic phenotypes [45][47]. However, some mutations identified in the compound heterozygous state in mildly affected patients appear to be profoundly impaired when expressed in model systems, suggesting that these naturally-occurring mutations could also result in severe disease if inherited together with another severe allele [42].

6.2.4 Diagnostic Tests for GALE Deficiency

Infants with GALE deficiency may be detected by NBS on the basis of elevated total galactose or Gal-1P but normal GALT [42]. However, many newborn screening programs only measure total blood galactose in follow-up to an abnormal GALT activity result; by definition, these programs will not detect cases of GALE deficiency [8].Diagnosis of GALE deficiency is confirmed by quantitative assay of GALE in freshly drawn erythrocytes or other cells. Further studies of GALE activity in transformed lymphoblasts, and red cell Gal-1P or urinary galactitol measured while on and off dietary galactose, may help characterise the disorder further [45]. If the familial *GALE* mutations are known, DNA analysis may be the fastest method of determining whether or not an infant is affected.

6.2.5 Treatment and Prognosis for GALE Deficiency

Newborns at risk for generalized GALE deficiency should be maintained on low galactose formula until the diagnosis can be confirmed or excluded. Once confirmed, patients with generalized GALE deficiency should be treated and followed much like patients with classic galactosemia, though less stringent dietary galactose restriction may be advisable to ensure sufficient exogenous galactose for synthesis of galactoproteins and galactolipids. As with GALT deficiency, red cell Gal-1P levels tend to remain slightly elevated in treated patients. The oldest patients reported with generalized GALE deficiency are now in their 3rd decade; they have not shown evidence of progressive disease (JH Walter, personal communication).

True peripheral GALE deficiency does not require galactose restriction. However, since intermediate forms are now recognised, measurement of red cell Gal-1P and urine galactitol while the patient is on a normal galactose intake, and monitoring of psychomotor progress, may be advisable.

6.3 Galactokinase (GALK) Deficiency

6.3.1 Clinical Presentation of GALK Deficiency

Historically, untreated galactokinase deficiency has been considered largely benign except for diet-dependent cataracts and in rare cases pseudotumour cerebri [48]. However, a recent report detailing the outcomes of 18 patients with profound galactokinase deficiency identified by newborn screening in Germany [49] raised concern as many experienced long-term complications. The large majority of these patients were homozygous for the Romani founder mutation c.82C>A (p P28T). Most were clinically well as infants regardless of diet; however, complications were reported in close to 30%. These included hypoglycaemia, failure to thrive, microcephaly, intellectual disability, and hypercholesterolemia, with most symptoms seemingly more prevalent in those with poor dietary compliance. Complications did not appear to correlate with known consanguinity [49]. It remains unclear whether GALK deficiency was causal.

6.3.2 Metabolic Derangement in GALK Deficiency

Patients with profound GALK deficiency lack the ability to phosphorylate galactose (◘ Fig. 6.1) and consequently accumulate galactose and galactitol, but not Gal-1P [49]. As in classic galactosemia, these patients accumulate galactitol in the lens when consuming a high galactose diet, causing osmotic swelling, denaturation of proteins, and cataracts.

6.3.3 Genetics of GALK Deficiency

GALK deficiency is inherited as an autosomal recessive disorder. Historically, GALK deficiency was considered extremely rare (< 1/150,000) in most parts of Europe, the USA, and Japan, but higher in the Balkan countries [50], the former Yugoslavia, Rumania and Bulgaria. Among Roma people, the birth incidence is close to 1/ 2,500. Following the civil war in Yugoslavia, which caused a mass relocation of many Eastern Europeans, the birth incidence of GALK deficiency in Western Europe rose. Between 1991-2010 GALK deficiency was detected at 1/40,000 screened births in Germany [49], making it comparable in prevalence to classic galactosemia in the same population.

Two genes have been reported to encode human galactokinase [3]: *GK1* on chromosome 17q24 and *GK2* on chromosome 15. However, all mutations associated with clinical GALK deficiency map to *GK1*. The *GK2* gene product appears to function primarily as an *N*-acetylgalactosamine kinase. Of the many *GK1* mutations described in patients with GALK deficiency, P28T (c.82C>A) was identified as the founder mutation in most Roma patients and in German immigrants from Bosnia [51].

6.3.4 Diagnostic Tests for GALK Deficiency

Newborns with profound GALK deficiency may be discovered by NBS due to elevated total blood galactose following exposure to high levels of dietary galactose [3]. These infants will be missed if they have not been exposed to milk, or if the NBS protocol does not test total blood galactose, or only tests samples secondary to low GALT activity [8]. Older children and adults with nuclear cataracts should be tested for possible GALK deficiency by enzyme assay of freshly drawn red cells or another cell type. Elevated galactose and galactitol may also be detected in urine if the patient is on a high galactose diet.

6.3.5 Treatment and Prognosis for GALK Deficiency

Initial treatment of GALK deficiency involves elimination of milk and other high galactose foods from the diet. Cryptic sources of dietary galactose, such as fruits and vegetables, are generally allowed. Once a patient is on a galactose-restricted diet urinary levels of galactitol should normalize. When diagnosis and intervention occur within the first few weeks of life cataracts may be prevented or may resolve over time. However, when treatment is late and cataracts are already dense, they may require surgical removal. Patients who have had their lenses surgically removed remain at risk for recurrent cataracts originating from remnants of the posterior lens capsule. Recurrence can be avoided by continuing the galactose-restricted diet.

As in carriers with GALT deficiency, the speculation that heterozygosity for GALK deficiency predisposes to the formation of presenile cataracts remains unproven [52][53].

6.4 Fanconi-Bickel Syndrome

Fanconi-Bickel Syndrome is a rare, recessively inherited disorder of glucose and galactose transport resulting from deficiency of glucose transporter 2 (GLUT2). A few cases have been discovered during newborn screening for galactose in blood. The clinical features of the disorder are those of glycogen storage disease and renal tubular dysfunction. The diagnosis is confirmed by mutation analysis. For further details, see ▶ Chapter 10.

6.5 Portosystemic Venous Shunting and Hepatic Arteriovenous Malformations

Portosystemic bypass of splanchnic blood via ductus venosus or intrahepatic shunts causes alimentary hypergalactosaemia, which may be discovered during metabolic NBS [54][55][56].

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Disorders of Glycolysis and the Pentose Phosphate Pathway

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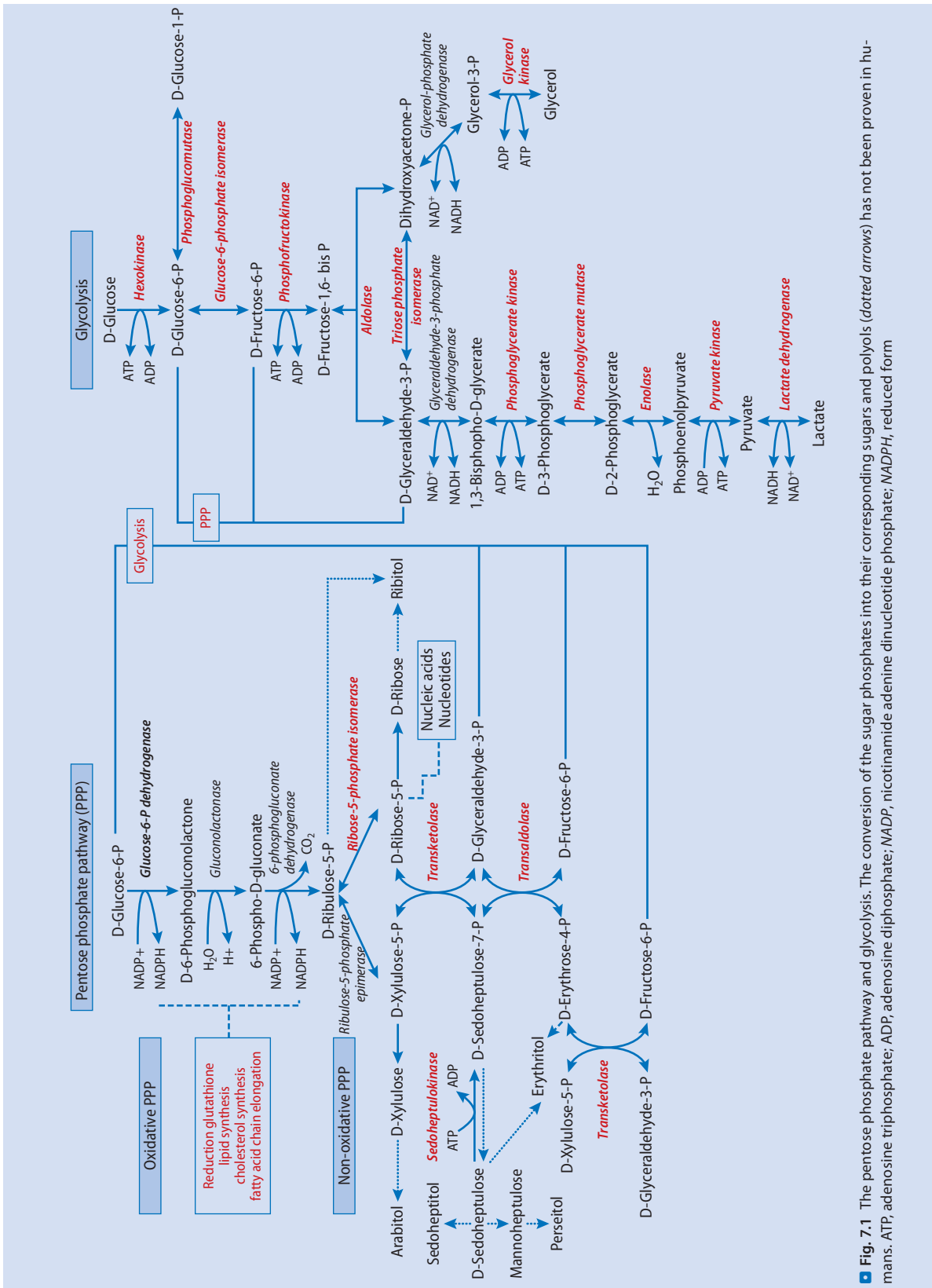

Fig. 7.1 The pentose phosphate pathway and glycolysis. The conversion of the sugar phosphates into their corresponding sugars and polyols (*dotted arrows*) has not been proven in humans. ATP, adenosine triphosphate; ADP, adenosine diphosphate; *NADP*, nicotinamide adenine dinucleotide phosphate; *NADPH*, reduced form

Glycolysis and the Pentose Phosphate Pathway

Glycolysis which converts each molecule of glucose to two of pyruvate (Fig. 7.1), consists of two phases and ten steps. The first five steps are the preparatory phase, consuming energy (ATP) to convert glucose into two, three-carbon sugar phosphate molecules. In the other five steps, the second »pay-off« phase, ATP and NADH are produced.

The pentose phosphate pathway (PPP; Fig. 7.1) consists of two distinct parts: the first part, an oxidative, non-reversible pathway, produces NADPH, and the second part, a non-oxidative, reversible pathway, produces ribose for nucleotide and nucleic acid synthesis and connects intermediates to glycolysis.

Because glycolysis is the most important source of energy in erythrocytes and in some types of skeletal muscle fibres, inherited diseases of glycolysis are mainly characterized by haemolytic anaemia and/or metabolic myopathy. Ten inborn errors of the glycolytic pathway are known, all inherited as an autosomal recessive trait except the X-linked phosphoglycerate kinase and glycerol kinase deficiencies. Hexokinase (HK), glucose-6-phosphate isomerase (GPI) and pyruvate kinase (PKD) deficiencies cause severe haemolytic anaemia. As these are exclusively haematological disorders they are not discussed further. Muscle phosphofructokinase (PFKM), aldolase A, triosephosphate isomerase (TPI) and phosphoglycerate kinase (PGK) deficiencies are characterized by haemolytic anaemia alone or coupled with neurological disease and/or myopathy. Phosphoglycerate mutase (PGAM), enolase and lactate dehydrogenase (LDH) deficiencies present with a purely myopathic syndrome characterized by exercise induced cramps and myoglobinuria. Glycerol kinase deficiency (GKD) is an X-linked disorder that is either an isolated condition presenting with hypoglycaemia and acidosis or part of a contiguous gene deletion where it also associated with congenital adrenal hypoplasia and/or Duchenne muscular dystrophy. Glucose-6-phosphate can also be formed by the conversion of the glycogen derived glucose-1-phosphate, a reaction catalysed by phosphoglucomutase (PGM). PGM1 deficiency is a congenital disorder of glycosylation CDG (▶ Chapter 41).

Four inborn errors in the pentose phosphate pathway (PPP) are known. Glucose-6-phosphate dehydrogenase deficiency is an X-linked defect in the first, irreversible step of the pathway. As this is an exclusive haematological disorder it is not discussed further. Ribose-5-phosphate isomerase (RPI) deficiency has been described in one patient, who presented with developmental delay and a slowly progressive leukoencephalopathy. Transaldolase (TALDO) deficiency often presents in the neonatal or antenatal period with hepatosplenomegaly, liver function problems, hepatic fibrosis and anaemia. In transketolase (TKT) deficiency, the main clinical symptoms are short stature, developmental delay and congenital heart defects. Sedoheptulokinase (SHPK) deficiency, a defect related to the PPP has been described as an isolated disorder and also as part of a 57-kb deletion in nephropathic cystinosis (▶ Chapter 41). Essential pentosuria is the result of a partial deficiency of L-xylulose reductase (xylitol dehydrogenase) an enzyme of the glucuronic acid pathway. Affected individuals excrete large amounts of L-xylulose in urine. Pentosuria, a benign disorder that occurs almost exclusively in Jewish people, is not discussed further here [1].

7.1 Muscle Phosphofructokinase (PFKM) Deficiency

7.1.1 Clinical Presentation

In its typical presentation, PFKM deficiency (GSD VII or Tarui Disease) is clinically indistinguishable from McArdle's disease (▶ Chapter 5). So far more than one hundred patients have been described with prominent clinical symptoms characterized by muscle cramps, exercise intolerance, rhabdomyolysis and myoglobinuria, often associated with haemolytic anaemia and hyperuricemia. The onset of the classic form is usually in the childhood but an infantile and a late onset form have also been reported. Patients with infantile onset may manifest as »floppy babies«. Symptoms include myopathy, psychomotor retardation, cataract, joint contractures, with death during early childhood. Curiously, none of the infantile cases had evidence of haemolytic anaemia [2].

7.1.2 Metabolic Derangement

PFK (EC 2.7.1.11) catalyses the ATP-dependent conversion of fructose-6-phosphate to fructose-1,6-biphosphate. Tissues deficient in PFK cannot use free or glycogen derived glucose as a fuel source and accumulate glycogen. Human PFK is a tetramer made up of various combinations of 3 subunits: muscle (M), liver (L) and platelet (P). Muscle and liver PFK are a homotetramers of 4M and 4L subunits, respectively. Erythrocytes contain both L and M subunits, which randomly tetramerize to form the various combinations.

7.1.3 Genetics

PFKM deficiency is an autosomal recessive disease with a predominance of cases among Ashkenazi American Jews and Japanese populations. About 25 distinct mutations in *PFKM* have been identified in patients of different ethnic origins. *PFKM* encodes the M subunit and is located to chromosome 12q13.3. It spans 30kb and contains 24 exons and at least 3

promoter regions. Among the detected mutations missense and splicing mutations are the most frequent [3].

7.1.4 Diagnostic Tests

A few simple laboratory test results, such as an increased bilirubin concentration and reticulocyte count, reflecting compensated haemolytic anaemia, are useful in distinguishing PFKM disease from McArdle disease. The forearm non ischemic exercise test is characterized by a flat lactate curve but with a normal increase of ammonia.

Definitive diagnosis requires biochemical documentation of PFK enzyme deficiency in muscle with a histochemical and/or biochemical assay and by sequence analysis of *PFKM* [2].

■ Treatment and Prognosis

No specific treatment or cure exists. Management primarily consists of avoiding strenuous exercise. Symptoms normally resolve with rest. Patients should avoid high-carbohydrate meals since glucose is not an alternative substrate in PFKM deficiency. Some patients have been helped by a high protein diet. A ketogenic diet has been proposed in children with the more severe variant of PFKM deficiency. Except for the very rare fatal infantile form, the disease does not progress to severe disability [2].

7.2 Aldolase A (ALDOA) Deficiency

7.2.1 Clinical Presentation

ALDOA deficiency (GSD XII) is a rare cause of haemolytic anaemia alone or in combination with neurological abnormalities and myopathy. Only 8 patients from five families have been reported so far [4]. Non-spherocytic haemolytic anaemia was present in five patients, with myopathy in two of them. Recently, three siblings from a Moroccan family were reported with isolated episodic rhabdomyolysis triggered by fever without haemolytic anaemia. Mental retardation was present in two patients. The onset is generally within the first months of life. If not promptly recognized, death from severe rhabdomyolysis can occur in myopathic patients.

7.2.2 Metabolic Derangement

Aldolase (EC 4.1.2.13) converts fructose-1,6-biphosphate to dihydroxyacetone phosphate and glyceraldehyde-3-phosphate. In mammals there are three tissue-specific aldolases encoded by distinct genes: ALDOA in erythrocytes and muscle, aldolase B in the liver, small intestine, and kidney (*Aldolase B deficiency*, ▶ Chapter 8) and aldolase C in the brain. Since ALDOA is the sole isozyme in erythrocytes and muscle it is predicted to affect these tissues more severely than others. The downstream effects of this enzyme block are the inhibition of glucose production and reduced regeneration of ATP.

7.2.3 Genetics

ALDOA deficiency is an autosomal recessive disease due to mutations in *ALDOA*. So far, four missense mutations (p.Asp128Gly, p.Glu206Lys, p.Cys338Tyr, p.Ala279Val) and a nonsense mutation (p.Arg303*) have been reported. Interestingly, functional studies of the p.Cys338Tyr and p.Ala279Val mutations associated with the myopathic phenotype showed that these mutations disrupt the enzyme in a temperature-sensitive fashion according the crucial role of fever as the trigger of rhabdomyolysis [4].

7.2.4 Diagnostic Tests

The diagnosis of ALDOA deficiency is made by measuring the enzymatic activity in erythrocytes or muscle biopsy. Notably, in erythrocytes ALDOA activity is dramatically reduced even in patients with myopathic symptoms without haemolytic anaemia. Genetic diagnosis is made by sequencing *ALDOA*.

7.2.5 Treatment and Prognosis

There is no specific treatment. Management primarily consists of avoiding strenuous exercise that may cause rhabdomyolysis. In patients with severe chronic anaemia, regular blood transfusions are required.

7.3 Triosephosphate Isomerase (TPI) Deficiency

7.3.1 Clinical Presentation

TPI deficiency is the most severe glycolytic enzymopathy and apart from the infantile form of PFK deficiency, is the only one that is lethal, with death occurring in childhood from respiratory distress. It is characterized by severe congenital haemolytic anaemia from birth, coupled with neurological dysfunction and progressive neuromuscular impairment, most often beginning in the first months of life. Neurological complications include dystonia, tremor, pyramidal tract signs and spinal motor neuron involvement. Affected individuals may also develop seizures. Cardiomyopathy and recurrent infections can occur [5].

7.3.2 Metabolic Derangement

TPI (EC 5.3.1.1) catalyses the interconversion of dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate, with the reaction favouring formation of DHAP by a ratio of 20:1. The triosephosphates, produced by aldolase, are interconnected to lipid metabolism, the glycerol-3-phosphate shuttle and to the pentose phosphate pathway (PPP). TPI deficiency causes elevation of DHAP concentration, particularly in red blood cells, which lack the capacity to metabolize

DHAP in the glycerophosphate shuttle via a glycerophosphate dehydrogenase. Patients show a 20- to 60-fold increased red cell DHAP concentration. DHAP is converted non-enzymatically to methylglyoxal, a toxic compound that causes oxidative stress, DNA damage and apoptosis [5].

7.3.3 Genetics

TPI deficiency is an autosomal recessive disorder due to mutations in *TPI*. The Glu104Asp mutation accounts for approximately 80% mutant alleles within patients. This mutation causes the most severe symptoms. Some additional missense or nonsense mutations have been identified so far, mostly in compound heterozygotes, coupled with Glu104Asp. The recombinant mutant Glu104Asp has been recently produced and characterized, showing that it causes instability of the TPI dimer and dissociation of the enzyme into inactive monomers. The unstable TPI monomers are thought to display high aggregation ability, further contributing to the formation of toxic aggregates from them is folded protein particularly in the brain, triggering neuronal dysfunction. Interestingly those patients bearing a null mutation (with no TPI protein) display a very severe haemolytic anaemia without any neurological signs. Moreover, recent knowledge suggests that this enzymopathy may be a conformational rather than a metabolic disease because no bioenergy deficit has been demonstrated in any case of TPI mutations [5].

7.3.4 Diagnostic Tests

The diagnosis is made by enzymatic assay of TPI activity in tissues (red blood cells, leucocytes, fibroblasts and muscle) and by sequence analysis of *TPI*.

7.3.5 Treatment and Prognosis

No effective therapy is available, but aggressive supportive care, especially assisted respiration, has appeared to prolong life in some instance. Usually death occurs in childhood.

7.4 Phosphoglycerate Kinase (PGK) Deficiency

7.4.1 Clinical Presentation

PGK deficiency (GSD IX) is an X-linked disorder and has three main clinical presentations: non-spherocytic haemolytic anaemia alone, myopathy alone, or the association of anaemia and central nervous system (CNS) involvement. The combined involvement of CNS and muscle or of all three tissues is much less frequent. Symptoms of CNS involvement can be mental retardation, behavioural abnormalities, seizures, strokes or parkinsonism. The myopathic form is clinically indistinguishable from PFK deficiency and is characterised by recurrent episodes of exercise-induced cramps and myoglobinuria. The onset is generally in childhood but an infantile onset has been also reported [6].

7.4.2 Metabolic Derangement

PGK (EC 2.7.1.11) catalyses the transfer of the high-energy phosphate from 1,3-diphosphoglycerate (1,3-DPG) to ATP, converting the 1,3-DPG to 3-phosphoglyceric acid. There are two human PGK isozymes: PGK1 and PGK2 encoded by two distinct genes. While PGK2 is expressed only in testis, PGK1 is expressed in all somatic cells and plays an important role in the generation of ATP during glycolysis. Although PGK1 is ubiquitously expressed, only tissues dependent on high-energy requirement are affected.

7.4.3 Genetics

PGK1 is encoded by *PGK1*, on chromosome Xq13.3. PGK deficiency is usually fully expressed in male hemizygotes, heterozygous females may have a variable degree of haemolytic anaemia. So far 27 mutations have been reported, most of which are of a missense type but frameshift and splicing mutations have been found. The relationship between the molecular alterations and the highly heterogeneous clinical features in PGK deficiency is still unclear. Mutations associated with haemolytic anaemia and CNS involvement are spread all along the gene, whereas the mutations causing isolated myopathy tend to cluster in the C terminal domain. Functional studies demonstrated that patients with unstable variants but only mildly affected in catalytic properties present haemolysis associated with neurological dysfunctions. Conversely, myopathy without haemolytic or neurological symptoms is mainly present in patients with variants heavily affected in both catalytic properties and protein stability [7].

7.4.4 Diagnostic Tests

The muscle form is characterized by a flat lactate curve with the forearm non ischemic exercise test. Muscle pathology is of little diagnostic help because glycogen storage is either undetectable or very mild. The differential diagnosis should include other causes of hereditary nonspherocytic haemolytic anaemia.

Definitive diagnosis requires biochemical assay of the PGK enzyme activity in muscle and/or erythrocytes and by identification of *PGK1* mutations by molecular analysis.

7.4.5 Treatment and Prognosis

No specific treatment or cure exists. Management primarily consists of avoiding strenuous exercise. Symptoms normally resolve with rest. Some patients have been helped by a high

protein diet. Except for the very rare fatal infantile onset form, the disease does not progress to severe disability. In patients with severe chronic anaemia, regular blood transfusions are required. Splenectomy has been shown to be beneficial in some cases. The prognosis is variable, depending on the severity of the anaemia and on the presence of the other manifestations [6].

7.5 Phosphoglycerate Mutase (PGAM) Deficiency

7.5.1 Clinical Presentation

To date, 15 patients with PGAM deficiency (GSD X) have been reported. Clinical symptoms are characterized by muscle cramps, exercise intolerance, and myoglobinuria. The onset of the disease is generally in adolescence but in two patients the onset was reported in the adulthood with muscle cramps triggered only by hard exercise. An unusual pathological feature of PGAM deficiency, described in about 36% of patients, is the association with tubular aggregates in muscle. The disease is more common in African-Americans [8].

7.5.2 Metabolic Derangement

PGAM (EC 5.4.2.11) catalyses the interconversion of 2-phosphoglycerate and 3-phosphoglycerate, by using 2,3-bisphosphoglycerate as a cofactor. Human PGAM is a dimeric enzyme, with normal mature muscle containing predominantly the muscle-specific M homodimer (PGAM-M) and the brain specific subunit (PGAM-B) that accounts for the residual activity (usually less than 7%) observed in PGAM deficient patients. Patients affected by PGAM deficiency have a genetic defect involving the muscle PGAM-M.

7.5.3 Genetics

PGAM is due to mutations in *PGAM*. *PGAM* contains 3 exons. The most common mutation is a nonsense mutation in exon 1 (c.233G>A, p.Trp78*) described exclusively in African-American patients. The p.Trp78* mutation was found homozygote in 7 cases, in association with Glu89Ala in one case and heterozygote in a patient with an asymptomatic hyper CKemia and only a partial reduction of PGAM activity. Two missense mutations (Arg90Trp, Arg10Gln) and a frameshift mutation (p.Gly178fs30*) have been described in four Italian patients. A nonsense mutation (Arg180*) was found homozygote in a patient of Pakistani ethnicity [8].

7.5.4 Diagnostic Tests

The forearm non ischemic test generally causes abnormally low, but not absent, venous lactate responses with normal increase of ammonia.

Definitive diagnosis requires biochemical documentation of PGAM enzyme defect in muscle and by sequence analysis of *PGAM*.

7.5.5 Treatment and Prognosis

No specific treatment or cure of the enzyme defect exists. Management primarily consists of avoiding strenuous exercise. The disease does not progress to severe disability.

7.6 Enolase Deficiency

7.6.1 Clinical Presentation

Enolase deficiency is a very rare inherited metabolic myopathy caused by enzymatic defect of distal glycolysis with only three patients described so far. All patients presented typical signs of myopathy with exercise intolerance, myalgia, cramps, and severe episodes of rhabdomyolysis followed by acute renal insufficiency with anuria in two of them [9].

7.6.2 Metabolic Derangement

Enolase (EC 4.2.1.11) catalyses the interconversion of 2-phosphoglyceric acid (PGA) and phosphoenolpyruvate. In mammals, enolase is composed of three subunits (α, β and γ) encoded by three different genes; the subunits associate to form five different isozymes as homo or heterodimers (αα, αβ, αγ, ββ, and γγ). The α-subunit is expressed in many tissues, γ primarily in neurons whereas β is prevalent in muscle. Enolase deficiency is due to reduction of β-enolase activity.

7.6.3 Genetics

Enolase deficiency is an autosomal recessive disorder due to mutations in *ENO3*, encoding β-enolase. *ENO3* is located on chromosome 7p13.2 and spans 12 exons. The first reported patient, an Italian man, carried two missense heterozygous mutations: pGly156Asp in exon 7 and p.Gly374Glu in exon 10. The other two patients, of Italian and Turkish origin, carried homozygous missense mutations in exon 7: p.Asn151Ser and p.Glu187Lys. All mutations change highly-conserved amino acid residues [9].

7.6.4 Diagnostic Tests

The forearm non ischemic test demonstrates no or a very mild increase of venous lactate with a normal increase of ammonia. Definitive diagnosis is made by enzymatic assay of enolase activity in muscle, which is less than 10% of normal values in the patients. Genetic diagnosis is made by sequencing *ENO3*.

7.6.5 Treatment and Prognosis

Management primarily consists of avoiding strenuous exercise. The disease does not progress to severe disability.

7.7 Lactate Dehydrogenase (LDH) Deficiency

7.7.1 Clinical Presentation

LDH deficiency (GSD XI) has been reported in five Japanese families and two Caucasian families. Clinical features are characterized by impaired ability to sustain exercise due to muscle pain and stiffness during heavy exercise. Rhabdomyolysis can occur. In addition to muscle symptoms, three affected Japanese women suffered from uterine stiffness necessitating caesarean sections, and a few patients had skin rashes [2].

7.7.2 Metabolic Derangement

LDH (EC1.1.1.27) converts pyruvate to lactate when oxygen is absent or in short supply. In muscles, LDH is involved in the breakdown of stored glycogen during anaerobic exercise. In human somatic tissues, five isozymes of tetrameric LDH are formed by a combination of the LDH-A (muscle) and LDH-B (heart) subunits encoded by two different genes (*LDH-A* and *LDH-B*). LDH-A4 is located primarily in skeletal muscle and LDH-B4 is located primarily in the myocardium. There is a third LDH-C subunit expressed only in mature testis and sperm. In patients with LDH-A deficiency, insufficient interconversion between pyruvate and lactate following exercise causes pyruvate and NADH accumulation and ATP reduction.

7.7.3 Genetics

To date, only patients with *LDH-A* mutations have been reported. The gene is located on chr11p15.4 and spans 7 exons. The disease is inherited with an autosomal recessive mode. In the majority of patients, a common 20-bp deletion in exon 6 has been reported [10].

7.7.4 Diagnostic Tests

In patients with myoglobinuria, LDH deficiency can be suspected in the presence of high values of serum CK but extremely low values of LDH. Electrophoretic analysis of serum LDH isozymes demonstrates only one activity band of LDH-B4 and complete lack of the LDH-A subunit. The definitive diagnosis is made by enzymatic assay of LDH activity in muscle and by sequence analysis of *LDH-A*.

7.7.5 Treatment and Prognosis

Treatment primarily consists of avoiding strenuous exercise. The disease does not progress to severe disability.

7.8 Glycerol Kinase Deficiency (GKD)

7.8.1 Clinical Presentation

GKD (▶ see also Chapter 34) is a rarely diagnosed X-linked recessive disorder, which occurs either together with congenital adrenal hypoplasia (CAH) and/or with Duchenne muscular dystrophy (DMD) or as an isolated form either symptomatic or asymptomatic. More than 100 male patients have been reported so far, while only a few cases of symptomatic female carriers are described. The first descriptions noted that there are 3 clinically distinct forms of GKD: infantile, juvenile, and adult. The infantile form is associated with severe developmental delay. Those with the adult form have no symptoms and are often detected fortuitously. Juvenile patients are often symptomatic [11].

Complex GKD presents in infants and is caused in general by partial deletion of Xp21, which includes the genes of glycerol kinase, congenital adrenal hypoplasia, Duchenne muscular dystrophy and intellectual disability (*IL1RAPL*). Symptoms depend on the size of deletion and appear almost exclusively in males. Usually the first and most severe are due to the adrenal hypoplasia, which, if untreated, may be rapidly fatal. The symptoms of GKD also occur early in life, but they may be masked by the mineralocorticoid deficiency. Duchenne muscular dystrophy appears in childhood and is always symptomatic. Developmental impairment and intellectual disability occur often with complex GKD. The reasons for it are heterogeneous, but usually, there is a connexion with the deletion of *DMD* or *IL1RAPL* genes [12].

Patients with isolated GKD are at risk from hypoglycaemia and hyperketonaemia. Juvenile patients present with episodic vomiting, metabolic acidosis, stupor, and coma. Patients with the adult form have no apparent clinical problems. They are usually identified through testing for hyperlipidemia when they are found to have pseudotriglyceridemia as a result of the increased glycerol in their blood being falsely identified as triglyceride [11].

7.8.2 Metabolic Derangement

GKD catalyses the phosphorylation of glycerol to glycerol phosphate and is essential for its further utilization as a gluconeogenetic precursor and for re-esterification of free fatty acids (FFA).

GKD is usually suspected in the laboratory from two findings: (a) high glycerol excretion in the urine found by gas chromatography-mass spectrometry (GC-MS) in the course of investigation of urinary organic acids and (b) falsely high serum triglyceride concentration as a result of the co-determi-

nation of glycerol in the method used to measure triglyceride. Further studies of glycerol metabolism are needed when urine and serum glycerol are elevated. In complex GKD, biological abnormalities may be due to the other genes involved, i.e signs of adrenal failure with sodium loss in urine, hypoglycaemia and hyperkalaemia; and elevated CK. In isolated GKD, ketoacidotic attacks with or without hypoglycemia have been reported.

7.8.3 Genetics

GKD is caused by mutations in *GK* alone or as part of a contiguous gene deletion syndrome [11][12]. The latter is due to microdeletions in the Xp21.2–p21.3 region often involving *GK*, *DAX1* (dosage-sensitive sex reversal locus and the adrenal hypoplasia congenita (AHC) locus on the X chromosome, gene 1) and/or part of the *DMD* (Duchenne muscular dystrophy) genes [11]. Point mutations or deletions in *DAX1* are responsible for AHC and hypogonadotropic hypogonadism. *DAX1* is closely linked to *GK* and a deletion often affects both loci [11]. Even though numerous aberrations in the *GK* gene have been reported, there does not seem to exist an apparent correlation between genotype and phenotype.

7.8.4 Diagnostic Tests

Determination of glycerol in urine is a rapid and simple way to diagnose GKD. Glycerol can also be detected by GC-MS when screening for organic acidurias. A few cases of hyperglycerolaemia not due to GKD have been reported and, if in doubt, a mutation in *GK* should be looked for [13]. This will then also make it possible to identify female carriers and symptomless males as required for family counselling. Both specific and indirect assays of GK activity in fibroblasts are available and are adequate to detect complete GKD (enzyme activity <1–5% of reference) [14]. Provocation with a ketogenic diet, fasting or an exercise test are unhelpful in making a diagnosis and dangerous since they may cause severe complications [13].

7.8.5 Treatment and Prognosis

Treatment for isolated or complex GKD is based on symptomatic therapies and prevention. Both forms of GKD carry a risk of acute acidosis, hypoglycemic episodes associated with impaired consciousness and neurological symptoms, triggered by fasting and catabolism. Metabolic crises should be avoided by providing an adequate supply of fluid, calorie and glucose during intercurrent illness [13]. In acute situations, IV glucose, in adequate amounts to block lipolysis and avoidance of lipid intake, is indicated. IV solutions containing glycerol or lipids (e.g some anaesthetic drugs) should be avoided. In complex GKD, adrenal insufficiency symptoms must be recognized and treated promptly with glucocorticoid and mineralocorticoid replacement therapy. Steroid replacement doses need to be adjusted in patients with CAH during intercurrent infections. Patients displaying Duchenne myopathy should be managed and supported accordingly. Patients are at risk of intellectual impairment and should be monitored to allow early intervention.

In isolated GKD, pseudo-hypertriglyceridemia responds poorly to conventional therapy which consequently should be avoided. A low-fat diet has reportedly been of value [15], alcohol should be avoided. In younger children, parents should be advised to give frequent complex carbohydrate-rich meals, and during infections and before intense physical activities, extra food or glucose. Since ketosis has been an early sign in many episodes, home testing for ketonuria can be useful. Most patients after puberty do not require dietary treatment but they should avoid situations that may cause extreme metabolic stress. In addition there appears to be an increased risk of glucose intolerance and for which patients need to be monitored. [13]. Special precautions must be taken during anaesthesia.

Prognosis is related to the early recognition of the disease, especially in complex GKD and to prompt treatment of associated adrenal insufficiency [15]. The frequency of metabolic decompensation generally declines after puberty [13]. With appropriate management the long-term prognosis for isolated GKD is very good [13].

7.9 Ribose-5-Phosphate Isomerase (RPI) Deficiency

7.9.1 Clinical Presentation

The one patient recorded with RPI deficiency had psychomotor retardation and developed epilepsy at the age of 4 years. From 7 years a slow neurological regression occurred, with prominent cerebellar ataxia, mild spasticity, optic atrophy and a mild sensorimotor neuropathy [16]. He is now in his thirties, wheelchair-bound but still communicative and interested in his environment. His epilepsy is well controlled with medication. Magnetic resonance imaging (MRI) of the brain at 11 and 14 years showed extensive abnormalities of the cerebral white matter with prominent involvement of the U-fibres and a slightly swollen appearance with some widening of the gyri. He has a slowly progressive leukoencephalopathy. Magnetic resonance spectroscopy (MRS) revealed highly elevated peaks in the 3.5-4.0 ppm region (the sugar and polyol region of the spectrum) and which were identified as representing ribitol and D-arabitol.

7.9.2 Metabolic Derangement

RPI (EC 5.3.1.6) is an enzyme of the reversible part of the PPP. In theory, this defect leads to a decreased capacity to interconvert ribulose-5-phosphate and ribose-5-phosphate and results in the accumulation of sugars and polyols: ribose and ribitol from ribose-5-phosphate or ribulose-5-phosphate, and xylulose and arabitol from ribulose-5-phosphate via xylu-

lose-5-phosphate. The concentrations of ribitol and arabitol displayed a steep descending brain/CSF/plasma gradient.

7.9.3 Genetics

RPIA is located at locus *2p11.2* and has nine exons. Human *RPIA* consists of a monomer of 311 amino acids. In the RPI-deficient patient two mutant alleles were demonstrated: a 1-bp deletion (c.540delG), resulting in a frameshift and a predicted truncated protein, and a missense mutation c.182C>T, (p.A61V). The molecular findings suggest autosomal recessive inheritance.

7.9.4 Diagnostic Tests

The diagnosis of RPI deficiency can be made by the analysis of sugars and polyols in urine, plasma or CSF. Urinary ribitol and arabitol, as well as xylulose, are more than 10 times elevated. Extremely high concentrations of these pentitols are also found in CSF. The myo-inositol concentration in CSF is decreased. In vivo brain MRS reveals elevated peaks in the 3.5- to 4.0-ppm region, corresponding to arabitol and ribitol.

The diagnosis can be confirmed by an enzyme assay in fibroblasts or lymphoblasts, and by sequence analysis of *RPIA*.

7.9.5 Treatment and Prognosis

Therapeutic options for RPI deficiency are not available. Supportive therapy and rehabilitation for the neurological complications are indicated. The prognosis is unclear given that a single patient has been described so far. We hypothesize that severe mutations in the gene cause a lethal phenotype. Finding other patients is needed to determine the phenotype.

7.10 Transaldolase (TALDO) Deficiency

7.10.1 Clinical Presentation

TALDO deficiency has now been diagnosed in more than 40 patients from more than 30 different families [16][17][18][19] (V Valayannopoulos/M Wamelink personal communication).

Wide phenotypic variability has been reported [16][17][18][19]. Most patients display the first symptoms of the disease in the neonatal or antenatal period while others may have milder or no symptoms at birth and may be diagnosed only later in life due to liver manifestations.

Among the early onset symptoms intrauterine growth retardation, oligohydramnios and hydrops fetalis have been described [16]. Neonates present with hepatomegaly and splenomegaly with portal hypertension, coagulopathy, abnormal liver function tests, cholestatic jaundice and elevated liver enzymes. Anaemia and thrombocytopenia are almost always present in the early onset forms of the disease and most showed dysmorphic features (antimongoloid slant, low-set ears, hypertrichosis and skin abnormalities (cutis laxa, spider telangiectasies and multiple haemangiomas), neonatal oedema and congenital heart defects (septal defects, cardiomyopathy, tetralogy of Fallot).

Hepatic fibrosis and cirrhosis are the histological liver hallmarks later in life and in late-onset patients. Early onset hepatocellular carcinoma was present as the only symptom of TALDO deficiency in one of the late onset patients [18]. One asymptomatic patient of 8 years old was diagnosed after molecular testing because of an affected sibling [18]. Given the variability in phenotype and outcome, TALDO deficiency could be considered in adults with cirrhosis of unknown aetiology.

Renal manifestations (tubulopathy, renal failure, nephrocalcinosis) and endocrine disorders have frequently been reported, leading to transient hypoglycaemia, and testicular or ovarian insufficiency leading to cryptorchidism, clitoris enlargement, secondary amenorrhea and bone development abnormalities with rickets.

Mild transient hypotonia was described in several patients, but mental and motor development were normal in the majority in whom assessment was possible (ten patients died before the age of 6 months). In contrast to those with RPI deficiency, brain MRI and MRS did not reveal abnormalities in patients with TALDO deficiency.

7.10.2 Metabolic Derangement

TALDO (EC 2.2.1.2) is located in the reversible part of the PPP and recycles pentose phosphates into glycolytic intermediates in concerted action with transketolase. Its deficiency results in the accumulation of polyols (erythritol, arabitol, ribitol, sedoheptitol and perseitol), erythronic acid and seven-carbon sugars derived from the pathway intermediates [16][20].

7.10.3 Genetics

TALDO is encoded by *TALDO1*, located on chromosome 11p15.5-p15.4, and a pseudogene, on chromosome 1p34.1-p33. The mode of inheritance is autosomal recessive. Mutations detected in *TALDO1* include missense mutations, deletions and a duplication.

7.10.4 Diagnostic Tests

Diagnosis of TALDO deficiency is achieved by detecting elevated urine concentrations of the seven-carbon sugars sedoheptulose, mannoheptulose, sedoheptitol, perseitol and sedoheptulose-7-phosphate and the polyols erythritol, arabitol and ribitol [16]. Elevations are most striking in the neonatal period and are more subtle in older patients. In plasma and CSF, there are only minor elevations of polyols or none at all. Elevated

concentrations of sedoheptulose-7-phosphate can be detected in blood spots, suggesting that newborn screening may be feasible [16]. MRS is not informative, and the gold standard of diagnosis is measurement of TALDO activity in fibroblasts, lymphoblasts, erythrocytes or a liver biopsy and sequence analysis of *TALDO1*.

Prenatal diagnosis is possible by sequence analysis of *TALDO1* in chorionic villi and amniocytes. In amniotic fluid from an affected fetus increased concentrations of sedoheptulose and ribitol were detected [16]. Prenatal diagnosis may therefore also be possible by measuring sedoheptulose and ribitol in amniotic fluid supernatant.

7.10.5 Treatment and Prognosis

For most patients the outcome seems to be correlated to the severity of the liver impairment. Nine patients died of acute liver failure at the onset of the disease or of chronic liver failure-related complications including bleeding and respiratory distress. However, a few patients with an initially severe liver disease associated with fibrosis and cirrhosis are currently stable. In our current knowledge of the disease, patients who present at birth with liver failure and who do not recover before the 1st month of life, and those who have deteriorating liver function within the first 6 months of life, have a high mortality close to 100%. These patients should be carefully monitored and considered promptly for liver transplantation. However, in one patient with neonatal liver failure, all liver symptoms, including hepatomegaly resolved, he is now 15 years old ([16], patient 5).

There is no specific treatment for TALDO deficiency. Liver manifestations may be treated symptomatically or require liver transplantation. Two patients have been reported in the literature to have received successfully liver transplants at age 1 year [19] and 16 months [18] before the diagnosis was known. One other patient age 6 months (V Valayannopoulos, personal communication) died in the course of liver transplantation.

Hormone disturbances are in general transient and can be addressed by specific replacement therapy.

Emerging pathophysiological insights into TALDO deficiency, namely the role of oxidative stress in liver pathology, have been described in the *Taldo1-/-* mouse model and seem to respond well to common antioxidant therapies such as N-acetylcysteine [21].

7.11 Transketolase (TKT) Deficiency

7.11.1 Clinical Presentation

TKT deficiency has been detected through whole exome sequencing in five affected individuals from three unrelated families, all of whom have proportional short stature and developmental delay [22][23]. Congenital heart disease (including atrium septal defects, ventricular septal defects, patent foramen ovale and patent ductus arteriosus) was present in 4 of the 5 patients. Their age at diagnosis varied between 5 and 24 years old. In the older patients bilateral cataracts and psychiatric symptoms (ADHD, obsessive compulsive disorder, stereotypic behaviour and self-injury) were present. In more than one patient there was also hypotonia, abnormalities of the eye: including juvenile bilateral cataract and inflammation in the eye. Three patients also had facial dysmorphism.

7.11.2 Metabolic Derangement

Transketolase is a reversible, thiamine-dependent enzyme in the pentose phosphate pathway (PPP). It catalyses the transfer of a two carbon unit from xylulose-5-phosphate to one of two substrates: ribose-5-phosphate producing glyceraldehyde-3-phosphate and sedoheptulose-7-phosphate or erythrose-4-phosphate producing glyceraldehyde-3-phosphate and fructose-6-phosphate. Its deficiency results in the accumulation of polyols (erythritol, arabitol and ribitol) in urine and plasma and accumulation of pent(ul)ose-5-phosphates in urine.

7.11.3 Genetics

Human *TKT* consists of 16 exons. The mode of inheritance is autosomal recessive. In two families from Ashekenazi heritage the c.769_770delins18 mutation was detected [23]. TKT deficiency may be more common in the Ashkenazi Jewish community.

7.11.4 Diagnostic Tests

Diagnosis of TKT deficiency is achieved by detecting elevated urine or plasma concentrations of erythritol, arabitol and ribitol. In urine also elevation of ribose-5-phosphate and ribulose+xylulose-5-phosphate can be detected. Confirmation is done by enzymatic analysis in fibroblasts, lymphoblasts or erythrocytes or DNA analysis.

7.11.5 Treatment and Prognosis

Therapeutic options for TKT deficiency have not yet been identified. Thiamine or benfotiamine (a synthetic derivative of thiamine) might in theory, be of benefit in patients with some residual TKT activity and should be investigated.

7.12 Sedoheptulokinase (SHPK) Deficiency

7.12.1 Clinical Presentation

SHPK (EC 2.7.1.14) deficiency was first described as part of a large 57-kb-deletion in infantile nephropathic cystinosis [16].

This deletion extends from exon 10 of *CTNS*, upstream through *CARKL/SHPK*, to intron 2 of *TRPV1*. In a previous small study no difference in the clinical phenotype between patients with other mutations causing the severe infantile nephropathic type or patients with the deletion could be found, suggesting that isolated SHPK deficiency would result in a benign or mild phenotype. In 2015, two patients were identified with isolated SHPK deficiency [24]. Clinical presentation in patient 1 showed neonatal cholestasis, hypoglycaemia, anaemia and dysmorphism, while patient 2 presented with congenital arthrogryposis multiplex, multiple contractures and dysmorphisms. Since both patients presented very differently and without a clear clinical overlap with cystinosis patients (caused by the 57-kb deletion), the biochemical defect of SHPK deficiency seems to be either unrelated to the clinical phenotypes or might have a broad phenotypic presentation, which is dependent on external factors and/or the genetic backgrounds of the individuals.

7.12.2 Metabolic Derangement

Strongly elevated excretion of sedoheptulose and erythritol were detected in the isolated SHPK deficient patients and the cystinosis patients with the homozygous 57-kb deletion. Kardon et al indicated that the accumulation of erythritol is likely derived from sedoheptulose through convertion to sedoheptulose-1-phosphate by fructokinase and to erythrose and dihydroxyacetone-phosphate by aldolase B. Erythrose would then finally be reduced to erythritol [25].

7.12.3 Genetics

Human *SHPK* (*CARKL*) consists of 7 exons. The mode of inheritance is autosomal recessive. Mutations detected in *SHPK* include stop mutations and a large 57-kb deletion.

7.12.4 Diagnostic Tests

Diagnosis of SHPK deficiency is achieved by detecting elevated urine concentrations of sedoheptulose and erythritol . With low-normal excretion of sedoheptulose-7-phosphate [16][17][18][19][20][21][22]. Confirmation is done by enzymatic analysis in fibroblasts or DNA analysis.

7.12.5 Treatment and Prognosis

Therapeutic options for SHPK deficiency have not yet been identified.

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Disorders of Fructose Metabolism

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Fructose Metabolism

Fructose is one of the main sweetening agents in the human diet. It is found in its free form in honey, fruits and many vegetables, and is associated with glucose in the disaccharide sucrose in numerous foods and beverages. Sorbitol, also widely distributed in fruits and vegetables, is converted into fructose in the liver by sorbitol dehydrogenase (Fig. 8.1). In recent years, increased consumption of fructose (particularly from sweetened beverages) has been associated with an increased prevalence of obesity, metabolic syndrome, type 2 diabetes and gout [1] which underscores the importance of understanding the metabolic consequences of fructose consumption. Fructose is mainly metabolised in the liver, renal cortex and small intestinal mucosa in a pathway composed of fructokinase, aldolase B and triokinase. Aldolase B is also involved in the glycolytic-gluconeogenic pathway (right hand part of the scheme).

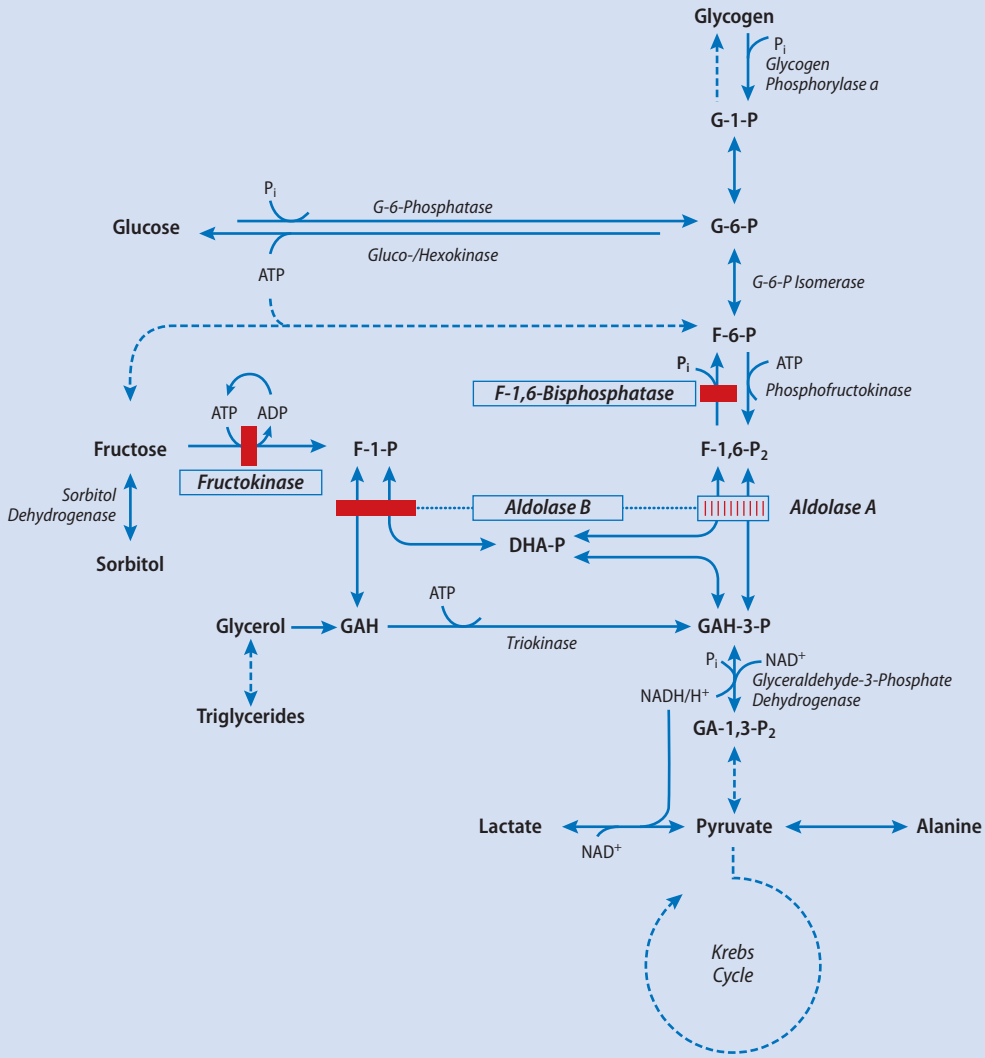

Fig. 8.1 Fructose metabolism. *DHA-P*, dihydroxyacetone phosphate; *F*, fructose; *G*, glucose; *GA*, glycerate; *GAH*, glyceraldehyde; *P*, phosphate; *Pi*, inorganic phosphate. The three enzyme defects in fructose metabolism are boxed and depicted by solid bars across the arrows; the diminished activity of aldolase B toward fructose-1,6-bisphosphate is depicted by a broken bar

Three inborn errors are known in the pathway of fructose metabolism (► Fructose Metabolism) depicted in ◘ Fig. 8.1. *Essential fructosuria* is a harmless anomaly characterised by the appearance of fructose in the urine after the intake of fructose-containing food. In *hereditary fructose intolerance (HFI)*, fructose may provoke prompt gastrointestinal discomfort and hypoglycaemia upon ingestion, symptoms that can vary from patient to patient and depend on the ingested dose. Fructose may cause liver and kidney failure when taken persistently, and its intake becomes life-threatening when given intravenously. *Fructose-1,6-bisphosphatase (FBPase) deficiency* is also usually considered an inborn error of fructose metabolism although, strictly speaking, it is a defect of gluconeogenesis. The disorder is manifested by the appearance of hypoglycaemia and lactic acidosis (neonatally, or later during fasting or induced by fructose) and may be life-threatening.

8.1 Essential Fructosuria

8.1.1 Clinical Presentation

Essential fructosuria is a rare non-disease; it is detected by routine screening of urine for reducing sugars. It is caused by a deficiency of fructokinase, also known as ketohexokinase (KHK), the first enzyme of the main fructose pathway (◘ Fig. 8.1).

8.1.2 Metabolic Derangement

In essential fructosuria, ingested fructose is partly (10–20%) excreted as such in the urine, the rest is slowly metabolised by an alternative pathway, namely conversion into fructose-6-phosphate by hexokinase in adipose tissue and muscle (◘ Fig. 8.1).

8.1.3 Genetics

The mode of inheritance is autosomal recessive and the frequency has been estimated at 1:130,000. However, since the condition is asymptomatic and harmless and since laboratories are abandoning tests for reducing substances in urine in favour of specific glucose tests, it may be more prevalent than reported.

Tissue-specific alternative splicing of *KHK* results in two isoforms, ketohexokinase A, widely distributed in most foetal and adult organs but with no clear physiological role, and ketohexokinase C, expressed in adult liver, kidney and small intestine, which is affected in essential fructosuria [2]. To date, only two mutations of *KHK*, p.G40R and p.A43T, both of which alter the same conserved region of fructokinase, have been detected in a family with three compound heterozygotes [3]; their functional effect has been characterised based on the crystallographic structure [4].

8.1.4 Diagnosis

Fructose gives a positive test for reducing sugars and a negative reaction with glucose oxidase. It can be identified by various techniques, such as thin-layer chromatography, and quantified enzymatically. Fructosuria depends on the time and amount of fructose, sorbitol and sucrose intake and, thus, is inconstant. Fructose-tolerance tests (► Section 8.2) neither provoke an increase in blood glucose as in normal subjects, hypoglycaemia or other changes as occur in HFI and FBPase deficiency, nor are metabolic changes in liver detectable by ^{31}P-magnetic resonance spectroscopy (MRS) [5].

8.1.5 Treatment and Prognosis

Dietary treatment is not indicated, and the prognosis is excellent.

8.2 Hereditary Fructose Intolerance

8.2.1 Clinical Presentation

Individuals with hereditary fructose intolerance (HFI) are perfectly healthy as long as they do not ingest food containing fructose, sucrose and/or sorbitol. Consequently, no metabolic derangement occurs during breast-feeding. The younger the child and the higher the dietary fructose load, the more severe the reaction. In the *acute* presentation of HFI, an affected newborn infant who is not breast-fed but receives a cow's milk formula sweetened and enriched with fructose or sucrose – formulas which should be obsolete today – is in danger of severe liver and kidney failure and death.

At weaning from breast-feeding or from a fructose/sucrose-free infant formula, the first symptoms appear with the intake of fruits and vegetables [6][7][8]. They are generally those of gastrointestinal discomfort, nausea, vomiting, restlessness, pallor, sweating, trembling, lethargy and, eventually, apathy, coma, jerks and convulsions. At this stage, laboratory signs may be those of acute liver failure and generalised dysfunction of the renal proximal tubules. If the condition is unrecognised and fructose not excluded from the diet, the disease may take a *chronic*, fluctuating course with failure to thrive, liver disease manifested by hepatomegaly, jaundice, bleeding tendency, oedema, ascites, and signs of proximal renal tubular dysfunction. Laboratory findings are those of liver failure, proximal renal tubular dysfunction and derangements of intermediary metabolism. Note that hypoglycaemia after fructose ingestion is short-lived and can be easily missed or masked by concomitant glucose intake.

HFI can be suspected in an asymptomatic infant, if the parents have excluded certain foods from the diet, having becoming aware that they are not tolerated. In older children, a distinct aversion towards foods containing fructose may develop; these

feeding habits protect them but are sometimes considered as neurotic behaviour. At school age, HFI is occasionally recognised when hepatomegaly or growth delay is found [9]. Some adults were diagnosed after developing life-threatening reactions with infusions containing fructose, sorbitol or invert sugar (a mixture of glucose and fructose obtained by hydrolysis of sucrose) when these IV solutions were still in use [10]. Because approximately half of all adults with HFI are free of caries, the diagnosis has also been suspected by dentists. Although several hundred patients with HFI have been identified since its recognition as an inborn error of metabolism in the 1950s [6][7], these observations indicate that affected subjects may remain undiagnosed and still have a normal life span.

8.2.2 Metabolic Derangement

HFI is caused by deficiency of the second enzyme of the fructose pathway, aldolase B (Fig. 8.1), which splits fructose-1-phosphate (F-1-P) into dihydroxyacetone phosphate and glyceraldehyde. As a consequence of the high activity of fructokinase, intake of fructose results in accumulation of F-1-P and trapping of phosphate. This has two major effects [11]: *(i)* inhibition of glucose production by blockage of gluconeogenesis (by inhibition of aldolase A, Fig. 8.1) and of glycogenolysis (by inhibition of glycogen phosphorylase A) which induces a rapid drop in blood glucose, and *(ii)* overutilization and diminished regeneration of ATP; this depletion of ATP results in an increased production of uric acid, a release of magnesium, and a series of other disturbances, including impaired protein synthesis and ultrastructural lesions which are responsible for hepatic and renal dysfunction. The accumulation of F-1-P has also been shown to result in deficient glycosylation of proteins, *e.g.,* serum transferrin, by inhibiting phosphomannose isomerase [12] (▶ Chapter 40).

Residual activity measurable with fructose-1,6-bisphosphate as substrate (see below) is mainly due to the isozyme aldolase A. Thus, glycolysis and gluconeogenesis are not impaired in the fasted state in HFI patients.

It should be noted that the IV administration of fructose to normal subjects also induces the metabolic derangements described above (including the drop in ATP and Pi, and rise in urate and Mg^{++}) to an equivalent extent, although they are more transient than in patients with HFI, as demonstrated by ^{31}P-MRS [5]. In normal subjects, IV fructose results in increased glycaemia because of its rapid conversion into glucose. However, the equally rapid conversion of fructose into lactate may provoke metabolic acidosis. For these reasons, the use of fructose, sorbitol and invert sugar has been strongly discouraged for parenteral nutrition in general [13].

8.2.3 Genetics

HFI is an autosomal recessive disorder. Three different genes coding for aldolases have been identified. While isozymes A and C are mainly expressed in muscle and brain, respectively, aldolase B is the major fructaldolase of liver, renal cortex, and small intestine. At present, according to different databases approximately 60 causative mutations of aldolase B gene (*ALDOB*) have been reported. Among them, three amino acid substitutions, p.A150P[1], p.A175D, and p.N335K are relatively common among patients of European descent [14].

Since the three most common mutations are responsible for more than 90% of HFI cases in some European regions and still more than 50% of cases from the more heterogeneous population in North America [http://www.bu.edu/aldolase/HFI/index.html], a non-invasive diagnostic approach using molecular genetic methods has been advocated [15][16]. Among these methods, multiplex ligation-dependant probe amplification (MLPA) assays can also detect copy number variations present on approximately 6% of mutant alleles not detectable by standard sequencing techniques [Santer, unpublished].

From molecular genetic neonatal screening studies in England and Germany, the prevalence of HFI has been calculated as 1:18,000 [1] and 1:29,600, respectively [15].

8.2.4 Diagnosis

Whenever HFI is suspected, fructose should be eliminated from the diet immediately. The beneficial clinical and chemical effects of withdrawal, usually seen within days, provide a first diagnostic clue. Laboratory findings will subsequently show a fall in the elevated serum transaminases and bilirubin, improved levels of blood clotting factors, and amelioration of proximal tubular dysfunction (proteinuria, glucosuria, generalised hyperaminoaciduria, hyperphosphaturia, hypophosphataemia, hyperuricuria, metabolic acidosis).

A cornerstone in the diagnosis of HFI is a careful nutritional history, with special emphasis on the time of weaning when fruits and vegetables were introduced [1][8][17]. If the nutritional history is suggestive or other aspects are indicative of HFI (*e.g.,* a positive family history), the disorder should be confirmed by molecular diagnosis (▶ above) on DNA from peripheral leukocytes. This is a non-invasive approach and has the advantage over enzymatic measurement in liver tissue in that it eliminates the complication of secondarily lowered aldolase B activity in a damaged liver.

If no mutation can be found despite a strong clinical and nutritional history suggestive of HFI, an enzymatic determination or a functional test should be undertaken after a few weeks of fructose exclusion. In liver biopsies from HFI patients, the capacity of aldolase to split F-1-P is reduced, usually to a few percent of normal (mean 5%, range 0–15%) [17], although residual activities as high as 30% of normal have been reported [10]. There is also a distinct (but less marked) reduction in the activity of aldolase B toward fructose-1,6-bisphosphate (mean 17%, range 5–30%). As a consequence, the ratio of V_{max} towards fructose-1,6-bisphosphate versus the V_{max} towards F-

1 Note that the initiation codon ATG for methionine in the *ALDOB* cDNA was ignored in previous designations and that, e.g., 'p.A150P' was originally termed 'A149P'

1-P, which is approximately 1 in control liver, is increased to 2 to ∞ in HFI patients [17]. Aldolase activity is normal in blood cells, muscle, and skin fibroblasts, which contain a different isozyme, aldolase A. The enzymatic determination of aldolase B in small intestinal mucosa is not recommended. For postmortem diagnosis, molecular studies and measurements of enzyme activity in liver and kidney cortex should be done.

It should be noted that the level of residual activity has never been shown to correlate with the degree of tolerance to fructose.

In vivo handling of fructose is best reflected by a fructose tolerance test, in which fructose (200 mg/kg b.w.) is injected as a 20% solution intravenously within 2 minutes. Blood samples are taken at 0 (2), 5, 10, 15, 30, 45, 60 and 90 minutes for determination of glucose and phosphate. In normal subjects, blood glucose increases by 0–40%, with no or minimal changes in phosphate [17]. In HFI patients, glucose and phosphate decrease within 10–20 minutes. As a rule, the decrease of phosphate precedes and occurs more rapidly than that of glucose. The test should be undertaken in a metabolic centre, with careful monitoring of glucose and an indwelling catheter for the (exceptional) case of symptomatic hypoglycaemia and its treatment by IV glucose administration. Oral fructose tolerance tests are not recommended, because they provoke more ill effects and are less reliable [17].

8.2.5 Differential Diagnosis

A high degree of diagnostic awareness is often needed in HFI because the spectrum of symptoms and signs is wide and nonspecific; HFI has been misdiagnosed as pyloric stenosis, gastro-oesophageal reflux, galactosaemia, tyrosinaemia, non-IgE-mediated gastrointestinal food hypersensitivity, intrauterine infection, glycogen and other storage disorders, ornithine transcarbamylase deficiency, and later in life as Wilson disease, leukaemia, and growth retardation. Fructosuria may be secondary to liver damage, *e.g.,* in tyrosinaemia.

HFI is frequently confused with fructose malabsorption [18], a condition caused by defective fructose transport in the small intestine whose metabolic basis, however, is not well understood. The ingestion of fructose, and to a considerably lesser extent of sucrose, leads to abdominal pain and diarrhoea. Since this condition is diagnosed by breath hydrogen analysis after an oral load of fructose, HFI has to be excluded before such a tolerance test is performed, otherwise deleterious effects may occur [19]. In sucrase-isomaltase deficiency, the ingestion of sucrose results in bloating, abdominal cramps and fermentative osmotic diarrhoea; free fructose, however, is well tolerated.

8.2.6 Treatment and Prognosis

In acute intoxication, intensive care may be required and supportive measures such as fresh frozen plasma may be needed. The main therapeutic step in HFI, however, is the immediate elimination of all sources of fructose from the diet. This involves the avoidance of all types of food in which fructose, sucrose and/or sorbitol occur naturally or have been added during processing. It should be borne in mind that fructose and sorbitol may be present in medications (*e.g.,* syrups, immunoglobulin solutions, rinsing fluids, enema solutions) and infant formulas (without adequate declaration of the carbohydrate composition). In this respect, it is deplorable that European Union regulations allow infant formulae to contain up to 20% of their total carbohydrate content as sucrose [20].

Sucrose should be replaced by glucose, maltose and/or starch to prevent the fructose-free diet from containing too much fat. Despite the availability of books and online information on food composition, a dietician should be consulted and practical aspects of the diet (*e.g.,* the considerable variability of the fructose content of different food types, and the influence of storage temperature or method of preparation and manner of cooking on bioavailability) be discussed. Substitution of vitamins, especially ascorbic acid and folates, in the form of a multivitamin preparation should be prescribed to make up for their diminished intake from fruits and vegetables.

After institution of a fructose-free diet most abnormalities disappear rapidly, except for hepatomegaly, which may persist for months or even years [21]; the reason for this is unclear. Different thresholds of fructose intake for the development of certain symptoms have appeared in the literature, ranging from 40–250 mg/kg b.w./day as compared with an average intake of 1–2 g/kg/day in Western societies [1]. Insufficient restriction of fructose has been reported to cause isolated growth retardation, as evidenced by catch-up growth on a stricter diet [9]. It has also to be kept in mind that recommendations for maximum doses have not been validated in different genotypes and that sensitivity is known to be different in individual patients. Thus, it should be suggested to parents that they keep fructose intake as low as possible and that, at least in childhood, it should not be determined by subjective tolerance. For dietary control, the regular taking of the nutritional history is still best, as there are no good sensitive chemical parameters except, perhaps, transaminases. Quantification of carbohydrate-deficient proteins, *e.g.,* transferrin, has been suggested for dietary monitoring [12]; however, the sensitivity of this procedure has not been validated. Needless to mention that patients (and their parents) should be made aware of the fact that infusions containing fructose, sorbitol or invert sugar are life-threatening. There are numerous reports in the literature of fructose ingestion by mistake and that is why HFI, if present, should be reported on any hospital admission by an emergency card.

The prognosis of HFI on diet is excellent with normal growth, intelligence and life span.

8.3 Fructose-1,6-Bisphosphatase Deficiency

8.3.1 Clinical Presentation

In about half of all cases, fructose-1,6-bisphosphatase (FBPase) deficiency presents in the first 1 to 4 days of life with severe

hyperventilation caused by profound lactic acidosis and marked hypoglycaemia. Later on, episodes of irritability, somnolescence or coma, apnoeic spells, dyspnea and tachycardia, muscular hypotonia, and moderate hepatomegaly may occur. Most affected children experience a number of acute attacks before the diagnosis is made. As reported in the first patient described [22], such episodes are typically triggered by a febrile episode accompanied by refusal to feed and vomiting. Attacks may also follow ingestion of large amounts of fructose (~1 g/kg b.w. in one dose) especially after a period of fasting. FBPase deficiency may be life-threatening and, as in HFI, administration of IV fructose is contraindicated and may lead to death. In between attacks, patients are usually well, although mild, intermittent or chronic acidosis may exist. The frequency of the attacks decreases with age, and the majority of survivors display normal somatic and psychomotor development [23].

In contrast to HFI, chronic ingestion of fructose does not lead to gastrointestinal symptoms – hence there is no aversion to sweet foods – or failure to thrive, and only exceptionally is there disturbed liver function.

Analysis of plasma during acute episodes reveals lactate accumulation (up to 15–25 mM) accompanied by a decreased pH and an increased lactate/pyruvate ratio (up to 40), hyperalaninaemia, an increase in glycerol which may mimic hypertriglyceridaemia [24], and glucagon-resistant hypoglycaemia. Hyperketonaemia may be found, but in several patients ketosis has been reported to be moderate or absent (▶ below and [25]). Increased levels of free fatty acids and uric acid may also be found. Urinary analysis reveals increased lactate, alanine, glycerol, and, in most cases, ketones and glycerol-3-phosphate.

8.3.2 Metabolic Derangement

Deficiency of hepatic FBPase, a key enzyme in gluconeogenesis, impairs the formation of glucose from all gluconeogenic precursors, including dietary fructose (Fig. 8.1). Consequently, maintenance of normoglycaemia in patients with the defect is exclusively dependent on glucose (and galactose) intake and degradation of hepatic glycogen and, to a minor degree, on glucose production by the muscle [26]. Thus, hypoglycaemia is likely to occur when glycogen reserves are limited (as in newborns) or exhausted (as when fasting). The defect provokes accumulation of the gluconeogenic substrates lactate, pyruvate, alanine, and glycerol. The lactate/pyruvate ratio is usually increased which is explained by secondary impairment of conversion of 1,3-bisphosphoglycerate to glyceraldehyde-3-phosphate; this results in accumulation of NADH/H^+ which shifts the equilibrium of pyruvate and lactate (Fig. 8.1). Attention has been drawn to the fact that hyperketonaemia and ketonuria, which usually accompany hypoglycaemia, may be absent in some patients with FBPase deficiency [25]. This may be explained by pyruvate accumulation resulting in an increase of oxaloacetate and, hence, in the diversion of acetyl-coenzyme A (CoA) away from ketone-body formation into citrate synthesis. This, in turn, results in increased synthesis of malonyl-CoA in the cytosol. Elevated malonyl-CoA, by inhibiting carnitine-palmitoyl transferase I, prevents the entry of long-chain fatty-acyl-CoA into the mitochondria and, thereby, further reduces ketogenesis. It also promotes accumulation of fatty acids in liver and plasma, as documented in some patients.

Children with FBPase deficiency generally tolerate sweet foods, up to 2 g fructose/kg b.w. per day, when given regularly distributed over the day and, in contrast to subjects with HFI, they thrive on such a diet [27]. Nevertheless, loading tests with IV fructose do induce hypoglycaemia, as in HFI. This is caused by the inhibitory effect of the rapidly formed but slowly metabolised F-1-P on liver glycogen phosphorylase a. That higher doses of fructose are required for hypoglycaemia to occur is explained by the fact that, in contrast to the aldolase B defect in HFI, FBPase deficiency still allows F-1-P to be converted into lactate. ^{31}P-MRS of the liver following IV administration of fructose (200 mg/kg b.w.) has documented a slower decrease in the fructose-induced accumulation of F-1-P and a delayed recovery of the ensuing depletion of Pi and ATP (both of which are signs of fructose toxicity) in patients with FBPase deficiency as compared with healthy controls [5].

8.3.3 Genetics

FBPase deficiency is a rare autosomal-recessive disorder with variable frequencies in different geographic regions. In addition to European and North American patients, many cases have been diagnosed in Japan. The high proportion of Turkish patients in our own series might simply be the result of the high rate of parental consanguinity.

There is evidence for the existence of more than one isozyme with FBPase activity in humans. The muscle isoform has different kinetic characteristics to the liver isoform and is not affected in FBPase deficiency. Only the liver-type isoform gene (*FBP1*) has been cloned and characterised. To date 35 different mutations in all regions of the gene have been published [28]. Among them, a gross deletion including exon 2 (c.-24-26_170+5192del) is common in patients of Turkish and Armenian descent [28]. The c.959dupG mutation has been reported to be responsible for 46% of mutated alleles in Japan [29] but only 14% in Central Europe [30]; c.841G>A has repeatedly been detected in Pakistani patients [24].

There are several patients in whom no mutation of the coding region of *FBP1* could be found. Therefore, we have supposed that these patients carry mutations within the promoter region of *FBP1* or, more hypothetically, in the gene for the bifunctional enzyme which controls the concentration of fructose-2,6-bisphosphate, the main physiological regulator of FBPase [31].

8.3.4 Diagnosis

Whenever possible, the diagnosis of FBPase deficiency should be made by molecular analysis on DNA from peripheral leukocytes. If no mutation is found despite highly suggestive clin-

ical and laboratory findings, the determination of enzymatic activity in a liver biopsy should be undertaken; the residual activity may vary from zero to 30% of normal, indicating genetic heterogeneity of the disorder. Obligate heterozygotes have intermediate activity. Diagnosis is not possible in mixed leukocytes [32] but seems to be reliable in isolated and stimulated monocytes [23]; however, cultured skin fibroblasts, amniotic fluid cells and chorionic villi do not express FBPase.

Loading tests with fructose (or with glycerol or alanine) or fasting tests should not be part of the initial investigations as they provide only a tentative diagnosis. However, such functional tests may be useful, and may point to a disturbance in the regulation of the fructose-6-phosphate – fructose-1,6-bisphosphate substrate cycle if mutation analysis and enzyme activity are normal despite a strong clinical and chemical suspicion of FBPase deficiency.

8.3.5 Differential Diagnosis

Other disturbances in gluconeogenesis and pyruvate oxidation have to be considered, including *(i)* pyruvate dehydrogenase deficiency characterised by a low lactate/pyruvate ratio, absence of hypoglycaemia and aggravation of lactic acidosis by glucose infusion; *(ii)* pyruvate carboxylase deficiency; *(iii)* respiratory chain disorders; *(iv)* glycogenosis type Ia and Ib presenting with the same metabolic profile (fasting hypoglycaemia and lactic acidosis) and hepatonephromegaly, hyperlipidaemia, and hyperuricaemia; *(v)* fatty acid oxidation defects presenting with fasting hypoketotic hypoglycaemia and hyperlactataemia.

8.3.6 Treatment and Prognosis

Whenever FBPase deficiency is suspected, adequate amounts of IV or oral glucose should be given. The acute, life-threatening episodes should be treated with an IV bolus of 20% glucose followed by a continuous infusion of glucose at high rates (10–12 mg/kg b.w./min for newborns) and bicarbonate to control hypoglycaemia and acidosis. If correction of acidosis is not really needed, recovery from it in response to glucose is a good (positive) indicator for the diagnosis of FBPase deficiency. Furthermore, the infusion of glycerol (that may even contain additional fructose), as frequently practiced in patients with brain oedema and hypoglycaemia in Japan, is extremely dangerous unless FBPase deficiency is excluded [27][33].

Maintenance therapy should be aimed at avoiding fasting, particularly during febrile episodes. This involves frequent feeding, the use of slowly absorbed carbohydrates (such as uncooked starch), and a gastric drip, if necessary. In small children, restriction of fructose, sucrose and sorbitol is also recommended, as are restrictions of fat (to 20–25%) and protein (to 10% of energy requirements). In the absence of any triggering effects leading to metabolic decompensation, individuals with FBPase deficiency are healthy and no carbohydrate supplements are needed.

Once FBPase deficiency has been diagnosed and adequate management introduced, its course is usually benign. Growth, psychomotor and intellectual development are unimpaired, and tolerance to fasting improves with age with the effect that the disorder in general does not present a problem in later life [23]. Pregnancies were reported to be uncomplicated [23][34][35]. Many patients, however, become obese because their concerned parents overfeed them and they continue these eating habits when older. Under carefully observed conditions, a hypocaloric diet (800–900 kcal/m^2/day) can lead to a considerable weight loss in obese patients without the development of lactic acidosis and hypoglycaemia [Steinmann, personal observation].

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Congenital Hyperinsulinism

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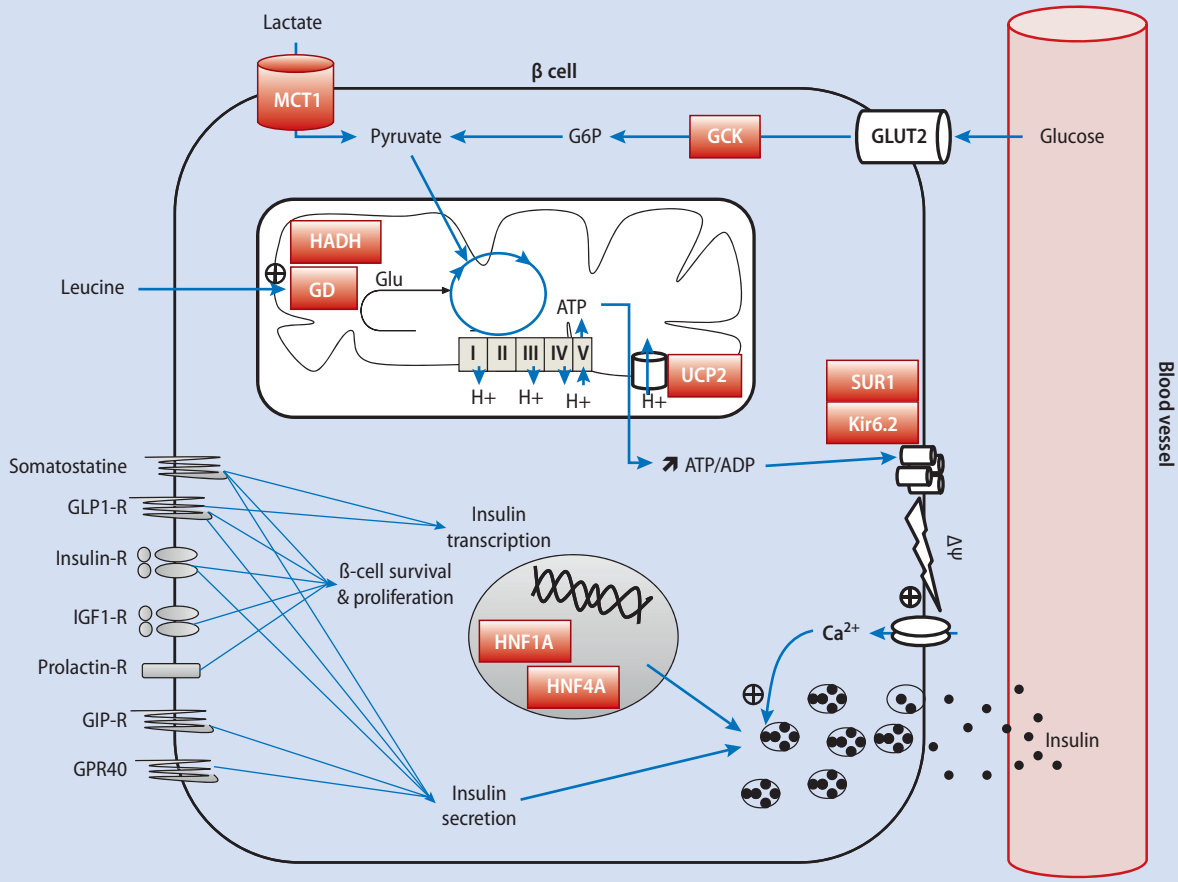
Glucose-induced insulin secretion and its modulation

When glucose is transported into the pancreatic β-cell it is phosphorylated to glucose-6-phosphate by glucokinase (GCK, ◘ Fig. 9.1). GCK has a K_m for glucose close to its concentration in blood and functions as a glucose sensor; any rise in blood sugar is followed by a proportional increase in the rate of glycolysis with phosphorylation of ADP to ATP by the glycolytic pathway and by the mitochondrial respiratory chain. At the plasma membrane, this increased ATP/ADP ratio is detected by ATP/ADP-sensitive potassium channels (K_{ATP}), leading to their closure, and subsequently to the depolarization of the plasma membrane. As a consequence of this depolarisation, voltage-sensitive Ca^{2+} channels open, and the influx of extracellular Ca^{2+}stimulates insulin secretion by exocytosis from storage granules.

In addition to glucose-induced insulin secretion (GSIS), other mechanisms are also involved that regulate the release of insulin to meet all physiological circumstances: (1) transcription factors, such as *HNF1A* and *HNF4A*, (2) metabolic factors which modulate the ATP production, namely leucine (through the activation of the glutamate dehydrogenase (GDH, *GLUD1*), short-chain L-3-hydroxyacyl-CoA dehydrogenase (SCHAD, *HADH*), the monocarboxylate transporter (MCT1, *SLC16A1*), and the mitochondrial uncoupling protein 2(*UCP2*), and (3) receptors for various hormones and neuropeptides including somatostatin, insulin, GLP1, GIP, etc.

Insulin, released into the blood, acts on the insulin receptors of insulin-sensitive cells. The activation of the downstream signaling pathway of the insulin receptor increases glucose utilization and inhibits lipid utilization, modulates cellular growth, and translocates the glucose transporter GLUT4 to the plasma membrane, causing an influx of glucose in the cell. Of note, cerebral cells are poorly insulin-sensitive and are highly dependent on circulating glucose to support brain metabolism, consequently, in hyperinsulinism, there is a significant risk of brain damage from neuroglucopenia.



◘ **Fig. 9.1** Glucose-induced insulin secretion and its modulation

Congenital hyperinsulinism (CHI) includes all genetic causes leading to hyperinsulinaemic hypoglycaemia (HI) that are due to a primary defect of the pancreatic β-cell (► Glucose-induced insulin secretion and its modulation). CHI can present throughout childhood but is most common in infancy. Severe CHI is responsible for recurrent severe hypoglycaemia in neonates, in whom a delayed diagnosis or inappropriate medical management is responsible for brain damage in about ⅓, underlining the importance of an early diagnosis and the use of glucagon. Most CHI patients are responsive to a first line oral treatment with diazoxide. If unresponsive, subcutaneous/intramuscular treatment with somatostatin analogues and/or frequent/continuous feeds, may be required with further exploration necessary to determine the histopathological form of the CHI. Two main histopathological variants of CHI exist: a diffuse form, where all the β-cells throughout the pancreas are involved, and a focal form, where hypersecreting β-cells are restricted to a small region of the pancreas. Focal forms can also be immediately and definitively cured by partial pancreatectomy, whereas alternative strategies for diffuse forms include subtotal pancreatectomy (leading to insulin-dependent diabetes in most cases) or onerous medical treatment for several years. The severity of CHI improves within a few years, allowing a progressive discontinuation of all therapies. This being the case and since surgery has not been proven to improve the long-term neurologic outcome of patients, several new medical treatments are in the process of being developed in order to ease the burden of conservative medical treatment and to limit the need for subtotal pancreatectomy.

9.1 Clinical Presentation

In adults and children, hyperinsulinemic hypoglycaemia (HI) is manifested by glucopenic symptoms (drowsiness, faints, seizures, hallucinations, or other neurological symptoms) and adrenergic symptoms (pallor, sweating, tachycardia). In some patients, unless glycaemia is sampled during/after the seizures, symptoms may be mistaken for epilepsy, or, in some others, hypoglycaemia can be asymptomatic. Hypoglycaemia may occur after a meal, following a fast, several times a day or only infrequently. There may be a family history of hypoglycaemia or Maturity Onset Diabetes of the Young (MODY). Rarely, the HI can be induced by a strenuous physical exercise as occurs in monocarboxylate transporter 1 deficiency (MCT1) (► Chapter 13).

In newborns and infants with CHI, the mean birthweight is 3.7 kg [7]. Hypoglycaemia may be diagnosed following a routine capillary blood sugar measurements, because of the macrosomia, or because of a seizure or coma (in about half of patients). In severely affected patients, hypoglycaemia is almost permanent and a high rate of continuous glucose (mean 17 mg/kg/min) by intravenous infusion or enteral feeding, is required to normalize glycaemia. Associated features may be a transient hypertrophic cardiomyopathy, and a discreet facial dysmorphism.

In neonates, HI can be associated with three different clinical scenarios [1][2]:

1. **Transient neonatal HI** can occur in newborns from diabetic mothers, those who are small for gestational age or as a consequence of a perinatal stress such as fetal distress or following birth asphyxia. Hypoglycaemia can be severe, but usually resolves within a few days or months. In this latter case, infants may require transient treatment with glucose enriched feeds and/or diazoxide.
2. **Syndromic HI**, occurs where HI is part of a developmental syndrome. Hypoglycaemia can be the initial manifestation of a number of different syndromes during the neonatal period including Beckwith Wiedemann Syndrome (BWS), congenital disorder of glycosylation PMM2-CDG and PMMI-CDG, Kabuki syndrome, Sotos syndrome, and chromosomal abnormalities or may be discovered later during follow-up, for example in Ondine syndrome. Dysmorphic features may be either absent or discreet at birth so that the precise diagnosis may remain unclear. HI may be severe during the neonatal period but can resolve within it (Sotos syndrome), during the first year of life (BWS), or within a few years (CDG, Kabuki syndrome). HI has been observed less frequently in the following syndromes: Timothy, Turner, Moebius, Cowden, Nager, Usher type C, ADK deficiency, Perlmann, Simpson-Golabi-Behmel.
3. **Isolated congenital HI**, is where HI is inherited but occurs primarily as an isolated abnormality. Hypoglycaemia can reveal the disease in all ages. There may be a family history of hypoglycaemia or neonatal and/or MODY (*ABCC8, HNF1A, HNF4A, GCK* mutations), or with mental retardation or epilepsy (*GLUD1* mutations). In neonates, the initial symptom is seizures in half of the patients ± macrosomia (especially with mutations in K_{ATP} genes and *HNF4A*). The age at presentation is earlier in *HNF4A* mutations (1 day), variable in K_{ATP} gene mutations (1 day to 1 year, mean: 27.4 days), and delayed in *GLUD1* and *HADH* mutations (157 days & 125 days) [3]. Hypoglycaemia occurs both in the fasting and the post-prandial states, requiring a mean glucose infusion rate of 16.4 mg/kg/min to avoid hypoglycaemia. Most neonates (86%) are resistant to the oral treatment with diazoxide. In diazoxide-unresponsive patients (mutations in *ABCC8*, *KCNJ11* and *GCK*), the disease may be either a diffuse form of CHI (CHI-D) involving the whole pancreas, or a focal form (CHI-F). The clinical differences between patients with diffuse and focal diseases are subtle and cannot be used to predict the histopathology. Despite isolated CHI being described as non-syndromic, patients may present with additional features:
   - *GLUD1* mutations lead to hyperammonemia-hyperinsulinism syndrome (HIHA). About half of patients may also present with various neurological symptoms including mild to moderate mental retardation, epilepsy (atypical, absence, & myoclonic seizures), pyramidal syndrome or dystonia.

- *HNF1A* and *HNF4A* mutations can down-regulate *GLUT2* gene thus leading to features of Fanconi-Bickel Syndrome (FBS), *HNF1A* mutations are also associated with liver adenomatosis.
- Focal forms of CHI have been reported in individual patients with either a septo-optic dysplasia, schizencephaly or with a hepatoblastoma.

9.2 Metabolic Derangement

CHI is the consequence of a primary functional defect of the pancreatic ß-cells. The inappropriate secretion of insulin will lead to hypoglycaemia both by inhibiting hepatic glucose release from glycogen and gluconeogenesis and by increasing glucose uptake in muscular and fatty tissues.

CHI is heterogeneous and can be caused by various defects in the regulation of insulin secretion by the pancreatic β-cell. These include (1) channelopathies affecting the K_{ATP} channel (*ABCC8* and *KCNJ11*mutations); (2) metabolic defects: enzymes deficiencies, such as glucokinase (*GCK* mutations), glutamate dehydrogenase (*GLUD1* mutations), or short-chain L-3-hydroxyacyl-CoA dehydrogenase (*HADHSC* mutations); transporter deficiencies such as the monocarboxylate transporter 1 (*SLC16A1* mutations) and the mitochondrial uncoupling protein 2 (UCP2); and (3) transcription factors impairment, such as HNF1A and HNF4A [2].

In some exceptional cases, hypoglycaemia can be the consequence of a defect in the signalling pathway of the insulin receptor. Mutations in the insulin receptor, and in the *AKT2* genes can indeed induce a primary defect of the insulin-sensitive cells by impairing the insulin clearance and/or over-activating the insulin pathway.

9.3 Genetics

The estimated incidence of severe CHI is 1 in 50,000 live births, but in countries with substantial consanguinity it may be as high as 1 in 2,500. To date, mutations in nine genes are known to cause isolated CHI-D, but these only explain a small proportion of all cases. The pattern of inheritance can be dominant or recessive, and the genetic abnormality sometimes occurs de novo. In isolated CHI, the inheritance is autosomal recessive for *ABCC8, KCNJ11* and *HADH* gene mutations; autosomal dominant or de novo for *GLUD1, GCK, UCP2, SLC16A1, HNF1A, HNF4A* mutations and in some cases for *ABCC8* and *KCNJ11* mutations. In syndromic CHI, the mode of inheritance depends on the diagnosis.

- In diazoxide-responsive patients a genetic abnormality is found in up to 47% of patients. *ABCC8* and *GLUD1* mutations are predominant (about 40% each), the other cases are equally distributed between *HNF4A, HNF1A, HADH, KCNJ11* and *UCP2* mutations.
- In diazoxide-unresponsive patients, 47% of patients have CHI-D and 53% CHI-F. A genetic cause is found in about 80–90% of cases. In CHI-D, *ABCC8* and *KCNJ11* mutations represent most cases (47–69% with a recessive inheritance and 20–34% with a dominant inheritance), and *GCK* only 2%. Finally, 10–20% of cases are genetically unexplained [3][4]. CHI-F is the consequence of an isodisomy of a paternally inherited recessive mutation in *ABCC8* (88%) or *KCNJ11* (11%), both located on the 11p15.5 chromosomal region, associated with a loss of the corresponding maternal allele, thus leading to an imbalance expression of imprinted growth factors and tumour suppressor genes (e.g. IGF2, H19). This somatic event, which is sporadic and spontaneous, probably occurs during the foetal period in a clone of β-cells. Rarely, some individuals with CHI-F have been reported without the paternally inherited K_{ATP} gene mutation (1% of cases) and thus were consistent with a diagnosis of BWS [5].

9.4 Diagnostic Tests

The investigation of CHI is sequential [6]:

1. **Diagnosis of HI**. Regardless the cause, the diagnosis of HI relies on 5 non-essential criteria:
   - Fasting and/or post-prandial hypoglycaemia (<2.5–3 mmol/l).
   - Inappropriate plasma insulin levels and c-peptide at the time of hypoglycaemia (potentially missed by a single sample because of the pulsatile secretion of insulin).
   - Absent/low blood & urine ketones bodies and non-esterified fatty acids (NEFA). However, in some cases, ketones bodies and NEFA are not totally suppressed.
   - An increase in blood glucose greater than 1.7 mmol/l (30 mg/dl) within 30–40 minutes after SC/IM or IV administration of 1 mg glucagon [2][6].
   - The need for a high glucose infusion rate (GIR) to keep blood glucose above 3 mmol/l is characteristic of an insulin related hypoglycaemia (e.g. >8 mg/kg/min in neonates, >10 in infants, >6 during childhood, and >3 in adults) [8].
2. **Diagnosis of CHI**. Once the diagnosis of HI has been made, some anamnestic, clinical and biological features can define the diagnosis more precisely: a family history of MODY (mutations in *GCK, HNF1A,*or *HNF4A*) or if hypoglycaemia occurs after strenuous physical exercise (mutations in *SLC16A1*); hyperammonaemia (usually between 80 and 180 µmol/l in HIHA syndrome), urine organic acids and plasma acylcarnitines (high 3-OH-glutarate in urine and C4 -OH-carnitine in plasma, in *HADH* mutations), western-blot of plasma glycoproteins (PMM2-CDG and PMMI-CDG; clinical syndromic features (hemihypertrophy, overgrowth, fat pads, cardiomyopathy or heart or vertebral malformations…); diazoxide-unresponsiveness (*ABCC8, KCNJ11, GCK* mutations). Finally, in some cases, the differential diagnosis of non-genetic HI should be considered, such as insulinoma, auto-immune syndromes and fabricated or induced illness in children.

3. **^{18}F-fluoro-L-DOPA positron emission tomography** fused with a contrast-enhanced CT angiography (DOPA-PET/CTA) has an 89% sensitivity and 98% specificity in differentiating CHI-F from CHI-D [9][10]. It should be performed in diazoxide-unresponsive patients, when genetic analysis has not diagnosed CHI-D. A single paternally inherited mutation in a K_{ATP} gene is typical of CHI-F but this might also be a dominantly inherited mutation responsible for CHI-D: this explains the positive predictive value of 66-94% in diagnosing CHI-F by DOPA-PET/CTA, when there is a single paternally inherited mutation.
4. **Pathology**. If a partial (CHI-F) or a subtotal (CHI-D) pancreatectomy is considered, the pancreatic biopsies sampled at the beginning of the surgery, will confirm the diagnosis intraoperatively. Three histopathological categories exist [11]:
   a. **CHI-D**. The pancreatic architecture is preserved, as well as the islets pattern. However, the latter contain very active β-cells with very abundant cytoplasm and highly abnormal nuclei (3 to 4 times the size of acinar nuclei).
   b. **CHI-F** (a consequence of a paternal uniparental disomy of the 11p15 associated with a paternally inherited K_{ATP} gene mutation in a subset of β-cells). Focal forms are small, 2 to 7 mm, within normal pancreatic tissue. The focal form contains focal adenomatous hyperplasia of islets composed of very large islets containing a heterogeneous population of endocrine cells of various sizes. Some of these cells have large cytoplasm and large nuclei of irregular shape. By contrast, normal islets observed outside the lesion have endocrine cells of usual (or shrunken) size without enlarged nuclei. The area of abnormal pancreatic development is multilobular and can have satellites in the nearby pancreas. CHI-F is observed in neonates and infants (not in adults).
   c. **CHI-A** (atypical focal CHI), is rare (about 4% of cases) [5]. It consists of a morphological mosaicism of the pancreatic β-cells. While most of the pancreas is normal (with endocrine cells of usual or shrunken size), one or some adjacent lobules contain abnormal β-cells showing evidence of hyper-activity (large nuclei and cytoplasm). There is no 11p15 involvement and no mutation in CHI genes in leucocytes. However, in some cases, either an excessive and unexplained expression of hexokinase I or a somatic activating mutation of *GCK* and *ABBC8* were found in the abnormal β-cells [12].

9.5 Treatment and Prognosis

Initial medical treatment [6]

In case of severe hypoglycaemia in neonates, the blood sugar must be normalized urgently by using 10% dextrose IV or orally, 2 ml/kg every 5 to 10 minutes, and a continuous glucose infusion considered. Glucagon 0.3 mg/kg IM, IV or SC can also be used as an emergency treatment. In severe HI, the glucose infusion rate (GIR) needed to normalize glycaemia may exceed the gastrointestinal tolerance of neonates. Thus a continuous glucose infusion is often required in addition to continuous enteral feeding. Continuous IV or SC glucagon (1–2 mg/24 h) may be used when glycaemia remains unstable despite a high GIR (e.g. >16 mg/kg/min).

If there is no significant spontaneous improvement in the GIR after a number days (e.g. 7–15 days), a specific treatment for HI must be considered. The first line treatment is oral diazoxide 10–15 mg/kg/d in three divided doses per day. Pulmonary hypertension (PHT) has been reported with diazoxide in infants up to 4 months old, especially in premature and syndromic patients, therefore we recommend diuretics and echocardiography before initiating this treatment and, in patients at risk of PHT, to start with lower doses (2–5 mg/kg/d). Fluid retention may require a transient diuretic treatment. Hyperpilosity is a frequent long-term side effect, but resolves after the discontinuation of this treatment. Diazoxide-responsiveness, defined by the absence of hypoglycaemia (e.g. threshold ≥3–3.8 mmol/l) while on a normal diet and during a fast of 8–12 hours, is assessed during a 5 day trial. If the neonate has a diazoxide-unresponsive HI, further treatments (octreotide ± feeding) and diagnostic tests (genetics ± FDOPA-PET/CT) must be performed to improve management and determine the histopathological form of CHI (diffuse or focal). Octreotide is started at an initial dose of 5–10 µg/kg/d either continuously (IV or SC) or SC t.i.d. The response to treatment is reassessed every 48–72 h because a phenomenon of tachyphylaxis may limit its efficacy after some hours. If unresponsive, the dose can be progressively titrated every 48 hours up to 30–50 µg/kg/d. The criteria for efficacy are the same as for diazoxide. Octreotide is usually well tolerated, but the gastrointestinal tolerance must be monitored daily in neonates since fatal enterocolitis has been reported. Diazoxide and/or octreotide may be stopped if they are considered to be of no benefit, in which case sufficient glucose must be given in order to maintain a normal blood sugar.

Surgery

Surgery is recommended for CHI-F & A, but maybe required in all forms (CHI-D, F & A) if medical and dietary therapies have been found ineffective in maintaining normoglycaemia. Because some patients may improve spontaneously within the first few months of life, the decision for surgery should not be made too hastily. The first part of surgery consists in intraoperative examination of frozen section of biopsies sampled from the head, body and tail of the pancreas, in order to confirm the diagnosis (CHI-F, or D or A). In CHI-F & A, the pancreatic area corresponding to the focal uptake of radiotracer on the PET imaging is removed. Additional pancreatic resection is performed until the intraoperative histology of the margins is clear from abnormal β-cells. In cases of CHI-F that are unresponsive to all medical therapies, a subtotal pancreatectomy may be considered. This surgery conserves the small portion of pancreas spanning from the duodenum to the common bile duct, which accounts for 2–5% of the total volume of the gland [13].

Post-operatively a Doppler ultrasound must be undertaken to check the integrity of the splenic vein and artery, since these can be damaged during surgery leading to splenic insufficiency.

9.6 Long-term Medical Management

When the patient requires long-term treatment (if surgery is not performed or is ineffective), the goal of the medical management is to maintain normoglycaemia with the least impact possible on the quality of life of patients and their families. Daily subcutaneous octreotide injections can be changed to a long-acting somatostatin analogue: lanreotide or long acting release (LAR) octreotide (given by injection every 4 weeks initially) [14][15]. Beside the usual side effects of somatostatin analogues, these long-term injections may be complicated by biliary stones, transient diarrhoea, and by subcutaneous nodules at the site of injection which disappear after a few months. Some other medical treatments are currently under evaluation (mTor inhibitors, soluble glucagon, exendin, insulin receptor inhibitors) [6]. Some patients may need long-term dietetic treatment, which encompasses frequent carbohydrate feeding, uncooked cornstarch (from age 1 year old), enteral feeding through a G-tube (continuously 24 h a day, or only at night).

This long-term treatment must be evaluated on a regular basis. Because of the progressive spontaneous remission of CHI, treatment can be progressively reduced and then stopped in most cases during childhood.

9.7 Prognosis

An intellectual disability is observed in 25–30% of patients [16]. Severe brain damage is the consequence of profound and prolonged hypoglycaemias presenting as coma and/or status epilepticus in neonates. The subsequent treatment strategy (conservative medical management vs. surgery) seems not to influence the neurological outcome. Occipital lobe injury is the commonest finding. Syndromic patients have a more unpredictable outcome since half have a progressive intellectual deficit and/or epilepsy unrelated to hypoglycaemia.

Surgery will cure immediately and definitively most patients with CHI-F with a limited risk of complication (there is a risk of exocrine pancreatic insufficiency in pancreatic head resection with Roux-en-Y pancreaticojejunostomy). However, CHI-D patients who have undergone a subtotal pancreatectomy, have an unpredictable short-term outcome with normoglycaemic, diabetes, or persistence of hypoglycaemia all possible. However, generally patients are more easily managed than before surgery. Thereafter there is an inextricable evolution to insulin-requiring diabetes, affecting 91% of patients within 14 years after their surgery [17].

Without surgery, hypoglycaemia, in most patients (CHI-F and D), will resolve slowly and spontaneously over several months to years, allowing the progressive withdrawal of most or all treatments, usually during childhood. However, because some genes are responsible for both CHI-D and monogenic diabetes (*HNF1A, HNF4A, GCK, ABCC8, KCNJ11*), we recommend lifelong glycaemic follow-up, in order to screen for diabetes in patients.

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Disorders of Glucose Transport

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Glucose Transporters

D-Glucose and other monosaccharides are hydrophilic substances that cannot easily cross the lipophilic bilayer of the cell membrane. Since these carbohydrates are most important for the energy supply of essentially all cell types, specific transport mechanisms have had to evolve: proteins embedded in the cell membrane function as hydrophilic pores that allow cellular uptake and release, transcellular transport, and sensing (generation of a membrane potential without transport) of these sugars.

Glucose transporter proteins can be divided into two groups. Sodium-dependent glucose transporters (SGLTs, symporter systems, 'active' transporters encoded by members of the *SLC5* gene family) couple sugar transport to the electrochemical gradient of sodium and hence can transport glucose against its own concentration gradient. Facilitative glucose transporters (GLUTs, uniporter systems, 'passive' transporters encoded by members of the *SLC2* gene family) can transport monosaccharides only along an existing gradient.

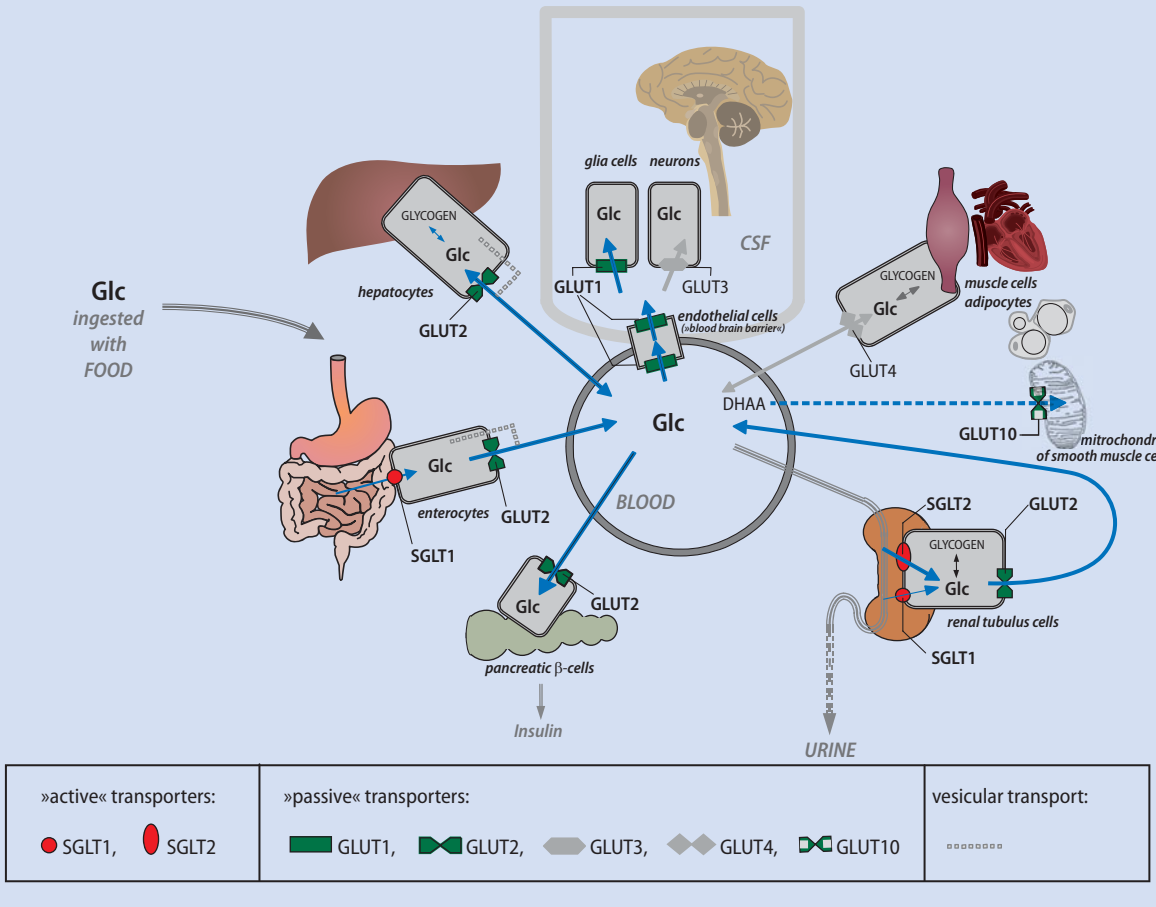

Fig. 10.1 Major routes of glucose transport. Transport across cell membranes is depicted by arrows, and specific transporters by symbols: round for sodium-dependent, 'active' transporters (SGLTs, encoded by members of the *SLC5* gene family), and angular for facilitative, ›passive‹ transporters (GLUTs, encoded by members of the *SLC2* gene family). Known defects are shown by coloured (red and green, respectively) instead of grey transporter symbols. For details see text. Glc, glucose; CSF, cerebrospinal fluid; DHAA, dehydro-ascorbic acid

To date, five congenital defects of monosaccharide transporters (► Glucose Transporters) are known (■ Fig. 10.1). Their clinical picture depends on tissue-specific expression and substrate specificity of the affected transporter (► Box). SGLT1 deficiency causes intestinal *glucose-galactose malabsorption*, a condition that presents with severe osmotic diarrhoea and dehydration soon after birth. *SGLT2* mutations result in isolated *renal glucosuria,* a harmless renal transport defect characterised by normal blood glucose concentrations and the absence of any other signs of renal tubular dysfunction. In GLUT1 deficiency, also termed *glucose transporter-1 deficiency*, clinical symptoms such as microcephaly, epileptic encephalopathies, paroxysmal movement disorders or different types of tremor, are caused by impaired glucose transport at the blood-brain barrier, but haemolytic anaemia has also been described in this condition. *Fanconi-Bickel syndrome* is the result of a deficiency of GLUT2, an important glucose and galactose carrier of hepatic, renal and pancreatic β-cells. Patients typically present with a combination of increased hepatic glycogen storage and generalised renal tubular dysfunction which includes severe glucosuria. Finally, *GLUT10 deficiency* is an entity characterised by hyperelastic connective tissue and generalised tortuosity and elongation of all major arteries including the aorta which is not related to impaired transport of glucose but of the structurally related dehydroascorbic acid at the mitochondrial membrane of smooth muscle cells and insulin-stimulated adipocytes.

10.1 Congenital Glucose/Galactose Malabsorption (SGLT1 Deficiency)

10.1.1 Clinical Presentation

Typically, children with congenital glucose-galactose malabsorption (GGM) caused by SGLT1 deficiency present with bloating and profuse watery diarrhoea within days after a normal birth and a normal pregnancy (with no polyhydramnios). Stools are very loose and may be mistaken for urine. Both breast- and bottle-fed infants are affected, but symptoms may have started even before milk feeds, if newborns are given tea sweetened with glucose or polymers of glucose. As a result of the diarrhoea, patients develop severe hypertonic dehydration, often with fever, which may be misinterpreted as a sign of a gastrointestinal infection. If the correct diagnosis is missed and glucose and galactose are not eliminated from the diet and if parenteral fluid administration is not available patients die from hypovolaemic shock. In typical cases, the diagnosis is considered after repeated frustrating attempts to switch from parenteral fluids to oral feeds [1]. The finding of an acidic stool pH and the detection of reducing substances in the stool are clues to the diagnosis, and most patients have mild intermittent glucosuria [2]. Chronic dehydration might be responsible for the nephrolithiasis and nephrocalcinosis that develop in a number of cases [3].

10.1.2 Metabolic Derangement

Congenital deficiency of SGLT1 is the basic defect in this disorder [4] which demonstrates the pivotal role of this protein in intestinal transepithelial transport of glucose and galactose. SGLT1 is a high-affinity, low-capacity sodium-dependent transporter of the two monosaccharides, at the brush border of enterocytes. Whether it plays an additional role in the regulation of the postprandial expression of the facilitative transporter GLUT2 in the apical membrane is controversial [5][6]. At the basolateral membrane, glucose transport is mediated by facilitative diffusion and/or by a membrane vesicle-associated transport process [7]. Fructose is not a substrate for SGLT1 and is considered to be mainly absorbed by GLUT5 (although genetic defects of this transporter have never been detected in individuals with intestinal fructose malabsorption; ► Chapter 8).

Both truncating and missense mutations of *SGLT1* (*SLC5A1*) have been shown to result in the absence of a functioning transporter protein within the apical plasma membrane [7]. The fact that patients with glucose-galactose malabsorption show mild glucosuria points to an additional physiological role of this transporter in renal glucose reabsorption.

10.1.3 Genetics

In most populations, GGM is a relatively rare autosomal recessive disorder. Its exact prevalence is unknown, but the fact that approximately 65% of reported patients are homozygous (in contrast to compound heterozygosity in the remainder) confirms its rarity [1]. A high mutation carrier rate (1:20) was recently reported for the Amish population in the United States [8]. *SGLT1* codes for a protein of 664 amino acids that form 14 transmembranous loops. To date, approximately 70 different mutations have been found [4][8][9], scattered all over the gene; the existence of a mutational hot spot is controversial.

10.1.4 Diagnostic Tests

Owing to its life-threatening character, GGM must be suspected clinically and treatment started before the diagnosis can be confirmed. The clinical stabilisation of patients on parenteral nutrition with no foods given orally or those on a fructose-based formula, are in favour of the diagnosis. Oral monosaccharide tolerance tests (measuring stool pH, reducing substances and blood glucose) combined with a hydrogen breath test can be performed, but some of the test parameters may be unreliable owing to antibiotics, which are frequently given to sick neonates. In these tests, glucose and galactose, but not fructose, may induce severe clinical symptoms in affected infants. Glucose and galactose uptake studies on intestinal biopsies are possible, but they are invasive and time consuming. Although costly, molecular genetic studies on

genomic DNA are recommended, particularly if prenatal diagnosis is likely to be requested for in a future pregnancy.

10.1.5 Treatment and Prognosis

Whenever GGM is considered, glucose and galactose should be omitted from the diet. A formula containing fructose as the only carbohydrate is well tolerated by infants with GGM. Such a formula is easily prepared by addition of this monosaccharide to commercially available carbohydrate-free dietary products. The preparation of the diet becomes more complicated when additional foods are introduced, but it has repeatedly been reported that glucose tolerance improves with age by an, as yet, unknown mechanism [10]. To date, there are no long-term studies on the outcome of patients with GGM and it is not clear how strict the adherence to the glucose- and galactose-restricted diet must be for the patients not to have an increased risk of nephrolithiasis. Similarly, there is no information on long-term sequelae of a high-fructose diet on liver function (▶ Chapter 8).

10.2 Renal Glucosuria (SGLT2 Deficiency)

10.2.1 Clinical Presentation

Most individuals with renal glucosuria, a congenital defect of SGLT2, are detected during a routine urine examination. Only a small number present with polyuria and/or enuresis. Thus, renal glucosuria is an important differential diagnosis when diabetes mellitus is considered, but is easily excluded by the presence of normal blood glucose concentrations. Renal glucosuria is an isolated defect of tubular glucose reabsorption at the proximal tubules and, in general, does not affect other glomerular or tubular kidney functions [11].

Mild renal glucosuria [0.4–5(–10) g/1.73m^2/day] is relatively common. Individuals with a higher glucose excretion or a virtual absence of tubular glucose reabsorption (termed renal glucosuria type 0) are extremely rare.

10.2.2 Metabolic Derangement

In most cases renal glucosuria is a non-disease. Only individuals with massive glucose excretion may have a propensity to hypovolaemia and hypoglycaemia (with an activation of counter regulatory hormones [12]); they can present with a delay of somatic maturation [13]. In patients with massive glucosuria mild secondary hyperaminoaciduria has been described [14].

10.2.3 Genetics

Most individuals with renal glucosuria have been found to carry mutations within *SGLT2* (*SLC5A2*) [15]. Its product is a low-affinity carrier that transports glucose but not galactose. Homozygosity or compound heterozygosity for *SGLT2* mutations results in the severe types of renal glucosuria whereas heterozygosity is associated with mild glucose excretion, albeit not in all of the carriers [15]. Therefore, inheritance of renal glucosuria is best characterised by a codominant trait with variable expressivity. To date, approximately 80 *SGLT2* mutations have been described which are scattered all over the gene [15][16]. Most recently, it has been shown that MAP17 is necessary for proper SGLT2 function and its deficiency is another, very rare cause of renal glucosuria [56].

10.2.4 Diagnostic Tests

Diagnosis is straight forward in patients with glucosuria and normoglycaemia who do not show any other evidence of renal tubular dysfunction.

10.2.5 Treatment and Prognosis

For most cases dietary treatment is not indicated, and the prognosis, even in individuals with type 0 glucosuria, is excellent [13].

10.3 Glucose Transporter-1 Deficiency (GLUT1 Deficiency)

10.3.1 Clinical Presentation

GLUT1 deficiency (GLUT1D) in its classic form presents as an early-onset epileptic encephalopathy with three cardinal features: severe epilepsy, a complex movement disorder and developmental delay.

Following an uneventful fetal and neonatal period (with immature tight junctions of endothelial cells at the blood-brain barrier allowing paracellular glucose transport), first seizures occur within the first year of life. In this age group, seizures often present as cyanotic spells or peculiar eye movements. In later childhood, they can be of various types and frequency, often refractory to anticonvulsants. GLUT1D has been reported to account for up to 10% of cases with early-onset absence epilepsy [17] and for approximately 5% of patients with myoclonic astatic epilepsy [18]. In some cases of GLUT1D, seizures can be aggravated by fasting.

With increasing age, a complex motor disorder becomes apparent, including signs of dystonia, ataxia, chorea and spasticity. Patients may develop an ataxic-spastic gait, action limb dystonia, mild chorea and cerebellar action or dystonic tremor [19]. In addition, patients often develop non-epileptic paroxysmal events with episodes of ataxia, weakness, Parkinsonism, alternating hemiplegia, and non-kinesogenic dyskinesias. These episodes may be triggered by poor dietary intake [20].

Global developmental delay becomes apparent in almost all patients with GLUT1D. Impaired language development is

often the most prominent feature. Severe cases may develop secondary microcephaly [19][20].

Several GLUT1D variants have been recognised in recent years. Such patients present with only one or two of the cardinal features as described above, for instance isolated early-onset absence epilepsy or an isolated movement disorder without epilepsy. Additional features may be myoclonus and dyspraxia. Manifestations with only minimal symptoms in adults have also been described [20][21][22]. Paroxysmal exertion-induced dystonia (PED) has been found to be allelic to classic GLUT1D. PED is characterised by onset beyond childhood, a normal head circumference, a normal interictal neurologic examination, and a less prominent decrease of CSF glucose concentration when compared with classic GLUT1D [23][24].

10.3.2 Metabolic Derangement

GLUT1 is a membrane-spanning, glycosylated protein that facilitates glucose transport across the blood-brain barrier (Fig. 10.1). A GLUT1 defect results in a low CSF glucose concentration (hypoglycorrhachia). As glucose is the principal fuel for brain energy metabolism, the GLUT1 defect impairs glucose supply to both neurons and glial cells, leading to clinical symptoms, deceleration of brain growth, and reactive astrocytosis [25]. GLUT1 is also highly expressed in erythrocytes where 5% of the membrane proteins are GLUT1. This explains why an exercise induced energy deficit can be accompanied by haemolytic anaemia which may result from alterations in intracellular electrolytes caused by a cation leak through mutant GLUT1 [24].

10.3.3 Genetics

Approximately 80% of patients are heterozygous for mutations within *GLUT1* (*SLC2A1).* Both autosomal dominant and autosomal recessive inheritance have been described [26][27][28][29]. Mutations are mostly *de novo,* of various character (missense, nonsense, and splice-site mutations, haploinsufficiency, compound heterozygosity, and paternal mosaicism), and randomly distributed [20][29]. Emerging mutational hot spots have implications for GLUT1 function and recently the GLUT1 three dimensional structure was unravelled including the disease mechanisms of certain *SLC2A1* mutations [30]. The type of mutation and the extent of hypoglycorrhachia are related to the phenotype; missense mutations and higher CSF glucose concentrations appear to be associated with a milder clinical presentation [19][20]. In approx. 10-15% of cases of GLUT1D no *SLC2A1* mutation can be detected even with additional use of MLPA analysis to detect copy number variations [31].

10.3.4 Diagnostic Tests

GLUT1D illustrates the importance of CSF evaluation in children with undiagnosed epilepsy and/or movement disorders. GLUT1D should be suspected in any child with a CSF glucose concentration below 2.5 mmol/l (normal >3.3 mmol/l). Values may vary considerably in affected patients (range 0.9–2.9 mmol/l) and appear to be higher in milder phenotypes and paroxysmal movement disorders [19][20]. A CSF to blood glucose ratio, which normally is >0.6, should be obtained in a non-ictal, metabolic steady state. A ratio of <0.5 (range 0.19–0.52) in the absence of hypoglycaemia or a CNS infection is diagnostic. Typically, CSF cell count, protein and lactate concentrations are normal [19][20][32].

Other routine laboratory analyses are unremarkable and interictal EEGs are often normal. If abnormal, an improvement in the EEG with glucose intake may be of diagnostic value. Ictal EEGs may show focal slowing or epileptiform discharges in infants and a generalised 2.5- to 4-Hz spike-wave pattern in older children. No structural brain abnormalities are detected by neuroimaging, but PET studies may demonstrate a diminished cortical glucose uptake with more severe hypometabolism in the mesial temporal regions and thalami, accentuating a relative signal increase in the basal ganglia [33]. In addition to molecular genetic investigations, GLUT1D may be confirmed by western blot analysis and studies on glucose uptake into erythrocytes, cells abundantly expressing GLUT1. Glucose uptake and GLUT1 expression measured in erythrocytes of GLUT1D patients are generally reduced to about half of control values [34] and do not correlate with disease severity.

10.3.5 Treatment and Prognosis

During fasting, ketone bodies provide an alternative fuel to the brain and this metabolic state can be mimicked by a high-fat, low-carbohydrate ('ketogenic') diet (▶ Chapter 13). This type of diet may restore brain energy metabolism in patients with GLUT1D since ketone body transport at the blood brain barrier is not dependent on GLUT1. Classic ketogenic diets (3:1 and 4:1 ratios of calories from fat and non-fat sources, respectively) and the modified Atkins diet may effectively control seizures and improve movement disorders and development [35] in GLUT1-deficient patients. Multivitamin and calcium supplements are necessary on such a diet [36]. In contrast to intractable childhood epilepsy, the ketogenic diet in GLUT1D should be maintained throughout childhood and into adolescence, when cerebral glucose requirements decrease.

Substances known to inhibit GLUT1, such as anticonvulsive drugs (phenobarbital, chloralhydrate, diazepam), methylxanthines (theophyllin, caffeine), alcohol and green tea should be avoided. If an antiepileptic medication is required, one has to be aware that no data on the specific effectiveness of antiepileptic drugs in this condition is available. Valproate interferes with GLUT1 function *in vitro*, but may be used in GLUT1D [37][38]. Ethosuximide should be considered in

early-onset absence epilepsy. The use of dietary antioxidants, such as α-lipoic acid (thioctic acid), has been discussed but currently it cannot be considered an accepted therapy. Artificial ketones, such as triheptanoin and ketoesters, provide an alternative energy source as well as anaplerotic substances for the citric cycle. Thus, they may provide a novel therapeutic option. A trial of treatment in patients presenting with recurrent paroxysmal attacks is underway [F Mochel and E Roze, personal communication].

GLUT1D patients on treatment have a favourable prognosis: patients continue to make progress, they generally acquire speech and mobility, and the disease generally stabilizes after puberty. However, seizures and a variable degree of impairment may persist in some individuals despite adequate treatment [30], impairment of expressive language and paroxysmal events appear prominent and difficult to treat in adults, and a sheltered environment is often required.

10.4 Fanconi-Bickel Syndrome (GLUT2 Deficiency)

10.4.1 Clinical Presentation

Patients with Fanconi-Bickel syndrome (FBS), which is caused by GLUT2 deficiency, typically present at 3–10 months of age with a combination of hepatomegaly, a Fanconi-type nephropathy with severe glucosuria, a propensity to hypoglycaemia in the fasted state and glucose and galactose intolerance in the fed state [39][40]. A few patients have come to attention because of neonatal diabetes mellitus [41], others owing to the finding of hypergalactosaemia on newborn screening [42], and occasionally because of the identification of early cataracts [43]. Initially, hepatomegaly, which is caused by massive accumulation of glycogen, may not be present, and non-specific symptoms such as fever, vomiting, chronic diarrhoea and failure to thrive may predominate. With increasing age, the clinical presentation with a protuberant abdomen, moon-shaped face, and short stature becomes increasingly similar to that of patients with hepatic glycogen storage diseases (▶ Chapter 5). The kidneys also accumulate glycogen and their enlargement can be detected by ultrasound. Hypophosphataemic rickets is the major manifestation of tubular dysfunction, resulting in joint swelling, bowing of legs and pathological fractures. Patients with FBS have entirely normal mental development but growth and puberty are severely retarded [39][40].

Recently, GLUT2 deficiency has been reported in patients with very mild clinical signs and symptoms, including cases presenting with isolated glucosuria [44].

10.4.2 Metabolic Derangement

Fanconi-Bickel syndrome is caused by congenital deficiency or variably impaired function of GLUT2, a high-K_m monosaccharide carrier that can transport both glucose and galactose [45]. This facilitative glucose carrier is expressed in hepatocytes and at the basolateral membrane of reabsorbing cells of the proximal tubule. Furthermore, GLUT2 is found on both sides of the intestinal epithelium although the role of a diffusive component at the apical membrane has recently been questioned [5][6]. GLUT2 has been detected within the cell membrane of pancreatic β-cells, however, in humans the major facilitative transporter in β-cells has been shown to be GLUT1 and the rate-limiting step in sensing glucose concentration is glucokinase (▶ Chapter 9).

Intestinal uptake of glucose and galactose appear unimpaired in FBS; this has been explained by additional transport systems for glucose, SGLT1 in the apical membrane and a membrane vesicle-associated pathway at the basolateral membrane [7]. Postprandial hyperglycaemia and hypergalactosaemia are caused by impaired hepatic uptake of the two sugars. To what extent hyperglycaemia is further exaggerated by a diminished insulin response caused by an impairment of glucose sensing of β-cells has remained controversial [46][47][48]. In FBS hepatocytes, GLUT2 has the effect of a malfunctioning glucose sensor: in the fasted state, when extracellular glucose concentration declines, the concentrations of glucose and glucose-6-phosphate within hepatocytes are inappropriately high in FBS patients. This stimulates glycogen synthesis, inhibits gluconeogenesis and glycogenolysis, and ultimately predisposes to hypoglycaemia and hepatic glycogen accumulation [39].

Impaired transport of glucose out of renal tubular cells results in the accumulation of glycogen and free glucose within these cells. This impairs other transport functions, resulting in a generalised tubulopathy with disproportionately severe glucosuria. The extreme amounts of glucose lost with the urine (even at times when blood glucose is low) may contribute to the propensity to develop hypoglycaemia.

10.4.3 Genetics

FBS is a very rare autosomal recessive condition caused by mutations of *GLUT2* (*SLC2A2*). More than 70% of cases come from consanguineous families [49]. *GLUT2* codes for a 524 amino acid protein with 55% amino acid identity to GLUT1. In contrast to SGLTs, all GLUT proteins form 12 transmembranous loops within the cell membrane. The genomic structure of *GLUT2* encompasses 11 exons and to date, more than 100 different mutations scattered throughout the gene have been detected [39][49].

10.4.4 Diagnostic Tests

Diagnosis of FBS is suggested by the characteristic combination of an altered glucose homeostasis, hepatic glycogen accumulation, and the typical features of a Fanconi-type tubulopathy. Elevated biotinidase activity in serum has been found to be a useful screening test for hepatic glycogen storage disorders including FBS [50]. Fasting hypoglycaemia and impaired

glucose and galactose tolerance may be documented during oral loading tests. Laboratory signs include mildly elevated transaminases without signs of an impaired hepatic protein synthesis or a diminished secretory function. Plasma lipids, uric acid and lactate are elevated. If a liver biopsy is performed, both histologic and biochemical methods show an increased glycogen content; enzymatic studies of all glycogenolytic enzymes, however, give normal results. Hyperaminoaciduria, hyperphosphaturia, hypercalciuria, renal tubular acidosis, mild tubular proteinuria and polyuria are indicative for a generalised proximal tubular dysfunction. A hallmark of the diagnosis of FBS is the relatively severe glucosuria. Calculated tubular glucose reabsorption is dramatically reduced or even zero in most patients [40].

The diagnosis of FBS is ultimately confirmed by the detection of homozygosity or compound heterozygosity for *GLUT2* mutations [49].

10.4.5 Treatment and Prognosis

Only symptomatic treatment is available. Measures are directed towards an improvement of glucose homeostasis and an amelioration of the consequences of renal tubulopathy. FBS patients should receive a diet with adequate caloric intake compensating for the renal glucose losses. Frequent feeds using slowly absorbed carbohydrates are recommended. Continuous carbohydrate supply by tube feeding of oligosaccharide solutions during the night may be indicated. The administration of uncooked corn starch has been demonstrated to have a beneficial effect on metabolic control, particularly on growth [51].

Regarding tubulopathy, water and electrolytes must be replaced in appropriate amounts. Administration of alkali may be necessary to compensate for renal tubular acidosis. Hypophosphataemic rickets requires supplementation with phosphate and vitamin D preparations. With these measures, prognosis is fairly good and some of the originally described paediatric patients have reached adulthood. The main subjective problem for these adult patients are short stature and orthopaedic problems from hypophosphataemic rickets and osteomalacia. Hepatic adenomas or tumours, as described for other glycogen storage diseases, have never been observed in FBS. Metabolic decompensation with severe acidosis and renal insufficiency similar to that seen in diabetic glomerulosclerosis have been exceptional complications causing death in childhood [40].

10.5 Arterial Tortuosity Syndrome (GLUT10 Deficiency)

10.5.1 Clinical Presentation

Arterial tortuosity syndrome, caused by congenital GLUT10 deficiency, is characterised by generalised tortuosity and elongation of all major arteries, including the aorta. Intracranial involvement of blood vessels may result in acute infarction owing to ischaemic stroke or an increased risk of thromboses. Aortic regurgitation and multiple pulmonary artery stenoses are typical intrathoracic manifestations. Additional clinical signs include telangiectasias of the cheeks, high palate, excessively stretchable skin and diaphragmatic abnormalities. These vascular problems are the consequence of the disruption of elastic fibres in the medial layer of the arterial wall.

10.5.2 Metabolic Derangement

The role of a glucose transporter is not yet ultimately clear in this condition, which more closely resembles a connective tissue disorder in presentation. It could be demonstrated that not glucose but a structurally related substance is transported by GLUT10. This transporter facilitates dehydroascorbic acid import into mitochondria of smooth muscle cells and insulin-stimulated adipocytes and protects cells against oxidative stress [52], and connects mitochondrial function to TGF-beta signalling [53]. Deficiency of GLUT10 has been found to be associated with up-regulation of the TGF-β pathway in the arterial wall, a finding which might explain clinical similarities to individuals with TGF-β receptor mutations [54].

10.5.3 Genetics

Arterial tortuosity syndrome is a rare, recessively inherited condition. *GLUT10* (*SLC2A10*), is a member of the so-called type III *GLUTs*, which are older and more distantly related to the previously mentioned facilitative glucose transporter genes. To date, approximately 20 different mutations have been described [54][55].

10.5.4 Diagnostic Tests

Echocardiography, angiography, and/or CT scan are important to demonstrate vascular changes. The ultimate diagnosis is based on the detection of the basic defect by molecular genetic methods.

10.5.5 Treatment and Prognosis

No curative treatment is available. Surgical measures for correction of single blood vessels, *e.g.,* pulmonary stenosis, have been reported.

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Section III
Disorders of Mitochondrial Energy Metabolism

Disorders of Pyruvate Metabolism and the Tricarboxylic Acid Cycle

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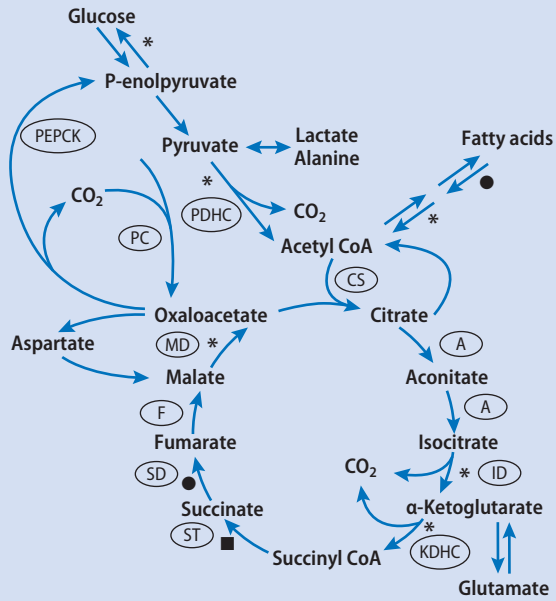
Pyruvate Metabolism and the Tricarboxylic Acid Cycle

Pyruvate is formed from glucose and other monosaccharides, from lactate, and from the gluconeogenic amino acid alanine (◘ Fig. 11.1). After entering the mitochondrion, pyruvate can be converted into acetyl-coenzyme A (acetyl-CoA) by the pyruvate dehydrogenase complex, followed by further oxidation in the TCA cycle. Pyruvate can also enter the gluconeogenic pathway by sequential conversion into oxaloacetate by pyruvate carboxylase, followed by conversion to phosphoenolpyruvate by phosphoenolpyruvate carboxykinase. Acetyl-CoA can also be formed by fatty acid oxidation or used for lipogenesis. Other amino acids enter the TCA cycle at several points. One of the primary functions of the TCA cycle is to generate reducing equivalents in the form of reduced nicotinamide adenine dinucleotide (NAD) and reduced flavin adenine dinucleotide (FAD), which are utilised to produce energy under the form of ATP in the electron transport chain.



◘ **Fig. 11.1 Overview of glucose, pyruvate/lactate, fatty acid and amino acid oxidation by the tricarboxylic acid cycle**. *A*, aconitase; *CS*, citrate synthase; *F*, fumarase; *ID*, isocitrate dehydrogenase; *KDHC*, α-or 2-ketoglutarate dehydrogenase complex; *MD*, malate dehydrogenase; *PC*, pyruvate carboxylase; *PDHC*, pyruvate dehydrogenase complex; *PEPCK*, phosphoenolpyruvate carboxykinase; *SD*, succinate dehydrogenase; *ST*, succinyl coenzyme A transferase. Sites where reducing equivalents and intermediates for energy production intervene are indicated by the following symbols: *, reduced nicotinamide adenine dinucleotide; ●, reduced flavin adenine dinucleotide; ■, guanosine triphosphate

Owing to the role of pyruvate and the TCA cycle in energy metabolism (► Pyruvate Metabolism and the Tricarboxylic Acid Cycle), as well as in gluconeogenesis, lipogenesis and amino acid synthesis, defects in pyruvate metabolism and in the tricarboxylic acid (TCA) cycle almost invariably affect the central nervous system. The severity and the different clinical phenotypes vary widely among patients and are not always specific, the range of manifestations extending from overwhelming neonatal lactic acidosis and early death to relatively normal adult life and variable effects on systemic functions. The same clinical manifestations may be caused by other defects of energy metabolism, especially defects of the respiratory chain (► Chapter 14). Diagnosis depends primarily on biochemical analyses of metabolites in body fluids, followed by definitive enzymatic assays in cells or tissues, and DNA analysis. Pyruvate carboxylase (PC) deficiency constitutes a defect both in the Krebs cycle and in gluconeogenesis, but generally presents with severe neurological dysfunction and lactic acidosis rather than with fasting hypoglycaemia. Deficiency of Phosphoenolcarboxykinase (PEPCK) is now considered to be a secondary phenomenon. Deficiency of Pyruvate dehydrogenase complex (PDHC) impedes glucose oxidation and aerobic energy production, and ingestion of carbohydrate aggravates lactic acidosis. Treatment of disorders of pyruvate metabolism comprises avoidance of fasting (PC deficiency) or minimising dietary carbohydrate intake (PDHC deficiency) and enhancing anaplerosis (restoration of pools of intermediate metabolites). Dihydrolipoamide dehydrogenase (E3) deficiency affects PDHC and also the 2-ketoglutarate dehydrogenase complex (KDHC) and the branched-chain 2-ketoacid dehydrogenase (BCKD) complex (► Chapter 18), with biochemi-

cal manifestations of all three disorders. The deficiencies of the TCA cycle enzymes, KDHC and fumarase, interrupt the cycle, resulting in accumulation of the corresponding substrates. Succinate dehydrogenase deficiency represents a unique disorder affecting both the TCA cycle and the respiratory chain. Defects of mitochondrial transport of pyruvate and ketoglutarate have also been identified. Treatment strategies for the TCA cycle defects are limited.

11.1 Pyruvate Carboxylase Deficiency

11.1.1 Clinical Presentation

PC deficiency is an autosomal recessive disorder with an incidence of around 1 in 250,000. Several dozen patients have been described in detail, allowing the definition of three overlapping phenotypes that probably constitute a continuum from the most severe (type B) to the less severe form (type C). For a recent review see [1].

1. Patients with the French phenotype (type B) become acutely ill 3–48 h after birth with hypothermia, hypotonia, lethargy and vomiting [1][2][3]. These children manifest a severe neurological dysfunction with initially a preserved level of consciousness but then rapid deterioration with rigidity, hypokinesia and tremor (resembling infantile parkinsonism) and abnormal ocular movements [2]. Hepatomegaly, seizures and failure to thrive may occur. Most die in the neonatal period. Some survive, but they remain unresponsive and severely hypotonic and finally succumb from respiratory infection before the age of 5 months.
2. Patients with the North American phenotype (type A) become severely ill between 2 and 5 months of age. They develop progressive hypotonia and are unable to smile. Numerous episodes of acute vomiting, dehydration, tachypnoea, facial pallor, cold cyanotic extremities and metabolic acidosis, characteristically precipitated by metabolic or infectious stress are a consistant finding. Clinical examination reveals pyramidal tract signs, ataxia and nystagmus. All patients are severely mentally retarded and most of them have seizures. Hepatomegaly and renal dysfunction (tubular acidosis) may also be present. Neuroradiological findings (also found in type B) include subdural effusions, severe antenatal ischaemia-like brain lesions and periventricular haemorrhagic cysts, followed by progressive cerebral atrophy and delay in myelination. Leigh syndrome has been also reported but its frequency remains uncertain. The course of the disease is generally progressive, with death in infancy.
3. The Type C phenotype is more benign and has only been reported in a few patients without clear ethnic predilection [1]. The clinical course is dominated by the occurrence of acute episodes of lactic acidosis and ketoacidosis, usually responding rapidly to hydration and bicarbonate therapy. Despite the important enzymatic deficiency, the patients have a near-normal cognitive and neuromotor development. However, dystonia, episodic ataxia, dysarthria, transitory hemiparesis, seizures and subcortical leukodystrophy have been described in some cases.

11.1.2 Metabolic Derangement

PC is a biotinylated mitochondrial matrix enzyme that converts pyruvate and CO_2 to oxaloacetate. It plays an important role in gluconeogenesis, anaplerosis and lipogenesis. For gluconeogenesis, pyruvate must first be carboxylated into oxaloacetate, because the last step of glycolysis, conversion of phosphoenolpyruvate to pyruvate is irreversible and pyruvate cannot be used for gluconeogenesis. Oxaloacetate, which cannot diffuse freely out of the mitochondrion, is translocated into the cytoplasm via the malate/aspartate shuttle. Once in the cytoplasm, oxaloacetate is converted into phosphoenolpyruvate by PEPCK, which catalyses the first committed step of gluconeogenesis.

The anaplerotic role of PC, i.e. the generation of Krebs cycle intermediates from oxaloacetate, is even more important. In severe PC deficiency, the lack of Krebs cycle intermediates lowers reducing equivalents in the mitochondrial matrix. This drives the redox equilibrium between 3-OH-butyrate and acetoacetate in the direction of acetoacetate thereby lowering the 3-OH-butyrate/acetoacetate ratio. Aspartate, formed in the mitochondrial matrix from oxaloacetate by transamination, also decreases. As a consequence, the translocation of reducing equivalents between cytoplasm and mitochondrial matrix by the malate/aspartate shuttle is impaired. This drives the cytoplasmic redox equilibrium between lactate and pyruvate in the direction of lactate, and the lactate/pyruvate (L/P) ratio increases. Reduced Krebs cycle activity also plays a role in the increase of lactate and pyruvate. Since aspartate is required for the urea cycle, plasma ammonia and citrulline can increase. The low 2-ketoglutarate production explains the low plasma level of glutamate. The energy deprivation induced by PC deficiency has been postulated to impair astrocytic buffering capacity against excitotoxic insults and to compromise microvascular morphogenesis and autoregulation, leading to degeneration of white matter [1].

The importance of PC for lipogenesis derives from the condensation of oxaloacetate with intramitochondrially produced acetyl-CoA into citrate. Deficient lipogenesis explains the widespread demyelination of the cerebral and cerebellar white matter and symmetrical periventricular cavities around the frontal and temporal horns of the lateral ventricles, reported in the few detailed neuropathological descriptions of PC deficiency. PC is present in oligodendrocytes (glial cells essential for the formation of myelin sheets), where it plays an anaplerotic role [4]. PC deficiency in the oligodendrocytes should result in insufficient fatty acid synthesis and myelin malformation, whereas the impairment of oxidative metabolism in microglial cells is associated with an inflammatory response possibly contributing to neurodegeneration [5].

In a patient with the type B phenotype, muscle biopsy disclosed nemaline rods that probably will occur due to defective energy metabolism. Mitochondrial accumulation in type 1 fibers raises the possibility that the thin filaments may become the target structures of mitochondrial dysfunction [6].

PC requires biotin as a cofactor. Metabolic derangements of PC deficiency are thus also observed in biotin-responsive multiple carboxylase and in carbonic anhydrase VA deficiency (▶ Chapter 19 and ▶ Chapter 26).

11.1.3 Genetics

PC deficiency is an autosomal recessive disorder. PC is a tetramer formed by 4 identical subunits (α_4). More than half of the patients with French phenotype have absence of PC protein and of the corresponding mRNA. The patients with North American phenotype generally have cross-reacting material (CRM-positive) [1], as did the patient with the benign variant of PC deficiency [2].

Mutations have been detected in patients of both types A and B. In Canadian Indian populations with type A disease, 11 Ojibwa and 2 Cree patients were homozygous for a 1828G → A missense mutation (Ala610Thr) in exon 13 of the PC gene. Several reports on mutations are now published [7][8][9]. Patients with at least one missense mutation have been described to have the milder type A form of PC deficiency. However, recently, two patients with the severe type B form, have been described to have novel homozygous missense mutations [3].

Mosaicism was found in five cases (one type A, three type B and one type C), and in four of these cases survival was prolonged.

11.1.4 Diagnostic Tests

The most severely affected patients typical exhibit an elevated L/P ratio, low hydroxybutyrate/acetoacetate (H/A) ratio with paradoxal postprandial ketosis, hypercitrullinemia and hyperammonemia, with low glutamine, parameters that often remain unaltered in types A and C.

Hence, the possibility of PC deficiency should be considered in any child presenting with lactic acidosis and neurological abnormalities, especially if associated with hypoglycemia, hyperammonemia or ketosis. In neonates, a high L/P ratio associated with a low H/A ratio is nearly pathognomonic [2]. Discovery of cystic periventricular leukomalacia at birth associated with lactic acidosis is also highly suggestive. Typically, blood lactate increases in the fasting state and decreases after ingestion of carbohydrate.

In patients with type B, blood lactate concentrations reach 10–20 mM (normal <2.2 mM) with L/P ratios between 50 and 100 (normal lower <28). In patients with type A, blood lactate is 2–10 mM with normal or only moderately increased L/P ratio (less than 50). In patients with type C, lactate can be normal and only increase (usually above 10 mM) during acute episodes. Overnight blood glucose concentrations are usually normal. Hypoglycaemia can occur during acute episodes of metabolic acidosis. The blood H/A ratio is decreased (less than 2, normal 2.5–3).

Hyperammonemia (100–600 μmol/L, normal <60), and an increase of blood citrulline (100–400 μmol/L, normal <40), lysine and proline, contrasting with low glutamine, are constant findings in patients with type B [2]. Plasma alanine is usually normal in type B, but increased (500–1400 μmol/L, normal <455) in all reported patients with type A. During acute episodes, aspartate can be undetectably low [10]. An elevation of total cholesterol or its precursors (mevalonic acid) may occur in type A and B forms [1].

In cerebrospinal fluid (CSF), lactate, the L/P ratio and alanine are increased and glutamine is decreased. High levels of homovanillic acid (a metabolite of dopamine) in the CSF and low staining of GABAergic markers in the substantia nigra have been found in a type B patient [11].

Measurement of PC activity is preferentially performed on cultured skin fibroblasts [2] although it can also be done in lymphocytes and other tissues except muscle. Assays can also be performed in post-mortem liver, in which the activity of PC is 10-fold that in fibroblasts, but must be interpreted with caution because of rapid post-mortem degradation of the enzyme. Although in general the lowest activities correspond to the most severe phenotypes, residual PC enzymatic activity is of limited value for the distinction among the 3 phenotypes because enzymatic analysis often yields activities below 5% of normal regardless of PC deficiency type [1].

Prenatal diagnosis of PC deficiency is possible by measurement of PC activity in cultured amniotic fluid cells, direct measurement in chorionic villi biopsy specimens or DNA analysis when the familial mutations are known.

11.1.5 Treatment and Prognosis

The outcome of treatment for the severe type A and B forms is very disappointing. Patients should be promptly treated with intravenous 10% glucose and may require bicarbonate to correct acidosis. In one patient with the French phenotype who was treated with high doses of citrate and aspartate [10] lactate and ketones diminished and plasma amino acids normalized, except for arginine. However, in the CSF, glutamine remained low and lysine elevated. An orthotopic hepatic transplantation completely reversed ketoacidosis and the renal tubular abnormalities and decreased lactic acidaemia in a patient with a severe phenotype, although concentrations of glutamine in CSF remained low [12]. A patient with type B was started on early treatment with triheptanoin, a triglyceride containing three 7-carbon fatty acids (4 g of triheptanoin/kg body weight, providing 35% of total caloric intake) in order to restore anaplerosis by providing the intramitochondrial source of both oxaloacetate and acetyl-CoA [13]. Lactate, the L/P ratio, ammonia, and citrulline decreased rapidly with a progressive increase in glutamine. Although there was a clinical improvement without evidence of neurodegeneration, the patient died during an episode of acute decompensation at

Structure and Activation/Deactivation System of the Pyruvate Dehydrogenase Complex

PDHC, and the two other mitochondrial α- or 2-ketoacid dehydrogenases, KDHC and the BCKD complex, are similar in structure and analogous or identical in their specific mechanisms. They are composed of three components: E1, α- or 2-ketoacid dehydrogenase; E2, dihydrolipoamide acyltransferase; and E3, dihydrolipoamide dehydrogenase. E1 is specific for each complex, utilizes thiamine pyrophosphate, and is composed of two different subunits, E1α and E1β. The E1 reaction results in decarboxylation of the specific α- or 2-ketoacid. For the PDHC, the E1 component is the rate-limiting step and is regulated by phosphorylation/dephosphorylation catalysed by two enzymes, E1 kinase (inactivation) and E1 phosphatase (activation). E2 is a transacetylase that utilises covalently bound lipoic acid. E3 is a flavoprotein common to all three 2-ketoacid dehydrogenases. Another important structural component of the PDHC is E3-binding protein (E3BP), formerly protein X. This component has its role in attaching E3 subunits to the core of E2 (Fig. 11.2, Fig. 11.3).

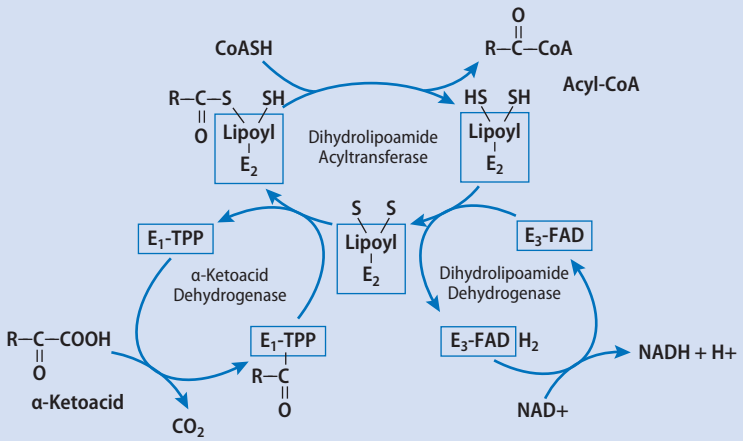


Fig. 11.2 Structure of the α- or 2-ketoacid dehydrogenase complexes, pyruvate dehydrogenase complex (PDHC), 2-ketoglutarate dehydrogenase complex (KDHC) and the branched-chain α-ketoacid dehydrogenase complex (BCKD). *CoA*, coenzyme A; *FAD*, flavin adenine dinucleotide; *NAD*, nicotinamide adenine dinucleotide; *R*, methyl group (for pyruvate, PDHC) and the corresponding moiety for KDHC and BCKD; TPP, thiamine pyrophosphate

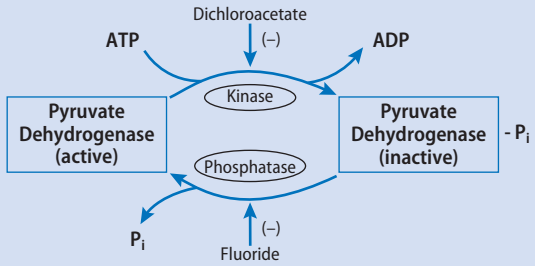


Fig. 11.3 Activation/deactivation of PDHE1 by dephosphorylation/phosphorylation. Dichloroacetate is an inhibitor of E1 kinase, and fluoride inhibits E1 phosphatase. *ADP*, adenosine diphosphate; *P*, phosphate

8 months of age. Two further patients with type B have been reported to be unsuccessfully treated with triheptanoin, continuously from 11 and 21 days of age. They also received citrate, aspartate and dichloroacetate. No clinical or biochemical improvement was observed and the patients died at 7 and 8 months of age with a severe neurological impairment [14].

Neither biotin, thiamine, dichloroacetate, nor a high-fat or high-carbohydrate diet has been shown to provide clinical benefit. PC deficiency is a contraindication for a ketogenic diet.

Symptomatic treatment of seizures must be undertaken with caution since valproic acid and barbiturates have adverse effects in patients with energy defects. Exacerbation of lactic

acidosis and increase of alanine, lysine and leucine have been reported with the use of ACTH in a type B patient with infantile spasms [1].

The prognosis depends on the severity of the defect. Patients with minimal residual PC activity usually do not live beyond the neonatal period, but some children with very low PC activity have survived to more than 5 years. Those with milder defects or somatic mosaicism [8] may survive longer with varying degrees of neurological disease.

11.2 Phosphoenolpyruvate Carboxykinase Deficiency

Only six patients have been reported in the literature, and none since 1986 [15]. It now appears that deficiency of PEPCK is more likely to be a secondary phenomenon. Separate mitochondrial and cytosolic isoforms of the enzyme exist. The cDNA encoding the cytosolic isoform of PEPCK in humans has been sequenced; no mutations have yet been identified. Synthesis of this isoform is repressed by hyperinsulinism, a condition which was also present in a patient with reported deficiency of cytosolic PEPCK [16]. Deficiency of mitochondrial PEPCK has also been disputed: a sibling of a patient developed a similar clinical picture but had normal enzyme activity [16]. Further studies showed a depletion of mitochondrial DNA in this patient [17] caused by defective DNA replication [18] (▶ Box). Recently PEPCK1 homozygous mutation has been found in a patient with hepatic liver failure [91].

11.3 Pyruvate Dehydrogenase Complex Deficiency

11.3.1 Clinical Presentation

More than 200 cases of PDHC deficiency have been reported [19][20], the majority of which involved the X-encoded α subunit of the dehydrogenase component (E1) of the complex (◘ Fig. 11.2). The most common features of PDHE1α deficiency are delayed development, hypotonia, seizures and ataxia.

In hemizygous males, three presentations are encountered: neonatal lactic acidosis, Leigh's encephalopathy and intermittent ataxia. These correlate with the severity of the biochemical deficiency and the location of the gene mutation. In the first presentation, neonatal lactic acidosis is often associated with brain dysgenesis, such as agenesis of the corpus callosum. In Leigh's encephalopathy, quantitatively the most important group, the initial presentation is usually within the first 5 years of life and includes respiratory disturbances or episodic weakness and ataxia with absence of tendon reflexes. Respiratory disturbances may lead to apnoea, dependence on assisted ventilation or sudden unexpected death. Intermittent dystonic posturing of the lower limbs occurs frequently. A moderate to severe developmental delay becomes evident within the next few years. A very small subset of male patients are initially much less severely affected, with intermittent episodic ataxia after carbohydrate-rich meals, progressing slowly over years into mild Leigh's encephalopathy. Sperl et al [20] reviewed the literature and their own patients and found that 75.5% had mutations in PDHC, mostly PDHE1α, with similar clinical presentations and 13.5% were due to a thiamine cofactor deficiency (▶ Chapter 28).

A number of patients have developed an acute peripheral neuropathy during infancy or an acute episodic ataxia [21][22], without mental retardation. Isolated optic atrophy with acute peripheral neuropathy can also be a late presenting finding [23]. Females with PDHE1α deficiency tend to have a more uniform clinical presentation, although with variable severity, depending on variable lyonisation [24]. This includes dysmorphic features, microcephaly, moderate to severe mental retardation and spastic di- or quadriplegia, resembling non progressive encephalopathy. Dysmorphism comprises a narrow head with frontal bossing, wide nasal bridge, upturned nose, long philtrum and flared nostrils and may suggest fetal alcohol syndrome. Other features are low-set ears, short fingers and short proximal limbs, simian creases, hypospadias and an anteriorly placed anus. Seizures are encountered in almost all female patients. These appear within the first 6 months of life and are diagnosed as infantile spasms (flexor and extensor) or severe myoclonic seizures. Brain MRI frequently reveals severe cortical/subcortical atrophy, dilated ventricles, partial to complete corpus callosum agenesis and in boys basal, ganglia and midbrain abnormalities [25]. Severe neonatal lactic acidosis can be present. Males [26][27][28] and females [29] who are mosaic for PDHE1α deficiency have been reported with an attenuated phenotype. An isolated paroxysmal exercise dystonia responding to thiamine was also seen in a female [30].

Neuropathology can reveal various degrees of dysgenesis of the corpus callosum usually associated with other migration defects, such as the absence of the medullary pyramids, ectopic olivary nuclei, abnormal Purkinje cells in the cerebellum, dysplasia of the dentate nuclei, subcortical heterotopias and pachygyria [31].

Only a few cases with PDHE1β deficiency have been reported [32][33][34][35]. These patients present either with early-onset lactic acidosis and severe developmental delay or with a moderate clinical course with slowly progressive neurological features reflecting basal ganglia and brain stem involvement associated with typical findings of Leigh syndrome [34]. Seven cases of E1-phosphatase deficiency [36] (◘ Fig. 11.3) have been identified, including two brothers with hypotonia, feeding difficulties and delayed psychomotor development [37] and a lethal infantile phenotype [38].

A few cases of PDHE2 (dihydrolipoamide transacetylase) deficiency have been reported with mutations in *DLAT* [39][40]. The main clinical manifestations of E3BP (formerly protein X) deficiency are hypotonia, delayed psychomotor development and prolonged survival [20][41][42][43]. Often more slowly progressive, it also comprises early-onset neonatal lactic acidosis associated with subependymal cysts and thin corpus callosum. A founder mutation was found in a group of Roma children with a cerebral palsy like clinical picture [44].

Activating mutations in the pyruvate dehydrogenase kinase isoenzyme 3 (PDK3) gene have been found to cause X-

linked dominant Charcot-Marie-Tooth disease type 6. Findings suggest a reduced pyruvate flux due to R158H mutant PDK3-mediated hyper-phosphorylation of the PDHC as the underlying pathogenic cause of peripheral neuropathy [45].

11.3.2 Metabolic Derangement

Defects of PDHC provoke conversion of pyruvate into lactate and alanine rather than to acetyl-CoA, the gateway for complete oxidation of carbohydrate via the TCA cycle (◘ Fig. 11.1). The conversion of glucose to lactate yields less than one tenth of the ATP that would be derived from complete oxidation of glucose via the TCA cycle and the respiratory chain. Deficiency of PDHC thus specifically interferes with production of energy from carbohydrate oxidation, and hyperpyruvicaemia, lactic acidaemia and hyperalaninaemia are aggravated by consumption of carbohydrate.

PDHC deficiency impairs production of NADH but, unlike respiratory chain defects, does not hamper oxidation of NADH. PDHC deficiency thus does not modify the NADH/NAD^+ ratio in the cell cytosol, which is reflected in a normal L/P ratio. In contrast, deficiencies of respiratory chain complexes I, III, and IV are generally characterised by a high L/P ratio (▶ Chapter 1 and ▶ Chapter 14).

11.3.3 Genetics

All components of PDHC are encoded by nuclear genes, and synthesised in the cytoplasm as precursor proteins that are imported into the mitochondria, where the mature proteins are assembled into the enzyme complex. Most of the genes that encode the various subunits are autosomal, except the E1α-subunit gene, which is located on chromosome Xp22.11. Therefore, most cases of PDHC deficiency are X-linked. To date, over 120 different mutations of the E1α subunit of PDHC have been characterised [35][46][47][48]. About half of these are small deletions, insertions or frame-shift mutations, and the other half are missense mutations. Premature termination codons, mostly resulting from frame-shift insertions or deletions in exons 10 and 11, are frequently noted in females, while missense mutations predominate in males [46]. No null E1α mutations have been identified in males, except in a mosaic state [25], suggesting that such mutations are likely to be lethal. Rare splicing mutations involve exonic [49] or intronic [50] regulatory sequences. A few large rearrangements have been identified, such as a large intragenic 5 kb deletion [51].

Only 17 E1β-deficient patients have been described, with mutations in *PDHB* in eight of them [20][33]. A deficiency in a patient was due to an increased proteasome-mediated degradation of the ubiquinated E1β subunit [32]. The molecular basis of E3-binding protein (E3BP) deficiency has been characterised in 47 cases. Half of these patients have splicing errors, while others have frameshift or nonsense mutations [52]. Two large rearrangements have been identified, one of them due to retrotranspositional insertion of a full-length LINE-1 element [53]. Mutations in E2 [39][40] and in the pyruvate dehydrogenase phosphatase gene *(PDP1)* [37][38] have also been identified.

In only about 25% of cases was the mother of a child with PDHE1α deficiency a carrier of the mutation. Therefore, since most cases of PDHC deficiency appear to be the consequence of new E1α mutations, the overall rate of recurrence within any one same family is low. Based on measurement of PDHC activity in chorionic villus samples and/or cultured amniocytes obtained from some 30 pregnancies in families with a previously affected child, three cases of reduced activity were found. However, PDHC activities in affected females might overlap with normal controls. Therefore, prenatal testing of specific mutations determined in the proband is the most reliable method. Molecular analysis is also the preferred method for prenatal diagnosis in families at risk for E1β and E3BP deficiency.

11.3.4 Diagnostic Tests

The most important laboratory test for initial recognition of PDHC deficiency is the measurement of lactate and pyruvate in blood and CSF. In PDHC deficiency CSF lactate concentrations are generally raised and in excess of blood lactate [54]. Quantitative analysis of plasma amino acids and urinary organic acids may also be useful. Blood lactate, pyruvate and alanine can be intermittently normal, but characteristically an increase is observed after an oral carbohydrate load. While the L/P ratio is usually normal, a high ratio can be found if the patient is acutely ill, if blood is very difficult to obtain, or if the measurement of pyruvate (which is unstable) is not done reliably. The practical solution allowing avoidance of these artefacts is to obtain several samples of blood, including samples collected under different dietary conditions (during an acute illness, after overnight fasting, and postprandially after a high-carbohydrate meal) (▶ Chapter 1 and ▶ Chapter 3). Glucose tolerance or carbohydrate loading tests are not necessary for a definite diagnosis. In contrast to PC deficiency, fasting hypoglycaemia is not an expected feature of PDHC deficiency, and blood lactate and pyruvate usually decrease after fasting.

The most commonly used material for assay of PDHC is cultured skin fibroblasts [20]. PDHC can also be assayed in lymphocytes, separated from EDTA-blood, after less than 2 days. Molecular analysis of *PDHE1α* in girls is often more efficient than measuring the enzyme activity. If available, skeletal muscle and/or other tissues are useful [55]. PDHC is commonly assayed by measuring the release of $^{14}CO2$ from [1-^{14}C]pyruvate in cell homogenates and tissues. PDHC activity should be measured at low and high TPP concentrations to detect thiamine-responsive PDHC deficiency [56].

PDHC must also be activated (dephosphorylated; ◘ Fig. 11.3) in some of the cells, which can be done by preincubation of whole cells or mitochondria with dichloroacetate (DCA, an inhibitor of the kinase; ◘ Fig. 11.3). In E1-phospha-

tase deficiency there is a deficiency in native PDH activity, but on activation of the PDH complex with DCA, activity becomes normal. The three catalytic components of PDHC can be assayed separately. Immunoblotting of the components of PDHC can help distinguish whether a particular protein is missing. In females with PDHE1α deficiency, X inactivation can interfere with the biochemical analysis. E3BP, which anchors E3 to the E2 core of the complex, can only be evaluated using immunoblotting, since it has no catalytic activity [41].

11.3.5 Treatment and Prognosis

The general prognosis for individuals with PDHC deficiency is poor, and treatment is not very effective. Experience with early prospective treatment to prevent irreversible brain injury is lacking. Perhaps the most rational strategy for treating PDHC deficiency is the use of a ketogenic diet [57] (▶ Chapter 13). Oxidation of fatty acids, 3-hydroxybutyrate and acetoacetate are providers of alternative sources of acetyl-CoA. In a series of males with PDHC deficiency caused by identical E1 mutations it was found that the earlier the ketogenic diet was started and the more severe the restriction of carbohydrates, the better was the outcome for mental development and survival [58]. Thiamine has been given in variable doses (500–2,000 mg/day), with lowering of blood lactate and apparent clinical improvement in some patients [23][59] (▶ Chapter 28).

DCA offers another potential treatment for PDHC deficiency. DCA, a structural analogue of pyruvate, inhibits E1 kinase, thereby keeping any residual E1 activity in its active (dephosphorylated) form (Fig. 11.3). DCA can be administered without apparent toxicity (about 50 mg/kg/day). Over 40 cases of congenital lactic acidosis attributable to various defects (including PDHC deficiency) were treated with DCA in uncontrolled studies, and most of these patients appeared to have some limited short-term benefit [60]. Chronic DCA treatment was shown to be beneficial in some patients, improving the function of PDHC, and this has been related to specific DCA-sensitive mutations [61]. Sporadic reports have also shown a beneficial effect of concomitant DCA and high-dose thiamine (500 mg). A ketogenic diet and thiamine should thus be tried in each patient. DCA can be added if lactic acidosis is important, in acute situations. Phenylbutyrate in combination with DCA has shown to increase PDH activity in mice [62]. Fibroblasts of patients with PDHC deficiency and missense mutations responded with increase of activity when incubated with phenylbutyrate [63].

11.4 Dihydrolipoamide Dehydrogenase Deficiency (DLD)

11.4.1 Clinical Presentation

Approximately 28 cases of DLD (E3) deficiency have been reported [20][64][65][66][67]. Since this enzyme is common to all the 2-ketoacid dehydrogenases (Fig. 11.2), E3 deficiency results in multiple 2-ketoacid-dehydrogenase deficiency and should be thought of as a combined PDHC and TCA cycle defect. E3 deficiency presents with severe and progressive hypotonia and failure to thrive, starting in the first months of life. Metabolic decompensations are triggered by infections. Progressively, hypotonia, psychomotor retardation, microcephaly and spasticity occur. Some patients develop a typical picture of Leigh's encephalopathy. A Reye-like picture with liver involvement and myopathy with myoglobinuria without mental retardation is seen in the Ashkenazi Jewish population [68].

11.4.2 Metabolic Derangement

DLD (E3) is a flavoprotein common to all three mitochondrial α-ketoacid dehydrogenase complexes (PDHC, KDHC and BCKD; Fig. 11.3). The predicted metabolic manifestations are the result of the deficiency state for each enzyme: increased blood lactate and pyruvate, elevated plasma alanine, glutamate, glutamine and branched-chain amino acids (leucine, isoleucine, valine and often alloisoleucine), and increased urinary lactic, pyruvic, 2-ketoglutaric, and branched-chain 2-hydroxy- and 2-ketoacids.

11.4.3 Genetics

DLD mutations [66], are inherited as an autosomal recessive trait. Mutation analysis in 13 unrelated patients has revealed 14 different mutations [65][66][67][68]. A G194C mutation is the major cause of E3 deficiency in Ashkenazi Jewish patients [69].

11.4.4 Diagnostic Tests

The initial diagnostic screening should include analyses of blood lactate and pyruvate, plasma amino acids, and urinary organic acids. However, the pattern of metabolic abnormalities is not seen in all patients or at all times in the same patient, making the diagnosis more difficult [70]. In cultured skin fibroblasts, blood lymphocytes, or other tissues, the E3 component can be assayed using a spectrophotometric method.

11.4.5 Treatment and Prognosis

There is no dietary treatment for E3 deficiency although restriction of dietary branched-chain amino acids was report-

edly helpful in one case. DL-Lipoic acid has been tried, but its efficacy remains controversial [69]. Riboflavin was succesfull in a patient with DLD deficiency and a chaperone effect is discussed [71].

11.5 2-Ketoglutarate Dehydrogenase Complex Deficiency (KDHC)

11.5.1 Clinical Presentation

Isolated deficiency of KDHC has been reported in several unrelated families [72][73]. As in PDHC deficiency, the primary clinical manifestations included developmental delay, hypotonia, ataxia, opisthotonos and, less commonly, seizures and extrapyramidal dysfunction. On magnetic resonance imaging (MRI) bilateral striatal necrosis can be found [74]. All patients presented in the neonatal period and early childhood.

In one patient the clinical picture was milder [73]. This patient had suffered from mild perinatal asphyxia. During the first months of life he developed opisthotonus and axial hypertonia, which improved with age. 2-Ketoglutaric acid (2-KGA) was intermittently increased in urine, but not in plasma and CSF. Diagnosis was confirmed in cultured skin fibroblasts. Three families with the clinical features of DOOR syndrome (onycho-osteodystrophy, dystrophic thumbs, sensorineural deafness) had increased urinary levels of 2-KGA and decreased activity of the E1 component of KDHC [75].

11.5.2 Metabolic Derangement

KDHC is a 2-ketoacid dehydrogenase that is analogous to PDHC and BCKD (Fig. 11.2). It catalyses the oxidation of 2-KGA to yield CoA and NADH. The E1 component, 2-ketoglutarate dehydrogenase, is a substrate-specific dehydrogenase that utilises thiamine and is composed of two different subunits. In contrast to PDHC, the E1 component is not regulated by phosphorylation/dephosphorylation. The E2 component, dihydrolipoyl succinyl-transferase, is also specific to KDHC and includes covalently bound lipoic acid. The E3 component is the same as for PDHC. An E3-binding protein has not been identified for KDHC. Since KDHC is integral to the TCA cycle, its deficiency has consequences similar to those of other TCA enzyme deficiencies.

11.5.3 Genetics

KDHC deficiency is inherited as an autosomal recessive trait. The *E1* gene has been mapped to chromosome 7p13-14 and the *E2* gene, to chromosome 14q24.3. The molecular basis of KDHC deficiencies has not yet been resolved. While prenatal diagnosis of KDHC should be possible by measurement of the enzyme activity in CVS or cultured amniocytes, this has not been reported.

11.5.4 Diagnostic Tests

The most useful test for recognising KDHC deficiency is urinary organic acid analysis, which can show increased excretion of 2-KGA with or without concomitantly increased excretion of other TCA cycle intermediates. However, mildly to moderately increased urinary 2-KGA is a common finding and not a specific marker of KDHC deficiency. Some patients with KDHC deficiency also have increased blood lactate with normal or increased L/P ratio. Plasma glutamate and glutamine may be increased. KDHC activity can be assayed through the release of $^{14}CO_2$ from [1-^{14}C] 2-ketoglutarate or [1-^{14}C] leucine in crude homogenates of cultured skin fibroblasts, muscle homogenates and other cells and tissues.

11.5.5 Treatment and Prognosis

There is no known effective treatment.

11.6 Fumarase Deficiency

11.6.1 Clinical Presentation

Fewer than 40 patients with fumarase deficiency have been reported. In the first reported case, onset started at 3 weeks of age with vomiting and hypotonia, followed by development of microcephaly (associated with dilated lateral ventricles), severe axial hypertonia and absence of psychomotor progression.

Most of the patients present in infancy with a severe encephalopathy and seizures, with poor neurological outcome and profound mental retardation [76]. Some patients display a relative macrocephaly (in contrast to previous cases) and large ventricles. Dysmorphic features such as frontal bossing, hypertelorism and depressed nasal bridge were noted. Milder cases with developmental delay and epilepsy resembling non progressive cerebral palsy have also been reported.

Neuropathological changes include agenesis of the corpus callosum with communicating hydrocephalus and also cerebral and cerebellar heterotopias. Polymicrogyria, open operculum, colpocephaly, angulations of frontal horns, choroid plexus cysts, decreased white matter and a small brain stem are considered characteristic [76].

11.6.2 Metabolic Derangement

Fumarase catalyses the reversible interconversion of fumarate and malate (Fig. 11.1). Its deficiency, like other TCA cycle defects, causes: (1) impaired energy production caused by interrupting the flow of the TCA cycle and (2) potential secondary enzyme inhibition associated with accumulation in various amounts of metabolites proximal to the enzyme deficiency such as fumarate, succinate, 2-KGA and citrate (Fig. 11.1).

11.6.3 Genetics

Fumarase deficiency is inherited as an autosomal recessive trait. A single gene, and the same mRNA encode alternately translated transcripts to generate a mitochondrial and a cytosolic isoform [77]. Mutations have been identified in several families [77][78]. Prenatal diagnosis is possible by enzyme assay and/or mutational analysis in CVS or cultured amniocytes [77]. Heterozygous mutations in the fumarase gene are associated with a predisposition to cutaneous and uterine leiomyomas and to kidney cancers [79].

11.6.4 Diagnostic Tests

The key finding is increased urinary fumaric acid, sometimes associated with increased excretion of succinic acid and 2-KGA. Mild lactic acidosis and mild hyperammonaemia can be seen in infants with fumarase deficiency, but generally not in older children. Other diagnostic indicators are an increased lactate in CSF, a variable leukopenia and neutropenia.

Fumarase can be assayed in mononuclear blood leukocytes, cultured skin fibroblasts, skeletal muscle or liver, by monitoring the formation of fumarate from malate or, more sensitively, by coupling the reaction with malate dehydrogenase and monitoring the production of NADH.

11.6.5 Treatment and Prognosis

There is no specific treatment.

11.7 Succinate Dehydrogenase Deficiency

11.7.1 Clinical Presentation

Succinate dehydrogenase (SD) is part of both the TCA cycle and the respiratory chain. This explains why SD deficiency resembles more the phenotypes associated with defects of the respiratory chain (▶ Chapter 14). The clinical picture of this very rare disorder [80][81][82] can include: Kearns-Sayre syndrome, isolated hypertrophic cardiomyopathy, combined cardiac and skeletal myopathy, generalised muscle weakness with easy fatiguability, and early-onset Leigh encephalopathy. It can also present with cerebellar ataxia and optic atrophy and tumour formation in adulthood. Profound hypoglycaemia was seen in one infant [83].

SD deficiency may also present as a compound deficiency state that involves aconitase and complexes I and III of the respiratory chain. This disorder, found only in Swedish patients, presents with life-long exercise intolerance, myoglobinuria, and lactic acidosis, with a normal or increased L/P ratio at rest and paradoxically a decreased L/P ratio during exercise. It can start with early-childhood-onset fatigue; episodic weakness and rhabdomyolysis may occur [84][85] (See also ▶ Chapter 14).

11.7.2 Metabolic Derangement

SD is part of a larger enzyme unit, complex II (succinate-ubiquinone oxidoreductase) of the respiratory chain. Complex II is composed of four subunits. SD contains two of these subunits, a flavoprotein (Fp, SDA) and an iron-sulfur protein (Ip, SDB). SD is anchored to the membrane by two additional subunits, C and D. SD catalyses the oxidation of succinate to fumarate (◘ Fig. 11.1) and transfers electrons to the ubiquinone pool of the respiratory chain.

Theoretically, TCA cycle defects should lead to a decreased L/P ratio, because of impaired production of NADH. However, too few cases of SD deficiency (or other TCA cycle defects) have been evaluated to determine whether this is a consistent finding. Profound hypoglycaemia, which was reported once, might have resulted from the depletion of the gluconeogenesis substrate, oxaloacetate [84]. The combined SD/aconitase deficiency found only in Swedish patients appears to be caused by a defect in the metabolism of the iron-sulfur clusters common to these enzymes [84] (▶ Chapter 14).

Complex II is unique among the respiratory chain complexes in that all four of its subunits are nuclear encoded. The flavoprotein and iron-sulfur-containing subunits of SD (A and B) and the two integral membrane proteins (C and D) have been mapped to different chromosomes. Homozygous and compound heterozygous mutations of SDA have been identified in several patients [80][83][84], as well as mutations in a separate iron-sulfur cluster encoding gene . In two sisters with partial SDA deficiency and late-onset neurodegenerative disease with progressive optic atrophy, ataxia and myopathy only one mutation was found, suggesting a dominant pattern of transmission [86].

Mutations in *SDB*, *SDC* or *SDD* cause susceptibility to familial phaeochromocytoma and familial paraganglioma [87]. This suggests that *SD* genes may act as tumour suppression genes.

In contrast to the other TCA cycle disorders, SD deficiency does not always lead to a characteristic organic aciduria. Many patients, especially those whose clinical phenotypes resemble the patients with respiratory chain defects, do not exhibit the expected succinic aciduria and can excrete variable amounts of lactate, pyruvate and the TCA cycle intermediates fumarate and malate.

Diagnostic confirmation of a suspected SD deficiency requires analysis of SD activity itself, as well as of complex-II (succinate-ubiquinone oxidoreductase) by using standard spectrophotometric procedures.

No effective treatment has been reported. Although SD is a flavoprotein, riboflavin-responsive defects have not been described.

11.8 Other Krebs Cycle Disorders

Deficiency of mitochondrial NAD^+-specific isocitrate dehydrogenase (IDH3) was found to be associated with retinitis pigmentosa [88]. Some patients with mutations in the gene for

the cytosolic $NADP^+$-specific IDH or in the gene for the mitochondrial $NADP^+$-specific IDH2 have presented with malignant gliomas and acute myeloid leukaemia. Mutations in *IDH2* have also been identified in half of those patients with D-2-hydroxyglutaric aciduria not found to be deficient in D-2-hydroxyglutarate dehydrogenase (▶ Chapter 22).

11.9 Pyruvate Transporter Defect

MPC1 mutations have been described in 5 patients [20][89][90]. Neonatal lactic acidosis in a female baby born to consanguineous parents was associated with generalised hypotonia and facial dysmorphism. MRI of the brain revealed cortical atrophy, periventricular leukomalacia and calcifications. Progressive microcephaly, failure to thrive and neurological deterioration led to death at the age of 19 months. Two other patients with a mild progressive encephalopathy have recently been identified in consanguineous families of North African descent.

The pyruvate carrier mediates the proton symport of pyruvate across the inner mitochondrial membrane. Consequently, the metabolic derangement should be the same as in pyruvate dehydrogenase deficiency.

As in PDHC deficiency, high lactate and pyruvate are found with normal L/P ratio. To evidence the transport defect, [2-^{14}C] pyruvate oxidation is measured in digitonin-permeabilised versus disrupted fibroblasts. Digitonin induces outer cell membrane permeabilisation, leaving intracellular mitochondrial membranes intact. Oxidation of ^{14}C-pyruvate is severely impaired in digitonin-permeabilised fibroblasts but not in disrupted cells.

No treatment is known at this moment.

11.10 Protein-bound lipoic acid defect and defects in cofactors

See ▶ Chapter 23 and ▶ Chapter 28.

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Disorders of Mitochondrial Fatty Acid Oxidation & Riboflavin Metabolism

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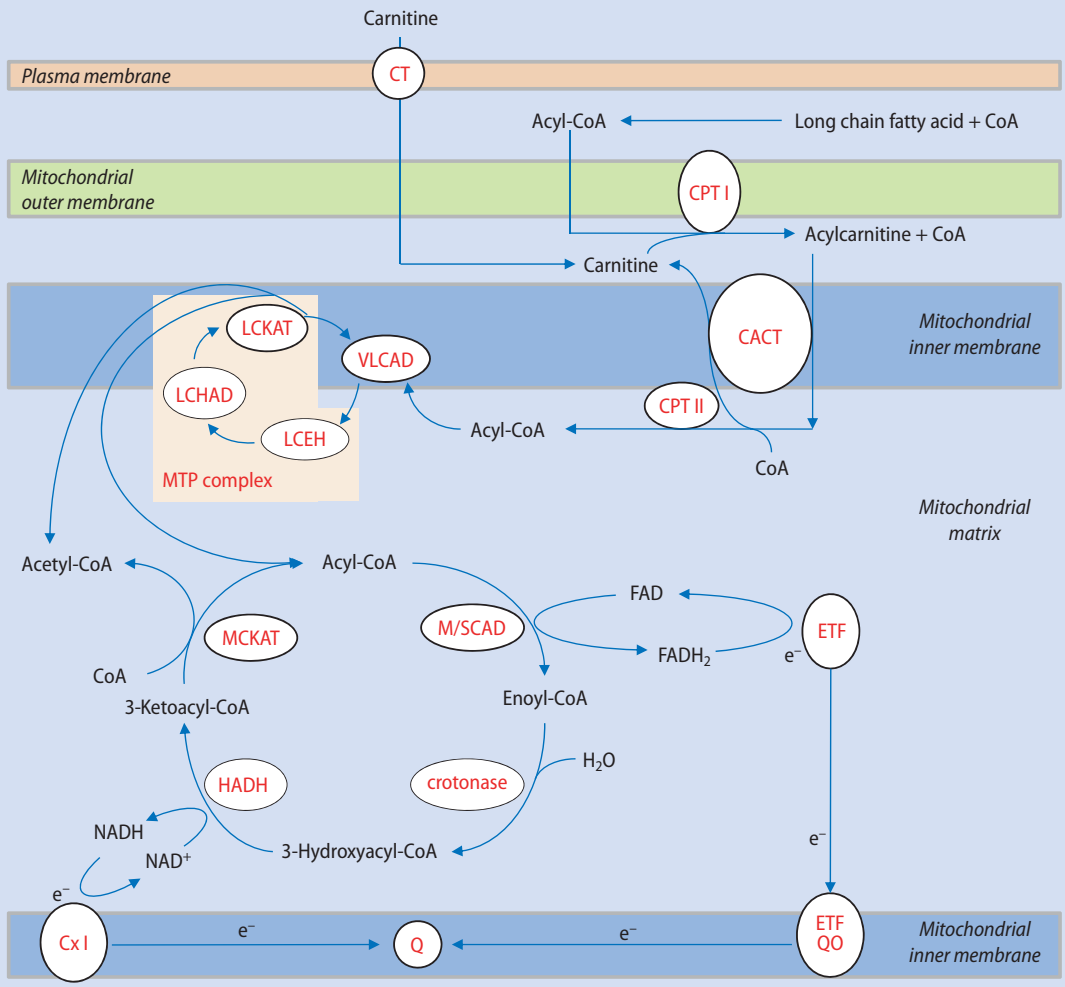
Mitochondrial Fatty Acid Oxidation and Riboflavin Metabolism

Mitochondrial fatty acid oxidation
Mitochondrial fatty acid oxidation involves three processes (■ Fig. 12.1).

1. **Entry of fatty acids into mitochondria**. Long-chain fatty acids are activated to coenzyme A (CoA) esters in the cytoplasm but they need to be transferred to carnitine in order to cross the inner mitochondrial membrane; they are transferred back to CoA within the mitochondria. Carnitine palmitoyltransferase I is the main site for the regulation of fatty acid oxidation by cytoplasmic malonyl-CoA. Medium and short-chain fatty acids enter mitochondria independent of carnitine and are activated to CoA esters in the matrix.
2. **β-Oxidation via a spiral pathway**. Each turn of the spiral shortens the



■ **Fig. 12.1 Pathway of mitochondrial fatty acid oxidation.** *CACT*, carnitine acylcarnitine translocase; *CoA*, coenzyme A; *CPT*, carnitine palmitoyltranferase; *CT*, carnitine transporter; *Cx I*, complex I of respiratory chain; *e-*, electrons ; *ETF*, electron transfer flavoprotein; *ETFQO*, ETF ubiquinone oxidoreductase; *FAD*, flavin adenine dinucleotide; *FADH2*, reduced FAD; *HADH*, 3-hydroxyacyl-CoA dehydrogenase; *LCEH*, long-chain enoyl-CoA hydratase; *LCKAT*, long-chain ketoacyl-CoA thiolase; *LCHAD*, long-chain 3-hydroxyacyl-CoA dehydrogenase; *MCKAT*, medium-chain ketoacyl-CoA thiolase; *MTP*, mitochondrial trifunctional protein; *M/SCAD*, medium-chain acyl-CoA dehydrogenase or short-chain acyl-CoA dehydrogenase; *NAD^+*, nicotinamide adenine dinucleotide; *NADH*, reduced NAD; *Q*, ubiquinone; *VLCAD*, very-long-chain acyl-CoA dehydrogenase. Some substrates and products are omitted for VLCAD and MTP. Extra enzymes are required for the oxidation of unsaturated fatty acids (dodecenoyl-CoA delta isomerase and 2,4-dienoyl-CoA reductase, not shown).

acyl-CoA by two carbons and involves four steps. These include two dehydrogenation reactions, linked respectively to flavin adenine dinucleotide (FAD) and nicotinamide adenine dinucleotide (NAD). β-Oxidation is catalysed by enzymes of different chain length specificities. The enzymes for long-chain substrates are membrane-bound; three of the reactions are catalysed by the mitochondrial trifunctional protein (MTP). Medium and short-chain enzymes are located in the matrix.

3. **Electron Transfer.** Electrons are passed to the respiratory chain either directly (from NADH to complex I) or via two transfer proteins (from FADH2 to ubiquinone).

Acetyl-CoA released by β-oxidation can either be oxidised in the Krebs cycle or, in the liver, used to synthesize ketone bodies (► Chapter 13).

Riboflavin

Riboflavin (vitamin B2) is the precursor of flavin mononucleotide (FMN) and FAD. FMN is a cofactor for complex I of the respiratory chain and FAD is a cofactor for many dehydrogenation reactions. Three riboflavin transporters have been identified. RFVT1 and RFVT3 are responsible for intestinal riboflavin uptake whereas RFVT2 is involved in transport into tissues, particularly brain. Within cells, riboflavin is phosphorylated to FMN, which can then be converted to FAD by FAD synthase. This enzyme has 2 isoforms, mitochondrial (FADS1) and cytoplasmic (FADS2), generated by alternative splicing. FAD can also enter mitochondria on the FAD transporter.

The fatty acid oxidation pathway is summarsed in ► Mitochondrial fatty acid oxidation and Riboflavin Metabolism and ◘ Fig. 12.1. Fatty acid oxidation disorders have a high incidence, particularly in populations of European origin. Many countries now have newborn screening programs for these disorders. Before screening was introduced, the commonest clinical presentations were hypoketotic hypoglycaemia and sudden death, usually precipitated by an infection or fasting in the neonatal period or early childhood. Older children or adults may present with exercise-induced rhabdomyolysis. Patients can remain asymptomatic throughout life if they have mild defects and are not exposed to the necessary stress. Treatment should be tailored to the severity of the disorder.

This chapter also considers defects of riboflavin metabolism and transport, including Brown-Vialetto-van Laere syndrome.

12.1 Disorders of Mitochondrial Fatty Acid Oxidation

Fat is an important source of energy and, because of its high energy density, it is the body's principal fuel store. Fatty acids are used by heart muscle in preference to glucose and are the main fuel for skeletal muscle during sustained exercise. During prolonged fasting, most tissues derive energy from fatty acids, allowing glucose to be spared for the brain. As well as releasing energy, hepatic fatty acid oxidation provides acetyl-CoA for ketone body synthesis. By using ketone bodies, even the brain can derive energy indirectly from fatty acids.

12.2 Clinical Presentations

Mitochondrial fatty acid oxidation disorders (FAODs) have three characteristic clinical presentations.

- Acute hypoketotic hypoglycaemia and encephalopathy, accompanied by hepatomegaly and liver dysfunction but seldom jaundice. Problems are precipitated by fasting or an infection with vomiting. Some patients die unexpectedly during a minor illness. This is often described as a hepatic presentation or a 'Reye-like illness'.
- Cardiomyopathy (usually hypertrophic), arrhythmias or conduction defects.
- Myopathy, presenting either with weakness or with acute rhabdomyolysis, which may be precipitated by exercise or infection.

Some disorders are only associated with one of these presentations, whereas others can cause all three problems (◘ Table 12.1), depending on the residual enzyme activity, the age of the patient and exposure to stress. Thus, patients may suffer hypoglycaemia in infancy and rhabdomyolysis as adults. Additional problems occur in specific disorders, as described below. A number of patients never develop symptoms, either because they have a mild defect or because they are not exposed to the necessary environmental stress.

12.2.1 Fatty Acid Transport Defects

The mechanisms of fatty acid transport across the plasma membrane are still not completely clear [1]. Impaired uptake has been reported in 2 boys who presented with liver failure [2]. The molecular basis was not identified and the diagnoses remain uncertain.

12.2.2 Carnitine Cycle Defects

Carnitine transporter deficiency

The organic cation/carnitine transporter OCTN2 is responsible for carnitine uptake across the plasma membrane, particularly in heart, muscle and kidney. Defects lead to primary carnitine deficiency with increased renal loss of carnitine, low plasma concentrations and sufficiently low intracellular concentrations to impair fatty acid oxidation [3].

Table 12.1 Inherited disorders of mitochondrial fatty acid oxidation

Defect	Gene(s)	Potential clinical manifestations*				
		Hypoglycaemia & acute liver dysfunction	Cardio-myopathy	Rhabdo-myolysis	Chronic weakness	Other problems
Carnitine defects						
CT	*SLC22A5*	+	+		+	
CPT I	*CPT1*	+				RTA
CACT	*SLC25A20*	+	+		+	
CPT II	*CPT2*	+	+	+	+	Malformations
β-oxidation defects						
VLCAD	*ACADVL*	+	+	+	+	
MCAD	*ACADM*	+				
SCAD	*ACADS*					
ACAD9	*ACAD9*		+		+	
Crotonase	*ECHS1*		+			Neurodegeneration
LCHAD & MTP	*HADHA, HADHB*	+	+	+	+	Retinopathy, Neuropathy
HADH	*HADHSC*	+				Hyperinsulinism
Dienoyl-CoA reductase	*NADK2*					Neurodegeneration
Electron transfer defects						
MAD**	*ETFA, ETFB, ETFDH*	+	+		+	Malformations

*Features depend on the residual enzyme activity and exposure to environmental stress. **MAD can also result from defects of riboflavin transport or metabolism Table 12.3. *MAD*, multiple acyl-CoA dehydrogenase deficiency; *RTA*, renal tubular acidosis; other abbreviations in Fig. 12.1

Symptoms may be precipitated by an infection, fasting, pregnancy or antibiotics containing pivalate, which is excreted bound to carnitine further reducing carnitine concentrations [4]. Some patients present in infancy with hypoglycaemia, liver dysfunction and hyperammonaemia, usually before 2.5 years of age. Other children develop heart failure due to cardiomyopathy, with thickened ventricular walls and reduced contractility. This is often accompanied by skeletal muscle weakness. Adults may suffer fatigue or arrhythmias [4] but screening has shown that many subjects with low plasma carnitine remain asymptomatic (for example in the Faroe Islands, where the prevalence is 1:300).

Carnitine palmitoyltransferase I (CPT I) deficiency

Different isoforms of CPT I have been found in liver and kidney (CPT Ia), muscle and heart (CPT Ib) and brain (CPT Ic). Only CPT Ia deficiency has been identified in man. Patients usually present by the age of 2 years with hypoketotic hypoglycaemia, induced by fasting or illness. This is accompanied by hepatomegaly, liver dysfunction and occasionally cholestasis that may take several weeks to resolve. There may also be transient lipaemia and renal tubular acidosis [5]. Paradoxically, a few patients have had cardiac problems or raised plasma creatine kinase (CK) [5].

CPT I deficiency is extremely common in the Inuit population of Canada and Greenland. A few of these patients present with hypoglycaemia as neonates or young children but most remain asymptomatic [6].

Carnitine acylcarnitine translocase (CACT) deficiency

This rare disorder usually presents in the neonatal period, with death by 3 months of age [7]. Problems include severe hypoglycaemia and hyperammonaemia, cardiomyopathy, atrioventricular block and ventricular arrhythmias. A few more mildly affected patients present later with hypoglycaemic encephalopathy, precipitated by fasting or infections.

Carnitine palmitoyltransferase II (CPT II) deficiency

The commonest form of this disorder is a partial deficiency that presents with episodes of rhabdomyolysis. Attacks are usually precipitated by prolonged exercise, particularly in the

cold or after fasting, and start in adolescents or young adults. In childhood, episodes may be brought on by infections. Muscle pain may begin during or after exercise and can spread from muscles that have been working to those that have not. In moderate or severe episodes there is myoglobinuria, which may lead to acute renal failure and require dialysis for a few days. Plasma CK is markedly raised; it often normalises between episodes but may remain moderately elevated, with chronic weakness.

Severe neonatal onset CPT II deficiency is usually lethal. Patients become comatose within a few days of birth, due to hypoglycaemia and hyperammonaemia. In addition, they may have cardiomyopathy, arrhythmias and congenital malformations, principally renal cysts and neuronal migration defects [8]. There is also an intermediate form of CPT II deficiency that causes episodes of hypoglycaemia and liver dysfunction, sometimes accompanied by cardiomyopathy and arrhythmias.

12.2.3 β-Oxidation Defects

▪ Very-long-chain acyl-CoA dehydrogenase (VLCAD) deficiency

Mildly affected patients present as adolescents or adults with exercise-induced rhabdomyolysis. Other patients present in childhood with hypoglycaemia but suffer exercise- or illness-induced rhabdomyolysis or chronic weakness at a later age. Severely affected patients present in early infancy with cardiomyopathy, in addition to the problems seen in milder patients. Neonatal screening has shown that VLCAD deficiency is the second commonest fatty acid oxidation disorder in Europe and the USA, with a prevalence between 1:50,000 and 1:100,000 [9]. This is much higher than was detected clinically. Undoubtedly, the diagnosis was missed in some symptomatic patients, particularly those presenting as adults, but it is likely that many patients diagnosed by screening would remain asymptomatic without intervention.

▪ Long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) & Mitochondrial trifunctional protein (MTP) deficiencies

MTP is composed of four α-subunits and four β-subunits; the α-subunit has long-chain enoyl-CoA hydratase (LCEH) and LCHAD activities and the β-subunit has long-chain ketoacyl-CoA thiolase (LCKAT) activity. Patients may have isolated LCHAD deficiency or a generalised deficiency of all three enzyme activities.

Patients with isolated LCHAD deficiency usually present acutely before 6 months of age with hypoglycaemia, accompanied by liver dysfunction and lactic acidosis [10]. Many patients have cardiomyopathy and some have hypoparathyroidism or acute respiratory distress syndrome [11]. Other patients present with chronic symptoms, such as failure to thrive, hypotonia or, occasionally, cholestasis or cirrhosis. Subsequently, episodes of rhabdomyolysis are common. Many patients develop retinopathy, which may start as early as 2 years of age. Granular pigmentation, especially in the macular region, is followed by chorioretinal atrophy with deteriorating central vision [12]. Some patients develop cataracts [12]. Peripheral neuropathy is rarer in LCHAD deficiency.

Generalised MTP deficiency is more heterogeneous [13]. Patients with severe deficiency present as neonates with cardiomyopathy, respiratory distress, hypoglycaemia and liver dysfunction; most die within a few months, regardless of treatment. Other patients resemble those with isolated LCHAD deficiency. There is also a milder neuromyopathic phenotype: the main problems in these patients are exercise-induced rhabdomyolysis and progressive peripheral neuropathy, which can present at any age from infancy to adulthood.

Mothers who are heterozygous for LCHAD or MTP deficiency have a high risk of illness during pregnancies when they are carrying an affected fetus [14]. The main problems are HELLP syndrome (Haemolysis, Elevated Liver enzymes and Low Platelets) and acute fatty liver of pregnancy [AFLP].

▪ Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency

MCAD deficiency is much the commonest fatty acid oxidation disorder in North-West Europe, with an incidence of approximately 1:10,000-20,000 in Europe, the USA and Australia [15][16]. Before newborn screening, patients usually presented between the ages of 4 months and 4 years with acute hypoglycaemic encephalopathy and liver dysfunction; some deteriorated rapidly and died. Problems are precipitated by prolonged fasting or, more often, by an infection with vomiting. A number of babies still present within 72 hours of birth, before newborn screening results are available, with hypoglycaemia and/or arrhythmias [17]; breast-fed babies are at higher risk, due to the small supply of breast milk at this stage.

Patients with MCAD deficiency only present clinically if exposed to an appropriate environmental stress. Even before newborn screening, many patients, probably 30–50%, remained asymptomatic [15][16]; with newborn screening and preventative measures, hypoglycaemia is rare. Patients do not develop cardiomyopathy or myopathy and few first present as adults [18].

When in good health, MCAD deficient children aged over 1 year can fast for 12–14 hours without problems. If fasting is continued, they deteriorate over a few hours with hypoglycaemia and inappropriately low ketone body concentrations. Shorter periods of fasting may cause problems in infancy, though some patients have fasted regularly for up to 12 hours from 4–6 months of age, prior to diagnosis. Encephalopathy may occur without hypoglycaemia [18], presumably due to the accumulation of free fatty acids and their carnitine and CoA esters.

▪ Short-chain acyl-CoA dehydrogenase (SCAD) deficiency

Various symptoms have been reported in SCAD deficiency, most frequently developmental delay. However, almost all patients diagnosed by screening or because of an affected relative remain asymptomatic [19]. The pathological significance

of SCAD deficiency is, therefore, unclear. It may confer susceptibility to disease or, more probably, it may be a non-disease whose association with symptoms results from ascertainment bias [20].

▪ 3-Hydroxyacyl-CoA dehydrogenase (HADH) deficiency

This defect, previously called SCHAD deficiency, causes hyperinsulinaemic hypoglycaemia (▶ Chapter 9).

▪ Short-chain enoyl-CoA hydratase (crotonase) deficiency

This defect presents with severe neurological problems, lactic acidosis and sometimes cardiomyopathy (▶ Chapter 18).

▪ Acyl-CoA dehydrogenase 9 (ACAD9) deficiency

ACAD9 is an assembly factor for respiratory chain complex I. It is also homologous to VLCAD and has dehydrogenase activity towards long-chain acyl-CoA esters. Patients with ACAD9 defects present in infancy or childhood with myopathy or hypertrophic cardiomyopathy and lactic acidaemia; some also have neurological problems. The myopathic patients often respond to treatment with riboflavin [21].

▪ Dienoyl-CoA reductase deficiency

Raised C10:2 acylcarnitine levels suggested this diagnosis in two infants with a fatal neurodegenerative disorder. They also had raised lysine concentrations. The abnormalities were secondary to mitochondrial NADP(H) deficiency, caused by *NADK2* mutations [22].

12.2.4 Electron Transfer Defects

▪ Multiple acyl-CoA dehydrogenase (MAD) deficiency

Electron transfer flavoprotein (ETF) and ETF ubiquinone oxidoreductase (ETFQO) carry electrons to the respiratory chain from multiple FAD-linked dehydrogenases (◘ Fig. 12.1). These include enzymes of amino acid and choline metabolism in addition to the acyl-CoA dehydrogenases of β-oxidation. Thus, defects of ETF or ETFQO lead to multiple acyl-CoA dehydrogenase (MAD) deficiency (also known as glutaric aciduria type II). MAD deficiency can also, less often, result from defects of riboflavin transport or metabolism (▶ Section 12.7).

ETF and ETFQO deficiencies have a wide range of clinical severity. Severely affected patients present in the first few days of life with hypoglycaemia, hyperammonaemia and acidosis accompanied by hypotonia and hepatomegaly. There is usually an odour of sweaty feet similar to that in isovaleric acidaemia. Some patients have congenital anomalies (including large cystic kidneys, hypospadias and neuronal migration defects that can be detected prenatally by fetal MRI) and facial dysmorphism (low set ears, high forehead and midfacial hypoplasia). The malformations resemble those seen in CPT II deficiency but the pathogenesis is unknown. Most patients with neonatal presentation die within a week of birth; many of the others develop cardiomyopathy and die within a few months.

Less severe cases can present at any age from infancy to adulthood with hypoglycaemia, liver dysfunction and weakness, usually precipitated by an infection [23]. Cardiomyopathy is common in infants. Rarer problems include stridor and leukodystrophy [24]. Mildly affected children may have recurrent bouts of vomiting. Muscle weakness is the commonest presentation in adolescents and adults. It predominantly affects proximal muscles and may lead to scoliosis, hypoventilation or an inability to lift the chin off the chest. The weakness can worsen rapidly during an infection or pregnancy but myoglobinuria is rare. Many of the milder defects respond to riboflavin (▶ Section 12.6.3) [23].

12.2.5 Other Potential Defects

Medium-chain 3-ketoacyl-CoA thiolase (MCKAT) deficiency has been reported in one patient, who died at 13 days of age with hypoglycaemia, hyperammonaemia, acidosis and myoglobinuria [25].

Long-chain acyl-CoA dehydrogenase (LCAD) appears to be involved in surfactant metabolism and LCAD deficiency has been reported in two cases of sudden infant death [26].

12.3 Metabolic Derangement

Fasting hypoglycaemia is the classic metabolic disturbance in FAODs and is primarily due to increased peripheral glucose consumption, though hepatic glucose output is also reduced under some conditions [27]. The hypoglycaemia is hypoketotic. Ketone bodies can be synthesised, particularly in medium- or short-chain FAODs [28] or if there is high residual enzyme activity, but the plasma concentrations are lower than expected for the degree of hypoglycaemia or the plasma free fatty acid concentrations. Hyperammonaemia occurs in some severe defects, with normal or low glutamine concentrations; it is thought to result from decreased acetyl-CoA production reducing the synthesis of N-acetylglutamate, which is the physiological activator of carbamoyl phosphate synthetase. Lactic acidaemia is seen in long-chain FAODs, particularly LCHAD and MTP deficiencies, and results from the inhibitory effects of metabolites on various steps in pyruvate metabolism [29]. Moderate hyperuricaemia is another frequent finding during acute attacks. Secondary hyperprolinaemia occurs in some babies with MAD deficiency [30] (▶ Chapter 21).

Accumulating long-chain acylcarnitines may be responsible for arrhythmias and may interfere with surfactant metabolism [11][26]. In LCHAD and MTP deficiencies, long-chain hydroxyacylcarnitine concentrations correlate with the severity of retinopathy [31] and may cause both this and the peripheral neuropathy.

12.4 Genetics

All mitochondrial FAODs show an autosomal recessive pattern of inheritance with the exception of transient neonatal MAD deficiency (▶ Section 12.7.2). The genes for individual enzymes are listed in ◘ Table 12.1. Heterozygosity seldom causes problems except for mothers heterozygous for LCHAD deficiency, who may develop AFLP or HELLP syndrome when carrying an affected fetus. Rhabdomyolysis has been reported in a few heterozygotes for CPT II deficiency. It has also been suggested that symptoms, such as myopathy, may occur in individuals who are heterozygous for more than one defect of fatty acid oxidation or related pathways [32]; this proposal has been termed 'synergistic heterozygosity'.

There is molecular heterogeneity in all these disorders but some prevalent mutations have been identified:

- *CPT I deficiency.* The high frequency of CPT I deficiency in the Inuit is due to a founder effect: in some regions of northern Canada, 70% babies are homozygous for c.1436C>T [6]. Though the mutation reduces CPT I activity to about 6% control values, fatty acid oxidation flux is only modestly decreased. The Inuit traditionally select a high fat diet leading to permanent ketosis; subjects feel unwell if ketogenesis stops abruptly. The mutant enzyme is less sensitive to inhibition by malonyl-CoA and this may confer a selective advantage by retaining ketosis.
- *CPT II deficiency.* The c.439C>T (p.S113L) *CPT2* mutation accounts for approximately 60% mutant alleles in myopathic Caucasian patients [33].
- *MCAD deficiency.* The c.985A>G mutation is common in populations originating from northern Europe: 80% symptomatic patients and 60% patients detected by screening are c.985A>G homozygotes [16].
- *SCAD deficiency.* There are two polymorphisms (c.625G>A and c.511C>T). In northern Europe, 6% of the general population have one of these variants on both alleles [19]. SCAD deficiency can be associated with these variants or with rare mutations.
- *LCHAD & MTP deficiency.* Most Caucasian patients are homozygous for the c.1528G>C mutation in the LCHAD domain of the α-subunit; this gives rise to isolated LCHAD deficiency. Patients with complete or partial deficiencies of all 3 enzyme activities are said to have generalised MTP deficiency. This can result from mutations affecting either subunit and includes most compound heterozygotes for c.1528G>C and a second α-subunit mutation [13][34].

The relationship between genotype and phenotype varies in different FAODs. In CPT II and VLCAD deficiencies, homozygous nonsense mutations are generally associated with severe early onset disease, whereas late onset rhabdomyolysis is associated with conservative missense mutations (such as the c.439C>T *CPT2* mutation and the c.848T>C *ACADVL* mutation) [33][35]. The latter is the commonest mutation in Caucasians with VLCAD deficiency and has only been found in mildly affected or asymptomatic patients. For patients with rare mutations, it is easier to predict the clinical course from the residual enzyme activity or fatty acid oxidation flux.

The genotype correlates less closely with phenotype in MCAD and carnitine transporter deficiencies. MCAD deficient patients with the same genotype may die or remain asymptomatic, depending on their exposure to fasting stress. Some *ACADM* mutations are, however, less likely to cause clinical problems. In particular, the c.199T>C mutation is associated with significant residual activity and is relatively benign: it accounts for >6% mutant alleles in most screened populations but there have only been a few reports of clinical problems [36].

12.5 Diagnostic Tests

The investigation of a suspected FAOD starts by looking for abnormal metabolites, particularly acylcarnitines. If the results suggest a specific diagnosis, this is confirmed by enzyme assays or mutation analysis. If the metabolite results are non-specifically abnormal or if they are normal despite strong clinical suspicion, it may be helpful to measure acylcarnitine production *in vitro* or flux through the pathway.

12.5.1 Abnormal Metabolites

Acylcarnitines

In most fatty acid oxidation disorders, acyl-CoA intermediates accumulate proximal to the defect and are transesterified to carnitine. The acylcarnitine abnormalities are best analysed by tandem mass spectrometry (TMS). The usual samples are plasma or dried blood spots on filter paper. ◘ Table 12.2 lists the typical abnormalities in different FAODs.

The diagnostic specificity can be increased by measuring the ratios of different acylcarnitines. For example, C8 acylcarnitine is raised in patients with MCAD and MAD deficiencies and in MCAD deficiency carriers at times of stress; the presence of a raised C8/C10 acylcarnitine ratio increases the specificity for MCAD deficiency, which is particularly useful in newborn screening programs. Severe CPT II and CACT deficiencies, however, cause identical acylcarnitine abnormalities, as do LCHAD and MTP deficiencies.

The clinical circumstances have a major effect on the acylcarnitine profile. Abnormalities are usually more marked in stressed patients but, if the plasma free carnitine concentration is very low, abnormal acylcarnitines may be hard to detect. Abnormalities may be reduced by intravenous glucose or dietary treatment, such as the use of medium-chain triglycerides (MCT) in long-chain FAODs. Interpretation is especially difficult for samples obtained terminally or post-mortem: these often show multiple raised acylcarnitine species, resembling MAD deficiency.

Acylcarnitine analysis can be completely normal in patients with high residual enzyme activity, such as mild VLCAD

Table 12.2 Abnormal metabolites seen in fatty acid oxidation disorders

Deficiency	Plasma acylcarnitines	Urinary acylglycines	Urinary organic acids*
CT	Low free carnitine		±(DCA)
CPT IA	Virtually absent long- & medium-chain acylcarnitines, high free carnitine		(Variable DCA)
CACT and CPT II severe	C18:1, C18:2, C16, C16-DC, C18:2-DC, C18:1-DC		Variable DCA
CPT II mild	↑(C16+C18)/C2**		
VLCAD	C16:1, C14:2, **C14:1**, C18:1**		Variable DCA
MCAD	C10:1, **C8**, C6	Hexanoyl-, suberyl-, phenylpropionyl-	DCA [suberic > adipic], (KB)
SCAD	C4	Butyryl-	Ethylmalonic, methylsuccinic, KB
LCHAD / MTP	C18:1-OH, C18-OH, C16:1-OH, C16-OH**		3-Hydroxydicarboxylic acids, DCA
HADH	±C4-OH		±(3-hydroxybutyric, 3-hydroxyglutaric)
MAD: severe	C4, C5, C5-DC, C6, C8, C10, C12, C14:1, C16, C18:1	Isobutyryl-, isovaleryl-, hexanoyl-, suberyl-,	Ethylmalonic, glutaric, 2-hydroxyglutaric, DCA
MAD: mild	C6, C8, C10, C12	Isobutyryl-, isovaleryl-, hexanoyl-, suberyl-	Ethylmalonic, adipic, DCA, KB

*These are typical organic acids during acute illness; those in parentheses are mildly elevated. Organic acids are often normal during anabolism. *DCA,* C6-C10 saturated straight-chain dicarboxylic acids; *Variable DCA,* C6-C12 saturated and unsaturated straight-chain dicarboxylic acids. **Acylcarnitines can be normal during anabolism (e.g. mild VLCAD and MTP deficiencies) or even during catabolism (e.g. mild CPT II deficiency). *KB,* ketone bodies; other abbreviations in Fig. 12.1

or MTP deficiencies. Abnormalities may, however, be detectable in samples collected after overnight fasting, exercise or loading with carnitine. Myopathic CPT II deficiency is particularly hard to diagnose; the sum of the C16:0 and C18:1 acylcarnitine concentrations may be raised relative to acetylcarnitine but this is not reliable.

There are no abnormal acylcarnitine species in patients with deficiencies of the carnitine transporter or CPT I but free carnitine concentrations are usually abnormal.

Free and total carnitine concentrations

Plasma free and total carnitine concentrations are best measured by an enzymatic radioisotope technique. Carnitine can be formed from acetyl- and acyl-carnitines during derivatisation for TMS. Nevertheless, with careful sample preparation, TMS can provide a reasonable estimate of the plasma free carnitine concentration. Measurement in dried blood spots is less reliable. Plasma free and total carnitine concentrations are usually <5 μmol/l in patients with carnitine transporter deficiency [3], though they may be higher in the newborn period, when they reflect the mother's carnitine status and carnitine transporter deficiency can be missed. Plasma free carnitine concentrations are often reduced in other FAODs, due to the accumulation of acylcarnitines which competitively inhibit the carnitine transporter and increase the renal loss of carnitine.

In CPT I deficiency, the ratio of free carnitine to long-chain acylcarnitines is increased. This abnormality is more reliably detected in blood spots than plasma and allows patients to be detected by newborn screening. Raised free carnitine concentrations are due to increased reabsorption from urine: because the defect prevents the formation of acylcarnitines, there is less competitive inhibition of the carnitine transporter than normal.

Urinary organic acids and acylglycines

Organic acid analysis is often normal in FAODs when the patient is well. Indeed, patients with mild CPT II, MTP or MAD deficiencies can have normal organic acids even during acute crises and patients with SCAD, MCAD and mild MAD deficiencies can have significant ketonuria. Many patients with FAODs, however, have elevated medium-chain (and sometimes long-chain) dicarboxylic acids during fasting or illness, with little or no increase in ketone bodies. Similar patterns can be seen in defects of ketogenesis or the respiratory chain and in patients recovering from ketosis or receiving MCT; a preponderance of sebacic acid helps to distinguish the latter.

Characteristic organic acid patterns are seen in certain FAODs (Table 12.2 and ▶ Chapter 3 ▶ Table 3.2) and abnormal glycine conjugates are found in some FAODs (Table 12.2). Using stable isotope-dilution mass spectrometry, these can be demonstrated even when patients are healthy.

12.5.2 *In Vitro* Studies

Enzyme assays

Suspected diagnoses need to be confirmed by enzyme assays or mutation analysis. Enzyme assays are generally performed on cultured fibroblasts or lymphocytes [37]. The latter can be prepared from as little as 1-2 ml fresh EDTA blood and results may be available within a few days. For the ß-oxidation spiral, the chain length specificities of different enzymes overlap. This problem can be overcome by finding a substrate that is specific for one enzyme (e.g. MCAD, HADH) or specific under the assay conditions (e.g. VLCAD). Alternatively, the interfering enzyme can be inhibited or immunoprecipitated before performing the assay (e.g. LCHAD, LCKAT). For some enzymes, such as MCAD and VLCAD, the residual activity *in vitro* correlates with the clinical severity: this may help in managing patients detected by screening. Few laboratories assay ETF or ETFQO and these defects are generally confirmed by mutation analysis.

Mutation analysis

Molecular studies, including gene panels, are often used to confirm the diagnosis, as an alternative to enzymology. This is usually satisfactory but the pathogenicity of sequence variants is sometimes hard to assess. Moreover, standard sequencing may miss some mutations, such as large deletions and those in introns that affect splicing. Mutation analysis facilitates carrier testing and prenatal diagnosis.

Whole cell techniques

Quantitative acylcarnitine profiling may indicate the site of a defect if this is not clear from metabolite results. Acylcarnitines are analysed by TMS after incubating fibroblasts or lymphocytes with fatty acids, labelled with stable isotopes [38].

Fatty acid oxidation flux is measured by incubating cells with radio-labelled fatty acids and collecting the oxidation products [39]. This is useful in evaluating the severity of a disorder but acylcarnitine profiling yields more diagnostic information.

12.5.3 Fasting Studies

For suspected FAODs, fasting studies have been supplanted by acylcarnitine analysis and *in vitro* studies. It is, however, still useful to collect blood (and urine) samples if a patient presents with hypoglycaemia: raised plasma free fatty acids with inappropriately low ketone body concentrations suggest an FAOD (▶ Chapter 3).

12.5.4 Prenatal Diagnosis

Mutation analysis is the preferred technique, if the molecular defect is known in the index case. All the enzymes of fatty acid oxidation are expressed in chorionic villus biopsies and amniocytes. Prenatal diagnosis is, therefore, also possible using enzyme assays.

12.5.5 Newborn Screening

Many countries now screen for FAODs, using acylcarnitine analysis by TMS. The free- and acyl-carnitine abnormalities and value of ratios are discussed in ▶ Section 12.5.1 and ◘ Table 12.2. The target conditions vary between countries, some only screening for MCAD deficiency. Screening for long-chain fatty acid oxidation defects is best performed when patients are catabolic on day 2 or 3 of life; acylcarnitines may be normal subsequently, so an initial abnormal profile should be followed by confirmatory enzyme or genetic testing rather than repeating acylcarnitine analysis. Screening tends to miss mild deficiencies of CPT II, MAD and MTP. For VLCAD and carnitine transporter deficiencies there is a high false positive rate (occasionally due to undiagnosed maternal carnitine transporter deficiency); moreover, many of the cases detected would not have caused symptoms during childhood.

12.6 Treatment and Prognosis

Most patients with FAOD need to avoid prolonged fasting and require careful management during acute illnesses to prevent metabolic decompensation. Long-term dietary treatment is needed in patients with severe long-chain FAODs. Carnitine and riboflavin are indicated in specific disorders and various forms of treatment have been proposed for exercise-induced symptoms.

12.6.1 Management of Acute Illness

The hormonal changes associated with acute illness lead to lipolysis and increased fatty acid oxidation. In most FAODs, this can lead to metabolic decompensation. The process can be prevented by providing sufficient glucose to stimulate insulin secretion and suppress lipolysis. Drinks containing an appropriate amount of glucose should be started at the first sign of illness and continued every 2–3 hours until the patient starts to improve; feeds should be reintroduced within 24–48 hours. If the drinks are vomited or the patient deteriorates, hospital admission is needed for intravenous glucose (at least the physiological hepatic glucose production rate, i.e. 3–12 mg/kg/minute, depending on age). Hypoglycaemia is a late event and management should be started without delay, regardless of the blood glucose concentration.

12.6.2 Long Term Dietary Management

Prolonged fasting should be avoided in all FAODs to prevent acute metabolic decompensation. Frequent, regular feeds are recommended during the first year of life but subsequently

overnight fasting can be tolerated in most disorders (including MCAD and most cases of VLCAD deficiency). In severe FAODs, overnight fasting is avoided until later in childhood, to reduce the risk of cardiomyopathy and long-term complications. These patients may be managed with continuous overnight tube feeding, with extra feeds during the night or, when older, with uncooked cornstarch before bed.

Dietary fat restriction is unnecessary in MCAD deficiency and breast feeding should be allowed. Top-ups of formula should, however, be given for the first 2–3 days, until the supply of breast milk improves. Unfortunately, this can only be implemented if there is a relevant family history, because screening results only arrive after the period of increased risk.

Long-chain fat is restricted in severe long-chain FAODs. Medium-chain fatty acids can enter mitochondria independent of carnitine and also bypass the long-chain ß-oxidation enzymes. Medium-chain triglyceride (MCT) can, therefore, be substituted for long-chain fat in patients with long-chain FAODs, such as VLCAD, MTP, LCHAD, CACT, CPT I and CPT II deficiencies. Dietary MCT has led to the resolution of cardiomyopathy in a number of patients with VLCAD and LCHAD deficiencies; its use has also been associated with the resolution of renal tubular acidosis in CPT I deficiency [5]. Anecdotal evidence suggests that a bolus of MCT before exercise can prevent rhabdomyolysis in patients with myopathic VLCAD deficiency [40].

For symptomatic infants with long-chain FAODs, a formula maximally enriched with MCT is recommended and breast feeding is avoided, at least initially [41][42]. After weaning, it has been suggested that MCT should provide 20% and long-chain fat only 10% energy intake; this is hard to achieve in older patients, particularly as the long-chain fat has to include adequate essential fatty acids (4% energy intake).

Newborn screening detects a number of mildly affected patients with long-chain FAODs. These do not need a special diet and breast feeding can be continued. Most asymptomatic patients with VLCAD deficiency fall into this category but some authorities recommend dietary modification (as above) if the mutations or enzyme studies predict a severe phenotype [42]. Dietary modification is recommended for all patients with LCHAD and MTP deficiencies [41].

MCT is contraindicated in MCAD, ETF and ETFQO deficiencies because medium-chain fatty acids enter mitochondria rapidly, bypassing the normal regulation at CPT I, and cannot be oxidised. Instead, patients with severe ETF and ETFQO deficiencies have been given large doses of 3-hydroxybutyrate, which has led to improvement in cardiomyopathy and leukodystrophy [24].

Triheptanoin has been substituted for MCT in a number of long-chain FAODs. Triheptanoin differs from conventional MCT in containing odd chain fatty acids, which generate propionyl-CoA as well as acetyl-CoA when they are oxidised. In FAODs, it is suggested that tricarboxylic acid cycle intermediates leak out of cells, and that propionyl-CoA can replenish this loss. Substituting triheptanoin for MCT was associated with fewer episodes of hypoglycaemia and hospitalisation, and improved cardiomyopathy in a number of patients [43] but initial results from a controlled trial have shown no improvement in muscle strength or the frequency of rhabdomyolysis [44].

12.6.3 Drug Treatment

Carnitine treatment is very effective in patients with carnitine transporter deficiency. The usual dose is 100 mg/kg/day, divided into 3 or 4 doses. Plasma concentrations may reach the lower part of the normal range but muscle carnitine concentrations remain less than 5% normal. Nevertheless, treatment prevents hypoglycaemia and arrhythmias; any cardiomyopathy or weakness resolves within a few months.

The value of carnitine therapy in other FAODs is controversial. Plasma free carnitine concentrations are often low, particularly after an acute illness, but tissue concentrations are seldom measured. It has been suggested that carnitine may promote the excretion of metabolites and prevent the sequestration of coenzyme A but this has not been proven. Indeed, carnitine treatment may be harmful in long-chain FAODs, as it increases the concentrations of long-chain acylcarnitines, which are potentially arrhythmogenic.

Patients with mild MAD deficiency due to *ETFDH* mutations often respond to treatment with *riboflavin* (100 mg/day). Benefit is seen in some children who presented with hypoglycaemia as well as adults who presented with weakness [23]. Symptoms may resolve completely or there may be some residual weakness.

Bezafibrate increases the expression of fatty acid oxidation enzymes by activating peroxisome proliferator-activated receptor (PPAR) α and PPARδ receptors. This allows it to enhance the residual enzyme activity in fibroblasts from patients with partial CPT II or VLCAD deficiencies. Conflicting results have been obtained in clinical trials. In one study, treatment for 6 months increased the enzyme activity in muscle biopsies from adults with CPT II deficiency [45] but a second randomised study found no increase in exercise tolerance or fatty acid oxidation [46].

12.6.4 Monitoring

Follow-up is required even for asymptomatic patients but the tests undertaken depend on the defect and its severity. The plasma transaminase levels indicate the recent metabolic status in patients with hepatic involvement, as does the plasma CK if muscle or heart is involved. In unsupplemented patients, the free carnitine concentration is another marker of metabolic status. Essential fatty acids and fat-soluble vitamins should be monitored in patients on fat-modified diets. Hepatic steatosis and cardiomyopathy can be assessed by ultrasound. Ophthalmological and neurophysiological studies are needed in LCHAD and MTP deficiencies.

Table 12.3 Riboflavin transport and metabolism defects

Transporter/ Enzyme	RFVT1	RFVT2	RFVT3	FAD synthase	FAD transporter
Gene	*SLC52A1*	*SLC52A2*	*SLC52A3*	*FLAD1*	*SLC25A32*
Maximum expression	Intestine, placenta	Brain, salivary glands	Intestine, testis		
Inheritance of defect	Transient problems in child of heterozygous mother	Autosomal recessive	Autosomal recessive	Autosomal recessive	Autosomal recessive
Clinical features	Neonatal hypoglycaemia & hyperammonaemia	Brown-Vialetto-van Laere syndrome		Myopathy ± cardiomyopathy	Exercise intolerance
Biochemical features	Mother: riboflavin deficiency, Child: MADD-like ACs & OAs	± Low riboflavin, ± MADD-like ACs		MADD-like ACs & OAs	

MADD, multiple acyl-CoA dehydrogenase deficiency; *ACs*, blood acylcarnitines; *OAs*, urine organic acids (Table 12.2 for ACs and OAs expected in MADD)

12.6.5 Prognosis

In the past, most FAODs had a significant mortality during the presenting illness but a good prognosis following diagnosis. Newborn screening improves the outcomes but, for many disorders, it identifies a number of patients who would never have developed symptoms. Most data are available for MCAD deficiency. Before screening programmes, approximately 4% patients died in the first 72 hours and a further 5–7% died over the next 6 years [47]. After an episode of encephalopathy, about 7% survivors have neuropsychological deficits. Newborn screening greatly reduces the morbidity and mortality, though it cannot eliminate early neonatal deaths [16]. Following diagnosis, the prognosis is also excellent for patients with carnitine transporter, CPT I and riboflavin-responsive MAD deficiencies.

Recurrent rhabdomyolysis is a long-term problem in mild CPT II deficiency and in some patients with VLCAD deficiency; no current form of treatment completely prevents this. LCHAD deficiency is a more serious condition: about a third of patients died in the presenting illness, prior to screening [10]. Moreover, in LCHAD and MTP deficiencies, there is a high long-term risk of retinopathy or peripheral neuropathy and pregnancy is hazardous. The neonatal onset forms of CACT, CPT II, MTP and MAD deficiencies often present before screening results are available; they are usually fatal within a few days or months.

12.7 Defects of Riboflavin Transport & Metabolism

Riboflavin is present in a wide variety of foods; milk and dairy products are major sources in Western diets. Riboflavin deficiency is associated with stomatitis and interferes with iron handling but it is hard to be sure of the true effects as human deficiency is usually accompanied by other dietary deficiencies and animal studies may not be relevant. Riboflavin deficient rats have impaired fatty acid oxidation and excrete dicarboxylic acids and acylglycines similar to those seen in MAD deficiency.

Riboflavin transport and metabolism are summarised in the Box on page 202. Defects of riboflavin metabolism cause myopathy or cardiomyopathy whereas defects of the RFVT2 and RFVT3 riboflavin transporters present with neurodegeneration (Brown-Vialetto-van Laere syndrome). The biochemical features often indicate MAD deficiency (Table 12.3).

12.7.1 Brown-Vialetto-van Laere Syndrome

This autosomal recessive disorder is caused by defects of the RFVT2 and RFVT3 transporters. It is characterised by progressive ponto-bulbar palsy and deafness; cases without deafness are called Fazio-Londe disease but have the same causes. Patients with RFVT2 defects usually present with sensory ataxia followed by weakness of the hands, wrists and neck, due to axonal sensorimotor neuropathy; some patients present with nystagmus due to optic atrophy. Patients with RFVT3 defects may present in infancy with hypotonia or later (occasionally as adults) with deafness or bulbar signs, such as stridor or dysphagia. In defects of either transporter, the initial signs are followed by rapidly progressive bulbar palsy with respiratory failure. Other features may include tongue fasciculation, facial weakness and ptosis or ophthalmoplegia.

A pattern of blood acylcarnitines suggesting mild MAD deficiency is seen in approximately 60% patients, regardless of the underlying defect. The organic acids may also suggest mild MAD deficiency and there may be low plasma flavin concentrations. *SLC52A2* and *SLC52A3* sequencing is needed for diagnosis.

Treatment with riboflavin leads to clinical stabilisation or improvement in patients with defects of either transporter [48]. Improvement may occur over a few days or several months but it is less likely if symptoms have been present for long. The biochemical abnormalities always resolve. In patients with RFVT3 defects, the usual dose is 10 mg/kg/day but higher doses are used for RFVT2 defects (up to 50 mg/kg/day, with a maximum of 1.5 g/day) [49].

12.7.2 RFVT1 Deficiency

This has been implicated in a *transient neonatal* fatty acid oxidation defect. The baby presented within 24 hours of birth with hypoglycaemia, hyperammonaemia and organic aciduria typical of MAD deficiency. The abnormalities resolved with riboflavin treatment and did not recur when this was withdrawn. The mother had riboflavin deficiency and a heterozygous mutation in *SLC52A1*, which encodes the RFVT1 riboflavin transporter [50]. RFVT1 is expressed in placenta as well as small intestine and haploinsufficiency may have caused severe riboflavin deficiency in the baby at birth.

12.7.3 FAD Synthase and Mitochondrial FAD Transporter Deficiencies

These disorders are rare causes of MAD deficiency, resembling ETF and ETFQO deficiencies and generally presenting with myopathy. Seven families with FAD synthase deficiency and one with mitochondrial FAD transporter deficiency have been identified. The clinical spectrum for FAD synthase deficiency has ranged from neonatal-onset myopathy or cardiomyopathy to adult-onset weakness; problems have included dysphagia, dysarthria, scoliosis, respiratory failure and arrhythmias [51]. The FAD transporter defect presented with exercise intolerance in late childhood [52]. In both disorders, the plasma acylcarnitines and urine organic acids were typical of MAD deficiency. Homozygous or compound heterozygous mutations were found in *FLAD1* (the gene for FAD synthase) or *SLC25A32* (which encodes the mitochondrial FAD transporter). Treatment with riboflavin led to clinical and biochemical improvement in the patient with the FAD transporter defect and in some patients with FAD synthase deficiency but other patients did not respond and many died in infancy.

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Disorders of Ketogenesis and Ketolysis

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Ketogenesis and Ketolysis

During fasting, ketone bodies (KB) are an important fuel for many tissues, including cardiac and skeletal muscle. They are particularly important for the brain, which cannot oxidise fatty acids. The principal KB, acetoacetate and 3-hydroxybutyrate, are maintained in equilibrium by 3-hydroxybutyrate dehydrogenase; acetone is formed from acetoacetate non-enzymatically and eliminated in breath. KB are formed in liver mitochondria, predominantly from fatty acids, but also from certain amino acids, such as leucine. KB can cross cell membranes (to leave hepatocytes and enter target cells) by diffusion or facilitated by monocarboxylate transporter 1 (MCT1); the latter appears to be important during catabolic stress, when the flux is much greater. For use as fuel, KB are converted to acetyl-CoA in the mitochondria of extrahepatic tissues. One of the ketolytic enzymes, mitochondrial acetoacetyl-CoA thiolase (also known as β-ketothiolase or T2), is also involved in the breakdown of isoleucine (Fig. 13.1).

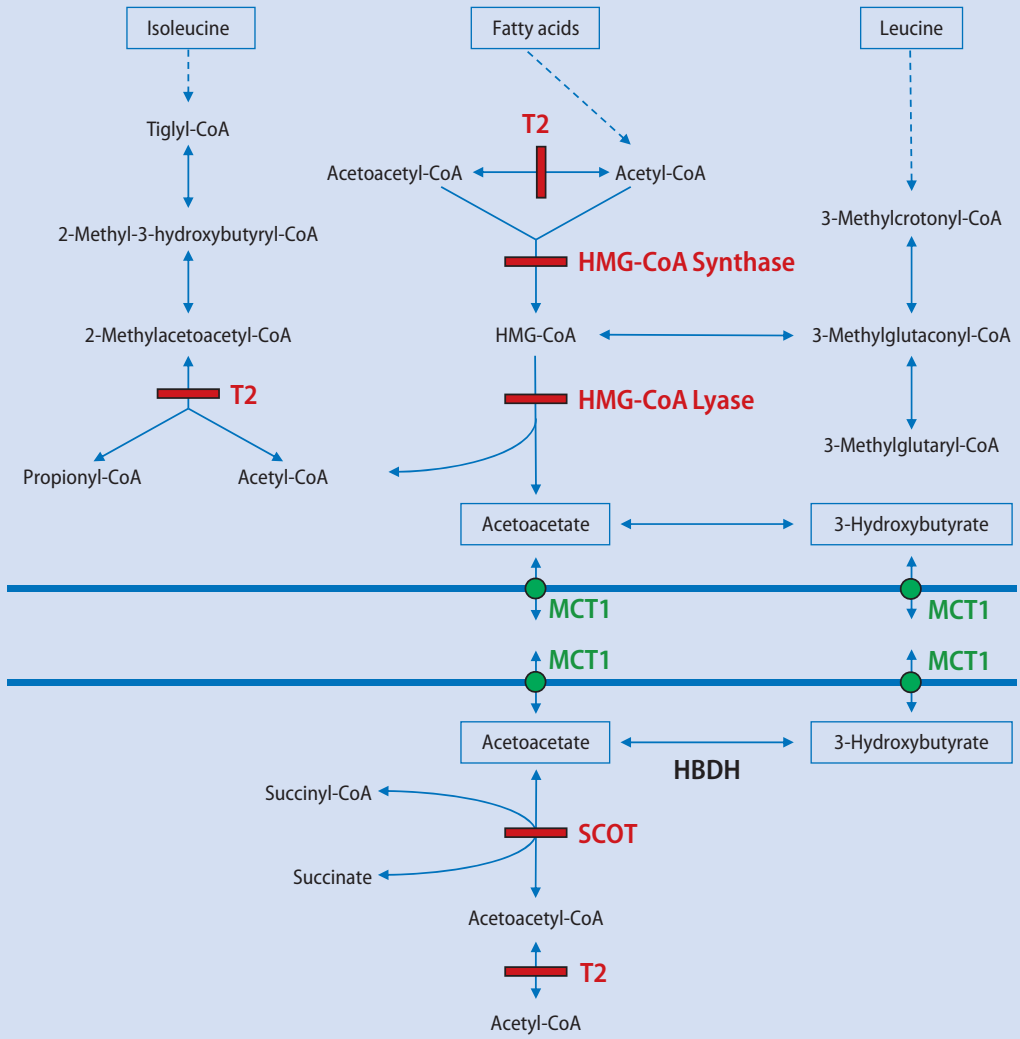

Fig. 13.1 Biochemical pathways involving enzymes of ketogenesis and ketolysis. *HMG-CoA*, 3-hydroxy-3-methylglutaryl coenzyme A; *SCOT*, succinyl-CoA 3-oxoacid CoA transferase; *T2*, mitochondrial acetoacetyl-CoA thiolase; *HBDH*, 3-hydroxybutyrate dehydrogenase; *MCT1*, monocarboxylate transporter 1. The thick blue lines represent the cell membranes of hepatocytes and of extrahepatic tissues that consume ketone bodies. Mitochondrial membranes are not shown. The enzyme defects discussed in this chapter are depicted by red bars across the arrows

Normal ketone body metabolism is described in ► Ketogenesis and Ketolysis and ◘ Fig. 13.1. Disorders of ketone body metabolism present either in the first few days of life or later in childhood, during an infection or some other metabolic stress.
There are two defects of ketogenesis, 3-hydroxy-3-methylglutaryl (HMG)-CoA lyase deficiency and HMG-CoA synthase deficiency. In these, decompensation leads to encephalopathy, with vomiting and a reduced level of consciousness, often accompanied by hepatomegaly. The biochemical features – hypoketotic hypoglycaemia, with or without hyperammonaemia – resemble those seen in fatty acid oxidation disorders. The organic acids are diagnostic in HMG-CoA lyase deficiency. In HMG-CoA synthase deficiency, the organic acids are characteristic during decompensation but normal at other times.
Ketone body utilisation is catalysed by succinyl-CoA:3-oxoacid CoA transferase (SCOT) and mitochondrial acetoacetyl-CoA thiolase (T2). Deficiencies of SCOT, T2 or the monocarboxylate transporter 1 (MCT1) present with episodes of ketoacidosis. This is often accompanied by dehydration and decreased consciousness. The organic acids usually show characteristic abnormalities in T2 deficiency but there are no specific findings in SCOT or MCT1 deficiencies and diagnosis relies on molecular analysis.
In all these disorders, the primary aim of treatment is to prevent decompensation. Fasting is avoided and a high glucose intake is maintained at times of metabolic stress, such as infections.
This chapter also briefly discusses the use of ketogenic diets in inherited metabolic disease.

13.1 Ketogenesis Defects

13.1.1 Clinical Presentation

- **Mitochondrial 3-Hydroxy-3-Methylglutaryl-CoA (HMG-CoA) Synthase Deficiency**

HMG-CoA synthase deficiency presents with hypoglycaemia, often accompanied by coma and metabolic acidosis [1]. Episodes are precipitated by infections with vomiting or poor feeding in early childhood (5 months to 6 years of age). There is usually hepatomegaly, which subsequently resolves. Hyperammonaemia is rare but (surprisingly) ketonuria may be present [2]. Most patients recover with intravenous glucose.

- **HMG-CoA Lyase Deficiency**

HMG-CoA lyase deficiency also presents with hypoglycaemia, metabolic acidosis, vomiting and a reduced level of consciousness. In 30% patients, the onset is within 5 days of birth, after a short symptom-free period [1]. In most others, symptoms are provoked by an infection in the first year. A few patients first present later, occasionally as adults [3].

KB levels are inappropriately low but blood lactate concentrations may be markedly elevated, particularly in neonatal onset cases. Patients often have hyperammonaemia, hepatomegaly and abnormal liver function tests and may develop pancreatitis or cardiomyopathy [4]. Most patients recover but many suffer neurological sequelae, including epilepsy, intellectual handicap, hemiplegia or cerebral visual loss, particularly after neonatal hypoglycaemia [5].

In asymptomatic patients, magnetic resonance imaging (MRI) shows diffuse mild signal changes in the cerebral white matter on T_2-weighted images with multiple foci of more severe signal abnormality; the combination is unique to this disorder [5]. There may also be abnormalities in the posterior limb of the internal capsule, the dentate nucleus and the pontine tegmentum. The cause of the changes is unknown; myelination may be impaired because KB are a substrate for the synthesis of myelin cholesterol. In patients with neurological damage, there may be additional abnormalities, for example in the basal ganglia and occipital lobes.

13.1.2 Metabolic Derangement

KB are synthesised in hepatic mitochondria, primarily using acetyl-CoA derived from fatty acid oxidation (◘ Fig. 13.1). HMG-CoA synthase catalyses the condensation of acetoacetyl-CoA and acetyl-CoA to form HMG-CoA, which is cleaved by HMG-CoA lyase to release acetyl-CoA and acetoacetate. HMG-CoA can also be derived from the amino acid leucine. Thus, HMG-CoA synthase and HMG-CoA lyase deficiencies both impair ketogenesis but HMG-CoA lyase deficiency also causes the accumulation of intermediates of the leucine catabolic pathway. During fasting, the lack of KB leads to excessive glucose consumption and hypoglycaemia.

13.1.3 Genetics

HMG-CoA synthase and HMG-CoA lyase deficiencies are inherited as autosomal recessive traits caused by homozygous or compound heterozygous mutations in *HMGCS2* and *HMGCL* respectively. HMG-CoA lyase deficiency is relatively common in Saudi Arabia, associated with the c.122G>A mutation in *HMGCL*; there is also a prevalent mutations in the Iberian peninsula (c.109G>A) [6]. The genotype correlates poorly with the clinical phenotype, which depends on exposure to environmental stress [6].

13.1.4 Diagnostic Tests

Samples collected during an episode of hypoglycaemia can be very valuable in disorders of KB metabolism. If the plasma free fatty acid concentration is raised with an inappropriately small rise in total KB (FFA/total KB >2.5) it implies a defect of ketogenesis or fatty acid oxidation [1]. These can be distinguished by analysing metabolites or measuring fatty acid oxidation flux in vitro.

- **HMG-CoA Synthase Deficiency**

During decompensation, urine contains saturated, unsaturated and 3-hydroxy-dicarboxylic acids, 5-hydroxyhexanoic acid

and other metabolites, of which 4-hydroxy-6-methyl-2-pyrone is the most specific [2]. Blood acylcarnitine analysis is normal when patients are well but acetylcarnitine may be raised during illness. The diagnosis is confirmed by mutation analysis. Enzyme assays require a liver biopsy and are complicated by a cytoplasmic isoenzyme, involved in cholesterol synthesis.

▪ HMG-CoA Lyase Deficiency

Even when healthy, patients excrete increased quantities of 3-hydroxy-3-methylglutaric, 3-hydroxyisovaleric, 3-methylglutaconic and 3-methylglutaric acids (Fig. 13.1); 3-methylcrotonylglycine may also be present. Blood acylcarnitine analysis shows raised 3-hydroxyisovalerylcarnitine concentrations. The diagnosis is confirmed by mutation analysis or measuring HMG-CoA lyase activity in leukocytes or cultured fibroblasts.

HMG-CoA lyase deficiency is included in the newborn screening programs for several countries, including the USA. Cases need to be distinguished from other causes of increased C5-hydroxyacylcarnitines (3-hydroxyisovalerylcarnitine or 2-methyl-3-hydroxybutyrylcarnitine, which have the same mass): 3-methylcrotonyl-CoA carboxylase deficiency (in the infant or mother), T2 deficiency, 2-methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency, multiple carboxylase deficiency (2 disorders) and various disorders associated with 3-methylglutaconic aciduria (▶ Chapter 18 and ▶ Chapter 26). Confirmatory tests include urine organic acid analysis (for the infant and mother), plasma acylcarnitine analysis and serum biotinidase assay. The American College of Medical Genetics has guidelines for the management of this and other positive screening tests (https://www.acmg.net/ACMG/Publications/ACT_Sheets_and_Confirmatory_Algorithms).

▪ Prenatal Diagnosis

Molecular techniques are used in families where the mutations are known. For HMG-CoA lyase deficiency, enzyme assays can be performed on chorionic villi or cultured amniocytes.

13.1.5 Treatment and Prognosis

Patients should avoid fasting and maintain a high carbohydrate intake during any metabolic stress, such as infections. An intravenous infusion of glucose is required if drinks containing glucose or glucose polymers are refused or vomited. Intravenous sodium bicarbonate may be needed if there is severe acidosis in HMG-CoA lyase deficiency.

A moderate protein restriction is usually recommended in HMG-CoA lyase deficiency because of its role in leucine catabolism [1]. There is less agreement about the need for a low fat diet [7]. Indeed, some patients have developed normally without any dietary restriction. Carnitine supplements are often given, though their value is unproven.

HMG-CoA synthase deficiency has a good prognosis after the presenting illness: most patients have no further episodes of encephalopathy. Neurological problems are commoner in HMG-CoA lyase deficiency, particularly in neonatal-onset cases. These patients are also more likely to have recurrent encephalopathy as older children or adults. Pregnancy carries a high risk in HMG-CoA lyase deficiency: the four reported pregnancies ended with two intrauterine deaths and one maternal death [8]. Pregnant women with either defect should be given intravenous glucose during labour and during illnesses with vomiting.

13.2 Defects of Ketone Body Utilization or Transport

Defects of ketone body utilization or transport include succinyl-CoA:3-oxoacid CoA transferase (SCOT), mitochondrial acetoacetyl-CoA thiolase (T2) and monocarboxylate transporter 1 (MCT1) deficiencies.

13.2.1 Clinical Presentation

Patients present with episodes of severe ketoacidosis in early childhood. Patients are healthy between episodes, with a normal blood pH. Decompensation is generally triggered by fasting or an infection with poor feeding and vomiting. Tachypnoea, due to acidosis, is accompanied by dehydration, due to vomiting and an osmotic diuresis; consciousness may be reduced if the acidosis is severe. Blood glucose, lactate and ammonia concentrations are normal in most cases but there may be hypo- or hyperglycaemia [9]. The high acetoacetate levels in blood and urine can cause screening tests for salicylate to give false positive results. A few patients have seizures or cardiomegaly at the time of presentation.

Approaching half of patients with SCOT deficiency become symptomatic within a few days of birth, the others presenting within the first two years [1]. Neonatal onset is rare in patients with T2 deficiency; most present during the first two years but some remain asymptomatic into adulthood [9]. Only three patients with homozygous MCT1 deficiency have been reported as yet [10]. Their presentations were indistinguishable from ketolysis defects and occurred by two years of age.

Most patients make a full recovery following episodes of acidosis but a few die and some have mental retardation, ataxia or dystonia [9]. Neuroimaging has shown abnormalities in the basal ganglia in a number of patients with T2 deficiency, sometimes without any preceding episodes of acidosis [11] [12]. The three initial patients with homozygous MCT1 deficiency all had intellectual impairment; it is not clear if this was due to the episodes of acidosis [10].

Ketoacidosis has been reported in some patients with heterozygous mutations in *SLC16A1*, the gene for MCT1 [10] [13]. The episodes have been less severe than in homozygotes and have occurred at an older age. Heterozygous *SLC16A1* mutations have also been associated with exercise-induced hyperinsulinism [14] or muscle injury [15].

13.2.2 Metabolic Derangement

KB utilisation occurs in extrahepatic mitochondria, starting with the transfer of coenzyme A from succinyl-CoA to acetoacetate, catalysed by SCOT. This forms acetoacetyl-CoA, which is converted to acetyl-CoA by T2. The second reaction can also be catalysed to some extent by medium-chain 3-ketoacyl-CoA thiolase (T1), which may explain why T2 deficient patients do not have permanent ketosis (unlike those with severe SCOT deficiency). SCOT is not expressed in liver and has no role other than ketolysis. In contrast, T2 is expressed in liver, where it participates in ketogenesis. Patients with T2 deficiency present with ketoacidosis, implying that the enzyme is more crucial in ketolysis than in ketogenesis. T2 also cleaves 2-methylacetoacetyl-CoA in the isoleucine degradation pathway and T2 deficiency causes the accumulation of isoleucine-derived acyl-CoA esters: these may be responsible for the neurodevelopmental abnormalities observed in some patients who have not had episodes of acidosis.

The occurrence of ketoacidosis in patients with MCT1 deficiency implies that these transporters are needed to facilitate the rapid entry of KB into target cells at times of stress. MCT1 transporters are also important for lactate transport and are expressed in the brain, particularly on oligodendroglia. Thus, the learning difficulties in MCT1 deficient patients may be due to the absence of MCT1 in the brain rather than the episodes of ketoacidosis.

13.2.3 Genetics

SCOT, T2 and MCT1 deficiencies are inherited as autosomal recessive traits with mutations in the *OXCT1*, *ACAT1* and *SLC16A1* genes, respectively. Heterozygous *SLC16A1* and *OXCT1* mutations have, however, been found in several patients investigated for ketoacidosis, suggesting that they can cause problems if subjects are exposed to sufficient stress [10][16]. Heterozygous *SLC16A1* mutations can also cause hyperinsulinism; these patients have promoter mutations that prevent the normal silencing of MCT1 expression in pancreatic β-cells (▶ Chapter 9) [14]. Apart from this, there is little genotype-phenotype correlation for these disorders. The frequency of ketoacidosis depends primarily on exposure to environmental stress [9].

13.2.4 Diagnostic Tests

SCOT & MCT1 Deficiencies

These conditions need to be considered in a number of patients because ketoacidosis is relatively common. A plasma free fatty acid : total KB ratio <0.3 suggests a defect of ketolysis [1]. Urine organic acid analysis reveals high concentrations of KB but no specific abnormalities. Patients with severe SCOT deficiency have persistent ketonuria in the fed state, but patients with a mild mutation do not [1]. The diagnoses are now usually made by mutation analysis, though SCOT enzyme assays can be undertaken on lymphocytes or cultured fibroblasts.

T2 Deficiency

Patients with T2 deficiency typically excrete increased amounts of 2-methylacetoacetate, 2-methyl-3-hydroxybutyric acid and tiglylglycine (◘ Fig. 13.1). However, 2-methylacetoacetate is unstable and patients with mild mutations may only show abnormalities when they are stressed [1][17], for example by an isoleucine load. 2-Methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency causes a similar pattern of organic acids but 2-methyacetoacetate is not excreted (▶ Chapter 18). The diagnosis must, therefore, be confirmed by mutation analysis or enzyme assay in fibroblasts. Assays are complicated by the presence of other thiolases that act on acetoacetyl-CoA. 2-Methylacetoacetyl-CoA is a specific substrate for T2 but it is difficult to synthesise. One solution is to repeat the assay in the presence and absence of potassium, which enhances the activity of T2 but not the other enzymes.

Blood acylcarnitine analysis in T2 deficiency generally shows raised 2-methyl-3-hydroxybutyrylcarnitine and tiglylcarnitine but the concentrations may be normal in patients with mild mutations [1][17]. T2 deficiency is included in some expanded newborn screening programs but a few patients are missed and cases need to be distinguished from HMG-CoA lyase deficiency and other inborn errors (▶ Section 13.1.4).

Prenatal Diagnosis

Prenatal diagnosis is possible using molecular techniques in families where the mutations are known. Alternatively, prenatal diagnosis can be performed by enzyme assays in chorionic villi (T2) or cultured amniocytes (T2 or SCOT).

13.2.5 Treatment and Prognosis

These patients can decompensate rapidly in early childhood. To prevent this, fasting must be avoided and a high carbohydrate intake must be maintained during any metabolic stress, such as surgery or infection (▶ Chapter 4). Drinks containing carbohydrate should be started at the first sign of illness; hospital admission is needed if these are vomited or if the patient develops moderate or heavy ketonuria. In hospital, patients require an intravenous infusion of glucose. Dehydration is common and must be corrected. An intravenous infusion of sodium bicarbonate is needed if there is severe ketoacidosis (pH<7.1); it may be given in milder acidosis but electrolytes must be monitored frequently as there is a risk of severe and potentially fatal hypernatraemia.

Dietary protein restriction is often recommended, particularly in T2 deficiency as neurological problems have occurred without episodes of acidosis [9]. On the other hand, a number of patients have developed normally without dietary modification [17]. Ketogenic diets should be avoided but a low fat diet is not needed. Carnitine supplements are often given if serum levels are low.

Patients with these disorders can die or suffer irreversible neurological damage during episodes of ketoacidosis. Problems are less likely following diagnosis and when patients become older: ketoacidosis is rare after mid-childhood [9]. Uncomplicated pregnancies have been reported in patients with SCOT and T2 deficiencies [17] but intravenous glucose should be given during labour and during illnesses with vomiting.

13.3 Cytosolic Acetoacetyl-CoA Thiolase Deficiency

Cytosolic acetoacetyl-CoA thiolase (CAT) is primarily involved in the synthesis of isoprenoid compounds, such as cholesterol (▶ Chapter 32), rather than ketone body metabolism. CAT deficiency has been reported in two patients with mental retardation and persistent ketosis [18] but the diagnosis remains uncertain.

13.4 Ketogenic Diets

Diets that induce ketosis are an effective treatment for GLUT1 deficiency (▶ Chapter 10) and for some patients with drug-resistant epilepsy; there have also been uncontrolled reports of benefit in pyruvate dehydrogenase (PDH) deficiency (▶ Chapter 11). The mechanism of the anti-epileptic effect remains uncertain [19] but there is a clear rationale for ketogenic diets in GLUT1 and PDH deficiencies. GLUT1 deficiency reduces the entry of glucose into the brain but ketone bodies cross the blood brain barrier on MCT1 transporters and can act as an alternative fuel. PDH deficiency interferes with the oxidation of carbohydrates but not of ketone bodies, which can supply acetyl-CoA and energy to the brain.

During prolonged fasting, fatty acid oxidation generates large amounts of acetyl-CoA. In the liver, this cannot enter the citric acid cycle because gluconeogenesis has depleted the supply of oxaloacetate, and instead it generates ketone bodies. The same process can be induced by a diet that is high in fat and low in protein and carbohydrate. In the classical ketogenic diet, there is a 3:1 or 4:1 energy ratio of fat to carbohydrate plus protein. Expert dietary supervision and supplements of calcium, trace elements and vitamins are needed; potential side effects include poor growth, constipation, acidosis, hyperlipidaemia and renal stones [20]. Compliance with the classical diet often deteriorates in the long-term, particularly in older patients.

Ketosis can be induced by diets that contain less fat (approximately 70% energy) if the fat is predominantly medium-chain triglycerides (MCT). After a meal containing MCT, high concentrations of medium chain fatty acids reach the liver because they are transported in the portal vein, rather than lymph vessels. Moreover, medium chain fatty acids are oxidised rapidly within hepatocytes, because they can enter mitochondria independent of carnitine, leading to high acetyl-CoA concentrations and ketogenesis.

Older patients generally find it easier to adhere to the modified Atkins diet, in which fat is encouraged and carbohydrate is restricted (usually to 10 g/day initially) but protein and energy are unrestricted. Ketone body concentrations are lower on this diet than on the classical or MCT-based diets but it has been used successfully in epilepsy and GLUT1 deficiency [21]. A low glycaemic index diet may lead to mild ketosis and it has been used for epilepsy but it is not recommended for GLUT1 deficiency.

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Disorders of Oxidative Phosphorylation

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Respiratory Chain and Oxidative Phosphorylation System

The respiratory chain (complexes I–IV) and oxidative phosphorylation (OXPHOS) system (complexes I–V) are embedded in the inner mitochondrial membrane and are responsible for ATP production by aerobic metabolism (Fig. 14.1). Complex I (NADH-ubiquinone oxidoreductase) contains 44 different polypeptide subunits, seven of which are encoded by mitochondrial DNA (mtDNA). Complex II (succinate-ubiquinone oxidoreductase) has only four subunits, all encoded by nuclear genes. Complex II includes succinate dehydrogenase (SDH) which catalyses the oxidation of succinate to fumarate and is a component of the Krebs cycle. Complex III (ubiquinol-cytochrome c oxidoreductase) is composed of 11 subunits only one of which (cytochrome b) is encoded by mtDNA. Complex IV (cytochrome c oxidase, COX) comprises 14 polypeptides including 3 encoded by mtDNA. Complex V (ATP synthase or F_1F_0 ATPase) has 16 subunits, of which two (ATP 6 and 8) are encoded by mtDNA. Reducing equivalents generated by the oxidation of pyruvate, fatty acids and the Krebs cycle are transferred to the respiratory chain via NADH (reduced nicotinamide dinucleotide) and $FADH_2$ (reduced flavin adenine dinucleotide). Electrons derived from oxidation of pyruvate and fatty acids are transferred via NADH to complex I, whilst electrons from succinate in the Krebs cycle are transferred to complex II via $FADH_2$. The electron carriers in the respiratory chain are flavins, iron-sulphur (FeS) complexes, quinones and the haem groups of cytochromes (Fig. 14.2). Ubiquinone (Coenzyme Q_{10}, CoQ) and cytochrome *c* function as mobile carriers of electrons between OXPHOS complexes. From complexes I and II, electrons are transferred to ubiquinone and then to complex III, and via cytochrome *c* (cyt *c*) to cytochrome *c* oxidase (COX), before finally reducing molecular oxygen to water. The energy released during this sequential electron transfer is used to generate an electrochemical gradient, by translocating protons from the matrix to the intermembrane space. Protons are pumped at three coupling sites (complexes I, III and IV) and the resulting membrane potential (proton motive force, ~150 mV) is used by complex V (ATP synthase or F_1F_0 ATPase) to drive ATP synthesis from ADP and inorganic phosphate by free energy transduction. ATP synthase is an enzyme complex that can either hydrolyse or synthesise ATP, according to the energy status of the cell.

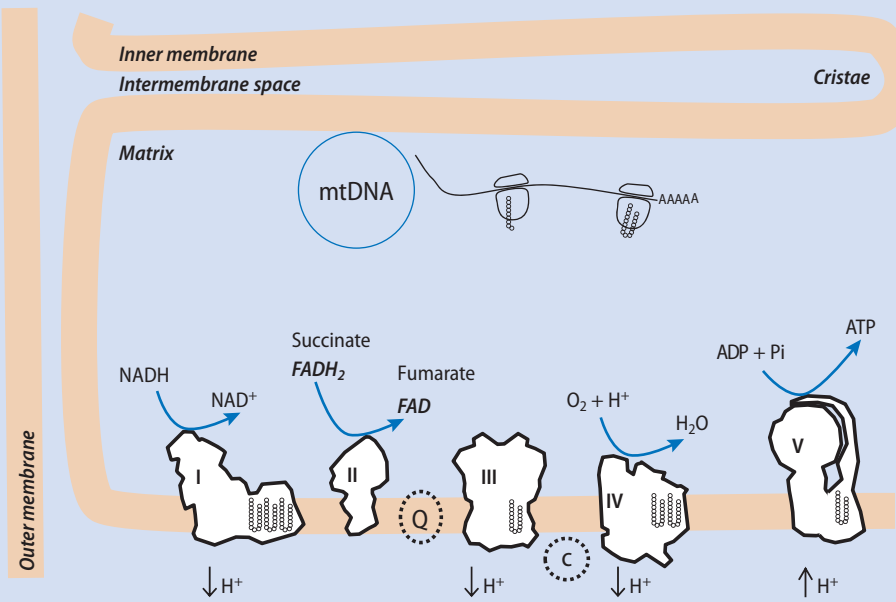

Fig. 14.1 Oxidative phosphorylation (OXPHOS) is the final part of aerobic energy metabolism. It takes place in mitochondria involving respiratory chain enzyme complex I (NADH dehydrogenase, I), complex II (succinate dehydrogenase), complex III (ubiquinol-cytochrome c oxidoreductase, III), complex IV (cytochrome *c* oxidase, IV), and the ATP synthase (complex V). These five enzymes contain multiple subunits, 13 subunits are encoded on the mitochondrial genome (mtDNA) and are produced by mitochondrial protein synthesis. Several cofactors including coenzyme Q_{10} (Q) and cytochrome *c* (*c*) are involved.

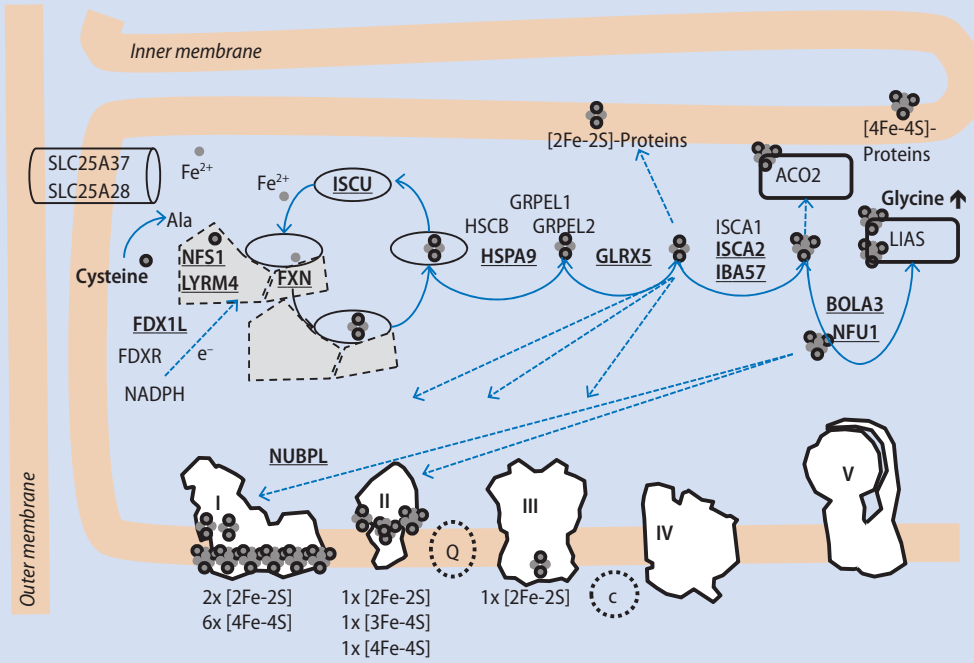

Fig. 14.2 The respiratory chain enzyme complexes I, II, and III depend on iron-sulphur (FeS) cluster cofactors, either [2Fe-2S], [3Fe-4S] or [4Fe-4S]. The formation of FeS clusters uses sulphur from cysteine, Fe^{2+}, NADPH, and ATP as substrates. A series of enzymatic steps are involved and mutations in 12 proteins (in bold and underlined) have been identified that cause human disease. Since FeS clusters are also required for lipoate synthesis, elevated glycine – due to glycine cleavage deficiency – can be indicative for FeS cluster defects. . *ISCU*, iron sulfur scaffold complex protein; *FXN*, frataxin; *ACO*, aconitase; *LIAS*, lipoic acid synthetase; *Ala*, alanine; *FDXR*, ferredoxin reductase; *HSP*, heat-shock protein; *ISCA,* Iron-sulfur cluster assembly.

Disorders of oxidative phosphorylation encompass heterogeneous infantile, childhood and adult onset diseases characterised by variable involvement of high energy requiring organs. The central and peripheral nervous systems, skeletal and cardiac muscle, eyes, ears, kidneys and liver are frequently involved. Some well-characterised mitochondrial syndromes are recognised, but many patients have overlapping features not corresponding to a specific syndrome. Theoretically any organ or tissue or combination of organs may be affected, with onset at any age. Owing to the involvement of two distinct genomes, the nuclear genome and that located within the mitochondrion itself, a range of modes of inheritance of mitochondrial disease has been observed: maternal (mitochondrial DNA), autosomal recessive, autosomal dominant, X-linked and sporadic. Disease mechanisms include mutations affecting OXPHOS subunits and assembly factors, and disorders of mitochondrial DNA maintenance, protein synthesis, cofactor biosynthesis and lipid metabolism. The complexity of underlying disease mechanisms, together with clinical, biochemical and genetic heterogeneity, creates enormous diagnostic challenges (► Respiratory chain and oxidative phosphorylation system). Most mitochondrial diseases, especially childhood-onset forms, are characterised by relentless progression. Specific treatments are available for some extremely rare forms of mitochondrial disease, but the majority of cases lack curative treatments, and the mainstay of treatment is supportive.

14.1 Clinical Presentation

Clinical diagnosis of mitochondrial disease is extremely challenging since the presence of mitochondria in virtually all cells (except mature erythrocytes) means that mitochondrial dysfunction can affect any organ or combination of organ systems. Moreover, clinical presentation can occur at any time, ranging from antenatal presentations (e.g. intrauterine growth retardation, birth defects) to isolated myopathy in the elderly. The spectrum of clinical features associated with mitochondrial disease is listed in Table 14.1.

Clinical suspicion of a mitochondrial disorder usually arises in one of three scenarios:

1. A constellation of symptoms and signs that falls within a recognised mitochondrial 'syndrome'.
2. A complex multisystem presentation involving two/more unrelated organ systems that can best be explained by an underlying disorder of energy generation.

Table 14.1 Clinical features of mitochondrial disease

Organ/tissue	Recognised symptoms/signs in mitochondrial diseases
Brain	Stroke-like episodes, seizures, ataxia, encephalopathy, hypotonia, spasticity, dystonia, extrapyramidal movement disorder, parkinsonism, developmental delay and/or regression, cognitive decline
Eye	Ptosis, progressive external ophthalmoplegia, cataracts, pigmentary retinopathy, optic neuropathy and atrophy
Ear	Sensorineural hearing loss (SNHL), auditory neuropathy
Heart	Hypertrophic cardiomyopathy, dilated cardiomyopathy, conduction defects (Wolff-Parkinson-White, complete heart block)
Lungs	Pulmonary hypertension
Kidney	Fanconi-type tubulopathy, nephritis (focal segmental glomerulosclerosis), steroid-resistant nephrotic syndrome
Liver	Acute hepatic dysfunction/failure, hepatomegaly, hypoglycemia
Gut	Enteropathy, dysmotility, pseudo-obstruction, pancreatic exocrine insufficiency
Endocrine organs	Diabetes mellitus, hypo/hyperthyroidism, growth hormone deficiency, hypoparathyroidism, adrenal insufficiency
Gonads	Primary ovarian failure, hypo/hypergonadotrophic hypogonadism
Bone marrow	Sideroblastic anaemia, neutropaenia, pancytopaenia, dyserythropoiesis
Skeletal muscle	Myopathy (often proximal, but may be distal or generalised), rhabdomyolysis
Peripheral nerve	Axonal sensorimotor or demyelinating neuropathy
Skin and hair	Hypertrichosis

3. The presence of lactic acidosis, or other investigation result leading to suspicion of a mitochondrial disease (e.g. characteristic neuro-imaging, 3-methylglutaconic aciduria, ragged red fibre myopathy).

In the 21st century, a fourth category should be added to this list, namely pathogenic mutations in a known mitochondrial disease gene revealed by a genome-wide next generation sequencing (NGS) approach, in a patient in whom clinical suspicion of a mitochondrial disorder was low prior to the genetic testing.

In the following, the clinical features of some of the more well-recognised mitochondrial syndromes are described, grouped by age at presentation. Other syndromic mitochondrial presentations are summarised in Table 14.2. However, it should be noted that many patients do not have clinical presentations fitting neatly into recognised syndromes, but instead have overlapping constellations of symptoms, so the reality is that mitochondrial disorders comprise hundreds of different clinical entities. Furthermore, the range of mitochondrial phenotypes is continuing to expand at a very rapid pace as more and more gene defects are being linked to mitochondrial dysfunction by NGS technologies.

14.1.1 Neonatal and Infantile Presentations

Congenital lactic acidosis typically presents in the newborn period with tachypnoea and sepsis-like nonspecific illness, and is a relatively frequent manifestation of mitochondrial disease [1]. Lactic acidosis may be associated with features of Leigh syndrome (see below) and/or multisystem involvement including cardiomyopathy or renal tubulopathy. Congenital lactic acidosis may be caused by deficiency of pyruvate dehydrogenase, pyruvate carboxylase and biotinidase, as well as isolated or combined OXPHOS deficiencies. Lactate elevation may also be observed in patients with Krebs cycle enzyme deficiencies, long chain fatty acid oxidation defects and organic acidurias (▶ Chapter 1).

Leigh syndrome is the most frequent clinical presentation of mitochondrial disease in childhood. It was originally a neuropathological diagnosis, consisting of bilateral symmetrical focal lesions in the basal ganglia and brainstem characterised by spongiform change, vacuolation of the neuropil, demyelination, gliosis, necrosis, capillary proliferation and relative preservation of neurons. However since neuropathology is rarely performed nowadays, diagnosis is currently usually based on a characteristic clinical history associated with typical brain magnetic resonance imaging (MRI) features (T2-weighted bilateral symmetrical hyperintense lesions affecting the basal ganglia and/or brainstem) and compatible biochemical findings (lactate elevation in blood and/or cerebrospinal fluid and/or documented respiratory chain enzyme deficiency) [2]. Onset is typically in infancy or early childhood with neurodevelopmental regression following an intercurrent viral illness (which may be mild) or other metabolic stress. There is frequently a preceding history of feeding difficulties and vomiting. Neurological findings include bouts of hyper- or hypo-

Table 14.2 Recognised mitochondrial syndromes

Syndrome	Clinical features	Associated gene defect(s)
ADOA	Optic atrophy type 1: progressive vision loss starting in first decade, variably associated with SNHL	*OPA1* (AD)
Alpers-Huttenlocher	Infantile/early childhood onset of developmental delay/regression, intractable epilepsy, +/- liver failure	*POLG*, *FARS2*, *NARS2*, *PARS2* (AR)
Amish lethal microcephaly	Severe microcephaly, micrognathia, hepatomegaly	*SLC25A19* (AR)
ARSACS	Autosomal recessive spastic ataxia of Charlevoix-Saguenay: spasticity, ataxia, muscle wasting, nystagmus, dysarthria	*SACS* (AR)
Ataxia neuropathy spectrum	Ataxia, epilepsy, cognitive impairment, psychiatric symptoms, eye movement disorders, involuntary movements, peripheral neuropathy	*POLG*, *C10orf2*, *OPA1* (AR)
Barth	Cardiomyopathy, skeletal myopathy, short stature, (cyclical) neutropaenia	*TAZ* (X-linked)
Bjornstad	Pili torti, congenital SNHL	*BCS1L* (AR)
Cowchock	Early childhood onset slowly progressive axonal sensorimotor neuropathy +/– SNHL and learning difficulties	*AIFM1*, *COX7B* (X-linked)
DCMA	Dilated cardiomyopathy with ataxia syndrome	*DNAJC19* (AR)
Ethylmalonic encephalopathy	Hypotonia, seizures, abnormal movements, petechiae, acrocyanosis, chronic diarrhoea	*ETHE1* (AR)
GRACILE	Growth retardation, aminoaciduria, cholestasis, iron overload, lactic acidosis, early death	*BCS1L* (AR)
HUPRA	Hyperuricaemia, pulmonary hypertension, renal failure, alkalosis	*SARS2* (AR)
Kearns-Sayre syndrome (KSS)	Onset <20y of progressive external ophthalmoplegia, pigmentary retinopathy, cardiac conduction defects, ataxia, high CSF protein	mtDNA deletion (sporadic)
LBSL	Leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation: movement disorder starting in childhood/adolescence, spasticity, ataxia	*DARS2* (AR)
Leigh	Subacute necrotising encephalomyelopathy: infantile/early childhood onset of vomiting/feeding difficulties, stepwise neurodevelopmental regression following infection/metabolic stress, dysphagia, hypotonia, dystonia, ataxia, ophthalmoparesis, nystagmus, optic atrophy; bilateral symmetrical MRI lesions involving basal ganglia and/or brainstem	>75 genes (maternal, AR, X-linked)
LHON	Leber hereditary optic neuropathy: subacute painless vision loss affecting both eyes sequentially, onset teens/20s, male preponderance	mtDNA point mutations (maternal)
LSFC	Leigh syndrome French Canadian variant: hypotonia, developmental delay, mild facial dysmorphism, chronic well-compensated metabolic acidosis, episodes of severe acidosis and coma associated with high mortality	*LRPPRC* (AR)
LTBL	Leukoencephalopathy with thalamus and brainstem involvement and high lactate: infantile onset hypotonia, developmental delay/regression, characteristic MRI brain changes, some cases have milder course with improvement after 2y	*EARS2* (AR)
MDDS	Mitochondrial DNA depletion syndromes (hepatocerebral, myopathic and encephalopathic variants)	*POLG*, *DGUOK*, *MPV17*, *C10orf2*, *TK2*, *SUCLA2*, *SUCLG1*, *RRM2B*, *MGME1* (AR)
MEGDEL	3-methylglutaconic aciduria, deafness (SNHL), encephalopathy, Leigh-like disease: hypotonia, feeding difficulties and hepatopathy in infancy, later dystonia and spasticity	*SERAC1* (AR)

Table 14.2 (continued)

Syndrome	Clinical features	Associated gene defect(s)
MELAS	Mitochondrial encephalomyopathy, lactic acidosis, stroke-like episodes: childhood-onset muscle weakness, migraine headache, vomiting and seizures; stroke-like episodes before 40y (seizures, altered consciousness, hemiparesis, hemianopia); cognitive decline	*MT-TL1* m.32423A>G (80% cases) and other mtDNA point mutations (maternal)
MEMSA	Myoclonic epilepsy, myopathy, sensory ataxia	*POLG* (AR)
MERRF	Myoclonic epilepsy with ragged-red fibres: myoclonus, myopathy, spasticity, epilepsy, ataxia, peripheral neuropathy, cognitive decline, multiple symmetrical lipomata	*MT-TK* and other mtDNA point mutations (maternal), *POLG* (AR)
MIDD	Maternally inherited diabetes and deafness: adult onset SNHL, insulin-dependent diabetes mellitus, macular retinal dystrophy	*MT-TL1* m.32423A>G (maternal)
MIRAS	Mitochondrial Recessive Ataxia Syndrome: Ataxia neuropathy spectrum	*POLG* (AR)
MLASA	Myopathy, lactic acidosis, sideroblastic anaemia	*PUS1*, *YARS2* (AR)
MNGIE	Mitochondrial neurogastrointestinal encephalopathy: adolescent/early adult onset of gastrointestinal dysmotility, peripheral neuropathy, leukoencephalopathy	*TYMP* (AR), may be mimicked by *POLG, RRM2B* (AR) and *MT-TL1* and *MT-TV* (maternal)
Mohr-Tranebjærg	Deafness (SNHL), dystonia, optic neuronopathy (DDON), cognitive decline, psychiatric symptoms	*TIMM8A* (X-linked)
NARP	Neuropathy, ataxia, and retinitis pigmentosa; childhood onset sensory neuropathy, muscle weakness, learning difficulties, visual impairment	*MT-ATP6* (maternal)
PCH6	Pontocerebellar hypoplasia type 6: neonatal onset seizures, hypotonia, severe lactic acidosis at birth (later resolves), progressive microcephaly, developmental stasis from birth	*RARS2* (AR)
Pearson	Infantile onset transfusion-dependent sideroblastic anaemia (later recovers), variably associated with neutropaenia and thrombocytopaenia, exocrine/endocrine pancreatic failure, severe lactic acidosis and hepatic impairment; those who survive early childhood subsequently develop KSS	mtDNA deletion (sporadic)
PEO	Progressive external ophthalmoplegia +/- skeletal myopathy	mtDNA deletion (sporadic), mtDNA point mutations (maternal), *POLG*, *C10orf2*, *RRM2B*, *SLC25A4* (AD)
Perrault	Premature ovarian failure, SNHL	*C10orf2*, *CLPP*, *HARS2*, *LARS2*, *HSD17B4* (AR)
RIRCD	Reversible infantile respiratory chain deficiency: 'Benign reversible' mitochondrial myopathy causing hypotonia, severe muscle weakness leading to feeding difficulties or respiratory failure, recovery by 12–18 months or	*MT-TE* (maternal inheritance, incomplete penetrance)
	Acute liver failure	*TRMU* (AR)
SANDO	Sensory ataxia, neuropathy, dysarthria, ophthalmoplegia: see Ataxia neuropathy spectrum	*POLG*, *C10orf2*, *OPA1* (AR)
SCAE	Spinocerebellar ataxia with epilepsy: see MEMSA	*POLG* (AR)
Sengers	Congenital cataract, hypertrophic cardiomyopathy, muscle weakness, lactic acidosis	*AGK* (AR)
Wolfram	Diabetes insipidus, diabetes mellitus, optic atrophy, deafness (DIDMOAD)	*WFS1* (AR)

ventilation, hypotonia, spasticity, dystonia, ataxia, tremor, ophthalmoparesis and optic atrophy. Multisystem involvement may include cardiomyopathy, renal tubulopathy and gastrointestinal dysfunction (vomiting, diarrhoea, constipation, faltering growth). Periods of stability are interspersed by episodes of further neurodevelopmental regression, often without obvious triggers. Progressive brainstem involvement eventually leads to death from central respiratory failure. Late onset may occur, including in adulthood in rare cases. Leigh syndrome is biochemically and genetically heterogeneous and can be caused by mutations in more than 75 genes (on two genomes, mitochondrial and nuclear) encoding mitochondrial proteins [3]. One of the most recently described forms of Leigh syndrome is caused by *ECHS1* mutations affecting valine metabolism [4] (▶ Chapter 18). Some geographical isolates may allow a more rapid diagnosis in some communities, such as the Leigh syndrome French Canadian variant originating from the Saguenay-Lac-Saint-Jean region of Quebec [5].

3-Methylglutaconic aciduria, deafness, encephalopathy and Leigh-like disease (MEGDEL) is a form of Leigh syndrome caused by recessive *SERAC1* mutations leading to defective phosphatidylglycerol remodelling in the mitochondrial membrane [6]. A full description is given in ▶ Chapter 34.

The **Pearson marrow-pancreas syndrome** typically presents shortly after birth with lactic acidosis and a severe transfusion-dependent sideroblastic anaemia, variably associated with neutropaenia and/or thrombocytopaenia. Transfusion requirement usually resolves by 2 years of age, reflecting clearance of the responsible large-scale mitochondrial DNA (mtDNA) deletion from rapidly dividing blood cells. There is a 'common' ~4.9kb heteroplasmic mtDNA deletion but many other mtDNA deletion species have been reported. A high mortality in the first 5 years of life is related to liver failure and/or overwhelming acidosis. Those who survive have a progressive multisystem disease course (associated with accumulation of mtDNA deletions in non dividing tissues), including renal tubulopathy (leading to severe electrolyte losses and sometimes progressing to end-stage kidney disease), cardiomyopathy, cardiac conduction defects (complete heart block), pancreatic exocrine and/or endocrine insufficiency, hypothyroidism, hypoparathyroidism and adrenal insufficiency, and eventually develop the neurological features of Kearns-Sayre syndrome (see below) [7].

The **mitochondrial DNA depletion syndromes** (**MDDS**) are a group of encephalomyopathic, myopathic and hepatocerebral syndromes which usually present in infancy or early childhood and in most cases are rapidly progressive, leading to death in infancy or childhood. These are autosomal recessive disorders of mtDNA maintenance caused by mutations in genes involved directly in mtDNA replication or in mitochondrial nucleoside salvage, resulting in progressive mtDNA depletion in affected tissues [8]. Infants with **hepatocerebral MDDS** (caused by mutations in *DGUOK* (▶ Chapter 35), *POLG*, *C10orf2, MPV17* or *SUCLG1*) present with hepatic dysfunction manifesting as persistent vomiting, hypoglycaemia and sometimes hepatomegaly, with associated lactic acidosis. Neurological involvement may present as roving eye movements or developmental delay and/or regression. Alpers-Huttenlocher syndrome (see below) is a form of hepatocerebral MDDS. **Myopathic MDDS** (caused by *TK2* mutations leading to thymidine kinase deficiency, ▶ Chapter 35) presents in infancy with hypotonia and muscle weakness, often with involvement of the bulbar musculature leading to feeding difficulties. Affected infants also have lactic acidosis and markedly elevated creatine kinase. Death from respiratory failure occurs in early childhood owing to progressive respiratory muscle weakness, although survival to teenage years has been reported. **Encephalomyopathic** MDDS (caused by mutations in *SUCLA2*, *SUCLG1*, *RRM2B*, *ABAT* or *MGME1*) presents with global developmental delay, hypotonia and muscle weakness in infancy, variably associated with sensorineural hearing loss (SNHL), dystonia, Leigh-like MRI lesions and methylmalonic aciduria (*SUCLA2*), fatal infantile lactic acidosis with methylmalonic aciduria (*SUCLG1*) (▶ Chapter 18), or prominent renal involvement (*RRM2B*).

Alpers-Huttenlocher syndrome (progressive neuronal degeneration of childhood with epilepsy, PNDE) is a form of hepatocerebral MDDS usually caused by recessive *POLG* mutations [9], and rarely by mutations in the mitochondrial phenylalanyl-, asparaginyl- and prolyl-tRNA synthetases encoded by *FARS2, NARS2* and *PARS2* respectively [10][11]. There is a classical clinical triad of intractable seizures often resistant to multiple antiepileptic drugs, developmental regression and (terminally) liver failure. Seizures may be focal, multifocal or generalised. Disease progression may be rapid, with intractable seizures or liver failure leading to death within weeks or months, or may follow a more indolent course. Rarely there may be no further progression for several years. Liver failure may be triggered by sodium valproate treatment, or occur spontaneously without exposure to valproate. EEG in the early stages of disease may be pathognomonic, showing unilateral occipital rhythmic high-amplitude delta with superimposed (poly)spikes (RHADS) [12].

Reversible infantile respiratory chain deficiency (RIRCD) may present as a myopathy or a hepatopathy. Infants with so-called 'benign reversible' mitochondrial myopathy develop a rapidly progressive myopathy associated with hypotonia, profound muscle weakness and severe lactic acidosis at a few weeks of age. Nasogastric or gastrostomy feeding is usually required, and some affected infants need ventilatory support for up to 12-18 months. Gradual recovery starts from ~6 months. Two maternally inherited homoplasmic mtDNA point mutations, m.14674T>C/G in the *MT-TE* gene, have been linked to benign reversible mitochondrial myopathy [13]. In other infants transient acute liver failure is caused by recessive mutations in *TRMU*, encoding an enzyme responsible for 2-thiolation of uridine on the wobble positions of the mitochondrial tRNAs for lysine, glutamate and glutamine, an essential post-transcriptional modification needed for accurate and efficient synthesis of the 13 mtDNA-encoded OXPHOS proteins [14]. Spontaneous recovery has been noted in some infants with *TRMU* mutations following supportive care, but others need liver transplantation. The underlying molecular mechanisms responsible for spontaneous remission of RIRCD have not yet been unravelled.

Infantile onset coenzyme Q_{10} (CoQ_{10}) biosynthetic defects typically present with multisystem disease including a rapidly progressive nephropathy (frequently progressing to end-stage kidney disease) variably associated with SNHL, optic atrophy, ataxia, dystonia, weakness and stroke-like episodes [15]. Other children with CoQ_{10} deficiency may present with steroid-resistant nephrotic syndrome, either in isolation or associated with seizures and/or learning difficulties. Primary CoQ_{10} deficiency is clinically heterogeneous and other reported phenotypes in later childhood include encephalomyopathy with seizures and recurrent myoglobinuria, cerebellar ataxia and isolated myopathy. Prompt diagnosis and treatment of disorders of CoQ_{10} biosynthesis with high-dose exogenous CoQ_{10} supplementation may result in a good outcome.

Barth syndrome [16] and **Sengers syndrome** [17] are two **syndromic cardiomyopathies** presenting in infancy with methylglutaconic aciduria. Both are fully described in ▶ Chapter 34.

■ Isolated organ involvement

Not all infants with mitochondrial disease present with classical syndromes or multisystem disease. Some have isolated organ involvement, e.g. epileptic encephalopathy [18], hypertrophic cardiomyopathy [19] or acute liver failure [20]. Patients with mutations in *LARS* (encoding a *cytoplasmic* leucyl-tRNA synthetase) also present with acute liver failure in the first few months of life, which may be mistaken for mitochondrial disease. Additional symptoms include anaemia, renal tubulopathy, developmental delay, seizures, failure to thrive and deterioration of liver function with minor illness [21].

14.1.2 Presentation in Childhood and Adolescence

Kearns-Sayre syndrome (**KSS**) is usually sporadic and caused by single large-scale rearrangements of mtDNA (most commonly the ≈4.9kb 'common' deletion that is also frequently seen in Pearson syndrome) [7], but may occasionally be autosomal recessive when it is caused by nuclear-encoded defects of mtDNA maintenance (e.g. *RRM2B* mutations) associated with multiple mtDNA deletions [22]. Clinically there is a triad of progressive external ophthalmoplegia (PEO), pigmentary retinopathy and onset <20 years, with at least one of the following: heart block, cerebellar ataxia or raised cerebrospinal fluid protein (>1 g/l). Multisystem disease manifestations include cardiac conduction defects, short stature, renal tubulopathy, dysphagia, gastrointestinal dysmotility, pancreatitis, diabetes mellitus, sensorineural hearing loss and cognitive deficits (learning difficulties or dementia) [7].

Mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (**MELAS**) is a maternally inherited multisystem disorder usually manifesting in mid-late childhood [23]. Most (≈80%) cases have the common m.3243A>G mtDNA point mutation in *MT-TL1*, although most individuals with this mutation (which is present in ≈1 in 400 of the White European population) never develop symptoms of MELAS [24]. Stroke-like episodes typically first occur in late childhood or adolescence, but may commence in adult life. Migraine-like headache with vomiting and seizures may herald the stroke-like episodes, which may be associated with hemianopia or cortical blindness. Other clinical features include myopathy, cognitive decline, myoclonus, ataxia, episodic coma, optic atrophy, short stature, sensorineural hearing loss and hypertrophic cardiomyopathy. Intrafamilial variability is well recognised [23].

Myoclonic epilepsy with ragged red fibres (**MERRF**) is a maternally inherited disorder frequently causing myoclonus and/or generalised seizures and ataxia, with onset usually in childhood or adolescence. Approximately 80% of cases have a 'common' mtDNA mutation, m.8344A>G in *MT-TK*. There is enormous clinical heterogeneity, even within families, and other features include sensorineural hearing loss, optic atrophy, pigmentary retinopathy, nystagmus, ophthalmoparesis, dysarthria, exercise intolerance, cardiomyopathy, multiple symmetrical lipomas and psychiatric disturbance [25].

Neuropathy, ataxia and retinitis pigmentosa (NARP) is a maternally inherited disorder caused by m.8993T>G/C mutations in the *MT-ATP6* gene also associated with maternally inherited Leigh syndrome [26]. Symptoms of sensory neuropathy, muscle weakness, epilepsy and ataxia usually start in late childhood or early adult life. Later features include retinitis pigmentosa and cognitive decline. Short stature, sensorineural hearing loss, progressive external ophthalmoplegia and cardiac conduction defects may occur. The severity and extent of disease depend (at least partly) on the percentage and distribution of mutant mtDNA, as discussed below. Other mtDNA point mutations in the *MT-ATP6* and *MT-ATP8* genes may also cause NARP, and the clinical spectrum extends to isolated peripheral neuropathies indistinguishable from Charcot-Marie-Tooth disease [27].

Leber hereditary optic neuropathy (LHON) presents in adolescence or early adulthood with bilateral painless subacute loss of central vision due to optic neuropathy [28]. LHON is maternally inherited with incomplete penetrance and an extreme male preponderance. In most cases optic neuropathy is the only clinical feature, but in rare 'LHON-plus' families associated symptoms may include dystonia, tremor, cardiac conduction defects or psychiatric disturbance. Three usually homoplasmic mtDNA mutations (m.11778G>A, m.3460G>A and m.14484T>C) in complex I subunit genes account for greater than 90% of cases, but other mtDNA mutations have also been reported (www.mitomap.org).

Myoclonic epilepsy, myopathy, sensory ataxia (MEMSA) includes recessive *POLG*-related epilepsy syndromes previously known as spinocerebellar ataxia with epilepsy (SCAE) and mitochondrial recessive ataxia syndrome (MIRAS) [29]. Clinical features include ataxia and epilepsy, with or without myoclonus, and epilepsia partialis continua. Seizures are difficult to treat and episodes of acute encephalopathy or status epilepticus may occur.

Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) typically presents in adolescence or early adult

life with gastrointestinal dysmotility (dysphagia, early satiety, post-prandial nausea and vomiting, bloating, constipation, diarrhoea or repeated episodes of pseudo-obstruction), severe cachexia, ptosis and/or ophthalmoplegia, proximal myopathy and demyelinating peripheral neuropathy [30]. A florid leukoencephalopathy revealed by brain MRI is usually relatively asymptomatic. The main underlying cause is thymidine phosphorylase deficiency (▶ Chapter 35) leading to impaired mtDNA maintenance because of intramitochondrial nucleoside imbalance, resulting in accumulation of multiple mtDNA deletions and point mutations and progressive mtDNA depletion. Mutations in *POLG*, *RRM2B*, *MT-TL1* and *MT-TV* have also been reported to present with a MNGIE-like phenotype including chronic intestinal pseudo-obstruction and neuropathy.

Isolated organ involvement in childhood and adolescence may include skeletal myopathy (exercise intolerance +/- rhabdomyolysis) and steroid-resistant nephrotic syndrome (in disorders of coenzyme Q_{10} biosynthesis).

14.1.3 Adult-Onset Disorders

Adult-onset mitochondrial disorders are frequently (in >50% of cases) caused by mtDNA mutations. Many of the disorders which typically present in late childhood or adolescence (including KSS, MELAS, MERRF, MEMSA, MNGIE, NARP and LHON) may have onset in early adult life. The full MELAS syndrome is seen in fewer than 10% of patients with the m.3243A>G point mutation [24]. The most frequent presentation associated with this mutation is **maternally inherited diabetes and deafness** (**MIDD**). Diabetes (often progressing to insulin requirement) and sensorineural hearing loss usually start in the fourth decade [31]. Other frequent problems experienced by individuals with the m.3243A>G mutation include cardiomyopathy (which may be a cause of sudden unexpected death), nephropathy (focal segmental glomerulosclerosis), gastrointestinal dysmotility, short stature and macular retinal dystrophy (frequently diagnosed in the diabetic retinopathy screening clinic in patients with undiagnosed MIDD) [24].

One of the most frequent adult presentations of **mitochondrial myopathy** is **progressive external ophthalmoplegia (PEO)**, which may or may not be accompanied by a proximal skeletal myopathy or extend to complete KSS. PEO may be caused by sporadic mtDNA deletions, be maternally inherited as a result of mtDNA point mutations, or be dominantly inherited (due to mutations in one of several nuclear genes involved in mtDNA maintenance, most commonly *POLG*). Patients with *POLG* mutations with adult onset may present with dominant PEO, recessive epilepsy syndromes or with **sensory ataxic neuropathy, dysarthria and ophthalmoparesis** (**SANDO**) also known as **ataxia neuropathy syndrome (ANS)** [29].

Isolated organ involvement in adults with mitochondrial disease includes myopathy (rarely rhabdomyolysis) [32], epilepsy (especially myoclonic or epilepsia partialis continua) [18] and peripheral neuropathy (e.g. Charcot-Marie-Tooth hereditary neuropathy type 2A2, an autosomal dominant axonal peripheral sensorimotor neuropathy caused by mutations in *MFN2* encoding an outer mitochondrial membrane GTPase essential for mitochondrial fusion) [33]. Mutations in two dually localised aminoacyl tRNA synthetases KARS and GARS, which act in both the mitochondria and the cytosol, as well as four cytosolic aminoacyl tRNA synthetases AARS, HARS, MARS and YARS, have also been reported to cause peripheral neuropathies, mostly axonal and affecting both motor and sensory function [34]. Sometimes the presence of additional clinical features such as acute epileptic encephalopathy may lead to confusion with primary mitochondrial disorders.

14.2 Metabolic Derangement

Oxidative phosphorylation (OXPHOS) is a central and essential part of mitochondrial energy metabolism [35]. One function of OXPHOS is the oxidation of reduced REDOX molecules NADH and $FADH_2$ by using molecular oxygen (O_2) as electron acceptor. These REDOX molecules are formed by numerous oxidation reactions in the cell including glycolysis, but predominantly by the degradation of pyruvate, fatty acids and amino acids in mitochondria. By harnessing a proton gradient that is generated by respiratory chain enzyme complexes I, III, and IV, mitochondrial ATP synthase (complex V) produces the majority of the cellular energy molecule ATP (◘ Fig. 14.1). Insufficient ATP supply affects highly energy dependent tissues most severely. Owing to the importance of mitochondrial energy metabolism in multicellular organisms, a complete loss of OXPHOS is not observed in systemic human disease; there is always some residual function left. The amount of residual activity is often variable in different tissues, which can complicate the diagnosis of OXPHOS diseases.

In the absence of oxidative mitochondrial metabolism, human cells can survive by using ATP from anaerobic glycolysis. This fermentative pathway has two major disadvantages: it is approximately 20-fold less efficient and it generates the reduced molecule lactate as a final product, which has to be excreted from the cell. Depending on glutamate availability, pyruvate can be metabolised to alanine by a specific transaminase and secreted as well. Lactate, pyruvate and alanine are the typical products of anaerobic glycolysis and elevation of these compounds in body fluids (e.g. blood, urine and cerebrospinal fluid (CSF)) is a typical but not a specific finding in OXPHOS defects.

Each OXPHOS enzyme consists of **multiple polypeptide subunits**, in total approximately 90. OXPHOS complexes I, III, IV, and V also contain subunits that are encoded by the **mitochondrial DNA**, which is a unique feature of the mitochondrial organelle within the cell. The formation of these 13 protein subunits depends on a complex machinery required for **replication** and **transcription** of mitochondrial DNA and **translation** on mitochondrial ribosomes [36]. These processes necessitate in total more than 200 enzymes, ribosomal RNAs (rRNAs) and transfer RNAs (tRNAs). Furthermore, OXPHOS depends on numerous **cofactors**, e.g. coenzyme

Q_{10}, iron-sulphur clusters that are also involved in lipoic acid synthesis (Fig. 14.2 and ▶ Chapter 23), haem and copper, that all need to be synthesised and/or transported to the OXPHOS system. Furthermore specialised membrane lipids such as mitochondrion-specific cardiolipin also play an essential role in mitochondrial health, being important for the formation of cristae structure and activation of OXPHOS enzymes [37] (▶ Chapter 34). In addition, the formation of all of the OXPHOS enzymes depends on numerous **assembly factors**. Proper **maintenance of the organelle** including import of the cytosolically formed subunits, protein turnover, fission, and fusion are prerequisites for normal OXPHOS function. Last but not least, OXPHOS is sensitive to **toxic metabolites**, which might be formed by other mitochondrial pathways or perturbations of mitochondrial reactive oxygen species (ROS)-defence [4][38][39].

The list of factors that influence OXPHOS is continuously expanding and the final number can only be estimated at present. Taking the number of more than 1500 mitochondrial proteins in the human mitochondrial proteome and the expanding list of other relevant factors, up to 10% of the human proteome might be involved in this metabolism [40]. In summary, mitochondria are complex organelles with multitudinous functions, and consequently mitochondrial disease may be associated with diverse metabolic derangements.

14.3 Genetics

OXPHOS disorders comprise defects of two genomes, the maternally inherited mitochondrial ~16.6 kb genome with known mutations in all 37 encoded genes, and currently 193 nuclear encoded genes, a number that is increasing exponentially. Mitochondrial disease may be inherited by any mode of inheritance: maternal (mtDNA), autosomal recessive, autosomal dominant, X-linked or sporadic (*de novo* mutations). Furthermore, somatic mutations may occur in both genomes.

14.3.1 Mitochondrial DNA Mutations

Any type of mutation can occur in mtDNA, including point mutations, small rearrangements, large-scale rearrangements (single deletions and duplications, multiple deletions), or copy number reduction known as mtDNA depletion. Several hundred different pathogenic mtDNA mutations have been reported and catalogued in the MITOMAP online database (www.mitomap.org). Mutations can either affect single OXPHOS subunits, tRNAs, rRNAs, or a combination of these. In contrast to single OXPHOS subunit defects, mutations affecting the tRNA and rRNA genes usually result in a decrease of multiple OXPHOS enzymes.

Peculiarities of mitochondrial genetics include the presence of hundreds or even thousands of copies of mtDNA molecules in individual cells, leading to the phenomena of heteroplasmy (co-existence of mutant and wild-type mtDNA in variable percentages) and homoplasmy (100% wild-type or mutant mtDNA). Pathogenic relevance of a mtDNA mutation depends on the proportion of mutated DNA. A minimal amount of wild-type mtDNA is necessary to maintain OXPHOS function in cells in a particular tissue (threshold effect). This minimal amount varies between different mtDNA mutations. Some mutations can be found in a homoplasmic or nearly homoplasmic state (e.g. LHON mutations). For many of the more common mt DNA mutations the threshold is typically between 80-95% mutation load (e.g. MELAS, MERRF mutations), while for large deletions the level is often between 50-70% deleted mtDNA. For a few mutations, e.g. anticodon mutations of mitochondrial tRNAs, a dominant-negative effect has been observed, with pathogenic relevance at a mutation load of less than 20% [41].

14.3.2 Nuclear Gene Defects

Mutations in approaching 200 nuclear genes have been identified that result in either single or combined OXPHOS defects [42]. A classification of nuclear gene defects causing mitochondrial disease is presented in Table 14.5.

Mutations of OXPHOS structural subunits or OXPHOS assembly factors typically result in single OXPHOS enzyme defects (Table 14.5). Mutations of complex I structural subunits and complex IV assembly factors appear to be relatively frequent causes of isolated complex I or complex IV deficiency, respectively.

Defects of mtDNA maintenance include mutations in factors needed for mtDNA replication and enzymes involved in the metabolism of nucleotides necessary for mtDNA replication [8]. Defects in this group of genes result either in mtDNA depletion (*SUCLA2*, *SUCLG1*) or in mtDNA depletion and/or multiple deletions of mtDNA (*POLG*, *POLG2*, *C10orf2* (*Twinkle*), *MGME1*, *DNA2*, *DGUOK*, *TYMP*, *MPV17*, *SLC25A4*, *RRM2B*, *TK2*, *MFN2*, *OPA1*, *SPG7*, *AFG3L2*, *CHCHD10*, *SAMHD1*) (Table 14.5). The consequence of depletion of mtDNA or accumulation of multiple mtDNA deletions is usually a reduction of OXPHOS complexes I, III, IV and V, although in the early stages of disease there may be an isolated deficiency of complex I or complex IV.

Defects of mitochondrial gene expression (RNA processing, mRNA and tRNA modification) also lead to reduction of OXPHOS enzyme complexes I, III, IV and V, as do the large group of **defects of mitochondrial translation,** including mutations in mitochondrial ribosomal proteins, aminoacyl tRNA synthetases and factors needed for initiation, elongation and regulation of translation (Table 14.5) [36].

Defects of cofactors and their biosynthesis comprise a fast growing subgroup of OXPHOS defects, including mutations in factors required for biosynthesis and/or transport of CoQ_{10}, Fe-S clusters (Fig. 14.2 and ▶ Chapter 23), haem, riboflavin, copper and iron [36] (Table 14.5).

Defects of mitochondrial membrane lipids constitute another expanding disease group that includes *TAZ*, *AGK* and *SERAC1* mutations, leading to Barth, Sengers and MEGDEL syndromes respectively (Table 14.5 and ▶ Chapter 34) [37].

An increasing number of defects are related to the maintenance of mitochondrial organelles, which is a prerequisite for OXPHOS function. This group includes defects of the mitochondrial protein import machinery, mitochondrial solute import, mitochondrial dynamics, and quality control (Table 14.5). It is anticipated that next generation sequencing will lead to a rapid increase in recognition of this group of disorders, since several of these defects mainly affect the central nervous system, which is difficult to assess using conventional functional studies.

Toxic metabolites of several mitochondrial metabolic pathways can cause either isolated or combined OXPHOS deficiency (Table 14.5) [4][38][39].

X-chromosomal OXPHOS defects reported to date include mutations in eight X-chromosomal genes (*NDUFA1, NDUFB11, COX7B, HSD17B10, HCCS, TAZ, TIMM8A, AIFM1*). Remarkably, X-chromosomal OXPHOS defects group into three patterns of disease manifestation: (i) only heterozygous females are affected and presumably disease is embryonic lethal in affected males (*NDUFB11*, *COX7B*, *HCCS*); (ii) defects occur in both sexes (*HSD17B10*); or (iii) disease only reported in males or with very mild phenotype in females (*NDUFA1*, *TAZ*, *TIMM8A*, *AIFM1*).

14.3.3 Frequency of Mutations

Precise data on the frequency of individual OXPHOS defects are not available since patient registries often contain incomplete information and the genetic diagnosis is missing in numerous patients. Several studies have indicated that mtDNA mutations account for only 15–30% of OXPHOS defects in childhood. In adult patients the proportion of patients with mtDNA mutations is much higher. Among mtDNA mutations, the 'common' ~4.9kb deletion m.8483_13459del4977 is frequently found in patients with Pearson and Kearns-Sayre syndromes or PEO. The m.3243A>G mutation, which is the most frequent cause of MELAS, is also associated with other clinical features as discussed above and is found at low mutational load in 0.2% of the European population, as is the homoplasmic m.1555A>G mutation that predisposes to profound hearing loss following exposure to aminoglycoside antibitiotics [43]. The m.8993T>G mutation causing maternally inherited Leigh syndrome and NARP and the m.8344A>G mutation causing MERRF are also relatively frequent. The frequent mutations for LHON (m.3460G>A, m.14484T>C, and m.11778G>A) are detectable in 0.3% of all mitochondrial genomes [44].

The frequency of nuclear DNA mutations is often related to founder events and differs between populations. In our experience mutations in *POLG* and *SURF1* are most prevalent [29][45].

14.4 Diagnostic Tests

The starting point in diagnostics of OXPHOS defects should be a detailed evaluation of the clinical and family history including analysis of a three-generation pedigree. Diagnosis can be extremely challenging because of the heterogeneous clinical presentations associated with many OXPHOS defects, with a wide differential diagnosis. This means that a high index of clinical suspicion is needed. Signs of developmental regression, often associated with infectious disease, are typical findings. Precise clinical investigations, metabolic tests, and screening for multi-system involvement should be performed. Depending on availability, the use of genetic screening tests, especially whole exome sequencing (WES) or whole genome sequencing (WGS), should be considered early in the diagnostic workup [46][47]. Functional studies of OXPHOS enzymes in tissue biopsies could either come later in the diagnostic workup or can be performed in parallel in acutely ill patients.

14.4.1 Screening Tests

Metabolic investigations

Simple standard tests for categorising a metabolic disorder including full blood count, pH, bicarbonate and lactate should be performed as first line investigations (▶ Chapter 1).

Interpretation of lactate measurements needs to consider that lactate is the product of anaerobic ATP production via glycolysis, which is a physiological means to provide energy rapidly. Therefore, the patient's exercise load at the time of sample collection needs to be considered, since elevated lactate may be appropriate in a crying child or following epileptic seizure activity (▶ Chapter 1).

Other metabolic investigations include measurement of **pyruvate**, the end product of glycolysis. Pyruvate can be increased in patients with OXPHOS defects, but a caveat is that the preanalytical requirements for pyruvate investigation are demanding. Perchloric acid needs to be added to deproteinise the sample, which has to be cooled immediately, otherwise pyruvate will continue to be metabolised in the blood sample leading to a falsely low result. Therefore, pyruvate has not become a widely used metabolite. Alanine correlates to the concentration of pyruvate, since transamination occurs especially during amino acid catabolism and excess glutamate levels. Amino acid analysis can provide helpful data, particularly if ratios between certain amino acid concentrations are analysed, e.g. alanine/lysine (normally < 3:1). Glycine may be elevated in defects of lipoic acid biosynthesis [48] (▶ Chapter 23), whilst low levels of citrulline and arginine have been reported in maternally inherited Leigh syndrome, NARP, MELAS and Pearson syndrome [49].

Urinary organic acid analysis frequently reveals elevation of lactate, pyruvate, and Krebs cycle intermediates (succinic, malic, fumaric, 2-oxoglutaric and citric acids), which are all indicative of an OXPHOS defect. Elevation of urinary **3-methylglutaconic acid** has been found in a heterogeneous group of

Table 14.3 Metabolites in mitochondrial disease

Metabolite	Sample	Investigation	MRS	OXPHOS defect	Category
Lactate	P, U, CSF	CC, OA	^{1}H	all	all
Pyruvate	P, U, CSF	CC, OA		all	all
Alanine	P, U, CSF	AA		all	all
Glycine +/- lactate, BCAA, 2KG and 2KA	P, U, CSF	AA		CII>CI>CIII	Cofactor/Fe-S cluster (BOLA3, GLRX5, IBA57, ISCA2, NFU1)
Acylcarnitine	P, BS	MS		CII, CI, all	Inhibitors (ECHS1, HIBCH), Cofactor/flavins (FLAD1)
Coenzyme Q_{10}	PBMC, M, F	HPLC, MS		CI+III, CII+III	Cofactor/Coenzyme Q_{10}
Thymidine, deoxyuridine	P, U	PP		CI, CIII, CIV, CV	Replication (TYMP)
Creatine	P	MS	^{1}H (total creatine)	all	all
FGF-21	P	ELISA		all	all
Krebs cycle metabolites	U	OA		all	all
3-Methylglutaconic acid	U	OA		CI, CIII, CIV, CV	Defects affecting IMM integrity (AGK, ATP5E, CLPB, DNAJC19, OPA3, SERAC1, TAZ, TMEM70)
Methylmalonic acid	U	OA		CI, CIII, CIV, CV	Replication/nucleotide (SUCLA2, SUCLG1)
Succinate	U	OA	^{1}H	CII, in lower amount for all	Subunit/Assembly/Cofactors of CII
Ethylmalonic acid	U	OA		CIV	Inhibitor (ETHE1)
2-Methyl-2,3-dihydroxy-butyrate	U	OA		all	Inhibitors (ECHS1, HIBCH)

^{1}H, proton; *2KA,* 2-ketoacids; *2KG,* 2-ketoglutarate; *AA,* amino acids; *BCAA,* branched chain amino acids; *BS,* dried blood spot; *CC,* clinical chemistry; *CI-V,* complexes; I-V, *CSF,* cerebrospinal fluid; *F,* fibroblasts; *MS,* mass spectrometry; *M,* muscle; *OA,* organic acids; *P,* plasma; *PBMC,* peripheral blood mononuclear cells; *PP,* purines and pyrimidines; *U,* urine

OXPHOS disorders, including Barth, Sengers and MEGDEL syndromes (▶ Chapter 18 and ▶ Chapter 34), as well as ATP synthase deficiency and the recently described CLPB deficiency, all of which seem to have abnormalities of mitochondrial cristae in common [50][51]. Elevated excretion of **ethylmalonic acid** is found in ETHE1 deficiency [38] (▶ Chapter 20) but is also present in other conditions. Mild elevation of **methylmalonic acid** is associated with succinyl-CoA-ligase deficiency [52] (▶ Chapter 18).

Investigation of acylcarnitines can be helpful in defects of flavin cofactor metabolism (e.g. *FLAD1* mutations) and defects of inhibitors originating from the valine degradation pathway (ECHS1 and HIBCH deficiencies) [4][39] (▶ Chapter 18). **Investigation of purines and pyrimidines** in the plasma or urine of MNGIE patients shows an elevation of thymidine and deoxyuridine [30] (▶ Chapter 35). Specific investigation of **coenzyme Q_{10}** in peripheral blood mononuclear cells or tissue biopsies is of diagnostic relevance in patients with suspected CoQ_{10} biosynthetic disorders and can also be useful in identifying secondary CoQ_{10} deficiency [53]. In myopathic patients, elevation of the three biomarkers FGF-21, GDF15 and creatine correlates well with mitochondrial dysfunction [54][55][56] (Table 14.3). Recently metabolic profiling has been used to reveal a 'metabolic signature' of mitochondrial dysfunction in the French Canadian variant of Leigh syndrome [5]. It is anticipated that similar techniques will be used to study other mitochondrial disorders in the near future.

Neuroimaging

Certain mitochondrial syndromes are associated with characteristic lesions on brain magnetic resonance imaging (MRI), for example parieto-occipital stroke-like lesions not corresponding to vascular territories in MELAS, and bilateral focal symmetrical T2 hyperintense lesions in the basal ganglia,variably extending into the midbrain and brainstem, in Leigh syndrome (Table 14.4) (▶ Chapter 1) [57][58]. Recently

Table 14.4 Specific MRI brain findings in mitochondrial disorders

Syndrome	Characteristic MRI changes
Kearns-Sayre syndrome	Bilateral high-signal lesions in subcortical white matter, globus pallidus, thalamus and brain stem; cerebral, cerebellar and brainstem atrophy; basal ganglia calcification (CT)
LBSL	T2-weighted and FLAIR high signal intensity in cerebral subcortical, periventricular and deep white matter, posterior limbs of internal capsules, centrum semiovale, medulla oblongata, intraparenchymal trajectory of trigeminal nerves, deep cerebellar white matter, and spinal cord dorsal column and lateral cortico-spinal tracts
Leigh	T2-weighted focal symmetrical hyperintensities affecting basal ganglia, variably extending into midbrain and brainstem
LTBL	T2-weighted symmetrical hyperintensities in deep cerebral white matter (sparing periventricular rim), thalami, midbrain, pons, medulla oblongata and cerebellar white matter; increased lactate on proton MRS
MEGDEL	Bilateral basal ganglia involvement, especially putamina, but early sparing of dorsal putamina leading to a characteristic putaminal 'eye' without signal alteration; later progressive putaminal involvement
MELAS	T2-weighted hyperintense lesions in grey and subcortical white matter of temporal, parietal, and occipital lobes, sparing deep white matter and crossing vascular boundaries, +/- basal ganglia calcification, generalised cerebral atrophy
PCH6	Severe progressive atrophy of cerebellum and pons; cerebral cortex may also be affected
SDH deficiency	Succinate peak on ^{1}H-magnetic resonance spectroscopy

LBSL, leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation; *LTBL,* leukoencephalopathy with thalamus and brainstem involvement and high lactate; *PCH6,* pontocerebellar hypoplasia type 6

some of the newly reported mitochondrial translation defects (tRNA aminoacyl synthetase deficiencies) have been linked to specific brain MRI 'signatures' (Table 14.4), whilst cavitating leukoencephalopathies are a feature of some mitochondrial disorders, particularly Leigh syndrome caused by mutations in nuclear-encoded complex I subunits. Magnetic resonance spectroscopy may be used to visualise increased cerebral lactate (a nonspecific finding) or elevated succinate (deficiencies of succinate dehydrogenase (complex II) subunits or assembly factors).

Screening for multi system involvement

Involvement of multiple organs is a typical finding in OXPHOS disease, especially in infantile and childhood onset disease. It is important to systematically screen for multisystem involvement for two reasons. Firstly, the pattern of organ involvement may point to a specific syndromic/genetic diagnosis. Secondly, it is imperative to search for organ involvement for which there may be supportive therapy (▶ Section 14.5.2). Investigations looking for multisystem disease may include echocardiogram, electrocardiogram, measurement of renal tubular and endocrine (pituitary, thyroid, parathyroid, pancreatic, adrenal) function, and ophthalmological and audiological assessment. Reassessment over time is important since clinical evolution of mitochondrial disorders is typical, with disease progression and involvement of new organ systems.

14.4.2 Muscle and Other Tissue Biopsies

Usually, the best tissue to be investigated by biochemical or genetic techniques is a clinically affected tissue. Investigation of a skeletal muscle biopsy is frequently helpful in identifying the underlying OXPHOS defect in patients with neuromuscular symptoms. If results are normal, consideration should be given to whether the correct tissue has been investigated, e.g. patients with *POLG* mutations may have normal OXPHOS results in muscle but a clear biochemical phenotype in liver. The samples and conditions detailed in the following paragraph should be considered.

Tissue sampling and storage

1. Fresh tissue (muscle, liver) for investigation of mitochondrial respiration, and for cell culturing (skin fibroblasts, lymphocytes, myoblasts).These tissue samples should be sent on water-ice [not necessary for skin] in an appropriate transport medium.
2. Snap-frozen tissue (muscle, liver, heart, kidney) for enzymatic investigations, histochemical staining, western blot and blue-native gel electrophoresis (send packed with dry ice).
3. Fixed tissue for electron microscopic investigations.
4. Blood for genetic investigations: EDTA blood, stable for several days (temp 4–25°C).
5. Other useful samples for mtDNA investigations: urine, buccal swab, hair roots.

Types of investigations

Numerous biochemical techniques have been established for the analysis of mitochondrial energy metabolism. By investigating intact mitochondria from fresh tissue or cultured cells, all kinds of OXPHOS defects can be identified, including disorders that depend on the integrity of the mitochondrial membranes, for which it is essential to measure the activity of ATP synthase. In frozen tissue it is still possible to measure the activity of OXPHOS complexes I-IV and the hydrolytic activity of complex V.

Histopathology

Histochemical investigation of complex II (succinate dehydrogenase, SDH) and complex IV (cytochrome *c* oxidase, COX) can be performed on frozen tissue sections. Furthermore, Gömöri trichrome staining identifies cells with mitochondrial accumulation (e.g. »ragged-red« muscle fibres) and sequential COX/SDH staining can reveal so-called »ragged-blue« fibres (COX-negative, SDH-positive). A major advantage of histological investigation is the ability to detect a heterogeneously affected tissue, especially muscle fibres with different mutation load due to heteroplasmic mtDNA mutation. Microdissection of single muscle fibres either positive or negative for COX staining, and quantification of the mutation load, can be used to determine the pathogenic relevance of novel mtDNA mutations. Tissue sections from cryo-tissue or formalin-fixed paraffin-embedded (FFPE) samples can also be used for immunohistochemical staining, which may show reduced immuno-staining of subunits in cases with OXPHOS deficiency. Histopathological examination of cardiac or renal tissue may reveal 'giant' mitochondria, although these are not specific for mitochondrial disease. Analysis of bone marrow aspirates in Pearson syndrome demonstrates vacuolation of myeloid precursors and the presence of ringed sideroblasts. Post-mortem brain pathology may reveal characteristic features in Leigh or Alpers-Huttenlocher syndromes.

Electron microscopy

Electron microscopy (EM) can reveal abnormal ultrastructure of mitochondria, e.g. paracrystalline inclusions or concentric cristae, or extremely elongated mitochondria in the case of mitochondrial fission defects. These findings are often helpful in combination with other results but might be misleading if they are interpreted out of context, since subtle ultrastructural abnormalities of mitochondria (e.g. alterations in number or size or abnormal cristal morphology) can also be found in other disease processes.

Investigation of OXPHOS activity

Spectrophotometric assays have been established for all OXPHOS enzymes: complexes I, II, III, IV and the oligomycin-sensitive ATPase activity of complex V. Furthermore the combined activities of complex I+III (NADH:ferricytochrome *c* oxidoreductase) and complex II+III (succinate:ferricytochrome *c* oxidoreductase) may be measured. These combined activity assays can enable the detection of defects in coenzyme Q_{10}, which is an essential electron carrier in these reactions (Fig. 14.1). These enzyme investigations allow the identification of isolated or combined OXPHOS deficiencies. Combined OXPHOS defects are most frequent, and typical combinations of enzyme deficiencies can point to the underlying genetic defect. For example, combined reduction of complexes I, III, IV and V is a typical pattern of defects in mtDNA-dependent disorders including mtDNA mutations (point mutations and rearrangements involving tRNA or rRNA genes) and nuclear-encoded defects of mtDNA maintenance and mitochondrial gene expression (Table 14.5). Combined decrease of complexes I, II, and eventually III together with PDHc deficiency and glycine elevation is suggestive of a defect of iron-sulphur cluster biosynthesis (Table 14.5 and Fig. 14.2). Such findings allow direction of subsequent genetic investigations to pinpoint the precise genetic defect. Reduced activities of complexes I+III and II+III in muscle biopsy, with normal activities of the individual complexes I, II and III when assayed separately, suggests CoQ_{10} deficiency. However other patterns of respiratory chain enzyme deficiency have been observed in primary CoQ_{10} deficiency, therefore direct measurement of CoQ_{10} in muscle biopsy is the preferred screening test.

Investigation of the **oxidation of different substrates by intact mitochondria** isolated from fresh tissue biopsies or cultured patient cells provides the best characterisation of mitochondrial function, and allows identification of decreased activity of ATP synthase, pyruvate oxidation, the Krebs cycle, and substrate transport. Available techniques include measurement of oxygen consumption (by polarography or fluorimetry), ATP formation (luciferase-coupled assay), and the use of radiolabelled substrates in flux assays.

Blue or clear native gel electrophoresis allows further characterisation of OXPHOS defects by enabling the separation of intact OXPHOS enzymes and even supercomplexes [59]. Abnormal OXPHOS assembly or absence of single enzymes can be identified by this method.

In a research setting, functional complementation can be used to investigate the pathogenic relevance of novel genetic defects by complementing an OXPHOS defect in patient cells using stable transfection with the wild-type candidate gene, e.g. with a lentiviral vector.

Tissue-specificity

Defects of OXPHOS enzymes can occur in a tissue-specific manner. There are several reasons for this phenomenon including different threshold for minimal residual activities in different organs but also expression of different isoforms of involved enzymes, different degrees of X-inactivation in X-chromosomal disorders, different percentages of mtDNA heteroplasmy, or somatic mosaicism of an affected gene. Re-investigation of a clinically affected tissue (usually skeletal muscle, liver or heart) should be considered in cases where genetic investigations in blood do not identify the underlying cause.

Table 14.5 Genetic defects resulting in OXPHOS deficiency (37 mitochondrial and 193 nuclear genes)

Type	Subtype	Inheritance (affected genes)	OXPHOS defect (typical)
OXPHOS subunit	Complex I	**AR** *(NDUFA2, NDUFA9, NDUFA10, NDUFA11, NDUFA12, NDUFA13, NDUFB3, NDUFB9, NDUFS1, NDUFS2, NDUFS3, NDUFS4, NDUFS6, NDUFS7, NDUFS8, NDUFV1, NDUFV2);* **mtDNA** *(MT-ND1, MT-ND2, MT-ND3, MT-ND4, MT-ND4L, MT-ND5, MT-ND6);* ***X*-linked** *(NDUFA1, NDUFB11)*	CI
	Complex II	***AR*** *(SDHA, SDHB, SDHD);* **somatic in tumours** *(SDHC)*	CII
	Complex III	**AR** *(CYC1, UQCRB, UQCRC2, UQCRQ);* **mtDNA** *(MT-CYB)*	CIII, (CI)
	Complex IV	**AR** *(COX4I2, COX6A1, COX6B1, COX8A, NDUFA4);* **mtDNA** *(MT-CO1, MT-CO2, MT-CO3;* ***X*-linked** *(COX7B)*	CIV
	Complex V	**AR** *(ATP5A1, ATP5E);* **mtDNA** *(MT-ATP6, MT-ATP8)*	CV
Assembly factor	Complex I	**AR** *(ACAD9, FOXRED1, NDUFAF1, NDUFAF2, NDUFAF3, NDUFAF4, NDUFAF5 (=C20orf7), NDUFAF6 (=C8orf38), TMEM126A)*	CI
	Complex II	**AR** *(SDHAF1);* **somatic in tumours** *(SDHAF2)*	CII
	Complex III	**AR** *(BCS1L, LYRM7, TTC19, UQCC2, UQCC3)*	CIII, (CI)
	Complex IV	**AR** *(CEP89, COA5, COX14 (=C12orf62), COX20 (=FAM36A), FASTKD2, PET100, SURF1)*	CIV
	Complex V	**AR** *(ATPAF2, TMEM70)*	CV
mtDNA replication	Nucleotide metabolism	**AR** *(***ABAT, *DGUOK, *MPV17, **SAMHD1, ***SUCLA2, ***SUCLG1, *TK2, *TYMP);* **AR and AD** *(*RRM2B, *SLC25A4)*	CI, CIII, CIV, CV
	mtDNA replication	**AD** *(**DNA2, **POLG2);* **AR** *(*MGME1, *RNASEH1);* **AR and AD** *(*C10orf2 (=Twinkle), *POLG)*	CI, CIII, CIV, CV
mtDNA transcription	Regulation	**AR** *(LRPPRC)*	CIV
	RNA processing	**AR** *(MTPAP, ELAC2);* **X-linked** *(HSD17B10)*	CI, CIII, CIV, CV
Mitochondrial translation	Transfer RNA	**mtDNA** *(MT-TA, MT-TC, MT-TD, MT-TE, MT-TF, MT-TG, MT-TH, MT-TI, MT-TK, MT-TL1, MT-TL2, MT-TM, MT-TN, MT-TP, MT-TQ, MT-TR, MT-TS1, MT-TS2, MT-TT, MT-TV, MT-TW, MT-TY)*	CI, CIII, CIV, CV
	tRNA modification	**AR** *(GTPBP3, MTO1, PNPT1, PUS1, TRIT1, TRMT5, TRMU, TRNT1)*	CI, CIII, CIV, CV
	tRNA loading	**AR** *(AARS2, CARS2, DARS2, EARS2, FARS2, GARS, HARS2, IARS2, KARS, LARS2, MARS2, NARS2, QRSL1, PARS2, RARS2, SARS2, TARS2, VARS2, YARS2)*	CI, CIII, CIV, CV
	Start-tRNA	***AR*** *(MTFMT)*	CI, CIII, CIV, CV
	Ribosomal RNA	**mtDNA** *(MT-RNR1, MT-RNR2)*	CI, CIII, CIV, CV
	Ribosomal protein	**AR** *(MRPS7, MRPS16, MRPS22, MRPS23, MRPL3, MRPL12, MRPL44)*	CI, CIII, CIV, CV
	Regulation	**AR** *(C12orf65, GFM1, GFM2, RMND1, TACO1, TSFM, TUFM)*	CI, CIII, CIV, CV
Cofactor	Coenzyme Q_{10}	**AR** *(ADCK3, ADCK4, COQ2, COQ4, COQ6, COQ7, COQ9, PDSS1, PDSS2)*	CI+III, CII+III
	Copper	**AR** *(COA6, COX10, COX15, SCO1, SCO2)*	CIV
	Haem	**AD** *(CYCS, PPOX);* **AR** *(SLC25A38);* ***X*-linked** *(HCCS)*	Cii, CIII, CIV
	Iron-sulphur cluster	**AR** *(BOLA3, FDX1L, FXN, GLRX5, HSPA9, IBA57, ISCA2, ISCU, LYRM4, NFS1, NFU1, NUBPL)*	CI, CII, (CIII)
	Iron transport?	**AR** *(SFXN4)*	CI
	Niacin	**AR** *(NADK2)*	CI-CV
	Riboflavin	**AR** *(FLAD1)*	CI, CII
	S-Adenosyl methionine	**AR** *(SLC25A26)*	CI, CIII, CIV, CV

Table 14.5 (continued)

Type	Subtype	Inheritance (affected genes)	OXPHOS defect (typical)
Mitochondrial homeostasis	Fission	**AD and AR** *(DNM1L);* **AR** *(GDAP1, MFF, STAT2)*	CI-CV
	Fusion	**AD** *(**OPA1);* **AR** *(SLC25A46);* **AR and AD** *(**MFN2)*	CI, CIII, CIV, CV
	Lipid	**AR** *(AGK, SERAC1);* **X-linked** *(TAZ)*	CI, CIII, CIV, CV, ANT
	Lipid?/Protein import?	**AR** *(DNAJC19)*	CI-CV
	Protein import	**AR** *(GFER, PMPCA, XPNPEP3);* **X-linked** *(AIFM1, TIMM8A)*	CI-CV
	Quality control	**AR** *(CLPB, CLPP, LONP1, PITRM1, SACS);* **AR and AD** *(**AFG3L2);* **AR and AD** *(HSPD1, **SPG7)*	CI-CV
Inhibitor	Protein import	**AD** *(HTT)*	CI-CV
	Toxic metabolite	**AR** *(D2HGDH, ECHS1, ETHE1, HIBCH, SLC25A1, TXN2);* **somatic in tumours** *(IDH2)*	CI-CV
Unknown	-	**AD** *(**CHCHD10);* **AR** *(APOPT1, FBXL4);* **AR and AD** *(OPA3)*	CI, CIII, CIV, CV

Mutations in these genes are associated with *mtDNA depletion and multiple mtDNA deletions; **multiple mtDNA deletions; ***mtDNA depletion
AD, autosomal dominant; *AR,* autosomal recessive; *CI-CV,* complexes I, II, III, IV, and V; *ANT,* adenine nucleotide translocator

14.4.3 Molecular Genetic Investigations

In recent years next-generation sequencing of the whole mitochondrial genome, together with high throughput genome-wide sequencing of the nuclear genome, has forged ahead in genetic testing for mitochondrial disorders. Since OXPHOS defects are an extremely heterogenous and fast expanding group of genetic disorders, the investigation of single mutations or single genes is only useful in a subgroup of patients with a clear clinical picture and few underlying mutations (e.g. LHON, MELAS, MNGIE). Most clinical manifestations of mitochondrial disease have been associated with a large number of different gene defects (Table 14.6). Leigh syndrome is a particular example of a genetically heterogeneous disorder (currently linked to >75 genes) [3] where targeted sequencing is indicated in only a few exceptions (e.g. maternally inherited Leigh syndrome due to *MT-ATP6* mutations). For this reason, whole exome sequencing (WES) is currently the state of the art for next-generation genetic screening although some centres use next-generation sequencing of large gene panels such as the 'MitoExome' [47]. Whole genome sequencing (WGS) is expected to be taken up into routine diagnostics in the near future. Interpretation of the resulting data will remain challenging, with variants of uncertain significance identified in many investigated patients. However, owing to growing numbers of available control and patient sequences, more and more variants will be classified. Screening the mitochondrial genome for large-scale rearrangements and/or depletion requires other techniques, such as long-range, real-time or digital PCR, and it is important to investigate a clinically relevant tissue such as muscle.

14.5 Treatment and Prognosis

14.5.1 Treatable Disorders

Currently no curative treatments correcting the underlying disease process exist for the vast majority of mitochondrial disorders [60][61]. However a few remarkable exceptions should be noted. Many patients with *ACAD9* mutations associated with exercise intolerance, lactic acidosis or infantile-onset cardiomyopathy appear to respond to oral riboflavin supplementation (100–400 mg/day) [62]. Disorders of CoQ_{10} biosynthesis may respond to high dose oral CoQ_{10} supplementation (at least 30 mg/kg/day in childhood, up to 3 g/day in adults), although response to treatment is highly variable [53]. Complete prevention of symptoms has been reported in some cases who received early treatment, whilst other cases have persistent ataxia or progressive renal impairment despite treatment.

Vigilance for reversible infantile respiratory chain deficiency is also associated with good outcomes, although some affected individuals with myopathy may require prolonged ventilatory support (up to 18 months), whilst those with acute liver failure caused by *TRMU* mutations may need liver transplantation [13][14].

Table 14.6 Tissue specificity of mitochondrial genetic defects

Tissue/organ	Gene defects
Leigh syndrome	*NDUFV1, NDUFV2, NDUFS1, NDUFS2, NDUFS3, NDUFS4, NDUFS7, NDUFS8, NDUFA1, NDUFA2, NDUFA9, NDUFA10, NDUFA12, NDUFAF2, NDUFAF5, NDUFAF6, FOXRED1, SDHA, SDHAF1, UQCRQ, BCS1L, TTC19, NDUFA4, SURF1, COX10, COX15, SCO2, PET100, LRPPRC, TACO1, FBXL4, POLG, SUCLA2, SUCLG1, GTPBP3, TRMU, EARS2, FARS2, IARS2, NARS2, GFM1, GFM2, TSFM, C12orf65, PNPT1, HIBCH, ECHS1, SERAC1, AIFM1, ETHE1, BTD, PDHA1, PDHB, PDHX, DLAT, DLD, LIPT1, LIAS, TPK1, SLC19A3, SLC25A19, MT-ND1, MT-ND2, MT-ND3, MT-ND4, MT-ND5, MT-ND6, MT-CO3, MT-ATP6, MT-TI, MT-TK, MT-TL1, MT-TV, MT-TW, MT-FMT,* mtDNA deletions
Epilepsy	*NDUFA1, NDUFA2, NDUFA10, NDUFA11, NDUFS2, NDUFS3, NDUFS4, NDUFS6, NDUFS7, NDUFS8, NDUFV1, NDUFAF1, NDUFAF2, NDUFAF3, NDUFAF4, NDUFAF5, NDUFAF6, FOXRED1, ACAD9, NUBPL, POLG, C10orf2, SUCLA2, SUCLG1, RRM2B, MPV17, DGUOK, TK2, TYMP, FBXL4, ABAT, SDHA, SDHD, SDHAF1, UQCRC2, UQCC2, BCS1L, HCCS, COX6B1, NDUFA4, SURF1, SCO1, SCO2, COX10, COX15, FASTKD2, PET100, ATP5A1, ATPAF1, ATPAF2, MTO1, GTPBP3, MRPS22, GFM1, TSFM, AARS2, CARS2, EARS2, FARS2, LARS2, NARS2, PARS2, RARS2, VARS2, MTFMT, LRPPRC, TACO1, RMND1, TRIT1, PNPT1, SLC25A12, SLC25A22, DNAJC19, PDSS2, COQ2, ADCK3, COQ4, COQ6, COQ9, BOLA3, LIAS, NFU1, NFS1, SERAC1, AGK, STAT2, AIFM1, ETHE1, APOPT1, HSPD1, AFG3L2, CLPB, SLC19A3, TPK1, NADK2, DARS, KARS, LARS, QARS*
Leukodystrophy	*NDUFS1, NDUFS8, NDUFV1, NUBPL, SDHA, SDHB, SDHD, SDHAF1, COX6B1, SURF1, COX10, COX15, COX20, PET100, TYMP, ABAT, TUFM, AARS2, DARS2, EARS2, MARS2, NFU1, BOLA3, LIAS, GLRX5, HSPD1, APOPT1, CLPB, DARS, NADK2*
Eye disease	*NDUFA1, NDUFA2, NDUFA9, NDUFA11, NDUFS1, NDUFS2, NDUFS3, NDUFS4, NDUFS6, NDUFS7, NDUFS8, NDUFV1, NDUFAF1, NDUFAF2, NDUFAF3, NDUFAF4, NDUFAF5, FOXRED1, NUBPL, SDHA, TMEM70, POLG, POLG2, C10orf2, SLC25A4, SUCLG1, RRM2B, DNA2, MGME1, FBXL4, MTO1, EARS2, IARS2, NARS2, RARS2, MTFMT, TSFM, MTPAP, TRNT1, C12orf65, LRPPRC, TACO1, PDSS1, BOLA3, LIAS, GLRX5, IBA57, AGK, GFER, TIMM8A, DNAJC19, MFN2, OPA1, DNM1L, TMEM126A, SPG7, HSPD1, CLPB, LONP1, SLC19A3,* mtDNA deletions
Deafness	*NDUFA1, NDUFA9, NDUFAF2, NDUFAF4, BCS1L, COX10, SUCLA2, SUCLG1, ELAC2, HARS2, IARS2, LARS2, NARS2, PNPT1, PDSS1, COQ6, BOLA3, LIAS, TIMM8A, GFER, SERAC1, MFN2, AIFM1, TMEM126A, LONP1, SDHD, MT-TL1,* mtDNA deletions
Cardiac disease	*NDUFA2, NDUFA10, NDUFA11, NDUFS2, NDUFS4, NDUFS8, NDUFV2, NDUFAF1, NDUFAF2, NDUFAF4, FOXRED1, ACAD9, SDHA, SDHD, SCO2, SCO1, COX10, COX15, COA5, COA6, LRPPRC, TMEM70, ELAC2, MTO1, GTPBP3, PUS1, MRPS22, MRPL3, MRPL44, AARS2, PARS2, YARS2, TSFM, SLC25A3, SLC25A4, SUCLA2, MGME1, AGK, TAZ, DNAJC19, PDSS1, COQ9, NFU1, BOLA3, LIAS, DNM1L, AIFM1, CLPB, TPK1, TMEM126A, FXN, GARS, MT-TI, MT-TL1, MT-ATP6, MT-ATP8,* mtDNA deletions
Pulmonary disease	*NFU1, SARS2*
Gastro-intestinal disease	*TYMP, POLG, RRM2B, ETHE1, MT-TL1, MT-TV,* mtDNA deletions
Pancreatic involvement (endocrine or exocrine)	*MT-TL1, MT-TK, MT-TS2, MT-TE,* mtDNA deletions*, POLG, RRM2B, OPA1, COX4I2*
Hepatic dysfunction	*POLG, C10orf2, DGUOK, MPV17, SUCLG1, ABAT, TRMU, GFM1, TSFM, TUFM, EARS2, FARS2, LARS2, NARS2, MRPL3, MRPL44, PUS1, SERAC1, SDHA, SDHD, UQCRB, BCS1L, SCO1, COX14, COX20, LRPPRC, GFER, STAT2, NFU1, BOLA3, GLRX5, LIPT1, ABCB,* mtDNA deletions
Renal disease	*NDUFAF2, UQCRC2, BCS1L, UQCC2, COX10, SCO1, SURF1, TMEM70, PDSS2, COQ2, COQ6, COQ9, ADCK4, RRM2B, TK2, DGUOK, SUCLA2, MPV17, FBXL4, MRPS22, SARS2, LARS2, NARS2, TFSM, TACO1, XNPEP3, STAT2, TRAP1, LARS, NADK2, MT-TF, MT-TL1,* mtDNA deletions
Endocrine involvement	*MT-TL1, MT-TK,* mtDNA deletions*, C10orf2, POLG, TYMP, MRPS7, GFER, STAR, NNT, TXNRD2, PTRH2*
Ovarian failure	*POLG, HARS2, LARS2, AARS2, CLPP, C10orf2*
Haematological disease	*YARS2, PUS1, TRNT1, SFXN4, GLRX5, FBXL4, COX4I2, TAZ, CLPB, CYCS, LARS, ABCB7,* mtDNA deletions
Myopathy (isolated)	*TK2, MT-CYB, MT-CO1, MT-CO2, MT-CO3,* mtDNA deletions*, FLAD1*
Peripheral neuropathy	*POLG, POLG2, C10orf2, TYMP, MPV17, SUCLG1, OPA1, NDUFA4, SURF1, COX20, ATP5E, C12orf65, TSFM, DARS2, HARS2, IARS2, PDSS1, COQ9, FXN, MFF, KARS, GARS*

This table is not exhaustive, as very few cases have been described so far for many of these gene defects

14.5.2 Supportive Management

Supportive measures remain the mainstay of management for most patients, and involve screening for and pro-actively treating known complications of mitochondrial disease when they occur. Such interventions may include the use of antiepileptic drugs (AEDs) for seizures (levetiracetam and clobazam appear to the most effective AEDs for mitochondrial epilepsy, and sodium valproate should be avoided, particularly in patients with *POLG* mutations, since its use may precipitate acute liver failure) [18]; ptosis surgery; hearing aids or cochlear implants; hormone replacement (growth hormone, thyroxine, insulin or hydrocortisone as needed); blood transfusions for anaemia (especially in Pearson and MLASA syndromes); fluid and electrolyte replacement for renal tubulopathy; medical treatment for cardiomyopathy; pacemaker insertion for cardiac conduction defects; and, occasionally, organ transplantation (heart, kidney, liver) in an appropriate clinical context such as isolated end-stage organ involvement in an otherwise 'healthy' child. L-arginine therapy may ameliorate the frequency and severity of stroke-like episodes in MELAS [23], whilst folinic acid supplementation has been reported to improve seizures and other neurological problems in patients with mitochondrial disease associated with cerebral folate deficiency [63] (▶ Chapter 27).

14.5.3 Vitamin and Cofactor Cocktails

Some centres (particularly in the United States) advocate 'cocktails' of vitamins and cofactors for patients with mitochondrial disease. Whilst some of these have a logical rationale, e.g. trials of riboflavin for complex I deficiency or thiamine for PDHc deficiency, many are nonspecific antioxidants and currently there is no evidence base to support or refute the use of these supplements [60]. An exhaustive list of the components of such cocktails is not given here, because of the lack of evidence.

14.5.4 Experimental Approaches

Numerous pharmacological and gene therapy approaches are currently under investigation but few have reached clinical trials [64]. This is partly because of the enormous difficulty in designing and executing effective clinical trials for these extremely heterogeneous multisystem disorders with an unpredictable disease course [61]. Pharmacological strategies currently in or close to clinical trial for mitochondrial disease fall into three main groups: 1) antioxidants (e.g. analogues of CoQ_{10} or N-acetylcysteine); 2) agents aiming to stimulate mitochondrial biogenesis (e.g. bezafibrate and vitamin B3 analogues); and 3) molecules that may 'stabilise' lipid components of the mitochondrial membranes [42].

14.5.5 Genetic Counselling and Prenatal and Preimplantation Genetic Diagnosis

■ Nuclear gene defects

Almost all childhood-onset nuclear-encoded mitochondrial disease is inherited in an autosomal recessive manner, with a few exceptions which are X-linked (as listed above). Nuclear gene defects causing mitochondrial disease presenting in adult life are frequently inherited as autosomal dominant traits, although some are recessive. Genetic counselling, prenatal and pre-implantation genetic diagnosis (PGD) are relatively straightforward for patients with nuclear gene defects in whom the underlying mutation/s is/are known, and follows the same principles as for other Mendelian disorders.

■ MtDNA defects

The situation is more complex for mtDNA mutations, not least because of the phenomenon of heteroplasmy arising from the multiple copy number of mtDNA molecules. Most large-scale rearrangements and some point mutations of mtDNA are sporadic, with a low risk of transmission. Heteroplasmic mtDNA point mutations are generally maternally inherited, but the factors that determine what percentage of mutation will be transmitted to the next generation are poorly understood. The genetic bottleneck for mtDNA means that there may be large shifts in the proportion of mutation transmitted from mother to offspring, and this varies between different mutations. It is therefore difficult to offer women who harbour heteroplasmic disease-causing point mutations accurate recurrence risks, although some mutations are better understood than others. The m.8993T>G mutation in *MT-ATP6* associated with maternally inherited Leigh syndrome and NARP appears to be particularly skewed to extremes of mutation (very low or very high) in oocytes, and this has allowed prenatal diagnosis for this mutation to be performed successfully on a number of occasions. Pre-implantation genetic diagnosis (PGD) has also been used successfully for some mtDNA mutations, particularly the m.8993T>G mutation [65]. The factors that determine clinical expression of homoplasmic point mutations associated with LHON are incompletely understood and so it is difficult to predict the recurrence risk, although it is approaching ten times higher for males than females who harbour LHON mutations.

Transmission of mtDNA point mutations may be avoided by donor egg in vitro fertilisation (IVF). The UK government has recently licenced two mitochondrial 'donation' IVF techniques (pronuclear transfer and spindle cell transfer) to be used with the aim of preventing the transmission of serious mitochondrial disease from a mother to her child, although concerns remain about the long-term efficacy and safety of these techniques [66].

14.5.6 Prognosis

Many early-onset mitochondrial disorders lead to death in infancy and early childhood, but natural history is extremely variable, even for patients with the same genetic defect.

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Creatine Deficiency Syndromes

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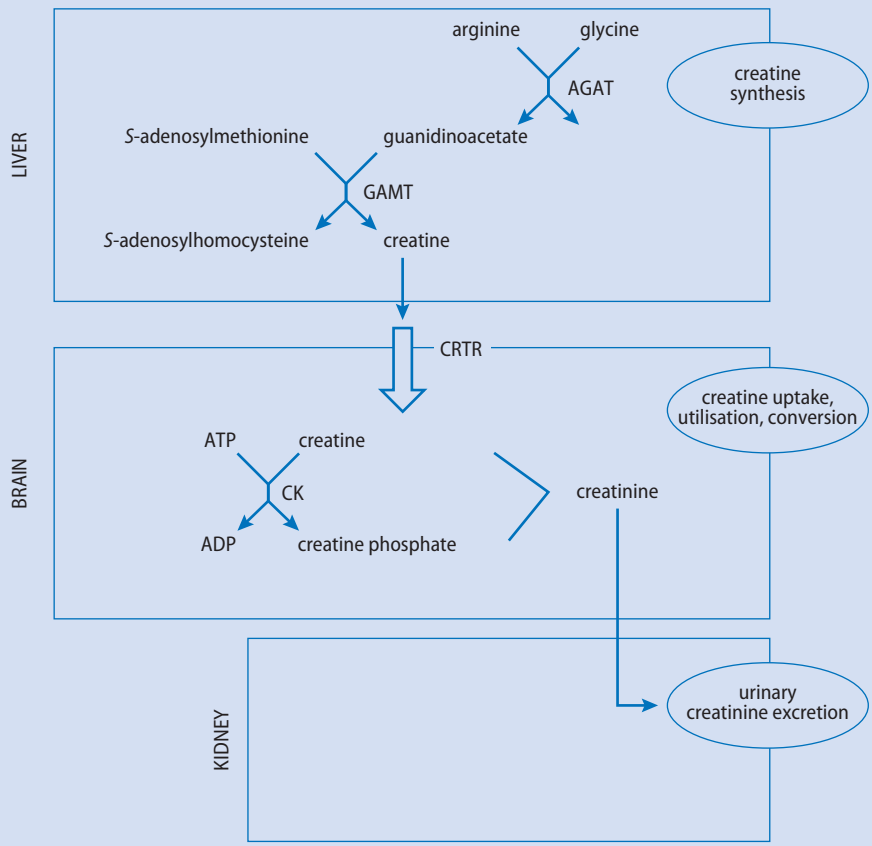
Creatine Synthesis and Transport

Creatine is synthesized by two enzymatic reactions (■ Fig. 15.1): 1) L-arginine:glycine amidinotransferase (AGAT, *GATM*) catalyses the transfer of an amidino group from arginine to glycine, yielding guanidinoacetate; 2) *S*-adenosyl-L-methionine: *N*-guanidinoacetatemethyltransferase (GAMT, *GAMT*) catalyses the methylation of the amidino group in the guanidinoacetate molecule, yielding creatine.
Creatine synthesis occurs mainly, but not exclusively, in the kidney and in the pancreas, which have high AGAT activity, and in the liver, which has high GAMT activity. From these organs of synthesis, creatine is transported via the bloodstream to the organs of utilization (mainly muscle and brain), where both the endogenously synthetized creatine as well as the creatine derived from dietary sources is taken up by a sodium and chloride dependent creatine transporter (CRTR, *SLC6A8*). The major proportion of brain creatine is taken up from the blood via CRTR expressed at blood-brain barrier. A minor proportion is synthesized in the brain.
Part of intracellular creatine is reversibly converted into the high-energy compound creatine-phosphate, by the action of creatine kinase (CK). Three cytosolic isoforms of CK (brain type BB-CK, muscle type MM-CK and the MB-CK heterodimer), and two mitochondrial isoforms exist. Creatine and creatine-phosphate are non-enzymatically converted into creatinine, with a constant daily turnover of 1.5% of body creatine. Creatinine is mainly excreted in urine and its daily excretion is directly proportional to total body creatine, and in particular to muscle mass (i.e. 20–25 mg/kg/24 h in children and adults, and lower in infants younger than 2 years).



■ **Fig. 15.1 Metabolic pathway of creatine/creatine phosphate that mainly but not exclusively occurs in the organs indicated.** *AGAT,* arginine:glycine amidinotransferase; *GAMT,* guanidinoacetate methyltransferase; *CRTR,* creatine transporter (SLC6A8); *CK,* creatine kinase

Creatine deficiency syndromes (CDS) are a group of inborn errors of creatine synthesis (arginine:glycine amidinotransferase (AGAT) deficiency (MIM 602360) and guanidinoacetate methyltransferase (GAMT) deficiency (MIM 601240)), and transport (the X-linked creatine transporter CRTR deficiency (MIM 300036) (► Creatine Synthesis and Transport) [1]. CDS typically present with global developmental delay/ intellectual disability along with various neurological manifestations. Diagnostic markers include cerebral creatine deficiency and high and low guanidinoacetate concentrations in body fluids in GAMT and AGAT deficiency, respectively, and increased urinary creatine/creatinine in CRTR deficiency. Oral supplementation of creatine leads to near complete restoration of cerebral creatine in creatine synthesis defects: In GAMT deficiency, reduction of guanidinoacetate is achieved by ornithine supplementation and/or dietary arginine restriction. In CRTR deficiency, creatine, arginine and glycine supplementation does not significantly improve outcome, although partial clinical improvement has been reported in single patients. Normal neurodevelopmental outcome has been reported in early treated patients with creatine synthesis defects.
Secondary changes in creatine metabolism have been described in disorders affecting arginine and ornithine metabolism including ornithine aminotransferase (OAT) deficiency, urea cycle defects, hyperammonemia, hyperornithinemia, homocitrullinuria (HHH) syndrome, Δ(1)-pyrroline-5-carboxylate synthetase deficiency, defects of methylcobalamin synthesis and mitochondrial disease.

15.1 Clinical Presentation

The common clinical hallmark of CDS is global developmental delay (GDD) /intellectual disability (ID) with prominent speech delay. GDD or ID ranges from mild to severe and is characteristically associated with challenging (hyperactive, autistic) behaviours. Movement disorders and basal ganglia changes are additional features in GAMT deficiency. Myopathy is an additional feature of AGAT deficiency. Epilepsy occurs in GAMT and CRTR deficiencies.

15.1.1 Arginine Glycine Amidinotransferase (AGAT) Deficiency

Since its first description [2], less than 20 patients have been diagnosed worldwide. In a recent study of 16 patients (age 3 weeks to 25 years) from 8 families and 8 different ethnic backgrounds [3], ID with speech and language delay was the most common clinical feature. Patients are prone to develop clinical, electrophysiological and histopathological signs of myopathy.

15.1.2 Guanidinoacetate Methyltransferase (GAMT) Deficiency

Since its first description [4], about 110 patients have been diagnosed worldwide. In a recent study on 48 patients (age 1 week to 34 years) from 38 families [5], ID with speech and language delay, behavioural problems and epilepsy were the most common clinical features, followed by movement disorder and basal ganglia changes. There seems to be a positive relationship between the age at start of treatment and severity of ID. Seizures vary from occasional to drug resistant. Presentations masquerading as Leigh-like syndrome and mitochondrial disease [6], late onset ballistic and dystonic movement disorder [7] and intermittent fever induced ataxia [5] have been reported in single patients.

15.1.3 Creatine Transporter (CRTR) Deficiency

Since its first description [8], more than 160 male patients from 140 families have been diagnosed (www.LOVD.nl/SLC6A8).

In a recent study of 101 patients from 85 families [9] ID was the most common clinical feature. Less than one third of patients were able to speak in sentences. Besides behavioural problems and seizures, spasticity, movement disorder, and gastrointestinal problems (vomiting, reflux, feeding difficulties) were frequently reported. Additional features reported in patients include muscular hypotonia, low muscle mass, hyperextensible joints, short stature, brain atrophy, cardiac arrhythmia and discrete facial dysmorphic features. Neurological and psychiatric problems can be progressive in adulthood [10].

Females heterozygous for the familial pathogenic variant in *SLC6A8* may have learning disability or mild ID [11], but others may be more severely affected, such as a patient described by Mercimek-Mahmutoglu et al. [12] who had intractable frontal lobe epilepsy, mild ID and behavioural problems.

15.2 Metabolic Derangement

Cerebral creatine deficiency is caused by reduced synthesis of creatine in AGAT and GAMT deficiency or by impaired uptake into the brain in CRTR deficiency.

Reduced muscle creatine levels have been described in AGAT [13] and in GAMT deficiency [14][33].

Low intracellular creatine and creatine-phosphate result in reduced production of creatinine. Thus plasma creatinine concentration and urinary creatinine excretion are low in patients with CDS.

Guanidinoacetate is depleted in AGAT deficiency and accumulates in GAMT deficiency. Guanidinoacetate is an alternative substrate to CK [15]. High or normal levels of guanidinoacetate-phosphate in GAMT and CRTR deficiency might serve as a high-energy phosphate carrier in compensation for reduced levels of creatine phosphate. In AGAT deficiency, guanidinoacetate and creatine are not formed. Deficiency of creatine-phosphate and guanidinoacetate-phosphate is likely to be the cause of myopathy in AGAT deficiency.

The neurotoxic effects of high guanidinoaceate levels in the central nervous system (CNS) might explain why severe epilepsy is more common in patients with GAMT deficiency than those with the other two CDS.

S-adenosylmethionine is required as a methylgroup donor for the methylation of guanidinoacetate to form creatine. Although up to 75% of the body methylgroup transfer is utilized for the formation of creatine out of guanidinoacetate [16], no major alterations of S-adenosylmethionine and metabolites of the methylation and remethylation cycle have been found in AGAT [3] and GAMT (unpublished) deficiency.

15.3 Genetics

The genes encoding for AGAT and GAMT (*GATM* and *GAMT*) are mapped to chromosome 15q21.1 and 19p13.3, respectively. Both disorders are inherited autosomal recessively.

Nine different pathogenic *GATM* variants (nonsense, splice, frame-shift, missense) have been identified in the 8 families known so far with AGAT deficiency. Each of the 8 families came from a different ethnic background and each had family specific pathogenic variants [3].

About 60 different pathogenic variants in *GAMT* have been identified. More than 60% are missense variants. c.327G>A and c.59G>A have been reported in 24% and in 21% of alleles. While c.327G>A occurs in all ethnicities, c.59G>A has been found in patients from Spain, Portugal and Turkey [5][17][18][19]. Carrier frequency of GAMT deficiency was between 1 in 250 in Dutch [20] and 1 in 1475 in British Columbian newborns [21] and 1 in 812 individuals in the general population [22].

At present, over 90 pathogenic *SLC6A8* variants have been found in CRTR deficiency (www.LOVD.nl/SLC6A8). Among 101 males from 85 families [9], one third of patients had a *de novo* pathogenic variant. The possibility of low level somatic or germline mosaicism [23] should be taken into account when counselling mothers of boys with seemingly *de novo* variants. Pathogenic missense variants with residual activity might be associated with a milder phenotype [9], whereas large deletions extending beyond the 3' end of *SLC6A8* were associated with a more severe phenotype in a recent study [24].

Compared to the other CDS, the prevalence of CRTR deficiencies is relatively high. Combined analysis of all studies published, including cohorts of males with ID and autism and ID and neurological disease, yielded a prevalence of 0.4% (CI 0.2–0.5) [25].

15.4 Diagnostic Tests

CDS present with non-specific developmental / speech delay. As they are amenable to treatment, biochemical / genetic screening for CDS should be considered in the list of investigations for every patient with unexplained GDD/ID [26]. Patients requiring a brain MRI as part of the diagnostic evaluation should also have MRS to screen for cerebral creatine deficiency.

15.4.1 *In Vivo* Brain MRS

Profound cerebral creatine deficiency is recognized by in vivo brain ^{1}H-MRS in patients with GAMT and AGAT deficiencies and in males with CRTR deficiency. Females with CRTR deficiency can have normal or mildly decreased creatine on brain ^{1}H-MRS. In patients with low brain creatine, further diagnostic tests including urinary guanidinoacetate and creatine to creatinine ratio measurements as well as mutation analysis are required for differentiation of the three CDS. Brain ^{1}H-MRS is not only employed for diagnostic purpose, but also for monitoring cerebral creatine upon treatment.

15.4.2 Metabolite Analysis

Analysis of urinary guanidinoacetate, creatine and creatinine is an important screening test for all CDS [5]. Low guanidinoacetate levels are characteristic of AGAT deficiency. In the majority of patients, urine and plasma guanidinoacetate levels are undetectable or ≤10% of lower range of the reference values [3].

Elevated guanidinoacetate in urine is a sensitive marker for GAMT deficiency. In untreated patients, urine and plasma guanidinoacetate levels are often more than 10 times higher than normal; in CSF, more than hundredfold elevations compared to normal are found [5][17].

Plasma creatine levels are largely influenced by nutritional factors [27] and normal plasma creatine levels do not exclude CDS.

In CRTR deficiency presumably impaired renal tubular [re]uptake results in urinary loss of creatine, hence in a high urinary creatine/creatinine ratio which serves as a valuable diagnostic marker in males [9][28]. Symptomatic and asymptomatic heterozygous females have normal or mildly elevated urinary creatine/creatinine ratio [11][12]. False positive values may be detected that may be explained by creatine rich diet and can be found in other disorders with muscle involvement [27].

Reference values have been established for urinary creatine and creatinine excretion [1][28][29] showing a strong dependence on age and sex.

An overall increased concentration of amino acids and organic acids in urine may be a result of a decreased creatinine excretion and suggest the possibility of CDS.

15.4.3 DNA Analysis

Mutation analysis of *GATM, GAMT* and *SLC6A8* is required to confirm the diagnosis. Single gene tests or CDS gene panels are available in clinical molecular genetics laboratories. Whole exome or genome sequencing will help to diagnose more patients.

15.4.4 Functional Tests

Enzymatic analyses in fibroblasts and/or lymphoblasts for AGAT and GAMT deficiency and creatine uptake in fibroblasts is necessary 1) if missense variants with unclear pathogenicity have been identified; or 2) in the case of a strong suspicion of CDS but where no pathogenic variants are detected. Overexpression of missense variants for functional characterization may be necessary to confirm the pathogenicity of the variant.

15.4.5 Prenatal Diagnosis

Prenatal diagnosis and preimplantation genetic diagnosis for at-risk pregnancies require prior identification of the disease-causing variant(s) in the family for all three CDS. In GAMT deficiency prenatal diagnosis can be made by determination of guanidinoacetate in amniotic fluid [30].

15.4.6 Newborn Screening

Elevated guanidinoacetate levels have been confirmed in blood spots of affected newborns with GAMT deficiency [21][31]. A two-tiered approach with the addition of a chromatographic step to remove the interfering chemicals has been successful in reducing the false positive rate [21][32].

Newborn screening for AGAT deficiency is more challenging, given that the normal range for guanidinoacetate is below the detection limit of assays suitable for blood spot analysis. Newborn screening for CRTR deficiency is so far not feasible due to lack of a suitable biomarker detectable in blood spots as well as insufficient evidence of treatability of this condition.

15.5 Treatment and Prognosis

15.5.1 *AGAT* Deficiency

The aim of treatment is to restore cerebral and muscular creatine levels. Treatment with creatine monohydrate (100–800 mg/kg/d) results in almost complete restoration of brain creatine levels and significant improvement of myopathy in most patients [3]. Early diagnosis and treatment may prevent ID and myopathy in patients [3].

15.5.2 GAMT Deficiency

The aim of treatment is to restore cerebral creatine levels and to suppress accumulation of guanidinoacetate. In a recent study long-term treatment outcome of 48 patients with GAMT deficiency was reported [5]. Creatine-monohydrate supplementation (400–800 mg/kg) resulted in the correction of reduced cerebral creatine levels. Suppression of guanidinoacetate production was achieved by additional L-ornithine supplementation (by competitive inhibition of AGAT activity) and dietary arginine restriction (substrate deprivation). The guanidinoacetate reducing effect of L-ornithine is best achieved by dosages of 400–800 mg/kg/d. Dietary arginine restriction allowing for only 0.2–0.3 grams/kg/day natural protein intake and supplementation of arginine-free amino-acid formula has been shown to effectively reduce, but not normalize, plasma and CSF guanidinoacetate levels [5][17].

Sodium benzoate has been recommended as an additional guanidinoacetate lowering approach via its ability to conjugate with glycine and thus reduce its availability for guanidinoacetate synthesis [33]. In one patient treated with L-ornithine and arginine restricted diet, additional sodium benzoate resulted in only 12% additional reduction of plasma guanidinoacetate levels [34].

Early initiation of combined treatments improves neurodevelopmental outcomes [5]. Normal neurodevelopment has been achieved in very early treated patients [5].

15.5.3 CRTR Deficiency

Among the three CDS, CRTR deficiency appears to be the most difficult to treat as cerebral creatine restoration has not been achieved thus far [25]. Besides high dose creatine supplementation, arginine, glycine and S-adenosylmethionine supplementation (substrates for creatine synthesis) have been employed with the aim to enhance intracerebral creatine synthesis. Improvements mainly in muscle mass, behaviour, communication, gross motor abilities, and epilepsy have been observed [35]. S-adenosylmethionine as an add-on to creatine, arginine and glycine supplementation [36] or supplementation of creatine ethylester [37] did not result in an increase of cerebral creatine levels.

High levels of homocysteine and reduced levels of folate have been reported during supplementation with arginine but may be prevented by simultaneous supplementation of folate and/or creatine [38].

A brain-specific *SLC6A8* knockout mouse was successfully treated with the cyclocreatine, a creatine analog [39]. Clinical trials are underway to prove its use in individuals with CRTR deficiency.

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Section IV Disorders of Amino Acid Metabolism and Transport

Hyperphenylalaninaemia

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Phenylalanine Metabolism

Phenylalanine (PHE), an essential aromatic amino acid, is mainly metabolised in the liver by the PHE hydroxylase (PAH) system (Fig. 16.1). The first step in the irreversible catabolism of PHE is hydroxylation to tyrosine (TYR) by PAH. This enzyme requires the active pterin, tetrahydrobiopterin (BH_4), which is formed in three steps from guanosine triphosphate (GTP). During the hydroxylation reaction BH_4 is converted to the inactive pterin-4a-carbinolamine. Two enzymes regenerate BH_4 via q-dihydrobiopterin (qBH_2). BH_4 is also an obligate co-factor for tyrosine hydroxylase and tryptophan hydroxylase, and thus necessary for the production of dopamine, catecholamines, melanin and serotonin, and for alkylglycerol monooxygenase (AGMO) and nitric oxide synthase [1]. The physiological role of AGMO, which is involved in ether lipid metabolism, is not yet fully characterised.

Defects in either PAH or the production or recycling of BH_4 may result in hyperphenylalaninaemia (HPA), as well as in deficiency of TYR, L-dopa, dopamine, melanin, catecholamines and 5-hydroxytryptophan (5HT). When hydroxylation to TYR is impeded, PHE may be transaminated to phenylpyruvic acid (a ketone excreted in increased amounts in the urine, whence the term phenylketonuria or PKU), and further reduced and decarboxylated.

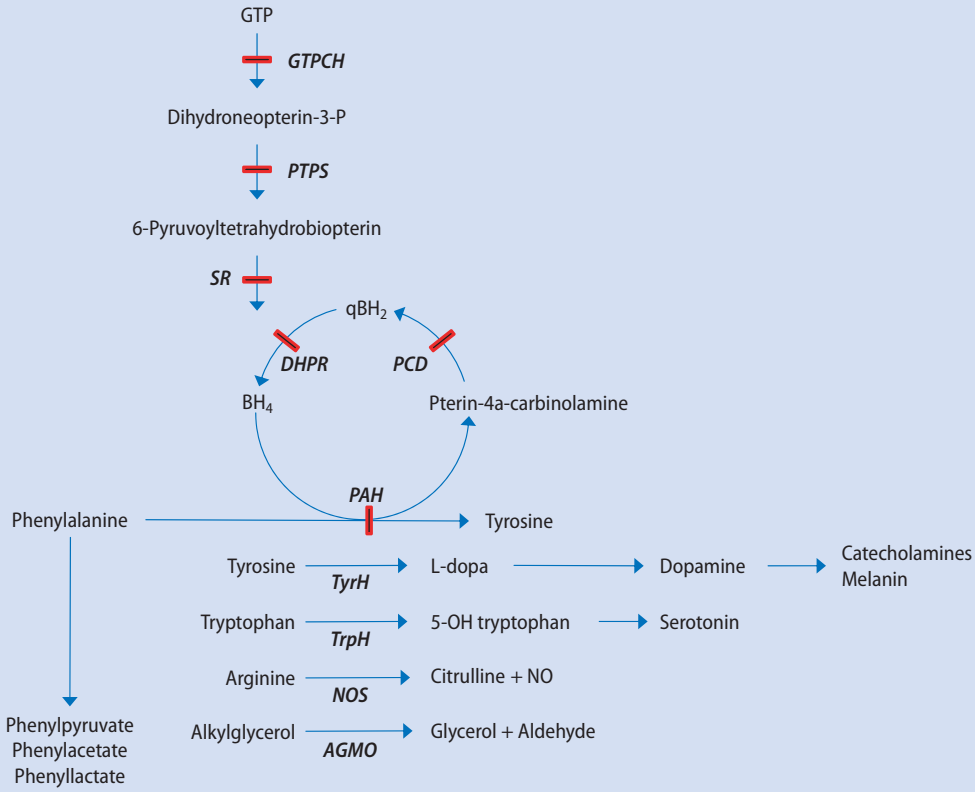

Fig. 16.1 The phenylalanine hydroxylation system, including the synthesis and regeneration of pterins and other pterin-requiring enzymes. *AGMO*, alkylglycerol monooxygenase; *BH_2*, dihydrobiopterin (quinone); *BH_4*, tetrahydrobiopterin; *DHPR*, dihydropteridine reductase; *GTP*, guanosine triphosphate; *GTPCH*, guanosine triphosphate cyclohydrolase; *NO*, nitric oxide; *NOS*, nitric oxide synthase; *P*, phosphate; *PAH*, PHE hydroxylase; *PCD*, pterin-4a-carbinolamine dehydratase; *PTPS*, pyruvoyl-tetrahydrobiopterin synthase; *SR*, sepiapterin reductase; *TrpH*, tryptophan hydroxylase; *TyrH*, tyrosine hydroxylase. The enzyme defects are depicted by solid bars across the arrows

Mutations within the gene for the hepatic enzyme phenylalanine hydroxylase (PAH) and those involving production or recycling of tetrahydrobiopterin metabolism are associated with hyperphenylalaninaemia (HPA). Severe PAH deficiency, which results in a blood phenylalanine (PHE) greater than 1200 µmol/l when individuals are on a normal protein intake, is referred to as classic phenylketonuria (PKU) or just PKU. Milder defects associated with levels between 600 µmol/l and 1200 µmol/l are termed hyperphenylalaninaemia (HPA), and those with levels less than 600 µmol/l but above 120 µmol/l, mild HPA (MHP). Disorders of biopterin metabolism have in the past been called malignant PKU or malignant HPA. However, such disorders are now best named according to the underlying enzyme deficiency. A comprehensive nomenclature is provided in [2]. PKU if left untreated leads to permanent central nervous system damage. Dietary restriction of PHE along with amino acid, vitamin and mineral supplements, started in the first weeks of life and continued through childhood, is an effective treatment and allows for normal cognitive development. Pharmacologic treatment with BH_4 can be effective in individuals with residual PAH activity. Continued dietary treatment into adulthood with PKU is now generally recommended but as yet there is insufficient data to know how necessary this is. Less severe forms of PAH deficiency may or may not require treatment, depending on the degree of HPA; however, there is no evidence-based level for blood PHE below which treatment is not required. High blood levels in mothers with PKU lead to foetal damage. This can be prevented by reducing maternal blood PHE throughout the pregnancy with dietary treatment. Disorders of pterin metabolism lead to both HPA and disturbances in central nervous system amines. Generally, they require treatment with oral BH_4 and neurotransmitters.

16.1 Phenylalanine Hydroxylase Deficiency

16.1.1 Clinical Presentation

The natural history of PKU is for affected individuals to suffer progressive, irreversible neurological impairment during infancy and childhood; untreated patients develop mental, behavioural, neurological and physical impairments. The most common outcome is a moderate to profound intellectual developmental disorder (IQ ≤50), often associated with a mousey odour (resulting from the excretion of phenylacetic acid), eczema (20–40%), reduced hair, skin and iris pigmentation (a consequence of reduced melanin synthesis), reduced growth and microcephaly, and neurological impairments (25% epilepsy, 30% tremor, 5% spasticity of the limbs, 80% EEG abnormalities). The brains of patients with PKU untreated in childhood have reduced arborisation of dendrites, impaired synaptogenesis and disturbed myelination. Other neurological features include pyramidal signs with increased muscle tone, hyperreflexia, Parkinsonian signs and abnormalities of gait and tics. Almost all untreated patients show behavioural problems, which include hyperactivity, stereotypy, aggressiveness, anxiety and social withdrawal. The clinical phenotype correlates with PHE blood levels, reflecting the degree of PAH deficiency.

16.1.2 Metabolic Derangement

Although the pathogenesis of brain damage in PKU is not fully understood, it is causally related to the increased levels of blood PHE. Tyrosine (TYR) becomes a semi-essential amino acid, with reduced blood levels leading to impaired synthesis of other biogenic amines, including melanin, dopamine and norepinephrine. Increased blood PHE levels result in an imbalance of other large neutral amino acids (LNAA) within the brain, resulting in decreased brain concentrations of TYR and serotonin. The ratio of PHE levels in blood/brain is about 4:1 [3]. In addition to the effects on amino acid transport into the brain, PHE impairs the metabolism of TYR hydroxylation to dopamine and tryptophan decarboxylation to serotonin. The phenylketones phenylpyruvate, phenylacetate and phenyllactate are not abnormal metabolites, but appear in increased concentration and are excreted in the urine.

16.1.3 Genetics

PAH deficiency is autosomal-recessively transmitted. At the time of writing 955 different *PAH* mutations have been described (http://www.biopku.org/home/pah.asp). Most patients are compound heterozygous. Although there is no single prevalent mutation, certain ones are more common in different ethnic populations: the R408W mutation accounts for approximately 30% of alleles in Europeans with PKU; in Orientals the R243Q mutation is the most prevalent (13% of alleles). The prevalence of PAH deficiency varies between different populations (e.g. 1 in 1,000,000 in Finland and 1 in 4,200 in Turkey). Overall global prevalence in screened populations is approximately 1 in 12,000, giving an estimated carrier frequency of 1 in 55.

Genotypes correlate well with biochemical phenotypes, pre-treatment PHE levels and PHE tolerance [4], which are determined by the milder mutation in compound heterozygotes. However, owing to the many other factors that affect clinical phenotype, correlations between mutations and neurological, intellectual and behavioural outcome are weak. Mutation analysis is of limited practical use in clinical management, but may be of value in determining genotypes associated with BH_4 responsiveness (http://www.biopku.org/BioPKU_DatabasesBIOPKU.asp) [5].

16.1.4 Diagnostic Tests

Blood PHE is normal at birth, but rises rapidly within the first days of life. In most Western nations PKU is detected by newborn population screening (NBS). There is variation between different countries and centres in the age at which screening

Table 16.1 Daily phenylalanine (*PHE*) tolerances and target blood levels, showing different targets aimed for in various countries

Patient age	PHE tolerance mg/day	Target blood PHE (µmol/l)					
		Germany	UK	USA	France	The Netherlands	ESPKU
0–2 years	130–400	40–240	120–360	120–360	120–300	120–360	120–360
3–6 years	200–400					120–480	
7–9 years	200–400		120–480				
10–12 years	350–800	40–900			<900		
13–15 years	350–800		120–700				120–600
Adolescent/adult	450–1000	40–1200			<1500	120–600	
Pregnancy	120–400*	120–360					

*tolerance will usually increase in later stages of pregnancy

is undertaken (day 1 to day 5), in the methodology used (Guthrie microbiological inhibition test, enzymatic techniques, HPLC, or tandem mass spectrometry) and the level of blood PHE that is taken as a positive result requiring further investigation (120–240 µmol/l, but with some laboratories also using a PHE/TYR ratio >3).

Co-factor defects must be excluded by investigation of pterins in blood or urine and dihydropteridine reductase (DHPR) in blood (▶ Section 16.3). HPA may be found in preterm and sick babies, particularly after parenteral feeding with amino acids and in those with liver disease (where blood levels of methionine, TYR, leucine/isoleucine and PHE are usually also raised), and in treatment with chemotherapeutic drugs or trimethoprim.

PAH deficiency may be classified according to the blood PHE concentration when patients are on a normal protein-containing diet, after a standardised protein challenge, or after standardised loading with BH_4 [2]:

- Classic PKU (PHE ≥1200 µmol/l; less than 1% residual PAH activity),
- Hyperphenylalaninaemia (HPA) or mild PKU (PHE >600 µmol/l and <1200 µmol/l; 1–5% residual PAH activity), and
- Non-PKU-HPA or mild hyperphenylalaninaemia (MHP) (PHE ≤600 µmol/l; >5% residual PAH activity),
- BH_4-Responsive PKU/HPA (blood PHE levels decrease substantially after oral administration of BH_4, thus increasing dietary PHE tolerance (▶ Section 16.1.5, »Treatment with BH_4«).

Although the spectrum of severity is continuous, such a classification has some use in terms of indicating the necessity for and type of treatment.

Prenatal diagnosis, rarely requested, is possible by means of *PAH* DNA analysis on chorion villus biopsy (CVB) or amniocentesis where the index case has mutations identified previously.

16.1.5 Treatment and Prognosis

Principles of Treatment

Dietary Treatment

Dietary treatment for PKU has proved highly successful and has provided a model for the dietary management of other aminoacidopathies, such as MSUD and classic homocystinuria. The principle of treatment in PAH deficiency is to reduce the blood PHE concentration sufficiently to prevent the neuropathological effects but also to fulfil age-dependent requirements for protein synthesis. Blood PHE is primarily a function of residual PAH activity and PHE intake. For the majority of patients with PKU the former cannot be altered, so that blood PHE must be reduced by restricting dietary PHE intake. The blood PHE concentration while on a normal protein-containing diet, defines whether treatment is indicated. There are some minor differences in the recommended cut-off above which PHE restriction is required: UK >400 µmol/l, Germany >600 µmol/l, USA >360 µmol/l [6][7], and 2016 European Society for PKU (ESPKU) guidelines >360 µmol/l [8]. To stay below these, patients with classic PKU will have to reduce nutritional PHE intake to 200–400 mg/day or 4–8 g natural protein per day. In all but the USA recommendations, treatment target blood PHE levels are age related but show substantial variation. Table 16.1 shows recommendations for UK, Germany, the USA, France, the Netherlands, and the 2016 ESPKU guidelines. Since PHE is an essential aminoacid, excessive restriction is also harmful and, particularly in infancy, will result in impaired growth and cognitive development. In order to prevent PHE deficiency a lower limit for blood PHE is also defined.

The degree of protein restriction required is such that in order to provide a nutritionally adequate supply a semi-synthetic diet is necessary. This is composed of the following:

- Unrestricted natural foods with a very low PHE content (<30 mg/100 g; e.g. carbohydrate; fat, some fruit and vegetables).

- Calculated amounts of restricted natural and manufactured foods with medium PHE content (30–100 mg/100 g; e.g. potato, spinach, broccoli; special bread and special pasta). In the United Kingdom a system of 'protein exchanges' is used, with each 1 g of natural protein representing a PHE content of approximately 50 mg.
- Calculated amounts of PHE-free amino acid mixtures (AAMs) supplemented with vitamins, minerals and trace elements. The biological value of AAMs is lower than that of natural protein; the equivalent daily protein from this source needs to be 20% higher than the age related reference values for natural protein.

Intake of these three components – including the PHE-free amino acid mixture – should be distributed as evenly as possible with meals during the day.

Those foods with a higher concentration of PHE (e.g. meat, fish, cheese, egg, milk, yoghurt, cream, rice, corn) are not allowed. Aspartame (L-aspartyl-L-phenylalanine methyl ester), a sweetener for foods (e.g. in soft drinks) contains 50% PHE and is therefore inappropriate in the PKU diet.

PHE-free amino acid infant formulas that also contain adequate essential fatty acids, minerals and vitamins are available. Human breast milk has relatively low PHE content; in breast-fed infants, PHE-free formulas are given in measured amounts followed by breast feeding to appetite. In the absence of breast feeding a calculated quantity of a normal formula is given to provide the essential daily requirement of PHE. Glycomacropetide, an intact whey protein containing 2.0 to 5.0 mg PHE per gram [9] may partly substitute AMMs thereby improving bioavailability and palatability.

With intercurrent illness, individuals may be unable to take their prescribed diet. During this period high-energy fluids may be given to counteract catabolism of body protein.

Treatment with BH_4

Pharmacological doses of BH_4 can reduce blood phenylalanine levels in some patients with PKU [10]; sapropterin dihydrochloride (Kuvan), a synthetic formulation of the active 6R-isomer of BH_4 is approved in Europe and the USA for the treatment of patients with HPA and PKU, of all ages, who have been shown to be responsive to such treatment. Most frequently BH_4 responsiveness is defined by a reduction of ≥30% in blood PHE level after a single dose of 20 mg BH_4/kg body weight, but there are alternative criteria [11]. It has been suggested that a more clinically relevant assessment is to initially determine BH_4-responsiveness with a screening test, measuring the decrease of blood PHE after a single BH_4 dose of 20 mg/kg, followed, if there has been a decrease ≥30%, by a further period of BH_4 treatment to assess the increase in PHE tolerance [2].

Studies on the PKU *Pah*^{enu1} mouse, a model of the mild hyperphenylalaninaemia phenotype, and expression studies of mutations found in BH_4-responsive patients have shown that reduced function of PAH can result from misfolding, aggregation and accelerated degradation of the enzyme. BH_4 may act as a chaperone, providing conformational stabilisation and augmenting the effective PAH concentration [12] [13], with different genotypes showing optimal responses at different PHE concentrations [14]. Treatment with BH_4 consists of single daily doses of 5-20 mg/kg body weight, with the aim of decreasing blood PHE levels or increasing dietary PHE tolerance. Both effects have been demonstrated in placebo controlled trials [15].

BH_4 responsiveness is most often found in those with mild PKU, who have a higher residual PHA activity. Except for where there are two null-mutations, the association between genotype and BH_4-responsiveness is probabilistic, and BH_4 responsiveness should always be tested clinically [5]. The manufacturer's prescribing information [16] and a US FDA drug review recommend that Kuvan be used in combination with a PHE-reduced diet, leaving open the question of BH_4 monotherapy for those patients who would with treatment have PHE levels sufficiently low not to require diet. There are no serious side effects in the short and midterm [17]. Given the different protocols and the limitations of the 30% criterion in determining BH_4 responsiveness, it is impossible to predict the proportion of patients who might benefit significantly from long-term treatment [15]. Limited data suggest that the use of BH_4 in pregnancy is effective and safe in controlling PHE levels in responsive patients [16][18] but diet remains the first-line treatment for pregnant women. Despite increased cost and regimen complexity, treatment with BH_4 can result in a substantial improvement in the quality of life in a subgroup of patients with PKU.

Monitoring of Treatment

A low-protein diet brings the risk of nutritional deficiency. Therefore, treatment is monitored by regular assessment of dietary intake and blood PHE levels, as well as neurological, physical, intellectual and behavioural development.

Alternative Therapies/Experimental Trials

Although dietary treatment is highly successful, it is difficult and compliance is often poor, particularly as individuals reach adolescence. Hence there is a need to develop more acceptable therapies.

- *Gene therapy*: A number of different PAH gene transfer vehicles have been tried in the *Pah*^{enu2} mouse. Vectors based on adeno-associated viruses (AAVs) expressed in either liver or muscle have been most promising. An AAV vector with genes for PAH and BH_4 synthesis injected into skeletal muscle of *Pah*^{enu2} mice resulted in correction of PHE for more than 1 year [19]. However, more experimental work is required before clinical trials in humans are undertaken.
- *Liver transplantation* fully corrects PAH deficiency [20], but the risks of transplantation surgery and post-transplantation immune suppressive medication are too high for it to be a realistic alternative to dietary treatment. The same is true for liver repopulation with PAH-expressing cells following hepatocyte or haematopoietic stem cell transplantation [21].

- *Phenylalanine ammonia lyase (PAL)*: Recombinant *Anabaena variabilis* PAL converts PHE to a harmless compound, transcinnamic acid. It can lower phenylalanine levels in a mouse model of PKU. Covalent attachment of polyethylene glycol polymer chains (PEGylation) ›mask‹ the agent from the host's immune system, reducing immunogenicity and antigenicity. rAvPAL-PEG is well tolerated by most human subjects, with only a minority having local or generalised reactions to the drug. Subcutaneous injection of the enzyme at 0.1 mg/kg body weight per dose was effective in reducing phenylalanine levels. Ongoing trials (phase III) are assessing the safety and efficacy of repeated doses in patients with PKU [22][23].
- The *large neutral amino acids (LNAA)* phenylalanine, tyrosine, tryptophan, leucine, isoleucine and valine compete for the same transport mechanism (the L-type amino acid carrier) to cross the blood-brain barrier as well as for the absorption by the intestinal mucosa [24]. Studies in the *Pah*^{enu2} mouse and in patients have shown a reduction in brain PHE levels and some positive effect on neuropsychological functions when LNAAs (apart from PHE) have been given enterally [25][26]. The greatest benefit may be to patients who are unable to comply with conventional dietary management but it is likely to be of limited efficacy.

Compliance with Treatment

Compliance with treatment is best in infancy and childhood. The diet severely interferes with culturally normal eating habits, particularly in older children and adolescents, and this often results in problems with keeping to treatment recommendations. Up to the age of 10 years only 40% of the German Collaborative Study of PKU patients could keep their PHE levels in the recommended range [27]. After the age of 10 years 50–80% of blood PHE levels measured in a British and Australian sample were above recommendation [28]. In the US, patient, social, and economic factors prevent >70% of adult patients from accessing treatment [29]. Dietary treatment of PKU is almost impossible without the support of a specialised team, which should include a dietitian, a metabolic paediatrician or physician for adult patients, a biochemist running a metabolic laboratory and a psychologist skilled in the behavioural management of a life-long diet. All professionals, and the families themselves, must fully understand the principles and practice of the diet. The therapeutic team should be trained to work in an interdisciplinary way in a treatment centre, which should care for at least 20 patients to have sufficient expertise [7][30].

Outcome

The outcome for PKU mainly depends upon the age at start of treatment, blood PHE levels in different age periods, duration of periods of blood PHE deficiency and the individual gradient for PHE transport across the blood-brain barrier. The most important single factor is the blood PHE level in infancy and childhood. Dietary treatment started within the first 3 weeks of life with average blood PHE levels ≤400 µmol/l in infancy and early childhood result in near-normal intellectual development. However, for each 300 µmol/l increase in blood PHE during the first 6 years of life, IQ is reduced by 0.5 SD, and during age 5–10 years the reduction is 0.25 SD. Furthermore, IQ at the age of 4 years is reduced by 0.25 SD for each 4 weeks of delay in the start of treatment and for each 5 months of insufficient PHE intake. After the age of 10 years all large studies show stable IQ performance, at least until mid-adulthood irrespective of PHE levels [31], and a normal school career if compliance during the first 10 years has been according to treatment recommendations [32][33][34][35]. A Bayesian meta-analysis covering the age range from 2 to 35 years distinguished long-term and concurrent blood PHE levels in a critical (<6 years) and non-critical period (≥6 years) as predictors for an IQ <85 [36]. Effects of long-term PHE were larger than for concurrent values and those for the critical period were stronger than for the later period. The associations of PHE with IQ were negative, with PHE measurements <400 µmol/l, predicting probabilities of IQ >85 close to the general population. However, correlations between concurrent and long-term PHE values, between the critical and non-critical period as well as age at start of treatment, degree of PAH activity, different IQ tests and socioeconomic status were not controlled. Compared with a matched control group over a 5 year period IQ of early-treated patients with classical PKU aged 10 to 41 years with blood PHE levels between 600 and 900 µmol/l remained stable. Older adults performed worse than younger ones, explained by higher PHE levels during childhood or adolescence [31]. IQs of early and well-treated adults with PKU are similar to those of their unaffected family members [32] however, longitudinal studies covering adulthood are still rare [37]. Quality of life (QoL) has become an important outcome. Despite the burden of strict dietary control, early treated patients can have a normal QoL [38][39]. QoL issues also apply to parents of patients with PKU [40].

Complications in Adulthood

The majority of early-treated patients are now adults. Dietary treatment has transformed their prospects: they can expect normal development, professional careers, to start families and to live independently. Nonetheless, when studied in detail, subtle neuropsychological deficits have been found. Neuroimaging reveals abnormalities in some and frank neurological disease has been reported in a few. This is concerning and it is important to define the precise phenotype of adults with early-treated PKU and determine which features relate to raised PHE concentrations, either historical or concurrent.

Neurological Abnormalities

Frank neurological disease is rare, and may not be related to PHE levels. Subacute combined degeneration of the spinal cord has been reported in adults after dietary relaxation. These patients had all developed profound vitamin B_{12} deficiency because they had stopped taking their amino acid supplements, but continued to follow diets low in high quality natu-

ral protein [41]. Early and well treated patients as well as those on relaxed diet can show tremor and brisk reflexes [42], the aetiology and clinical significance of which are unclear. Other complications such as cortical visual loss [43][44] are very infrequent and possibly associated with poor control in childhood or adolescence [45]. Reinstitution of dietary treatment and amino acid supplements can lead to improvement.

Neuropsychological Abnormalities

Subtle changes in executive function have been described in children, adolescents and adults with early-treated PKU. Effects remain stable in adulthood [31], but their clinical significance is unclear. Reaction time studies demonstrate a lifelong, but reversible, vulnerability of the brain to increased concurrent PHE levels [46]. Meta-analytic results suggest an upper threshold for PHE concentrations of 320 µmol/l for children (7–12 years) and 570 µmol/l for adolescents (13–18 years). In adults the negative effect remained stable between PHE concentrations of 750–1500 µmol/l [47]. Information processing was also stable in a 5 year longitudinal controlled study of adult patients with classical PKU [31]. The latency of visual saccades [48] in adults with PKU with concurrent PHE levels greater than 1200 µmol/l was significantly longer than in control patients, whilst no difference was detected with PHE levels below 800 µmol/l. Saccadic latencies normalised with improved metabolic control.

Neuroimaging Abnormalities

White matter abnormalities on brain MRI appear after longer periods of increased PHE levels, but are reversible after 3–6 months of strict dietary treatment [49]. In all but one study MRI did not correlate with intellectual or neurological abnormalities. In one study, 67% of patients under 10 years old had normal-appearing white matter. This decreased to only 4% in those over 20 [50], although there was no evidence of any neurological deterioration over this period. Alterations were associated with long-term PHE levels. Diffusion tensor imaging has demonstrated that patients with PKU have reduced diffusivity of water molecules with intact fractional anisotropy [51][52], suggesting that the axons in PKU white matter are structurally intact, but that water molecules move more slowly along them. The clinical relevance of any of these imaging findings needs to be demonstrated.

Neuropsychiatric Abnormalities

Although no association of PKU with psychiatric disease has been demonstrated, increased emotional and behavioural symptoms have been described [53]. Patients with poor dietary control during infancy show hyperactivity, temper tantrums, increased anxiety and social withdrawal, frequently associated with intellectual deficits. Well-treated subjects show increased risks of depressive symptoms and low self-esteem. However, without correlation to PHE levels, causality remains obscure, and such problems are also common in other chronic disease populations [54][55].

Dietary Deficiencies

Vitamin B_{12} deficiency is well recognised in patients who have stopped their vitamin supplements but continue to restrict their natural protein intake. For patients on a strict diet there have been concerns regarding deficiencies in vitamins and minerals, including selenium, zinc, iron, retinol and long-chain omega-3 polyunsaturated fatty acids (LC-PUFA). Such deficiencies are sometimes found but it is unclear whether they are of any clinical significance. Low calcium, osteopenia and an increased risk of fractures have also been reported, however despite individual studies reporting reduced bone mineral density, pooled data suggest that these reductions are not clinically important [56]. LC-PUFAs, already added in PHE-free infant formulas, have been shown to be low in children aged >4 years. Experimental supplementation has been tolerated well and resulted in increased visual evoked potentials and motor performance, but the optimal type and dose of supply still needs to be determined [57].

Diet for Life

Adults with PKU face different challenges from children, and it is much more difficult to be proscriptive about their dietary management. Although biochemically attractive, the concept of diet for life does pose substantial obstacles. For adolescents with PKU, the low-protein diet is restrictive, imposes a stark differentiation between them and their peers and is enforced by their parents: it is not surprising that many rebel against it. Those who wish to stay on diet when they leave the parental home may lack the skills, financial resources and time required. With suitable support these problems can be overcome, but the majority of adults are poorly compliant with dietetic advice with less than 30% of PHE levels falling within target ranges [28][58]. This is likely to be because most adults who have had a period when their diet has lapsed have noticed no ill effects.

Despite the lack of consistent evidence for any irreversible effects of PHE on the adult brain, recent guidelines are recommending much more stringent PHE control in adults than has historically been the case [6]. It remains to be seen how patients who have previously been models of compliance will respond to these new targets. Although experience with maternal PKU (see below) suggests that patients can obtain PHE levels below 360 µmol/l if they need to, pregnant women with PKU don't choose to maintain such a strict diet once their children have been born.

It has always been difficult to persuade the many patients who are leading a normal life and eating a normal diet of the need to return to the restrictions of their childhood. It is important, however, that these individuals remain under expert care by metabolic physicians and dietitians with a training in behavioural and adult medicine, to ensure that they are following a nutritionally adequate diet, to monitor their long-term outcome, and to keep them informed about new evidence and treatments. A pragmatic approach is likely to be most productive, recommending broader corridors for PHE target levels, and giving adults with PKU the support, training and resources they need to follow their own choices [59].

Management of Late-diagnosed PKU

Caring for adults with late-diagnosed PKU poses a unique set of problems. Although there is a wide spectrum, most of these patients will not be able to live independently. These older patients were either never treated, or treated late, when brain damage was already established, and often came off a low-protein diet at a young age. Although returning to diet does not affect established neurological disease, it can improve difficult behaviour. In a randomised double-blind cross-over trial of the reintroduction of diet in patients with late-diagnosed PKU, carers rated behaviour as significantly better when subjects were on a low-PHE diet [60]. A 6-month trial of dietary treatment is warranted in late-diagnosed patients with challenging behaviour.

For late-diagnosed patients who remain at home into adulthood, the major challenge is planning for what will happen when their parents are no longer able to care for them. Eventually, these individuals will need alternative arrangements for their long-term care. This is best done in good time and with parental participation. If arrangements have to be made in an emergency, because of ill health or death of a carer, the results can be disastrous.

16.2 Maternal PKU

16.2.1 Clinical Presentation

Before the introduction of NBS and early treatment, it was unusual for women with PKU to have children of their own: as with other women with learning difficulties, positive steps were often taken to control their fertility. Initial observations that some children of mothers with PKU also had learning difficulties and behavioural problems were interpreted as genetic transmission rather than an environmental problem. But the first description of maternal PKU syndrome (MPKUS) recognised the teratogenic effects of high maternal PHE levels [61]. Offspring of women with untreated classical PKU suffer developmental delay (92%), microcephaly (73%), cardiac defects (12%), low birth weight (40%) and dysmorphic features [62].

Although the pathogenesis of this condition is still poorly understood, much progress has been made and the MPKUS is now a preventable disease.

16.2.2 Metabolic Derangement

Teratogenic Effects of Phenylalanine

The Maternal PKU Collaborative Study (MPKUCS), initiated in 1984 investigated the efficacy of dietary treatment in preventing the MPKUS [63]. They showed that for maternal PHE levels below 360 μmol/l, there was no evidence of any deleterious effect on the foetus. For levels above 360 μmol/l, developmental indices decreased by about three points for every 60 μmol/l rise in average maternal PHE level. Congenital heart disease (CHD) was only seen in mothers with much higher PHE levels (≥900 μmol/l) [64]. The risk of CHD increases with increasing PHE exposure; 50% of mothers who had children with CHD had average PHE levels ≥1500 μmol/l.

16.2.3 Treatment and Prognosis

Prevention of the Maternal PKU Syndrome

Strict dietary control for pregnant women with PKU and, preferably those who are planning pregnancy, is necessary: many studies have clearly shown that the institution of strict metabolic control before conception and throughout pregnancy is associated with normal outcomes [65].

The plasma PHE targets used in maternal PKU have changed over time. Initially target levels of below 600 μmol/l led to improved outcomes and reduced incidence of CHD to background levels. The policy to aim for even lower levels was based on the fact that, in infancy, PHE levels below 360 μmol/l minimised the risk of brain damage and that active placental transport led to an enrichment of PHE in the foetal circulation [66]. In the UK, a target range of 100–250 μmol/l was set. With the emergence of data suggesting no adverse effect on neurodevelopment of average maternal PHE levels below 360 μmol/l, these target ranges have been relaxed somewhat.

Current Practice

Prevention of the MPKUS requires time and resources, and the best outcomes are obtained by the centres with the most experience [67]. In 109 pregnancies cared for in a single centre over a 30-year period, preconception diet was established in 69.5% [68]. This centre looks after 15–20 PKU pregnancies annually. Prospective mothers are offered dietary education with partners or families. PHE is monitored twice a week preconceptionally and three times a week in pregnancy, with next day reporting. This level of service requires clinicians trained in metabolic medicine, specialist dietitians, specialised laboratory services, foetal medicine services, and close cooperation with child neuropsychologists to monitor outcomes. These resources are only available in units caring for adults with PKU, and any woman with PKU already pregnant or considering pregnancy should be referred to the nearest such centre.

Outcome

All women who plan their pregnancies and start diet before conception can maintain excellent metabolic control throughout pregnancy irrespective of their baseline PHE levels [68]. Levels may rise transiently during morning sickness or intercurrent illness, but these episodes can be controlled by reducing natural protein intake and increasing amino acid supplements. With morning sickness it is important to maintain calorie and supplement intake, in order to prevent catabolism.

After the first trimester protein tolerance increases markedly as the baby grows. For women who remain on a low-protein diet after delivery, greater protein restriction is often required post partum.

Although PHE levels can usually be quickly brought under control, women who start diet after conception have significantly higher PHE levels throughout pregnancy [68]. A small

subgroup of women unable to fully comply with a low-protein diet, never obtain satisfactory metabolic control. Admission for full supervision of their diet will bring PHE levels down, but prolonged in-patient stays are neither practicable nor acceptable to the patients. Outcomes of such pregnancies remain poor. Often successive pregnancies are affected in the same way. In such pregnancies monitoring of PHE levels is infrequent, but often the absolute levels remain below 1000 µmol/l. For these women, new interventions are desperately required. BH_4, which is licensed for use in pregnancy, may have a role to play in responsive patients [18]; any significant improvements in IQ for the offspring would justify the cost.

Any effects of the low protein diet on the foetus are much less severe than those of PHE, but may still be significant. Maternal PHE below 120 µmol/l is associated with intrauterine growth retardation [69]. Low essential fatty acid intakes have led to the use of amino acid supplements fortified with DHA. Some centres use tyrosine supplements to maintain maternal TYR within the normal range.

The key to preventing MPKUS is planning, with dietary treatment being established prior to conception. This requires all women with HPA to be educated from an early age with the information repeated regularly thereafter.

16.3 HPA and Disorders of Biopterin Metabolism

Disorders of tetrahydrobiopterin (BH_4) associated with HPA and biogenic amine deficiency include deficiencies of GTP cyclohydrolase I (GTPCH), 6-pyruvoyl-tetrahydropterin synthase (PTPS), dihydropteridine reductase (DHPR) and pterin-4a-carbinolamine dehydratase (PCD) (primapterinuria). Dopa-responsive dystonia (DRD), which is due to a dominant form of GTPCH deficiency, and sepiapterin reductase (SR) deficiency, also lead to CNS amine deficiency but are associated with normal blood PHE (although HPA may occur in DRD after a PHE load); these conditions are not considered further here (▶ Chapter 29).

16.3.1 Clinical Presentation

The condition can present in any of three ways:

1. Asymptomatic, but with raised PHE found following NBS; as part of the standard screening protocol the infant is then investigated further for biopterin defects.
2. Symptomatic, with neurological deterioration in infancy despite a low-PHE diet. This will occur where no further investigations are routinely undertaken after a finding of HPA in NBS which is wrongly assumed to be PAH deficiency.
3. Symptomatic, with neurological deterioration in infancy on a normal diet. This will occur either where there has been no NBS for HPA or if the PHE level is not sufficiently raised to have resulted in a positive screen or to require dietary treatment.

Symptoms may be subtle in the newborn period and not readily apparent until several months of age. Birth weight and birth head circumference may be low in some infants, suggesting intrauterine involvement. All conditions apart from PCD deficiency are associated with abnormal and variable tone, abnormal movements, irritability and lethargy, seizures, poor temperature control, progressive developmental delay and microcephaly. An abnormal EEG and cerebral atrophy can occur in PTPS and in DHPR deficiency and basal ganglia calcification is reported in the latter [70]. There is a mild (peripheral) form of PTPS associated with HPA but without neurotransmitter deficiency, where there are usually no neurological symptoms. In PCD deficiency symptoms are mild and transient.

16.3.2 Metabolic Derangement

Disorders of pterin synthesis or recycling are associated with decreased activity of PAH, tyrosine hydroxylase, tryptophan hydroxylase and nitric oxide synthase (◘ Fig. 16.1). The degree of HPA is highly variable, with blood PHE concentrations ranging from normal to >2000 µmol/l. Central nervous system (CNS) amine deficiency is most often profound and responsible for the clinical symptoms. Decreased concentration of homovanillic acid (HVA) in cerebrospinal fluid (CSF) is a measure of reduced dopamine turnover, and similarly 5-hydroxyindoleacetic acid (5-HIAA) deficiency is a measure of reduced serotonin metabolism (see also ▶ Chapter 29).

16.3.3 Genetics

All disorders are autosomal recessive. Descriptions of the relevant genes and a database of mutations are available on http://www.biopku.org/biomdb/home.asp. In most series biopterin disorders account for 1-3% of infants found to have a raised PHE on newborn screening; PTPS deficiency is the most common disorder, followed by DHPR deficiency [53]. PTPS deficiency has a higher frequency in Chinese populations, and a genotype phenotype correlation has been reported [71].

16.3.4 Diagnostic and Confirmatory Tests

Diagnostic protocols and interpretation of results are as follows.

1. *Urine or blood pterin analysis and blood DHPR assay.* All infants found to have HPA on NBS should have blood DHPR and urine or blood pterin analysis. The interpretation of results is shown in ◘ Table 16.2.
2. *BH_4 loading test.* If dietary PHE restriction is in place this is stopped 2–3 days before the test. Blood PHE levels should be at least 400 µmol/l at the start. An oral dose of 20 mg BH_4/kg is given approximately 30 min before a feed. Blood samples are collected for PHE and TYR at 0,

Table 16.2 Interpretation of results of investigations in disorders of biopterin metabolism

Deficiency	Blood PHE µmol/l	Blood or urine biopterin	Blood or urine neopterin	Blood or urine primapterin	CSF 5HIAA and HVA	Blood DHPR activity
PAH	>120	↑	↑	–	N	N
GTPCH	50–1200	↓↓	↓↓	–	↓	N
PTPS	240–2500	↓↓	↑↑	–	↓	N
DHPR	180–2500	↓↓	N or ↑	–	↓	↓
PCD	180–1200	↓	↑	↑↑		N

CSF, cerebrospinal fluid; *DHPR*, dihydropterin reductase; *GTPCH*, guanosine triphosphate cyclohydrolase I; *5HIAA*, 5-hydroxyindole acetic acids; *HVA*, homovanillic acid; N, normal; *PAH*, phenylalanine hydroxylase; *PCD*, pterin-4a-carbinolamine dehydratase; *PHE*, phenylalanine; *PTPS*, 6-pyruvoyl-tetrahydropterin synthase

4, 8 and 24 h. The test is positive if plasma PHE falls to normal (usually by 8 h) with a concomitant increase in TYR. The rate of fall of PHE may be slower in DHPR deficiency. Blood for pterin analysis at 4 h will confirm that the BH_4 has been taken and absorbed. A combined PHE (100 mg/kg) and BH_4 (20 mg/kg) loading test may be used as an alternative. This combined loading test is reported to identify BH_4-responsive PAH deficiency and discriminate between co-factor synthesis or regeneration defects and is useful if pterin analysis is not available [72] [73].

3. *CSF neurotransmitters.* The measurement of HVA and 5-HIAA is an essential part of the diagnostic investigation and is also subsequently required to monitor amine replacement therapy with L-dopa and 5HT. CSF must be frozen in liquid nitrogen immediately after collection and stored at –70°C prior to analysis. If blood stained, the sample should be centrifuged immediately and the supernatant then frozen. The reference ranges for HVA and 5-HIAA are age related [74] (see also ▶ Chapter 29).
4. *Confirmatory tests.* Apart from DHPR measurement in erythrocytes, measurement of enzyme activity is not necessary for the initial diagnosis. For further confirmation DHPR activity can be measured in fibroblasts, PTPS activity in erythrocytes and fibroblasts and GTPCH activity in liver, cytokine-stimulated fibroblasts and stimulated lymphocytes. Mutation analysis is available for all conditions.
5. *Prenatal diagnosis.* If the mutation of the index case is already known prenatal diagnosis can be undertaken in the first trimester by mutation analysis following chorionic villus biopsy. Analysis of amniotic fluid neopterin and biopterin in the second trimester is available for all conditions. Enzyme analysis can be undertaken in foetal erythrocytes or in amniocytes in both DHPR deficiency and PTPS deficiency. GTPCH is only expressed in foetal liver tissue.

16.3.5 Treatment and Prognosis

For GTPCH deficiency, PTPS deficiency and DHPR deficiency the aim of treatment is to control the HPA and to correct CNS amine deficiency. In DHPR deficiency treatment with folinic acid is necessary to prevent CNS folate deficiency [57], and it may also be required in GTPCH and PTPS deficiency, where a reduction in CSF folate can be a consequence of long-term treatment with L-dopa. PCD deficiency does not usually require treatment, although BH_4 may be used initially if the child is symptomatic.

In PTPS and GPCH deficiency, blood PHE responds to treatment with oral BH_4. In DHPR deficiency, BH_4 may also be effective in reducing blood PHE, but higher doses may be required than in GTPCH and PTPS deficiency and may lead to an accumulation of BH_2 and a possible increased risk of CNS folate deficiency [75]. It is therefore usually recommended that in DHPR deficiency HPA should be corrected by dietary means and BH_4 should not be given. In a single case report, however, BH_4 up to a dose of 40 mg/kg/day did not cause a further increase in CSF BH_2 [76].

CNS amine replacement therapy is given as oral L-dopa with carbidopa (usually in 1:10 ratio, but also available in 1:4 ratio) and 5HT. Carbidopa is a dopa-decarboxylase inhibitor that reduces the peripheral conversion of L-dopa to dopamine, thus limiting side effects and allowing a reduced dose of L-dopa to be effective. Side effects (nausea, vomiting, diarrhoea, irritability) may also be seen at the start of treatment. For this reason L-dopa and 5HT should initially each be started in a low dose (Table 16.3), which is increased gradually to the recommended maintenance dose. Further dose adjustment depends on the results of CSF HVA and 5-HIAA levels.

Additional medications, developed primarily for treatment of Parkinson's disease, have been used as an adjunct to therapy, with the aim of reducing the dose and frequency of amine replacement medication and improving residual symptoms and preventing diurnal variation. These include selegiline (L-deprenyl), a monoamine oxidase-B inhibitor [77],

Table 16.3 Medication used in the treatment of disorders of biopterin metabolism

Drug	Dose (oral)	Frequency	GTPCH	PTPS	PCD	DHPR
BH_4	1–3 mg/kg/day	Once daily	+	+	±	-
5HT	1–2 mg/kg/day, increasing by 1–2 mg/kg/day every 4–5 days up to maintenance dose of 8–10 mg/kg/day	Give in four divided doses; final maintenance dose dependent on results of CNS neurotransmitters	+	+	-	+
L-Dopa (as combined preparation with carbidopa)	1–2 mg/kg/day, increasing by 1–2 mg/kg/day every 4–5 days up to maintenance dose of 10–12 mg/kg/day	Give in four divided doses; final maintenance dose dependent on results of CNS neurotransmitters	+	+	-	+
Selegiline (l-deprenyl)	0.1–0.25 mg/day	In three or four divided doses (as adjunct to 5HT and L-dopa; see text)	±	±	-	±
Entacapone	15 mg/kg/day	In two or three divided doses	±	±	-	±
Pramipexole	0.006 mg/kg/day increasing to 0.035 mg/kg/day	In two divided doses	±	±	-	±
Calcium folinate (folinic acid)	15 mg/day	Once daily	±	±	-	+

BH_4, tetrahydrobiopterin; *CNS*, central nervous system; *DHPR*, dihydropterin reductase; *GTPCH*, guanosine triphosphate cyclohydrolase I; *5HT*, 5-hydroxytrytophan; *PCD*, pterin-4a-carbinolamine dehydratase; *PTPS*, 6-pyruvoyl-tetrahydropterin synthase

entacapone, a catechol-*O*-methyltransferase (COMT) inhibitor and pramipexole, a dopamine agonist receptor [78][79].

Monitoring of Treatment

CSF amine levels should be monitored 3-monthly in the 1st year, 6-monthly in early childhood and yearly thereafter. Where possible, CSF should be collected before a dose of medication is given. CSF folate should also be measured.

Hyperprolactinaemia occurs as a consequence of dopamine deficiency and measurement of serum prolactin can be used as a method to monitor treatment, with normal values indicating adequate L-dopa replacement. It has been suggested that 3 blood prolactin measurements over a 6 hour period may be a more sensitive and less invasive marker than the CSF HVA level in deciding on dose adjustment [79].

Blood PHE must also be monitored, but this only needs to be undertaken frequently in DHPR deficiency where a low-PHE diet is used.

Outcome

Without treatment the natural history of GTPCH, 6PTPS and DHPR deficiency is poor, with progressive neurological disease and early death. The outcome with treatment depends upon the age at diagnosis and initiation of therapy and the phenotypic severity [70]. Most children with GTPCH deficiency have some degree of learning difficulties despite adequate control. Patients with PTPS deficiency may have a satisfactory cognitive outcome if detected early. Those with DHPR deficiency, if started on diet, amine replacement therapy and folinic acid within the first months of life, can show normal development and growth. Late diagnosis in all these conditions is associated with a much poorer outcome.

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Disorders of Tyrosine Metabolism

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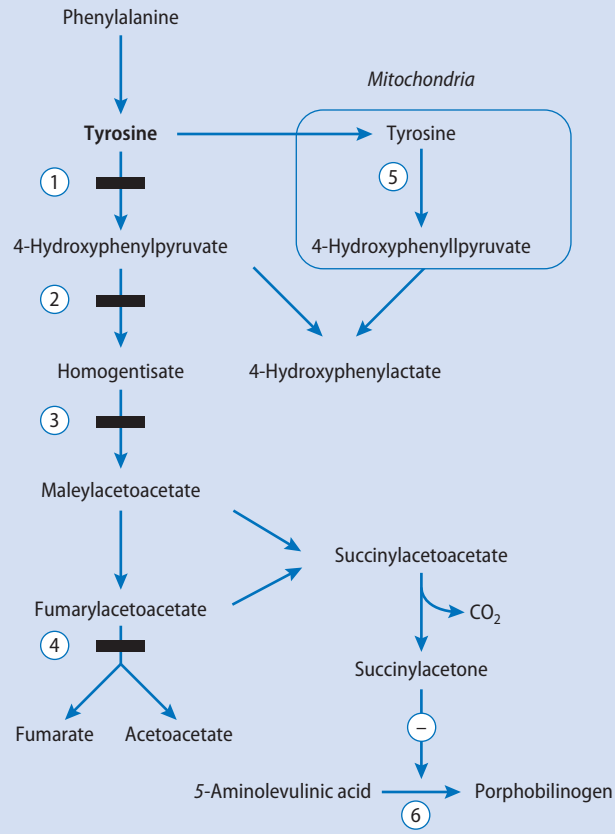
Tyrosine Metabolism

Tyrosine is a non-essential amino acid that is derived from two sources, diet and hydroxylation of phenylalanine (■ Fig. 17.1). Besides forming an integral part of proteins, it is a precursor of DOPA, thyroxine and melanin. Post- translational modifications of tyrosine residues in proteins by phosphorylation and sulfation have important roles in signal transduction and modulation of interaction between proteins. Tyrosine is both glucogenic and ketogenic, since its catabolism, which proceeds predominantly in the liver cytosol, results in the formation of fumarate and acetoacetate. The first step of tyrosine catabolism is conversion into 4-hydroxyphenylpyruvate by cytosolic tyrosine aminotransferase. Transamination of tyrosine can also be accomplished in the liver and in other tissues by mitochondrial aspartate aminotransferase, but this enzyme plays only a minor role under normal conditions. The penultimate intermediates of tyrosine catabolism, maleylacetoacetate and fumarylacetoacetate, are reduced to succinylacetoacetate, followed by decarboxylation to succinylacetone. The latter is the most potent known inhibitor of the heme biosynthetic enzyme, 5-aminolevulinic acid dehydratase (porphobilinogen synthase; ▶ Fig. 36.1).



■ **Fig. 17.1 The tyrosine catabolic pathway**. *1*, Tyrosine aminotransferase (deficient in tyrosinaemia type II); *2*, 4-hydroxyphenylpyruvate dioxygenase (deficient in tyrosinaemia type III, hawkinsinuria, site of inhibition by NTBC); *3*, homogentisate dioxygenase (deficient in alkaptonuria); *4*, fumarylacetoacetase (deficient in tyrosinaemia type I); *5*, aspartate aminotransferase; *6*, 5-aminolevulinic acid dehydratase (porphobilinogen synthase). Enzyme defects are depicted by solid bars across the arrows

Five inherited disorders of tyrosine metabolism are known (► Tyrosine Metabolism). Hereditary tyrosinaemia type I is characterised by progressive liver disease and renal tubular dysfunction with rickets. Hereditary tyrosinaemia type II (Richner-Hanhart syndrome) presents with keratitis and blistering lesions of the palms and soles and neurological complications. Tyrosinaemia type III may be asymptomatic or associated with mental retardation. Hawkinsinuria may be asymptomatic or present with failure to thrive and metabolic acidosis in infancy. In alkaptonuria, symptoms of osteoarthritis usually appear in adulthood. Other inborn errors of tyrosine metabolism include oculocutaneous albinism caused by a deficiency of melanocyte-specific tyrosinase, converting tyrosine into DOPA-quinone; deficiency of tyrosine hydroxylase, the first enzyme in the synthesis of dopamine from tyrosine; and deficiency of aromatic L-amino acid decarboxylase, which also affects tryptophan metabolism. The latter two disorders are covered in ► Chapter 29.

17.1 Hereditary Tyrosinaemia Type I (Hepatorenal Tyrosinaemia)

17.1.1 Clinical Presentation

The clinical manifestations of tyrosinaemia type I are very variable, and an affected individual can present at any time from the neonatal period to adulthood. There is considerable variability of presentation even between members of the same family.

Clinically, tyrosinaemia type I may be classified based on the age at onset of symptoms, which broadly correlates with disease severity: an acute form that manifests before 6 months of age (but rarely in the first 2 weeks of life) with acute liver failure; a subacute form presenting between 6 months and 1 year of age with liver disease, failure to thrive, coagulopathy, hepatosplenomegaly, rickets and hypotonia; and a more chronic form that presents after the 1st year with chronic liver disease, renal disease, rickets, cardiomyopathy and/or a porphyria-like syndrome. Treatment of tyrosinaemia type I with nitisinone in the last 15 years (► Section 17.1.5) has dramatically altered its natural history.

■ Hepatic Disease

The liver is the major organ affected in tyrosinaemia type I, and its involvement is a major cause of morbidity and mortality. Liver disease can manifest as acute hepatic failure, cirrhosis or hepatocellular carcinoma; all three conditions may occur in the same patient. The more severe forms of tyrosinaemia type I present in infancy with vomiting, diarrhoea, bleeding diathesis, hepatomegaly, mild jaundice, hypoglycaemia, oedema and ascites. Typically, liver synthetic function is most affected and, in particular, coagulation is markedly abnormal compared with other tests of liver function. Sepsis is common, and early hypophosphataemic bone disease may be present secondary to renal tubular dysfunction. Acute liver failure may be the presenting feature or may occur subsequently, precipitated by intercurrent illnesses, as hepatic crises which are associated with hepatomegaly and coagulopathy. Mortality is high in untreated patients [1].

Chronic liver disease leading to cirrhosis eventually occurs in most individuals with tyrosinaemia type I – both as a late complication in survivors of early-onset disease and as a presenting feature of the later-onset forms. The cirrhosis is usually a mixed micro and macronodular type with a variable degree of steatosis. Hepatocyte dysplasia is common, with a high risk of malignant transformation [1][2]. Unfortunately, the heterogeneity of the nodules make it difficult to detect malignant changes at an early stage (► Section 17.1.5).

■ Renal Disease

A variable degree of renal dysfunction is detectable in most patients at presentation, ranging from mild tubular dysfunction to renal failure. Proximal tubular disease is very common and may deteriorate during hepatic crises. Hypophosphataemic rickets is the most common manifestation of proximal tubulopathy, but generalised aminoaciduria, renal tubular acidosis and glycosuria may also be present [3]. Prior to the nitisinone era 40% developed nephrocalcinosis [4]. Rare renal manifestations include distal renal tubular disease and renal impairment.

■ Neurological Manifestations

Acute neurological crises can occur at any age. Typically, the crises follow a minor infection associated with anorexia and vomiting, and occur in two phases: an active period lasting 1–7 days characterised by painful paresthesias and autonomic signs that may progress to paralysis, followed by a recovery phase over several days to months [5]. Complications include seizures, extreme hyperextension, self-mutilation, respiratory paralysis and death.

■ Other Manifestations

Cardiomyopathy is an occasional incidental finding, but may be clinically significant [6]. Pancreatic cell hypertrophy may result in clinically significant hyperinsulinism [7].

17.1.2 Metabolic Derangement

Tyrosinaemia type 1 is caused by a deficiency of the enzyme fumarylacetoacetate hydrolase (FAH), which is mainly expressed in the liver and kidney. The compounds immediately upstream from the FAH reaction, maleylacetoacetate (MAA) and fumarylacetoacetate (FAA), and their derivatives, succinylacetone (SA) and succinylacetoacetate (SAA) accumulate and have important pathogenic effects. The effects of FAA and MAA occur only in the cells of the organs in which they are produced; these compounds are not found in body fluids of patients. On the other hand, their derivatives, SA and SAA are readily detectable in plasma and urine and have widespread effects.

FAA, MAA and SA disrupt sulfhydryl metabolism by forming glutathione adducts, thereby rendering cells susceptible to free radical damage [8]. Disruption of sulfhydryl metabolism is also believed to cause secondary deficiency of two other hepatic enzymes, 4-hydroxyphenylpyruvate dioxygenase and methionine adenosyltransferase, resulting in hypertyrosinemia and hypermethioninemia. Additionally, FAA and MAA are alkylating agents and can disrupt the metabolism of thiols, amines, DNA and other important intracellular molecules including inhibition of base excision repair by FAA, suggesting a mechanism for carcinogenesis in tyrosinaema type I [9]. As a result of these widespread effects on intracellular metabolism, hepatic and renal cells exposed to high levels of these compounds undergo either apoptotic cell death or a significant alteration of gene expression [10][11]. In patients who have developed cirrhosis, self-induced correction of the genetic defect and the enzyme abnormality occurs within some nodules. The clinical expression of hepatic disease may correlate inversely with the extent of mutation reversion in regenerating nodules [12][14].

SA is a potent inhibitor of the enzyme 5-aminolevulinic acid (5-ALA) dehydratase. 5-ALA, a neurotoxic compound, accumulates and is excreted at high levels in patients with tyrosinemia type I and is believed to cause the acute neurological crises seen during decompensation [5]. SA is also known to disrupt renal tubular function, heme synthesis and immune function [13][14][15].

▪ Newborn Screening

There is strong clinical evidence to support newborn screening for tyrosinemia type I, as the detection and treatment of patients in early life results in a dramatically better outcome than when treatment is initiated late [16][17]. Screening using tyrosine levels alone has been used in the past and has resulted in very high false-positive and false-negative rates [18]. SA is a highly sensitive and specific marker for tyrosinaemia type I, and assays based on the inhibitory effects of SA on 5-ALA dehydratase, either alone or in combination with tyrosine levels, have greatly improved diagnostic accuracy [18]. Recently, screening methods based on the direct measurement of SA in dried blood spots by tandem mass spectrometry have been developed and validated. Several laboratory-based methods have been described and commercial kit-based assays are also available, facilitating the routine inclusion of tyrosinaemia type I in many newborn screening programmes [18].

▪ Prenatal Diagnosis

The description of the geographical and ethnic distribution of causative mutations in many populations worldwide has enabled improved carrier detection, prenatal diagnosis and pre-implantation diagnosis [19]. Antenatal diagnosis is best performed by mutation analysis on chorionic villus sampling (CVS) or amniocytes. Alternative methods include FAH assay on CVS or amniocytes and determination of SA levels in amniotic fluid. However, FAH is expressed at low levels in chorionic tissue and interpretation of results may be difficult. Assay for elevated SA levels in amniotic fluid is very reliable and can be performed as early as 12 weeks; however, in occasional affected pregnancies normal SA amniotic fluid levels have been reported [20]. When mutation analysis is not available for prenatal diagnosis, we recommend a strategy combining initial screening for the common pseudodeficiency mutation and FAH assay on CVS at 10 weeks; in the case of low FAH activity revealed by CVS amniocentesis for amniotic fluid SA levels is subsequently performed at 11–12 weeks for confirmation.

17.1.3 Genetics

Tyrosinaemia type I is inherited as an autosomal recessive trait. *FAH* has been localised to 15q23–q25 and almost 100 mutations have been reported [21]. The most common mutation, I c.1062+5G>A, is found in about 25% of the alleles worldwide and is the predominant mutation in the French-Canadian population, in which it accounts for >90% of alleles. Another mutation, c.554-1G>T, is found in around 60% of alleles in patients from the Mediterranean area. Other *FAH* mutations are common within certain ethnic groups: W262X in Finns, D233V in Turks, and Q64H in Pakistanis. There is no clear genotype-phenotype correlation; spontaneous correction of the mutation within regenerative nodules may influence the clinical phenotype [12]. A novel mutation c.103G>A (Ala35Thr) was found in a patient with a mild phenotype who did not excrete succinylacetone and was successfully treated with diet alone [22]. A pseudodeficiency mutation, R341W, has been reported in healthy individuals who have in vitro FAH activity indistinguishable from that in patients with tyrosinaemia type I [23]. The frequency of this mutation in various populations is unknown, but it has been found in many different ethnic groups.

17.1.4 Diagnostic Tests

In symptomatic patients, biochemical tests of liver function are usually abnormal. In particular, liver synthetic function is severely affected – coagulopathy and/or hypoalbuminaemia are often present even if other tests of liver function are normal. In most acutely ill patients, α-fetoprotein levels are greatly elevated. A Fanconi-type tubulopathy is often present with aminoaciduria, phosphaturia and glycosuria, and radiological evidence of rickets may be present.

Elevated levels of succinylacetone in dried blood spots, plasma or urine are pathognomonic of tyrosinaemia type I. However, very rarely, urine succinylacetone elevation may be absent in mild cases [22]. Other metabolite abnormalities that are suggestive of the diagnosis include elevated plasma levels of tyrosine, phenylalanine and methionine, reduced erythrocyte 5-ALA dehydratase activity and increased urinary 5-ALA excretion.

Confirmation of the diagnosis is usually by mutation analysis. Failing this, FAH assays may be performed on liver

Table 17.1 Risk of hepatocellular carcinoma (HCC) in tyrosinaemia type I

	Reference	Age at start of treatment with NTBC	Number of patients	Patient age (in years) at assessment	Patients developing HCC (%)
Pre-NTBC	[2]	n/a	43	>2	16 (37%)
	[1]	n/a	55	2–12	10 (18%)
Post-NTBC	[25]	<6 months	180	2–13	1 (0.6%)
		6–12 months	61	2–12	1 (1.6%)
		1–2 years	44	2–12	3 (7%)
		2–7 years	65	2–19	14 (21%)
		>7 years	26	7–31	9 (35%)

HCC, hepatocellular carcinoma; *n/a*, not applicable; *NTBC*, 2-[2-nitro-4-(trifluoromethyl)benzoyl]cyclohexane-1,3-dione, or nitisinone

biopsy, fibroblasts, lymphocytes or dried blood spots. Falsely elevated enzyme results may be obtained on liver biopsy if a reverted nodule is inadvertently assayed. Enzyme assay results should therefore be interpreted in the context of the patient's clinical and biochemical findings.

17.1.5 Treatment and Prognosis

Nitisinone, also known as NTBC (2-[2-nitro-4-(trifluoromethyl)benzoyl]cyclohexane-1,3-dione), has revolutionised the treatment of type I tyrosinaemia and is now the recommended therapy, in combination with a tyrosine- and phenylalanine-restricted diet [17][24][25].

Nitisinone and Dietary Treatment

The rationale for the use of nitisinone is to block tyrosine degradation at an early step so as to prevent the production of toxic downstream metabolites such as FAA, MAA and SA; the levels of tyrosine, 4-hydroxyphenylpyruvate and 4-hydroxyphenylpyruvate concomitantly increase (Fig. 17.1). Nitisinone acts within hours of administration and has a long half-life of about 54 hours [26]. In patients presenting acutely with hepatic decompensation, rapid clinical improvement occurs in over 90%, with improvement of prothrombin time within days of starting treatment. Other biochemical parameters of liver function may take longer to normalise: α-fetoprotein concentrations should show a logarithmic fall but may not normalise for up to several months after the start of treatment. Nitisinone is recommended in an initial dose of 2 mg/kg body weight per day in liver failure or 1 mg/kg/day otherwise [24]. Individual dose adjustment is subsequently based on the biochemical response and the aim is to maintain a plasma nitisinone concentration of >50 μmol/l or a whole blood concentration of 20–40 μmol/l.

Dietary restriction of phenylalanine and tyrosine is necessary to prevent the known adverse effects of hypertyrosinaemia (▶ Section 17.2). We currently aim to maintain tyrosine levels between 200 and 400 μmol/l with a phenylalanine level of >30 μmol/l using a combination of a protein-restricted diet and phenylalanine- and tyrosine free amino acid mixtures. Occasionally specific phenylalanine supplementation is necessary.

A small proportion of acutely presenting patients (<10%) do not respond to nitisinone treatment; in these patients, coagulopathy and jaundice progress and mortality is very high without urgent liver transplantation. If encephalopathy or significant jaundice develops or if prothrombin time does not improve within 1 week, urgent liver transplantation should be considered.

Adverse events of nitisinone therapy have been few. Transient thrombocytopenia and neutropenia and transient eye symptoms (burning/photophobia/corneal erosion/corneal clouding) have been reported in a small proportion of patients [24]. The short to medium term prognosis in responders appears to be excellent. Hepatic and neurological decompensations are not known to occur on nitisinone treatment, and clear deterioration of chronic liver disease is rare. Renal tubular dysfunction responds quickly, and tubular function usually normalises within the 1st year of treatment, unless nephrocalcinosis is already established. Neurological crises have never been reported in patients compliant with nitisinone.

The risk of hepatocellular carcinoma (HCC) appears to be related to the age nitisinone is commenced. HCC has not been reported in those treated in the first month of life, with the relative risk increasing with age at treatment [22][23]. Where nitisinone is introduced after 2 years of age, the risk is similar to that in historical controls (Table 17.1) [25]. Long-term vigilance is however necessary in all patients as the lifelong risk of HCC is still unknown.

It has recently been recognized that many patients with tyrosinaemia type I appear to have significant learning difficulties; cognitive deficits affecting performance abilities more than verbal abilities have been found in many patients on psychological testing [27][28]. The aetiology of these cognitive

deficits is uncertain; whether they are related to nitisinone treatment, high tyrosine levels, low phenylalanine levels or liver failure, or are an intrinsic feature of tyrosinaemia type I per se, is unknown.

Monitoring of patients on nitisinone treatment should include regular blood tests for liver function, blood counts, clotting, plasma ALA dehydratase activity, 5-ALA, nitisinone levels and amino acid profile; tests of renal tubular and glomerular function; urinary SA and 5-ALA. Blood levels of phenylalanine and tyrosine should be frequently monitored and the diet supervised closely. While tyrosine levels are stable there appears to be considerable diurnal variation in phenylalanine levels and sampling should be done at a consistent time of the day. Monitoring for HCC consists of α-fetoprotein checked every 3 months, in combination with hepatic imaging by ultrasound every 6 months and by MRI annually. Lectin-reactive α-fetoprotein may be able to detect hepatocellular cancer earlier.

▪ Liver Transplantation

Liver transplantation provides a functional cure of tyrosinaemia type I and allows a normal unrestricted diet [29]. However, even in optimal circumstances, it is associated with approximately 5-10% mortality and necessitates lifelong immunosuppressive therapy. Therefore, at present liver transplantation in tyrosinaemia type I is restricted to patients with acute liver failure who fail to respond to nitisinone therapy, and patients with proven or suspected HCC.

The long-term impact of liver transplantation on renal disease in patients with tyrosinaemia type I relates to the era in which they were treated. Prior to nitisinone, all patients had tubular dysfunction and some had glomerular dysfunction before receiving transplants. In this group tubular function improved in most patients but they had higher rates of glomerular dysfunction owing to nephrotoxic immunotherapy [29][30]. Patients pre-treated with nitisinone usually have normal renal function at transplant, and this combined with the modern immunosuppression regimens ensures they have a much improved renal prognosis [31]. After transplantation, when nitisinone is discontinued, renal production results in significantly elevated plasma and urinary SA levels. The functional significance of these findings is unclear, but does not seem to be associated with renal dysfunction or malignancy. At present nitisinone treatment, which would probably necessitate reintroduction of dietary restriction, is not indicated.

▪ Supportive Treatment

In the acutely ill patient supportive treatment is essential. Clotting factors, albumin, electrolytes and acid/base balance should be closely monitored and corrected as necessary. Tyrosine and phenylalanine intake should be kept to a minimum during acute decompensation. Vitamin D is necessary to treat rickets. Infections should be treated aggressively.

▪ Pregnancy

A few pregnancies in patients on nitisinone treatment have been reported with encouraging outcomes and where pregnancy occurs Nitisinone should be continued [32][33]. Pregnancy is a realistic expectation for the majority of women who have had liver transplantation for any indication. Overall the outcome is excellent for both mother and infant, with a live birth rate of >70%. In our experience, a number of women have had successful pregnancies after liver transplant for tyrosinaemia type I.

17.2 Hereditary Tyrosinaemia Type II (Oculocutaneous Tyrosinaemia, Richner-Hanhart Syndrome)

17.2.1 Clinical Presentation

The disorder is characterised by ocular lesions (about 75% of the cases), skin lesions (80%), or neurological complications (60%), or by any combination of these [34]. The disorder usually presents in infancy but can become manifest at any age.

Eye symptoms are often the presenting problem and may start in the first months of life with photophobia, lacrimation and intense burning pain. The conjunctivae are inflamed and on slit-lamp examination herpetic-like corneal ulcerations are found. The lesions stain poorly with fluorescein. In contrast with herpetic ulcers, which are usually unilateral, the lesions in tyrosinaemia type II are bilateral. Neovascularisation may be prominent. Untreated, serious damage may occur with corneal scarring, visual impairment, nystagmus and glaucoma.

Skin lesions specifically affect pressure areas and most commonly occur on the palms and soles. They begin as blisters or erosions with crusts and progress to painful, nonpruritic hyperkeratotic plaques with an erythematous rim, typically ranging in diameter from 2 mm to 3 cm. Clinically, tyrosinemia II has to be differentiated from other severe forms of palmoplantar keratoderma such as Olmsted syndrome [35].

Neurological complications are highly variable: some patients are developmentally normal, whilst others have variable degrees of developmental retardation. More severe neurological problems, including microcephaly, seizures, self-mutilation and behavioural difficulties, have also been described [36].

It should be noted that the diagnosis of tyrosinaemia type II has only been confirmed by enzymatic and/or molecular genetic analysis in a minority of the early described cases and it is possible that some of these patients have actually had tyrosinaemia type III.

17.2.2 Metabolic Derangement

Tyrosinaemia type II is due to a defect of hepatic cytosolic tyrosine aminotransferase (◘ Fig. 17.1, ▶ enzyme 1). As a result of the metabolic block, tyrosine concentrations in serum and cerebrospinal fluid are markedly elevated. The accompa-

nying increased production of the phenolic acids 4-hydroxyphenylpyruvate, 4-hydroxyphenyllactate and 4-hydroxyphenylacetate (not shown in ◘ Fig. 17.1) may be a consequence of direct deamination of tyrosine in the kidneys, or of tyrosine catabolism by mitochondrial aminotransferase (◘ Fig. 17.1). Corneal damage is thought to be related to crystallisation of tyrosine in the corneal epithelial cells, which results in disruption of cell function and induces an inflammatory response. Tyrosine crystals have not been observed in the skin lesions. It has been suggested that excessive intracellular tyrosine enhances cross-links between aggregated tonofilaments and modulates the number and stability of microtubules [37]. As the skin lesions occur on pressure areas, it is likely that mechanical factors also play a role. The aetiology of the neurological manifestations is unknown, but it is believed that hypertyrosinaemia may have a role in pathogenesis.

17.2.3 Genetics

Tyrosinaemia type II is inherited as an autosomal recessive trait due to mutations in *TAT*, located at 16q22.1-q22.3. Several different mutations have so far been reported [21]. Prenatal diagnosis using mutation analysis on chorionic villus sampling has been reported.

17.2.4 Diagnostic Tests

Plasma tyrosine concentrations are usually above 1200 µmol/l. When the tyrosinaemia is less pronounced a diagnosis of tyrosinaemia type III should be considered (▶ Section 17.3). Urinary excretion of the phenolic acids 4-hydroxyphenylpyruvate, 4-hydroxyphenyllactate and 4-hydroxyphenylacetate is highly elevated, and *N*-acetyltyrosine and 4-tyramine are also increased. The diagnosis can be confirmed by mutation analysis. Patients diagnosed using tyrosine levels as part of expanded neonatal screening programmes have been reported. In a neonatally diagnosed patient early detection by screening facilitated presymptomatic treatment and identification of an affected 8-year old sibling who suffered with plantar hyperkeratosis [38].

17.2.5 Treatment and Prognosis

Treatment consists in a phenylalanine- and tyrosine-restricted diet, and the skin and eye symptoms resolve within weeks of treatment [39]. Generally, skin and eye symptoms do not occur at tyrosine levels <800 µmol/l; however, as hypertyrosinaemia may be involved in the pathogenesis of the neurodevelopmental symptoms, it may be beneficial to maintain much lower levels [38]. We currently aim to maintain plasma tyrosine levels of 200–500 µmol/l using a combination of a protein-restricted diet and a phenylalanine- and tyrosine-free amino acid mixture. Growth and nutritional status should be regularly monitored.

▪ Pregnancy

There have been several reports of pregnancies in patients with tyrosinaemia type II: some have suggested that untreated hypertyrosinaemia may result in fetal neurological abnormalities, such as microcephaly, seizures and mental retardation; however, other untreated pregnancies have been followed by normal fetal outcome [40], although these have only been associated with mild hypertyrosinaemia. In view of the uncertainty regarding possible fetal effects of maternal hypertyrosinaemia, dietary control of maternal tyrosine levels during pregnancy is recommended. In one pregnancy [34] treated with a low-protein diet to maintain plasma tyrosine levels of 100–200 µmol/l and phenylalanine levels of 200–400 µmol/l, a normal fetal and maternal outcome was reported.

17.3 Hereditary Tyrosinaemia Type III

17.3.1 Clinical Presentation

Only 13 cases of tyrosinaemia type III have been described, and the full clinical spectrum of this disorder is unknown [41]. Many of the patients have presented with neurological symptoms, including intellectual impairment, ataxia, increased tendon reflexes, tremors, microcephaly and seizures; some have been detected by the finding of a high tyrosine concentration on neonatal screening. The most common long-term complication has been intellectual impairment, found in 75% of the reported cases. None have developed signs of liver disease. Eye and skin lesions have not been reported so far, but as oculocutaneous symptoms are known to occur in association with hypertyrosinaemia it is reasonable to be aware of this possibility.

17.3.2 Metabolic Derangement

Tyrosinaemia type III is due to deficiency of 4-hydroxyphenylpyruvate dioxygenase (HPD) (◘ Fig. 17.1, ▶ enzyme 2), which is expressed in liver and kidney. As a result of the enzyme block there is an increased plasma tyrosine concentration and increased excretion in urine of 4-hydroxyphenylpyruvate and its derivatives 4-hydroxyphenyllactate and 4-hydroxyphenylacetate. The aetiology of the neurological symptoms is not known, but they may be related to hypertyrosinaemia, as in tyrosinaemia types I and II.

17.3.3 Genetics

Tyrosinaemia type III follows an autosomal recessive inheritance. *HPD* has been localised to 12q24-qter, and several mutations associated with tyrosinaemia III have been described [18]. There is no apparent genotype-phenotype correlation; some patients with enzymatically defined HPD deficiency do not have identifiable mutations in *HPD* [41][42].

17.3.4 Diagnostic Tests

Elevated plasma tyrosine levels of 300–1300 µmol/l have been found at diagnosis. Elevated urinary excretion of 4-hydroxyphenylpyruvate, 4-hydroxyphenyllactate and 4-hydroxyphenylacetate usually accompanies the increased plasma tyrosine concentration. Diagnosis can be confirmed by enzyme assay in liver or kidney biopsy specimens or by mutation analysis.

17.3.5 Treatment and Prognosis

At present, tyrosinaemia type III appears to be associated with intellectual impairment in some cases, but not in others. It is unknown whether lowering plasma tyrosine levels will alter the natural history. Amongst the patients described, the cases detected by neonatal screening and treated early appear to have fewer neurological abnormalities than those diagnosed on the basis of neurological symptoms [38][39]; whether this is due to ascertainment bias or to therapeutic intervention is unclear. Until there is a greater understanding of the aetiology of the neurological complications of tyrosinaemia type III, it is reasonable to treat patients with a diet that is low in phenylalanine and tyrosine, at least in early childhood. We currently recommend maintaining plasma tyrosine levels between 200 and 500 µmol/l. After infancy, many patients appear to be able to maintain these levels without dietary restriction or supplementation. No pregnancy data is available to date.

17.4 Transient Tyrosinaemia

Transient tyrosinaemia is one of the most common amino acid disorders, and is believed to be caused by late fetal maturation of 4-hydroxyphenylpyruvate dioxygenase (Fig. 17.1, ▶ enzyme 2). It is more common in premature infants than in full-term newborns. The level of protein intake is an important aetiological factor: the incidence of transient tyrosinaemia has fallen dramatically in the last 4 decades, with a concomitant reduction in the protein content of newborn formula milks. Transient tyrosinaemia is clinically asymptomatic. Tyrosine levels are extremely variable and can exceed 2000 µmol/l. Hypertyrosinaemia usually resolves spontaneously by 4–6 weeks; protein restriction to less than 2 g/kg/day, with or without vitamin C supplementation, results in more rapid resolution in most cases. Although the disorder is generally considered benign, some reports have suggested that it may be associated with mild intellectual deficits in the long term [43][44]. However, large systematic studies have not been performed.

The liver plays a central role in the metabolism of many amino acids, especially tyrosine, phenylalanine and methionine, and plasma levels of these and other amino acids are nonspecifically elevated in liver disease. In the context of newborn screening, elevated plasma tyrosine levels can occur secondary to neonatal liver disease; phenylalanine and methionine levels may also be elevated. Urgent investigations to evaluate liver function and to exclude treatable metabolic disorders such as galactosaemia and tyrosinaemia type I may be indicated in this situation.

17.5 Alkaptonuria

17.5.1 Clinical Presentation

Some cases of alkaptonuria are diagnosed in infancy due to darkening of urine when exposed to air. However, clinical symptoms first appear in adulthood. The most prominent symptoms relate to joint and connective tissue involvement; significant cardiac disease and urolithiasis may be detected in the later years [45].

The pattern of joint involvement resembles that of osteoarthritis. In general, joint disease tends to be worse in males than in females. The presenting symptom is usually either limitation of movement of a large joint or low back pain starting in the 3rd or 4th decade. Spinal involvement is progressive and may result in kyphosis, limited spine movements and height reduction. On X-ray examination, narrowing of the disc spaces, calcification and vertebral fusion may be evident. In addition to the spine, the large weight-bearing joints such as the hips, knees and ankles are usually involved. Radiological abnormalities may range from mild narrowing of the joint space to destruction and calcification. Synovitis, ligament tears and joint effusions have also been described. The small joints of the hands and feet tend to be spared. Muscle and tendon involvement is common: thickened Achilles tendons may be palpable, and tendons and muscles may be susceptible to rupture with trivial trauma. Abnormalities can be detected by ultrasound scanning [46]. The clinical course is characterised by episodes of acute exacerbation and progressive joint disability; joint replacement for chronic pain may be required. Physical disability increases with age and may become very severe by the 6th decade.

A greyish discolouration (ochre on microscopic examination, thus the name ochronosis) of the sclera and the ear cartilages usually appears after 30 years of age. Subsequently, dark colouration of the skin, particularly over the nose and cheeks and in the axillary and pubic areas, may become evident. Cardiac involvement probably occurs in most patients eventually; aortic or mitral valve calcification or regurgitation and coronary artery calcification is evident on CT scan and echocardiography in about 50% of patients by the 6th decade [45]. A high frequency of renal and prostatic stones has also been reported. Alkaptonuria is associated with secondary amyloidosis in many tissues [46].

17.5.2 Metabolic Derangement

Alkaptonuria was the first disease to be interpreted as an inborn error of metabolism in 1902 by Garrod [46]. It is caused by a defect of the enzyme homogentisate dioxygenase (Fig. 17.1, ▶ enzyme 3), which is expressed mainly in the liver and the kidneys. There is accumulation of homogentisate

and its oxidised derivative benzoquinone acetic acid, the putative toxic metabolite and immediate precursor to the dark pigment, which is deposited in various tissues. The relationship between the pigment deposits and the systemic manifestations is not known. It has been proposed that the pigment deposit may act as a chemical irritant; alternatively, inhibition of some of the enzymes involved in connective tissue metabolism by homogentisate or benzoquinone acetic acid and oxidative stress may have a role in pathogenesis [46][47].

17.5.3 Genetics

Alkaptonuria is an autosomal recessive disorder. The gene for homogentisate dioxygenase (*HGD*) has been mapped to chromosome 3q2, and over 90 mutations have been identified [21]. The estimated incidence is between 1:250,000 and 1:1,000,000 live births.

17.5.4 Diagnostic Tests

Alkalinisation of the urine from alkaptonuric patients results in immediate dark brown colouration of the urine. Excessive urinary homogentisate also results in a positive test for reducing substances. Gas chromatography-mass spectrometry (GC-MS)-based organic acid screening methods can specifically identify and quantify homogentisic acid. Homogentisate may also be quantified by HPLC and by specific enzymatic methods.

17.5.5 Treatment and Prognosis

A number of different approaches have been used in treatment. Dietary restriction of phenylalanine and tyrosine intake reduces homogentisate excretion, but compliance is a major problem as the diagnosis is usually made in adults. Ascorbic acid prevents the binding of ^{14}C-homogentisic acid to connective tissue in rats and reduces the excretion of benzoquinone acetic acid in urine [47]. Administration of the drug nitisinone also reduces urinary homogentisate excretion; the concomitant hypertyrosinaemia requires dietary adjustment to prevent ocular, cutaneous and neurological complications. Studies in a rat model of alkaptonuria showed that early administration of nitisinone prevented joint disease but ochronosis was not reversed [47]. A 3-year clinical trial of nitisinone has demonstrated a 95% reduction in urine and plasma homogentisic acid but no demonstrable effects on clinical symptoms. A further trial showed a dose-response relationship between the nitisinone dose and homogentisic acid excretion [47]. Although nitisinone has not been subjected to long-term clinical trials, due to the slow evolution of the manifestations of alkaptonuria, a sequential approach to treatment has been proposed. According to this approach, mild protein restriction is suggested in childhood to limit pigment deposition and to get the patients accustomed to the diet; in adulthood, nitisinone plus dietary treatment to limit plasma tyrosine levels is advised [47].

To date, no adverse effects on pregnancy have been reported patients with alkaptonuria.

17.6 Hawkinsinuria

17.6.1 Clinical Presentation

This rare condition, which has only been described in a few families [48][49], is characterised by failure to thrive and metabolic acidosis in infancy. After the 1st year of life the condition appears to be asymptomatic. Early weaning from breastfeeding seems to precipitate the disease; the condition may be asymptomatic in breastfed infants.

17.6.2 Metabolic Derangement

The abnormal metabolites produced in hawkinsinuria (hawkinsin (2-cysteinyl-1,4-dihydroxycyclohexenylacetate) and 4-hydroxycycloxylacetate) are thought to derive from incomplete conversion of 4-hydroxyphenylpyruvate to homogentisate caused by a defect in 4-hydroxyphenylpyruvate dioxygenase (HPD; ◘ Fig. 17.1, ▶ enzyme 2). Hawkinsin is thought to be the product of a reaction of an epoxide intermediate with glutathione, which may be depleted. The metabolic acidosis is believed to be due to 5-oxoproline accumulation secondary to glutathione depletion.

17.6.3 Genetics

Hawkinsinuria is thought to be a condition allelic to tyrosinaemia type III, and a heterozygous missense mutation predicting an alanine to threonine change at codon 33 (A33T) was found in the same *HPD* gene in the two patients with hawkinsinuria [48]. More recently, a patient who was heterozygous for a novel Asn241Ser mutation in *HPD* and also heterozygous in trans state for a known tyrosinaemia type III mutation Ile335Met in *HPD* displayed clinical and biochemical features of hawkinsinuria [49]. Using bioinformatic analysis of protein structure the authors concluded that hawkinsinuria is caused by mutations that lead to a retention of partial HPD function, which leads to the production of hawkinsin and 4-hydroxycyclohexylacetate.

17.6.4 Diagnostic Tests

Identification of urinary hawkinsin or 4-hydroxycyclohexylacetate by GC-MS is diagnostic [48]. Hawkinsin is a ninhydrin-positive compound, which appears between urea and threonine in ion-exchange chromatography of urine amino acids. Increased excretion of 4-hydroxycyclohexylacetate is detected on urine organic acids analysis. In addition to hawkinsinuria there may be moderate tyrosinaemia, increased urinary 4-hydroxyphenylpyruvate and 4-hydroxyphenyllac-

tate, metabolic acidosis and 5-oxoprolinuria during infancy. 4-Hydroxycyclohexylacetate is usually detectable only after infancy. The recent identification of mutations in the HPD gene in patients [48][49] makes molecular diagnosis possible in some cases.

17.6.5 Treatment and Prognosis

Symptoms in infancy respond to a return to breastfeeding or a diet restricted in tyrosine and phenylalanine along with vitamin C supplementation. The condition is asymptomatic after the 1st year of life, and affected infants are reported to have developed normally.

Addendum in proofs.
Maleylacetoacetate isomerase deficiency caused by mutations in *GSTZ1* has very recently been found in 6 individuals with mild hypersuccinylacetonemia. Those affected have so far remained asymptomatic despite receiving no specific treatment [Mitchell G, personal communication].

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Branched-chain Organic Acidurias/Acidaemias

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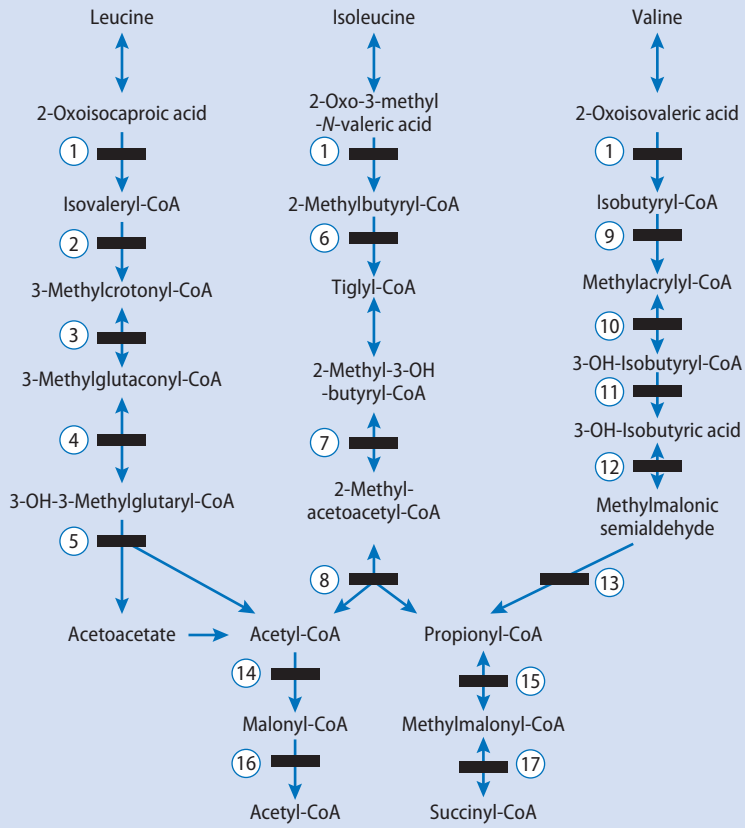
Catabolism of Branched-chain Amino Acids

The three essential branched-chain amino acids (BCAAs), leucine, isoleucine and valine, are initially catabolised by a common pathway (■ Fig. 18.1). The first reaction, which occurs primarily in muscle, involves reversible transamination to 2-oxo- (or keto) acids and is followed by oxidative decarboxylation to coenzyme A (CoA) derivatives by branched-chain oxo- (or keto) acid dehydrogenase (BCKD). The latter enzyme is similar in structure to pyruvate dehydrogenase (▶ Chapter 11, ▶ Fig. 11.2). Subsequently, the degradative pathways of BCAAs diverge. Leucine is catabolised to acetoacetate and acetyl-CoA, which enters the Krebs cycle. The final step in the catabolism of isoleucine involves cleavage into acetyl-CoA and propionyl-CoA, which also enters the Krebs cycle via conversion into succinyl-CoA. Valine is also ultimately metabolised to propionyl-CoA. Methionine, threonine, fatty acids with an odd number of carbons, the side chain of cholesterol, and bacterial gut activity also contribute to the formation of propionyl-CoA.



■ **Fig. 18.1 Pathways of branched-chain amino acid catabolism**. *1*, Branched-chain 2-ketoacid dehydrogenase complex; *2*, isovaleryl-coenzyme A (CoA) dehydrogenase; *3*, 3-methylcrotonyl-CoA carboxylase; *4*, 3-methylglutaconyl-CoA hydratase; *5*, 3-hydroxy-3-methylglutaryl-CoA lyase; *6*, short/branched chain acyl-CoA dehydrogenase deficiency; *7*, 2-methyl-3-hydroxybutyryl-CoA dehydrogenase, MHBD (HSD10); *8*, 2-methylacetoacetyl-CoA thiolase; *9*, isobutyryl-CoA dehydrogenase; *10*, enoyl-CoA hydratase, ECHS1; *11*, 3-hydroxyisobutyryl-CoA deacylase or hydrolase, HIBCH; *12*, 3-hydroxyisobutyric acid dehydrogenase; *13*, methylmalonic semialdehyde dehydrogenase; *14*, acetyl-CoA carboxylase (cytosolic); *15*, propionyl-CoA carboxylase; *16*, malonyl-CoA decarboxylase; *17*, methylmalonyl-CoA mutase. Enzyme defects are indicated by **solid bars**

Branched-chain organic acidurias or organic acidaemias are a group of disorders that result from an abnormality of specific enzymes involving the catabolism of branched-chain amino acids (BCAAs; ► Catabolism of Branched-chain Amino Acids). Collectively, the most commonly encountered are maple syrup urine disease (MSUD), isovaleric aciduria (IVA), propionic aciduria (PA) and methylmalonic aciduria (MMA). They can present clinically as a severe neonatal-onset form of metabolic distress, an acute and intermittent late-onset form, or a chronic progressive form presenting as hypotonia, failure to thrive, and developmental delay. Other rare disorders involving leucine, isoleucine, and valine catabolism are 3-methylcrotonylglycinuria, 3-methylglutaconic aciduria, short-/branched-chain acyl-CoA dehydrogenase deficiency, 2-methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency, isobutyryl-CoA dehydrogenase deficiency, enoyl-CoA hydratase (ECHS1) deficiency, 3-hydroxyisobutyric aciduria (3-hydroxy-isobutyryl-CoA hydrolase or deacylase, HIBCH, deficiency), malonic aciduria (malonyl-CoA decarboxylase deficiency) and combined methylmalonic and malonic aciduria (ACSF3 deficiency). All these disorders can be diagnosed by identifying acylcarnitines and other organic acid compounds in plasma and urine by tandem mass spectrometry (MS) or by gas chromatography-mass spectrometry (GC-MS) and all can be detected by newborn screening using tandem MS.

18.1 Maple Syrup Urine Disease, Isovaleric Aciduria, Propionic Aciduria, Methylmalonic Aciduria

18.1.1 Clinical Presentation

Children with maple syrup urine disease (MSUD), isovaleric aciduria (IVA), propionic aciduria (PA), or methylmalonic aciduria (MMA) have many clinical and biochemical symptoms in common. There are three main clinical presentations:

1. A severe neonatal-onset form with acute metabolic decompensation and neurological distress.
2. An acute, intermittent, late-onset form also with recurrent episodes of metabolic decompensation.
3. A chronic, progressive form presenting as hypotonia, failure to thrive, and developmental delay.

In addition, prospective data gathered by newborn screening programmes, mainly using tandem MS and the systematic screening of siblings of affected subjects, have demonstrated the existence of asymptomatic forms, especially for IVA.

■ Severe Neonatal-onset Form

■■ General Presentation

The general presentation is that of a toxic encephalopathy with either ketosis or ketoacidosis (type I or II in the classification of neonatal inborn errors of metabolism in ► Chapter 1). An extremely evocative clinical setting is that of a full-term baby born after a normal pregnancy and delivery who, after an initial symptom-free period, undergoes relentless deterioration with no apparent cause and is unresponsive to symptomatic therapy. The interval between birth and clinical symptoms may range from hours to weeks, depending on the nature of the defect, and may be related to the timing of the sequential catabolism of carbohydrates, proteins, and fats. Typically, the first signs are poor feeding and drowsiness, followed by unexplained progressive coma. There may be cerebral oedema with a bulging fontanelle, arousing suspicion of a central nervous system (CNS) infection. At a more advanced stage, neurovegetative dysregulation with respiratory distress, hiccups, apnoeas, bradycardia, and hypothermia may appear. In the comatose state, most patients have characteristic changes in muscle tone and exhibit involuntary movements. Generalised hypertonic episodes with opisthotonus, boxing or pedalling movements (typical of MSUD), and slow limb elevations, spontaneously or upon stimulation, are frequently observed. Another pattern is that of axial hypotonia and limb hypertonia with large-amplitude tremors and myoclonic jerks, which are often mistaken for convulsions and are more frequently observed in MMA and PA. In contrast, true seizures occur late and inconsistently. The electroencephalogram may show a burst-suppression pattern. In addition to neurological signs, patients may present with dehydration and mild hepatomegaly.

■■ Specific Signs

Maple syrup urine disease Concomitantly with the onset of the symptoms, the patient emits an intense (sweet, malty, caramel-like) maple-syrup-like odour. In general, neonatal (classic) MSUD does not lead to pronounced abnormalities seen on routine laboratory tests. Patients are not severely dehydrated, have no metabolic acidosis, no hyperammonaemia or only a slight elevation (<130 µmol/l), no blood lactate accumulation, and the blood cell count is normal. The main laboratory abnormalities are greatly increased branched-chain amino acids (BCAAs) in plasma and the presence of 2-ketoacids rapidly detectable in urine with organic acid analysis. Mild ketonuria and hypoglycaemia may also be present.

Isovaleric aciduria, propionic aciduria and methylmalonic aciduria In contrast to MSUD, dehydration is a frequent finding in patients with IVA, PA, or MMA, and moderate hepatomegaly may be observed. They have metabolic acidosis (pH <7.30) with increased anion gap and ketonuria (Acetest 2-3 positive). However, ketoacidosis can be moderate and is often responsive to symptomatic therapy. In MMA and PA, hyperammonaemia is a constant finding. When the ammonia level is very high (>500 µmol/l) it can induce respiratory alkalosis and lead to the erroneous diagnosis of an urea cycle disorder. Normal to low glutamine is a distinctive finding. Moderate hypocalcaemia (<1.7 mmol/l) and hyperlactataemia (3–6 mmol/l) are frequently found. Blood glucose can be normal, reduced, or elevated. When blood glucose level is very high (20 mmol/l) and is associated with glucosuria, ketoaci-

dosis, and dehydration, it may mimic neonatal diabetes. Neutropenia, thrombocytopenia, non-regenerative anaemia, and pancytopenia can occur and are frequently erroneously ascribed to sepsis. Among these disorders, IVA is easily recognized by an unpleasant sweaty feet odour. In some cases, the combination of vomiting, abdominal distension, and constipation may suggest gastrointestinal obstruction. Cerebral haemorrhages have been described in a few neonates, a complication that may be linked to inappropriate correction of acidosis and may explain some poor long-term neurological outcomes.

■ Acute Intermittent Late-onset Form

In approximately a quarter of patients, the disease presents after a symptom-free period, which is commonly longer than 1 year and sometimes lasts until adolescence or adulthood. Recurrent attacks may be frequent and, between them, the child may seem entirely normal. Onset of an acute attack may arise during catabolic stress such as infections or following increased intake of protein-rich foods, but sometimes there may be no overt cause.

■■ Neurological Presentation

Recurrent attacks of either coma or lethargy with ataxia are the main presentations of these acute late-onset forms. The most frequent variety of coma in MMA, PA and IVA is that presenting with ketoacidosis and mild hyperammonaemia, but in exceptional cases acidosis may be absent. There is no acidosis in MSUD.

Hypoglycaemia may be a presenting sign in patients with MSUD. Although most recurrent comas are not accompanied by focal neurological signs, some patients may present with acute hemiplegia, hemianopsia, or symptoms and signs of cerebral oedema mimicking encephalitis, a cerebrovascular accident, or a cerebral tumour. These acute neurological manifestations have frequently been preceded by other premonitory symptoms that had been missed or misdiagnosed. They include acute ataxia, unexplained episodes of dehydration, persistent and selective anorexia, chronic vomiting with failure to thrive, hypotonia, progressive developmental delay and abnormal behaviour.

■■ Hepatic Forms

In IVA, MMA or PA, some patients may present with a Reye syndrome-like illness characterised by onset of coma, cerebral oedema, hepatomegaly, liver dysfunction, hyperammonaemia and even macro- or microvesicular fatty infiltration of the liver.

■■ Haematological and Immunological Forms

Severe haematological manifestations are frequent, mostly concomitant with ketoacidosis and coma, and are sometimes the presenting problem. Neutropenia is regularly observed in both neonatal and late-onset forms of IVA, PA and MMA. Thrombocytopenia occurs mostly in infancy, and anaemia occurs only in the neonatal period. Various cellular and humoral immunological abnormalities have been described in patients presenting with recurrent infections, leading to erroneous diagnosis and management.

■ Chronic, Progressive Forms

■■ Gastrointestinal Presentation

Persistent anorexia, chronic vomiting, aversion to protein-rich food, failure to thrive and osteoporosis (evidence of a long-standing GI disturbance) are frequent manifestations. In infants, this presentation is easily misdiagnosed as gastro-oesophageal reflux, cow's milk protein intolerance, coeliac disease, late-onset chronic pyloric stenosis or hereditary fructose intolerance, particularly if these symptoms start after weaning and diversification of food intake. Later in life, recurrent vomiting with ketosis may occur. These patients may remain undetected until an acute neurological crisis with coma leading to the diagnosis.

■■ Chronic Neurological Presentation

Some patients present with severe hypotonia, muscular weakness and poor muscle mass that can simulate congenital neurological disorders or myopathies. Nonspecific developmental delay, progressive psychomotor retardation, dementia, seizures and movement disorders may also be observed during the course of the disease. However, these rather nonspecific findings are rarely the sole presenting symptoms.

■ Complications

■■ Neurological Complications

Maple syrup urine disease Acute cerebral oedema is a well-recognized complication in newborns with MSUD and encephalopathy. Brain ultrasonography [1] and magnetic resonance imaging (MRI) display a characteristic pattern that may be of help in the diagnosis. In older patients with metabolic decompensation it may cause brain stem compression and unexpected death, particularly following intensive rehydration [2]; it may also develop slowly due to long-standing elevations of BCAAs. Additionally, demyelination can occur over time in those patients with poor biochemical control and persistently raised BCAAs. The areas most commonly affected are the periventricular white matter of the cerebral hemispheres, the deep cerebellar white matter, the dorsal part of the brain stem, the cerebral peduncles, the dorsal limb of the internal capsule and the basal ganglia. The severity of dysmyelination does not correlate with signs of acute neurotoxicity, and the changes are reversible with appropriate treatment [3]. Acute axonal neuropathy may complicate late-onset decompensation [4].

Propionic aciduria and methylmalonic aciduria An increasing number of patients with PA and MMA have presented with an acute or progressive extrapyramidal syndrome associated with increased signal within the basal ganglia (mostly the globus pallidus in MMA). The basal ganglia involvement may be due to oedema that evolves to necrosis. In addition, MRI studies indicate cerebral atrophy and delayed myelination [5][6]. These dramatic complications are arguments for adequate life-long dietary control even if the patient

is free of symptoms. Even in well-treated patients with PA who are clinically and metabolically stable, brain lactate is elevated; this may indicate that aerobic oxidation and mitochondrial energy metabolism is persistently impaired from elevated intracellular propionic metabolites [6]. Late-onset optic neuropathy with visual dysfunction is another insidious complication in both MMA and PA [7]. In PA, late-onset psychiatric complications have been reported [8].

Renal Complications

Renal tubular acidosis associated with hyperuricaemia may be an early and presenting sign in some late-onset patients with MMA. This condition partially improves with metabolic control. Chronic renal failure is increasingly recognised in MMA patients older than 10 years of age [9]. Renal pathology is tubulo-interstitial nephritis with type-4 tubular acidosis and adaptative changes secondary to reduced glomerular filtration rate [10]. The course of the disease is usually indolent, but end-stage renal failure may develop, and dialysis and kidney transplantation are likely to be necessary by the end of the 2nd decade of life in many patients [11]. As the nephropathy is a likely complication from chronic glomerular hyperfiltration secondary to excessive MMA excretion, minimizing and deceleration of renal injury may require strict metabolic control. Of note, renal failure has also been reported in a few PA patients [12].

Skin Disorders

Large, superficial desquamation, alopecia, and corneal ulceration may develop in the course of late and severe decompensations in MSUD, PA or MMA. These skin lesions have been described as a staphylococcal scalded-skin syndrome with epidermolysis or as acrodermatitis enteropathica-like syndrome [13]. In many cases, these complications occur together with diarrhoea and can be ascribed to acute protein malnutrition, especially to isoleucine deficiency.

Pancreatitis

Acute, chronic or recurrent pancreatitis may complicate organic acidaemias (MMA, PA and IVA). It has been the presenting illness in some patients with late-onset forms of IVA. The pathophysiological mechanisms are unknown. However, ketoacidosis is assumed to play a role, as pancreatitis also complicates diabetic ketoacidosis. The condition may be difficult to diagnose and must be considered in the assessment of patients with acute deterioration. However, elevation of serum lipase and amylase alone does not confirm the diagnosis as pancreatitis being defined by inflammation on pancreas imaging. In contrast, isolated elevated lipase may normalize with the correction of the metabolic status [14].

Cardiomyopathy and Disturbances in Cardiac Electrophysiology

Cardiomyopathy is one of the major complications in PA and, less prominently MMA, and may be responsible for rapid deterioration or death [12]. It may develop as part of an acute decompensation or as a chronic deterioration even in patients who are metabolically stable. Both dilated and hypertrophic types have been reported, with an estimated prevalence of 23% in one cohort [15]. In another PA cohort, 70% of patients beyond infancy were found to have developed disturbance in cardiac electrophysiology, particularly prolonged QTc, which could contribute to cardiac complications [16]. The mechanism is uncertain but may result from energy deprivation and/or toxic accumulation. Investigation and follow-up may be useful to prevent irreversible damage and to help in decisions on therapeutic measures, as reversibility of cardiomyopathy with orthotopic liver transplantation has been described in rare cases [15][17].

18.1.2 Metabolic Derangement

Maple Syrup Urine Disease

MSUD is caused by a deficiency of the branched-chain 2-ketoacid dehydrogenase (BCKD) complex, the second common step in the catabolism of the three BCAAs (◘ Fig. 18.1, ▶ enzyme 1). Like the other 2-ketoacid dehydrogenases, BCKD is composed of three catalytic components (▶ Chapter 11, ▶ Fig. 11.2): a decarboxylase (E1), composed of E1α- and E1β-subunits and requiring thiamine pyrophosphate as a coenzyme, a dihydrolipoyl acyltransferase (E2) and a dihydrolipoamide dehydrogenase (E3). A deficiency of the E1 or E2 component can cause MSUD, whereas a deficiency of the E3 component produces a specific syndrome (dihydrolipoamide dehydrogenase [E3] deficiency) with congenital lactic acidosis, branched-chain 2-ketoaciduria and 2-ketoglutaric aciduria (▶ Chapter 11). However, E3 deficiency, particularly the neonatal-onset forms, may present with lactic acidaemia alone, with elevation of branched-chain amino acids only becoming apparent weeks or months later. Variants forms affecting the phosphatase and kinase that regulate the BCKD complex have been recently described. BCKD phosphatase deficiency has been reported in a mild MSUD-like patient [18] and BCKD kinase deficiency in patients with syndromic autism with intellectual disability and low plasma BCAA levels [19]. Additionally, patients affected with disorders in the synthesis of lipoic acid could theoretically exhibit high levels of BCAA due to a secondary defect in E3. However, elevation of BCAA seems to be a rare finding in lipoic acid synthesis defects, which is occasionally found in E3 subunit deficiency but not in the other defects [20]. BCKD dysfunction results in marked increases in the branched-chain 2-ketoacids in plasma, urine and cerebrospinal fluid (CSF). Owing to the reversibility of the initial transamination step, the BCAAs also accumulate. Smaller amounts of the respective 2-hydroxy acids are formed. Alloisoleucine, a diastereomer of isoleucine, is invariably found in the blood of all patients with classic MSUD and in those with variant forms, at least in those still without dietary treatment.

Among the BCAA metabolites, leucine and 2-ketoisocaproic acid appear to be the most neurotoxic. In MSUD, these compounds are always present in approximately equimolar concentrations in plasma, and may cause acute brain dysfunc-

tion when their plasma concentrations rise above 1 mmol/l. Isoleucine and valine are of lesser clinical significance. Their 2-ketoacid to amino acid ratios favour the less toxic amino acids, and cerebral symptoms do not occur even when the blood levels of both amino acids are extremely high.

Isovaleric Aciduria

IVA is caused by a deficiency of isovaleryl-CoA dehydrogenase (IVD; ◘ Fig. 18.1, ▶ enzyme 2), an intramitochondrial flavoenzyme which, in a similar way to the acyl-CoA dehydrogenases (▶ Chapter 12, ▶ Fig. 12.1), transfers electrons to the respiratory chain via the electron transfer flavoprotein (ETF)/ETF-ubiquinone oxidoreductase (ETF-QO) system. Deficiencies of the ETF/ETFQO system result in multiple acyl-CoA-dehydrogenase deficiency (MADD; synonym: glutaric aciduria type II) (▶ Chapter 12). The enzyme defect results in the accumulation of derivatives of isovaleryl-CoA, including free isovaleric acid, which is usually increased in both plasma and urine (although normal levels have been reported), 3-hydroxyisovaleric acid (3-HIVA) and *N*-isovalerylglycine. This glycine conjugate is the major derivative of isovaleryl-CoA, owing to the high affinity of the latter for glycine *N*-acylase. Conjugation with carnitine (catalysed by carnitine *N*-acylase) results in the formation of isovalerylcarnitine.

Propionic Aciduria

PA is caused by a deficiency of the mitochondrial enzyme propionyl-CoA carboxylase (PCC; ◘ Fig. 18.1, ▶ enzyme 15), one of the five biotin-dependent enzymes. PCC is a multimeric protein composed of two different subunits, α- (which binds biotin) and β-PCC subunits. So far, all patients with isolated PA have been biotin resistant.

PA is characterised by greatly increased concentrations of free propionic acid in blood and urine and the presence of multiple organic acid by-products, among which propionylcarnitine, 3-hydroxypropionate and methylcitrate are the major diagnostic metabolites. The first is formed by acylation to carnitine. The second is formed by either β- or ω-oxidation of propionyl-CoA. Methylcitrate arises by condensation of propionyl-CoA with oxaloacetate, which is catalysed by citrate synthase. During ketotic episodes, 3-HIVA is formed by condensation of propionyl-CoA with acetyl-CoA, followed by chemical reduction. High concentrations of organic acids derived from a variety of intermediates of the isoleucine catabolic pathway, such as tiglic acid, tiglylglycine, 2-methyl-3-hydroxybutyrate, 3-hydroxybutyrate and propionylglycine, can also be found. Owing to an abnormal biotin metabolism, propionyl-CoA accumulation also occurs in multiple carboxylase deficiency (biotinidase deficiency, holocarboxylase synthetase [HCS] deficiency), resulting in defective activity of all four biotin-dependent carboxylases (▶ Chapter 26) also observed in carbonic anhydrase VA deficiency (▶ Chapter 19).

Methylmalonic Aciduria

MMA is caused by a deficiency of methylmalonyl-CoA mutase (MCM; ◘ Fig. 18.1, ▶ enzyme 17), a vitamin B_{12}-dependent enzyme. Deficient activity of the MCM-apoenzyme leads to MMA. Because the apomutase requires adenosylcobalamin (AdoCbl) as a cofactor, disorders that affect AdoCbl formation cause variant forms of MMA (▶ Chapter 27).

Deficiency of MCM leads to accumulation of methylmalonyl-CoA, resulting in greatly increased amounts of methylmalonic acid in plasma and urine. Owing to secondary inhibition of PCC, propionic acid also accumulates, and other propionyl-CoA metabolites, such as propionylcarnitine, 3-hydroxypropionic acid, methylcitrate and 3-HIVA, are usually also found in urine. However, some mildly affected or asymptomatic patients, identified through urine organic acids screening in neonates but showing only slightly increased methylmalonic acid in blood and urine, have not shown constant excretion of metabolites derived from propionyl-CoA.

Recently, novel variants of MMA, also characterised by mild MMA, have been identified (▶ below).

Vitamin-B_{12} deficiency must be excluded when excessive urinary methylmalonic acid is found, particularly in a breast-fed infant whose mother either is a strict vegan or suffers from subclinical pernicious anaemia.

Secondary Metabolic Disturbances Common to PA and MMA

Accumulation of propionyl-CoA results in inhibitory effects on various pathways of intermediary metabolism, in increased levels of acylcarnitines (particularly propionylcarnitine) in blood and urine leading to a relative carnitine deficiency and in enhanced synthesis of odd-numbered long-chain fatty acids. Inhibition of various enzymes may explain some features such as hypoglycaemia, hyperlactataemia, hyperammonaemia and hyperglycinaemia. The increased ketogenesis that is a major cause of morbidity is not fully understood. Several pathomechanisms (e.g. accumulation of putatively toxic organic acids, inhibition of mitochondrial energy metabolism) have been suggested to explain acute and long-term organ damage [21].

Propionate, essentially in the form of propionyl-CoA, is produced in the body from three main sources: (1) catabolism of the amino acids isoleucine, valine, methionine and threonine, (2) anaerobic fermentation in the gut and (3) mobilization and oxidation of odd-chain fatty acids during prolonged fasting states. It has been estimated that catabolism of amino acids contributes approximately for 50% of the total propionate production, anaerobic gut bacteria 20%, and odd-chain fatty acids 30% [22]. These data, which are largely from stable isotope turnover studies, are based on a number of unproven assumptions and have not been reproduced in a more systematic manner. They are therefore questionable (for critical reviews, see [11][23]).

18.1.3 Genetics

Maple Syrup Urine Disease

MSUD is an autosomal-recessive disorder, with an incidence of 1 in 120,000 to 1 in 500,000. It is highly prevalent in the inbred Mennonite population in Pennsylvania, occurring in

approximately 1 in 176 newborns [2]. In countries where consanguineous marriages are common, the frequency is also higher (about 1 in 50,000 in Turkey). About 75% of those affected suffer from the severe classic form, and the remainder suffers from the milder intermediate or intermittent variants. Over 150 different causal mutations scattered among the three genes, *E1α, E1β* and *E2*, give rise to either classic or intermediate clinical phenotypes [24].

Isovaleric Aciduria

IVA is an autosomal recessive disorder, with extreme clinical variability for unknown reasons. Reported mutations in *IVD* are highly heterogeneous, and generally no genotype-phenotype correlation has been established. However, children with IVA diagnosed by newborn screening and carrying a c.932C>T mutant allele can exhibit a milder, potentially asymptomatic phenotype [25].

Propionic Aciduria

PA is an autosomal recessive disorder with an incidence of less than 1 in 100,000. PA can result from mutations in *PCCA* or *PCCB* encoding the α- and β-subunits, respectively, of propionyl-CoA carboxylase. To date, more than 50 different allelic variations in *PCCB* and more than 30 in *PCCA* have been identified in different populations [26]. Following the introduction of the newborn screening programme in Japan a number of infants with an apparently mild phenotype and the Y435C mutation in *PCCB* have been reported. The natural history of this phenotype is not yet clarified [27].

Methylmalonic Aciduria

Isolated MMA can be caused by mutations in the *MUT* locus encoding the methylmalonyl CoA mutase (MCM) apoenzyme, or by those in genes required for provision of its cofactor, 5'-deoxyadenosylcobalamin (AdoCbl). Isolated MMA is classified into several genotypic classes and complementation groups. These are designated either mut^- or mut^0 (together termed mut), according to whether there is minimal or no apoenzyme activity *in vitro*, respectively, or cobalamin A, B or D-variant 2 (cblA/B/D-MMA) for cofactor defects (see ▶ Chapter 27 for further details). MMA is an autosomal recessive disorder with an overall incidence of about 1 in 50,000. Approximately one half to two thirds of patients have a mutase apoenzyme defect; the remaining patients have cobalamin variants. To date more than 200 disease-causing mutations in patients with $mut^{0/-}$ MMA have been identified at the *MUT* locus [28]. Mutations in *MMAA* and *MMAB* encoding the cblA and cblB proteins respectively have been identified in cblA and cblB patient cell lines (▶ Chapter 27).

Newly described variants with mild MMA elevation include defects in succinyl-CoA synthase and methylmalonyl-CoA epimerase. Succinyl-CoA synthase catalyzes the conversion of succinyl-CoA to succinate in the Krebs cycle. Its deficiency causes mild MMA, variable lactic acidosis, accumulation of succinylcarnitine and mitochondrial DNA depletion (▶ Chapter 14). Succinyl-CoA synthase is composed of an α-subunit (encoded by *SUCLG1*) and two β-subunits (encoded by *SUCLA2* and *SUCLG2*). Patients with different genetic backgrounds have been found to have mutations in *SUCLA2*, and in *SUCLG1* [29]. The clinical picture in *SUCLA2* patients is highly homogeneous and comprises early-onset encephalomyopathy, dystonia, deafness and Leigh-like MRI abnormalities. Patients with *SUCLG1* mutations are clinically heterogeneous, showing either a severe form with neonatal multiorgan failure and early death or a phenotype similar to *SUCLA2* patients. Hypertrophic cardiomyopathy and liver involvement is exclusively found in patients with *SUCLG1* mutations [29].

A deficiency in methylmalonyl-CoA epimerase has been reported in individuals with mild MMA. This defect has a questionable clinical impact [30][31].

18.1.4 Diagnostic Tests

Only MSUD can be diagnosed by using plasma amino acid chromatography alone. IVA, PA and MMA are diagnosed by their specific urinary organic acid profiles using GC-MS or abnormal acylcarnitines on tandem MS, while amino acid chromatography displays nonspecific abnormalities, such as hyperglycinaemia and hyperalaninaemia. Owing to acidosis and its impact on glutamine metabolism, hyperammonaemia associated with organic acidurias leads to normal or even low plasma glutamine levels [32][33]. Whatever the clinical presentation, the diagnosis can be made by sending filter-paper blood specimens, fresh or frozen urine samples or 1- to 2-ml samples of fresh or frozen plasma to an experienced laboratory for analysis. Specific loading tests are not necessary. Newborn screening for this group of organic acidurias can be performed by tandem MS [34][35]. An increased leucine/isoleucine peak in blood spots taken at 24 or 36 h of age requires immediate notification. Similarly, the abnormal acylcarnitine profile found in PA and MMA with propionylcarnitine (C3-carnitine) and that in IVA with isovalerylcarnitine (C5-carnitine) also requires immediate notification [35].

Enzymatic studies and/or molecular DNA testing are useful for diagnostic confirmation. Around the 14th week of gestation (2nd trimester), reliable and rapid prenatal diagnosis of IVA, PA, and MMA can be performed by the direct measurement of metabolites in amniotic fluid using GC-MS, stable-isotope dilution techniques, or tandem MS. First-trimester diagnosis using direct enzyme assay or molecular DNA assays in families in which the mutations are known can be performed in fresh or cultured chorionic villi. This can also be done in cultured amniotic cells taken in the 2nd trimester. Prenatal diagnosis of MSUD relies exclusively on enzyme assays in chorionic villi or in cultured amniocytes and/or on mutational analysis.

18.1.5 Treatment and Prognosis

CNS dysfunction can be prevented or at least minimised by early diagnosis and emergency treatment. Neonatal-onset

forms frequently require early toxin removal (▶ Chapter 4). Thereafter dietary restriction, which is necessary to limit the production of organic acids and their metabolites and other specific treatments, is required both for survivors of the early-onset forms and for those with late-onset disease. For both groups it is essential that episodes of metabolic decompensation are recognised and treated sufficiently early; parents must be taught to recognise early warning signs and manage their child appropriately. Exhaustive recommendations on acute and long-term management of organic acidurias (MMA and PA) from the E-IMD consortium have recently been published [12].

Principles of Long-term Dietary Treatment

Long-term dietary treatment is aimed at reducing the accumulation of toxic metabolites while, at the same time, maintaining normal physical development and nutritional status and preventing catabolism. Some patients tolerate normal foods; others need only minimal restriction or can even regulate the diet themselves. However, many need very specific food allowances, implying stringent dietary restrictions that will be necessary for life.

The cornerstone of treatment is the limitation of one or more essential amino acids which, if present in excess, are either toxic or precursors of toxic organic acids. Precise prescriptions are established for the daily intake of amino acids, protein and energy. The diet must provide the recommended daily allowance (RDA) and the estimated safe and adequate daily dietary intakes of minerals and vitamins and follow the principles of paediatric dietetics [36].

Protein/Amino Acid Prescriptions

Requirements for BCAAs and protein vary widely from patient to patient and in the same patient, depending on the nature and severity of the disorder, other therapies prescribed (stimulation of an alternate pathway), growth rate, state of health and feeding difficulties. Individual requirements must be estimated for each child by frequent monitoring of clinical and metabolic status. The balance between protein malnutrition and metabolic disequilibrium can be difficult to maintain in severe PA and MMA and needs to be kept under regular review, especially after an acute metabolic decompensation or after a change in the diet.

Within this group of organic acidurias, only in MSUD is the diet directly related to the intake of an amino acid, which is leucine in milligram amounts. Natural protein, which contains leucine, must be severely restricted in an age-dependent manner to only one tenth to a half of the normal recommended daily requirement. Consequently, in order to meet the protein RDA for the patient's age, a large supplement of BCAA-free amino acid mixture as a protein substitute is necessary. In IVA it is generally sufficient to restrict natural protein to the recommended minimum daily requirements or just somewhat more; a special amino acid mixture free of leucine is rarely needed. In neonatal PA and MMA dietary protein is generally restricted to the adequate age-related safe levels. Restriction of specific amino acids has not proved to be useful. Although controversial, a limited, relatively small, amount of an amino acid mixture free of valine, isoleucine, methionine and threonine can be added to the diet to supply additional nitrogen and other essential and nonessential amino acids in order to promote a protein-sparing anabolic effect.

The prescribed amounts of leucine or natural protein are provided by natural foods. Breast milk or standard infant formula is used in young infants but breast milk should be preferentially used in the early infancy period. For toddlers and children solids are introduced, using serving lists and lists of amino acid content in foods. In all protein-restricted diets, high-protein foods (eggs, meat, dairy products), apart from milk, are generally avoided, since the lower percentage of amino acids in vegetable protein (compared with that in animal protein) makes it easier to satisfy the appetite of children.

Energy and Micronutrient Prescriptions

Energy and micronutrient prescriptions should follow the general rules common to all artificial medical diets, the model for which being PKU (▶ Chapter 16).

Evaluation of Clinical and Nutritional Status

The metabolic and nutritional statuses are both evaluated weekly during the 1st month of therapy, once a month during the 1st year, and later every 3–6 months [12]. In patients treated with a low-protein diet without an added amino acid mixture, measurement of urea excretion can be used to evaluate anabolism [37]. Regular assessment of developmental progress provides the opportunity for psychological support, as social and emotional needs are major issues of the overall therapy of the affected child and of the family's wellbeing.

Specific Adjustments

Maple Syrup Urine Disease

Acute phase management in the newborn Exogenous toxin removal procedures such as haemodialysis and haemofiltration together with high-energy dietary treatment are usually recommended for the reversal of acute metabolic decompensation in symptomatic newborns with the classic form of MSUD [38]. With these measures the plasma leucine level is reduced to 1 mmol/l or less within hours. During the recovery interval, oral intake of BCAA-free formula (by tube feeding) should be started early and BCAA intake adjusted according to the plasma levels, which are monitored daily until the optimal equilibrium is achieved. During this stage, plasma concentrations of valine and isoleucine may fall below normal and become rate limiting for protein synthesis, a situation which requires generous valine and isoleucine supplements in doses of 300–400 mg/day. Newborn screening for MSUD by tandem MS allows for early diagnosis and intervention and in some cases obviates the need for extracorporeal detoxification. In affected newborns found positive on screening the oral intake of BCAA-free formula (tube feeding) with adequate calorie supply (glucose polymer) and supplementation with isoleucine and valine (300–400 mg/day) can be sufficient to stimulate protein synthesis and to normalise plasma leucine levels within 2–3 days [2][39].

Long-term management Management of MSUD comprises a life-long strict and carefully adjusted semisynthetic diet, as well as acute-phase treatment during episodes of catabolic stress. The dietary treatment of MSUD differs from that of other organic acidurias, since the condition results in elevated plasma BCAA levels. In that respect MSUD can be regarded as an aminoacidopathy, and the principles of dietary treatment are essentially those that apply to PKU. The diet consists of measured proportions of BCAA-containing foods (as natural protein) and a synthetic BCAA-free amino acid supplement, which in most preparations also contains the recommended requirements for minerals, vitamins and other essential nutrients. Additional fat and carbohydrate are provided by protein-free products and additional supplements. The aim of such treatments is to maintain the 2–3 h postprandial plasma BCAAs at near-normal concentrations (leucine: 80–200 µmol/l; isoleucine: 40–90 µmol/l; valine: 200–425 µmol/l). Since leucine is the most toxic precursor, the diet can be based on the leucine requirement, with frequent adjustment according to plasma leucine levels.

In newborns with the classic severe form of MSUD, the leucine requirement is 300–400 mg/day (80–110 mg/kg/day), which is not far from the leucine intake in healthy breast-fed newborns. MSUD patients may be kept on breast milk in early infancy. Minimum valine and isoleucine requirements are 200–250 mg/day. Apart from considerable inter-individual variation, children, adolescents and adults with the classic form of MSUD tolerate about 500–700 mg of leucine per day. Individuals with variant forms tolerate greater amounts, and some do well on a low-protein diet.

Serial monitoring of blood BCAA levels is essential in the treatment of MSUD, and intakes of BCAAs must frequently be titrated against plasma concentrations. Additional small amounts of free valine and isoleucine may be needed to those provided by natural protein, because the tolerance for leucine is lower than that for the other two. When the plasma leucine levels are high and those of valine and isoleucine low, a rapid fall of leucine can only be achieved by combining a reduced leucine intake with a temporary supplement of valine and isoleucine.

In MSUD, unlike other organic acidurias, no abnormal acylcarnitines are formed and there is no increased carnitine loss; consequently no carnitine supplement is required. Although treatment with thiamine has often been advocated, its efficacy has not been confirmed in any form of MSUD.

Emergency regimen During maintenance treatment minor illnesses such as fever, vomiting, or diarrhoea result in an increase in catabolism and amino acid release from muscle protein. Neurotoxic levels of BCAAs and BCKAs are reached within hours, and patients may present with apathy, ataxia, hallucinations and, eventually, with fasting hypoglycaemia and convulsions. High energy intake and temporary removal of natural protein from the diet, and continuing supplements of BCAA-free amino acids (with the early addition of valine and isoleucine supplements) help to limit accumulation of the branched-chain compounds. Owing to its anabolic effect, intravenous insulin (0.05–0.20 IU/kg body weight/h) combined with large amounts of glucose and with continued enteral BCAA-free amino acids, can be successfully used to treat severe catabolic episodes. When enteral feeding is impossible, (gastric intolerance or enteral feeding refusal in adults), a parenteral BCAA-free amino acids mixture can be used [40]. Such therapy may prevent metabolic decompensation following major surgery and trauma and can obviate the necessity for extracorporeal toxin removal in critically ill children. The latter should be discussed in the event of very high leucine levels (>1000 µM) and/or rapid onset of neurological symptoms.

Maternal MSUD In pregnant women with MSUD, maintaining the plasma leucine level between 100 and 300 µmol/l and plasma valine and isoleucine in the upper normal ranges resulted in the delivery of healthy infants. Leucine tolerance increased progressively from the 22nd week of gestation from 350 to 2100 mg/day. The risk of metabolic decompensation in the mother during the catabolic postpartum period can be minimised by careful monitoring after delivery in a metabolic referral centre [41].

Liver transplantation Liver replacement results in a clear increase in whole-body BCKD activity to at least the level seen in the very mild MSUD variant; following transplant patients no longer require protein-restricted diets and the risk of metabolic decompensation during catabolic events is apparently abolished [42][43]. Explanted livers of MSUD patients have been successfully used in domino transplantation [44]. In neurocognitively-impaired patients prior to transplantation, liver transplant prevented further brain damage but did not reverse it [44].

Prognosis Patients with MSUD are now expected to survive; they are generally healthy between episodes of metabolic imbalance, and some attend regular schools and have normal IQ scores. However, the average intellectual performance is clearly below that of normal subjects [39][45]. The intellectual outcome is inversely related to how long after birth plasma leucine levels remained above 1 mmol/l and is dependent on the quality of long-term metabolic control [54]. This suggests that inclusion of MSUD in neonatal screening programmes by tandem MS may improve the prognosis in this disease. Normal development and normal intellectual outcome and performance can be achieved at least in prospectively treated patients [2] and if average long-term plasma leucine levels are not more than 1.5–2 times normal [46]. However, some patients may present mental health problems despite good metabolic control. Children may have inattention and hyperactivity, and older patients may show generalised anxiety, panic or depression, resulting in poor educational and social achievement. Both types of disorders may require specific treatment [39]. In addition, timely evaluation and intensive treatment of minor illnesses at any age is essential, as late death attributed to recurrence of metabolic crises with infections has occurred [2].

Isovaleric Aciduria

Acute phase management in the newborn Intensive treatment with nonspecific measures (glucose infusion to provide calories and reduce endogenous protein catabolism, possibly bicarbonate infusion to control the acidosis) including exogenous toxin (and ammonia) removal may be needed in newborns. Such infants are often in a poor clinical condition precluding the effective use of alternate pathways to enhance the removal of isovaleryl-CoA. In these circumstances, the administration of intravenous L-carnitine (100–400 mg/kg/day) and oral L-glycine (250–600 mg/kg/day) are effective means of treatment. Glycine can be provided as a 100-mg/ml-water solution delivered in four to eight separate doses. Carbamylglutamate has been successfully used to treat hyperammonaemia in acutely ill patients with IVA [47].

Dietary therapy The aim of treatment is to reduce the isovaleric acid burden to a minimum and to keep the urine free of IVA and 3-hydroxy-IVA. Such a therapy consists of a low-protein diet with supplemental glycine and carnitine and should be started as soon as possible after birth. In most patients the amount of protein tolerated meets the official protein requirements, and a special amino acid mixture free of leucine is rarely needed. Excessive protein intake should be avoided.

Carnitine and glycine therapy For supplemental therapy either oral L-carnitine (50–100 mg/kg/day) or oral L-glycine (150–300 mg/kg/day) can be used. Under stable conditions the need for both supplementations is still controversial, but it can be useful during metabolic stress when toxic isovaleryl-CoA accumulation increases the need for detoxifying agents [48]. Supplementation with large doses of carnitine gives rise to an unpleasant odour in many IVA patients.

Prognosis Prognosis is better than for the other organic acidurias. Even when a patient is compliant with treatment, metabolic crises can occur during catabolic stress, making a short hospitalisation for intravenous fluid (glucose/electrolytes/buffer) necessary. With puberty, metabolic crises rarely occur. Growth is normal; intellectual prognosis depends on early diagnosis and treatment and, subsequently, on long-term compliance [49]. According to this, inclusion of IVA into neonatal screening programmes by tandem MS should improve the prognosis. So far (8 pregnancies reported) there is no evidence that uncomplicated maternal IVA has any adverse effect on the unborn child [50].

In asymptomatic individuals identified by newborn screening and showing a mild biochemical phenotype it is crucial to follow the course of the inherited metabolic disturbance prospectively, as far as possible without any therapeutic regimen in order to better define the natural history.

Propionic Aciduria and Methylmalonic Aciduria

Recent recommendations and treatment guidelines have been published by the EIMD consortium [12].

Acute phase management in the newborn The urinary excretion of propionic acid is negligible, and no alternate urinary pathway is sufficient to effectively detoxify newborns with PA. However, this does not mean that exogenous toxin removal procedures are inevitably required. Extracorporal detoxification such as haemo(dia)filtration and haemodialysis (peritoneal dialysis is far less efficient), together with measures to promote anabolism, should be considered when neonatal illness is accompanied by severe hyperammonaemia (>400 μmol/l). In contrast to PA, the efficient removal of toxin in MMA takes place via urinary excretion, because of the high renal clearance of methylmalonic acid (22±9 ml/min per 1.73 m^2), which allows excretion of as much as 4–6 mmol MMA/day. Thus, in some cases not complicated by very high ammonia levels, emergency treatment may be limited to rehydration and promotion of anabolism [51].

When conservative measures with high energy supply are sufficient, hyperammonaemia (especially in PA) may be controlled by the use of sodium benzoate and/or carbamylglutamate [52]. The use of sodium phenylbutyrate is not recommended because in MMA and PA hyperammonemia is usually associated with decreased levels of glutamine [12][33]. Metabolic decompensation in PA may be complicated by severe lactic acidosis due to thiamine deficiency, requiring vitamin supplementation [53].

Long-term management The goal of treatment is to reduce the production of methylmalonic or propionic acid by means of

- Natural protein restriction
- Maintaining an optimal calorie intake
- Carnitine supplementation (100 mg/kg/day)
- Reduction of intestinal production of propionate by metronidazole

Dietary management The aim of dietary treatment is to reduce the production of propionate by both the restriction of precursor amino acids using a low-protein diet and avoidance of prolonged fasting to limit oxidation of odd-chain fatty acids, which are liberated from triglyceride stores during lipolysis. The low-protein diet must provide at least the minimum amount of protein, nitrogen and essential amino acids to meet requirements for normal growth. Figures for estimates of safe levels of protein intake for infants, children and adolescents are available [36], which can be used as a guide for low-protein diets. In early childhood this is often 1–1.5 g/kg/day. To improve the quality of this diet it may be supplemented with a relatively small amount of synthetic amino acids free from the precursor amino acids. However, the long-term value of these supplements remains uncertain, and metabolic balance can be achieved without them [11][36][37]. Some studies have shown that the addition of a special amino acid mixture to a severely restricted diet has no effect on growth or metabolic status and that these amino acids are mostly broken down and excreted as urea [37].

Long fasts should be avoided. In order to prevent fasting at night nocturnal tube feeding may be required in the early years of management.

In children with severe forms of PA and MMA, anorexia and feeding problems are almost invariably present, and in order to maintain a good nutritional status, feeds have to be given via nasogastric tube or gastrostomy at some stage. This is essential to provide adequate dietary intake, to prevent metabolic decompensation and to help the parents to cope with a child who may be difficult to feed [11][36][37].

Most patients with a late-onset form are easier to manage. Individual protein tolerance can be quite high. Even though this allows a less rigid protein restriction and leads to a lower risk of malnutrition, these patients must be taught to reduce their protein intake immediately during intercurrent illness to prevent metabolic imbalance.

Vitamin therapy Every patient with MMA should be tested for responsiveness to vitamin B12. Some late-onset forms (and, more rarely, neonatal-onset forms) are responsive to vitamin B12; thus, parenteral vitamin therapy, starting with hydroxocobalamin 1000–2000 µg/day for about 10 days, must be carefully tried during a stable metabolic condition. During this period 24-h urine samples are collected for an organic acid analysis. Vitamin B12 responsiveness leads to a prompt and sustained decrease of propionyl-CoA by-products, mainly MMA. However, as biochemical results may be difficult to assess, they must later be confirmed by *in vitro* studies. Most B_{12}-responsive patients need only mild protein restriction or none at all. Vitamin B12 is either given orally once a day or administered once a week (1000–2000 µg i.m.). In some cases, i.m. hydroxocobalamin therapy can be kept in backup for intercurrent infections.

Carnitine therapy Chronic oral administration of L-carnitine (100 mg/kg/day) appears to be effective not only in preventing carnitine depletion but also in allowing urinary propionylcarnitine excretion and with subsequent reduction of propionate toxicity [11].

Metronidazole therapy Microbial propionate production can be suppressed by antibiotics. Metronidazole, an antibiotic that inhibits anaerobic colonic flora, has been found to be specifically effective in reducing urinary excretion of propionate metabolites by 40% in MMA and PA patients. Long-term metronidazole therapy (at a dose of 10–20 mg/kg once daily for 10 consecutive days each month) may be of significant clinical benefit [11]. Such intermittent administration may prevent the known side effects of the drug, such as leukopenia, peripheral neuropathy and pseudomembranous colitis.

Growth hormone Growth hormone (GH) induces protein anabolism. It is contraindicated in the acutely ill patient but potentially useful in the long term for those in whom growth is poor [11]. There is a place for recombinant human GH treatment as an adjuvant therapy in some patients with MMA and PA, mainly in those with reduced linear growth, but controlled long-term studies are needed [54].

Biochemical monitoring During the course of decompensation, plasma ammonia, blood gases, electrolytes, calcium, phosphate, lactate, glucose, uric acid, amylase, lipase and ketones in urine should be monitored. Some groups prefer also to measure urea and urea to MMA molar ratio in urine [37]. Regular amino acid analysis (all essential amino acids, and in particular isoleucine) is important. Furthermore, MMA in plasma or urine should be controlled in order to define the lowest possible level in each individual patient on treatment. There may be little practical use for the measurement of acylcarnitines and of odd-chain fatty acids in terms of directing clinical management.

Prognosis Around 15% of patients with MMA are vitamin B12 responsive and have mild disease and a good long-term outcome [9][55][56]. Conversely, both vitamin B12-unresponsive patients with MMA and those with PA have severe disease and many encephalopathic episodes, mainly due to intercurrent infections [57]. Among all patients with all forms of MMA, mut^0 patients have the poorest prognosis, and vitamin B12-responsive cblA and mut^- patients, the best [9][12][55]. Owing to earlier diagnosis and better treatment, outcomes for PA and MMA patients have improved in the last decade [37][56][57]. Survival rates into early and mid-childhood can now exceed 70%. However, morbidity, in terms of cognitive development, remains high, with a majority of patients having DQ/IQ in the mildly to moderately retarded range [58][59]. With improved management, the frequency of growth retardation has decreased, and now most patients with PA and MMA have growth curves within the normal range [37]. Abnormal neurological signs (mainly movement disorders, chorea, dystonia) continue to increase with age [9][55][56][57]. Chronic progressive impairment of renal function is a frequent and serious complication that manifests in older patients with high MMA excretion [9][55]. Renal transplantation is likely to be necessary for many patients with MMA who survive into adolescence [60]. Including PA and MMA into newborn screening programmes by tandem MS may make it possible to identify the late-onset forms of the diseases in the newborn period and contribute to a further improvement in the outcomes in this group. Decreased early mortality, less severe symptoms at diagnosis and more favourable short-term neurodevelopmental outcomes were recorded in patients identified through expanded newborn screening. However, the short duration of follow-up so far does not allow drawing final conclusions about the effects of newborn screening on long-term outcome [57].

There are only a few reports of female patients with MMA who have carried a pregnancy to term [61]. The outcome was favourable despite high MMA levels in blood and urine However, the majority of pregnancies can be complicated by cesarean delivery and increased risk of prematurity. Among 17 reported pregnancies, only one was associated with mild metabolic decompensation in the mother [61].

Liver/kidney transplantation In MMA, liver transplantation or combined liver-kidney transplantation eradicates episodes

of hyperammonaemia and has resulted in excellent long-term survival in some patients suggesting stabilization of neurocognitive development [62]. However, some patients have developed acute decompensation and basal ganglia necrosis years after liver transplantation and while on a normal diet. Today, it is recommended that such patients be maintained on a relaxed diet and with continued carnitine supplementation. There are some examples that progressive renal failure and neurological dysfunction, including metabolic stroke, are not always prevented. Long-term follow-up will be mandatory to evaluate whether patients who undergo early liver transplant [63][64] need kidney transplantation later in life. Such an early liver transplant appears as a reasonable choice for treating MMA with favourable long-term outcome in two reported patients [65]. Successful isolated kidney transplantation has been performed in some MMA patients in end-stage renal failure, with significant improvement in their metabolic control [60]. For PA, cardiomyopathy, when present, is reversible following liver transplantation [15][17]. Liver transplantation experience in PA is still limited. Some studies reported clinical improvement and improved dietary protein tolerance [66][67]. However, others have reported a high mortality risk as well as high morbidity especially worsening of pre-existing renal failure [68].

Management of intercurrent decompensations Acute intercurrent episodes are prevented or minimised by awareness of the situations that may induce protein catabolism. These include intercurrent infections, immunisations, trauma, anaesthesia and surgery and dietary indiscretion. In all cases, the main response comprises a reduction in protein intake. All patients should have detailed instructions (sick day protocol), including information on a semi-emergency diet, in which natural protein intake is reduced by half, and an emergency diet, in which it is stopped. In both, energy supply is augmented using carbohydrates and lipids, such as solutions based on protein-free formula base powder or a mixture of glucose polymer and lipids diluted in an oral rehydration solution. For children treated with specific amino acid mixtures the usual supplements can be added, though one should be aware that they increase osmolarity and that their taste renders nasogastric tube feeding often unavoidable. Their use is contraindicated in MMA and PA in cases of severe hyperammonaemia. At home, the solution is given in small, frequent drinks during day and night or by nasogastric tube [36]. After 24–48 h, if the child is doing well the usual diet is resumed within 2 or 3 days.

In cases of clinical deterioration with anorexia and/or gastric intolerance or if the child is obviously unwell, the patient must be hospitalised to evaluate the clinical status, to search for and treat intercurrent disease and to halt protein catabolism. Emergency therapy depends on the presence of dehydration, acidosis, ketosis and hyperammonaemia. Most often, intravenous rehydration for 12–24 h results in sufficient clinical improvement to allow for progressive renutrition with continuous enteral feeding. During this step enough natural protein to at least cover the minimal dietary requirements should be introduced into the feeds. The energy intakes are supplied with carbohydrates and lipids. During this stage of management, close metabolic evaluation is recommended, as the condition is labile and may deteriorate, requiring adjustment of the therapy. Conversely, if the patient's condition improves quickly the usual diet should be initiated without delay.

During periods when enteral feeding is contraindicated or poorly tolerated, as can occur with severe or prolonged decompensation, the use of total parenteral nutrition may be an effective mean for improving metabolic control and preventing further deterioration [69].

18.2 3-Methylcrotonyl Glycinuria

18.2.1 Clinical Presentation

The clinical phenotype described in 3-methylcrotonyl-CoA carboxylase (3-MCC) deficiency (MCCD) has been highly variable ranging from neonatal onset with severe neurological involvement and even death to a complete lack of symptoms in adults [70]. In the past 15 years family studies and newborn screening have identified a number of totally asymptomatic newborn infants, siblings and mothers with MCCD who have very low carnitine concentrations in blood. Many symptoms and signs in consanguineous families, initially attributed to MCCD, are most likely due to rare homozygous disease causing mutations in other disease genes [71]. However, in a small number of affected individuals, MCCD does appear to cause metabolic decompensation with hypoglycaemia, ketonaemia and severe metabolic acidosis.

18.2.2 Metabolic Derangement

3-MCC is one of the four biotin-containing carboxylases known in humans (◘ Fig. 18.1, ▶ enzyme 3). Its deficiency leads to accumulation of 3-methylcrotonyl-CoA and 3-methylcrotonic acid. Most of the 3-methylcrotonyl-CoA is conjugated with glycine to form 3-methylcrotonylglycine (MCG) whereas acylation with carnitine leading to the formation of 3-hydroxyisovaleryl carnitine appears to be only a minor pathway. 3-Hydroxyisovalerate (3-HIVA), another major metabolite, is derived through the action of a crotonase on 3-methylcrotonyl-CoA and the subsequent hydrolysis of the CoA-ester.

18.2.3 Genetics

3-MCC is a heteromeric enzyme consisting of α- (biotin-containing) and β-subunits. MCCD results from loss of function mutations in *MCCA* and *MCCB* respectively encoding these subunits. More than 50 mutations have been identified in both genes [70][72]. They are associated with an almost total lack of enzyme activity in fibroblasts. The apparent biochemical severity of all the *MCC* mutations contrasts with the variety of

Table 18.1 Classification of disorders with significant 3-methylglutaconic aciduria

	Defect	Name	Gene	Previous classification	Reference
Primary	Leucine catabolism	3 HMG CoA hydratase deficiency	*AUH*	Type I	This chapter
Secondary	Phospholipid remodelling	TAZ defect or Barth Syndrome	*TAZ*	Type II	Chapter 34
		SERAC1 defect or MEGDEL syndrome	*SERAC1*	Type IV	
		Sengers Syndrome	*AGK*	Type IV	
	Mitochondrial membrane associated disorder	OPA3 defect or Costeff syndrome	*OPA3*	Type III	Chapter 14
		DNAJC19 defect or DCMA syndrome	*DNAJC19*	Type V	
		TMEM70 defect	*TMEM70*	Type IV	
	Unknown	CLPB defect	*CLPB*		This chapter
		NOS 3-MGC-aciduria	unknown	Type IV	

NOS: not otherwise specified

the clinical phenotypes. The introduction of tandem MS into newborn screening has revealed an unexpectedly high prevalence of this disorder, which in certain areas appears to be the most frequent organic aciduria, found in 1:40,000 newborns in Germany and Australia [34][35] and 1:2,400 in the Faroe Islands [73].

18.2.4 Diagnostic Tests

The diagnosis relies on a characteristic urinary profile of organic acids, with huge excretion of 3-HIVA and 3-methylcrotonylglycine and without the lactate, methylcitrate, and tiglylglycine found in multiple carboxlase deficiency (MCD) (▶ Chapter 26). Supplementation with pharmacological doses of biotin does not alter this pattern. Total and free carnitine concentrations in plasma are extremely low. The presence of 3-hydroxyisovaleryl carnitine (C5OH) in plasma and in dried blood spots is characteristic for MCCD. However, diagnostic approach based solely on detection of C5OH may lead to overdiagnosis. In view of it's generally benign nature, it is debatable whether or not MCCD should be included in newborn screening programmes [74].

18.2.5 Treatment and Prognosis

Asymptomatic individuals most probably do not require treatment. In those with metabolic crisis glycine and carnitine therapies directed at increasing the excretion of glycine and carnitine conjugates are complementary rather than competitive means of detoxification. Glycine supplementation (175 mg/kg/day) increases the excretion of 3-MCG. Carnitine supplementation (100 mg/kg/day) corrects the very low plasma carnitine levels and increases the excretion of 3-HIVA. Long-term treatment of symptomatic infants based on a mildly protein-restricted diet is debatable.

18.3 3-Methylglutaconic Aciduria

Primary 3-methylglutaconic aciduria caused by 3-methylglutaconyl-CoA hydratase deficiency (*AUH mutations*) has only been identified in very few individuals, who presented with a wide spectrum of clinical signs of a neurometabolic disease ranging from no symptoms (at 2 years of age) to mild neurological impairment, severe encephalopathy with basal-ganglia involvement, quadriplegia, athetoid movement disorder, severe psychomotor retardation and leukoencephalopathy in a 61-year-old woman. 3-Methylglutaconyl (MGC)-CoA is metabolised to 3-hydroxy-3-methylglutaryl-CoA by 3-MGC-CoA hydratase (Fig. 18.1, ▶ enzyme 4). Defective activity is characterised by urinary excretion of 3-MGC and 3-methylglutaric acids. Both metabolites derive from accumulated 3-methylglutaconyl-CoA, through hydrolysis and dehydrogenation, respectively. The combined urinary excretion of 3-MGC and 3-methylglutaric acids range from 500 to 1000 mmol/mol creatinine, of which 3-methylglutaric acid represents about 1%. The metabolic pattern also includes 3-HIVA, which differentiates it from the other secondary causes (▶ below). 3-MGC-CoA hydratase activity can be measured in fibroblasts. The role of the human 3-MGC-CoA hydratase in leucine metabolism has been elucidated, and different mutations in *AUH* have been identified. No clear therapeutic regimen has been described. Carnitine supplementation may have beneficial effects.

Secondary 3-MGC acidurias are a relatively common finding in a number of metabolic disorders, particularly mitochondrial disease. In most the excretion of 3-MGC acid is only slightly increased and accompanied by other disease specific

metabolites. However, there are some disorders where 3-MGC aciduria is a more significant and consistent finding with urinary excretion >40 μmol/mmol creatinine. Previously, 3-MGC acidurias had been classified into types I to V 3-MGC aciduria, but they are now reclassified and named according to their pathological mechanism and defective protein or historical name (Table 18.1) [75]. There remain, however, disorders for which the underlying pathological mechanism is still unclear. For example mutations in *CLPB* were recently found in individuals with intellectual disability, congenital neutropenia, progressive brain atrophy, movement disorder, cataracts, and 3-MGC aciduria without any obvious mitochondrial respiratory chain dysfunction [76].

18.4 Short/Branched Chain Acyl-CoA Dehydrogenase Deficiency

Isolated 2-methylbutyrylglycinuria, caused by 2-methylbutyryl-CoA dehydrogenase deficiency (MBD) and encoded by *ACDSB* (Fig. 18.1, ▶ enzyme 6), is an autosomal recessive disorder of isoleucine metabolism [77]. A few patients have been diagnosed following various clinical symptoms, and a set of asymptomatic subjects of Hmong descent were identified through newborn screening with elevated C5-acylcarnitine concentrations in blood spots. Detection of MBD deficiency in newborn screening is not limited to this population, and an increasing number of asymptomatic patients have been extensively investigated. Clinical relevance of this disorder remains in doubt and requires careful long-term follow-up of affected individuals. Theoretically, valproic acid should be avoided, as valproyl-CoA could be a substrate of MBD (▶ Chapter 3).

18.5 2-Methyl-3-Hydroxybutyryl-CoA Dehydrogenase Deficiency

Only a few patients with 2-methyl-3-hydroxybutyryl-CoA dehydrogenase (MHBD) deficiency (HSD10 disease) have been described. All male patients had an unusual neurodegenerative and progressive disease, and some affected females had psychomotor retardation and speech delay. Related women (mothers and grandmothers of patients) have shown mild to moderate developmental delay. In early childhood the severe neurodegenerative symptoms included rigidity, dystonic posturing, spastic diplegia, dysarthria, choreoathetoid movements, restlessness, cortical blindness, myoclonic seizures, brain atrophy, periventricular white matter and basal ganglia abnormalities. The majority of patients identified so far have had a severe progressive neurological phenotype rather than ketoacidotic attacks, in contrast to patients with a defect in the next step of isoleucine degradation attributable to 2-methylacetoacetyl-CoA thiolase deficiency. Nevertheless, one 6-year old boy has been diagnosed with MHBD deficiency in the course of a severe ketoacidotic crisis in the absence of any neurological symptoms thus mimicking 2-methylacetoacetyl-CoA thiolase deficiency [78].

MHBD deficiency (Fig. 18.1, ▶ enzyme 7) is a defect in the degradation of isoleucine but also in neurosteroid metabolism. MHBD is a multifunctional protein with an additional non-enzymatic role required for mitochondrial integrity and cell survival [78][79][80]. Laboratory findings include marked elevations of urinary 2-methyl-3-hydroxybutyrate and tiglylglycine without elevation of 2-methylacetoacetate. The organic acid excretion is more pronounced after a 100-mg/kg oral isoleucine challenge. Enzyme studies have shown markedly decreased activity of MHBD in fibroblasts and lymphocytes. MHBD deficiency is caused by mutations in *HADH2* on the X-chromosome. A short-term stabilisation of neurological symptoms and a biochemical response to an isoleucine-restricted diet have been observed in some patients [79][80].

The deficiency of 2-methyl-acetoacetyl-CoA thiolase (Fig. 18.1, ▶ enzyme 8), also known as 3-ketothiolase or T2, is discussed in ▶ Chapter 13.

18.6 Isobutyryl-CoA Dehydrogenase Deficiency

The mitochondrial enzyme isobutyryl-CoA dehydrogenase (IBD) catalyses the third step in the degradation of valine (Fig. 18.1, ▶ enzyme 9). It is encoded by *ACAD8* [81]. Fewer than 20 patients with IBD deficiency have been described. Only the first patient, a 2-year-old, was diagnosed following the investigation of anaemia and dilated cardiomyopathy. Other patients have been identified following the expansion of newborn screening [81][82]. This disorder can be detected on the basis of elevated butyrylcarnitine/isobutyrylcarnitine (C4-carnitine) concentrations in newborns' blood spots analysed by tandem MS. The presence of this metabolite, which is also present in short-chain acyl-CoA dehydrogenase deficiency, requires further investigation for precise diagnosis [82]. The possible clinical implication of this enzyme defect is not known, and to date most of the identified patients have remained asymptomatic. However, a few patients have moderate speech delay and careful follow-up is necessary.

18.7 3-Hydroxyisobutyric Aciduria

A few patients with increased excretion of 3-hydroxyisobutyric acid (3-HIBA), an intermediate of the catabolic pathways of valine and thymidine, have been identified. This condition may be linked to various enzymatic defects. Unfortunately, in most cases described, the enzymatic diagnosis has been speculative.

Clinical presentation is heterogeneous. Some patients present in infancy, with acute metabolic episodes with ketoacidosis, hypoglycaemia or hyperlactataemia. Muscle involvement and hypertrophic cardiomyopathy have been reported. CNS involvement is highly variable, ranging from normal development to brain dysgenesis observed in neonates.

Several enzyme defects may underlie 3-hydroxyisobutyric aciduria. However, only combined deficiency of malonic,

methylmalonic and ethylmalonic semialdehyde dehydrogenase (MMSDH) (Fig. 18.1, ▶ enzyme 13) [83] and 3-hydroxyisobutyryl-CoA deacylase also called hydrolase deficiency (Fig. 18.1, ▶ enzyme 11) have been identified [84]. Mutations in the *ALDH6A1* gene encoding MMSDH were found in two unrelated developmentally delayed patients with 3-hydroxyisobutyric aciduria [85]. Recently, 3-hydroxy-isobutyryl-CoA hydrolase or deacylase, HIBCH (Fig. 18.1, ▶ enzyme 11), deficiency was reported in patients presenting Leigh-like disease with elevated hydroxy-C4-carnitine and multiple mitochondrial respiratory chain defects and mutations in *HIBCH* [86] (▶ Chapter 14).

18.8 Malonyl-CoA Decarboxylase Deficiency

Malonyl-CoA decarboxylase (MLYCD) deficiency is a rare condition, with fewer than 30 cases reported, in which there is excessive excretion of malonic acid. MLYCD is usually expressed in fibroblasts or leukocytes, and various mutations have been reported in *MLYCD* [87]. A neonatal form has been described presenting with progressive lethargy, hypotonia, hepatomegaly, metabolic acidosis, and mild hyperammonaemia, variously associated with hypoglycaemia and/or hyperlactacidaemia. Cardiac failure due to cardiomyopathy was present in some patients at birth. In the late-onset forms, most have presented with acute metabolic episodes secondary to intercurrent infections. Some patients were previously known to be affected with a mild and nonspecific psychomotor retardation. Other children have been diagnosed following systematic screening for mental retardation and hypotonia. Cardiomyopathy was present in about 40%.

The physiological role of MLYCD, a cytosolic enzyme, could be in the regulation of cytoplasmic malonyl-CoA abundance and, thus, of mitochondrial fatty acid uptake and oxidation (Fig. 18.1, ▶ enzyme 16). Patients with MLYCD deficiency display a number of phenotypes that are reminiscent of mitochondrial fatty acid oxidation disorders [87]. However, in contrast to these, dicarboxylic aciduria together with ketonuria is found during catabolic episodes and the patients exhibit normal ketogenesis on acute fat-loading tests.

The disorder is autosomal recessive. More than 20 mutations in *MLYCD* have been reported. No hotspot mutations have been identified. No genotype-phenotype relationship was detected, and siblings may have different presentations [87].

Total and free carnitine concentrations in plasma are low. Documented accumulation of malonylcarnitine would allow tandem MS screening of newborn blood spots. MLYCD activity has been found to be reduced in cultured fibroblasts and/or leukocytes of most defective cell lines, with residual activity of less than 10% of control [87].

No rules for treatment and prognosis have been established. Carnitine supplementation corrects the carnitine deficiency and may improve the cardiomyopathy and muscle weakness. Conversely, some patients have worsened despite carnitine supplementation and have recovered with a long-chain triglyceride-restricted/medium-chain triglyceride-supplemented diet [88]. Long-term prognosis is unknown. Except for the two patients who developed extrapyramidal signs following an acute crisis, most patients have residual mild developmental delay. There are subjects identified by newborn screening who remained asymptomatic at least during preschool age.

18.9 ACSF3 Deficiency

ACSF3 deficiency is a rare disorder presenting with combined malonic and methylmalonic aciduria (CMAMMA) due to mutations in *ACSF3* deficiency, which encodes a putative methylmalonyl-CoA and malonyl-CoA synthetase, a member of the acyl-CoA synthetase family [89][90]. Diagnosis relies on a characteristic profile of urinary organic acids, in which malonic and methylmalonic acids are constant findings. Abnormal succinic aciduria has been found in about half the cases, as have various dicarboxylic and glutaric acidurias. The disorder has been detected in a number of asymptomatic infants in the Quebec newborn urine screening programme and appears to be benign [91].

18.10 Enoyl-CoA Hydratase or ECHS1 Deficiency

Enoyl-CoA hydratase (ECHS1) (Fig. 18.1, ▶ enzyme 10), a mitochondrial matrix enzyme, active in valine catabolism and short-chain fatty acid β-oxidation, is immediately upstream of HIBCH in the valine pathway (Fig. 18.1, ▶ enzyme 11). Similar to HIBCH, ECHS1 deficiency has been reported in patients with Leigh-like syndrome associated with a range of abnormalities of variable severity [92][93] (▶ Chapter 14). Urine metabolite testing can distinguish between ECHS1 and HIBCH deficiencies. Blood acylcarnitine profile has been found normal in ECHS1 deficiency [93].

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Disorders of the Urea Cycle and Related Enzymes

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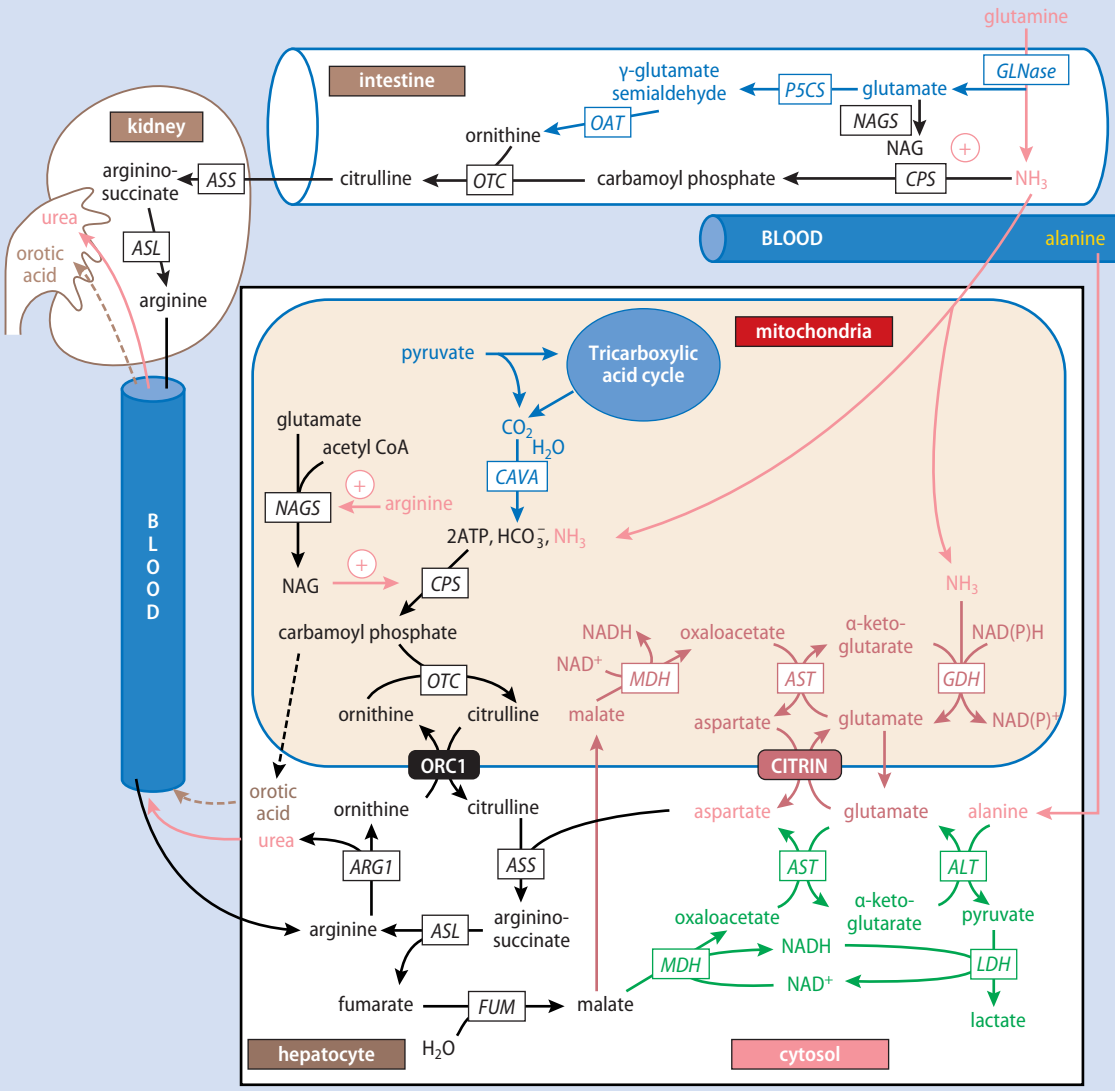

Fig. 19.1 The urea cycle. Metabolic scheme of the urea cycle (in *black*) and ancillary reactions (*coloured*). For simplicity not all the substrates and product of each reaction are shown. The contributions of the adult intestine and kidney to arginine synthesis are also shown in a highly simplified way. The major nitrogenous sources of the urea cycle, ammonia, glutamine, alanine and aspartate, as well as ornithine and the product urea are coloured *red*. Provision and excretion reactions for these compounds are symbolized with red arrows. The reactions involved in the provision of cytosolic aspartate by the mitochondria are coloured *darkred*. The reactions of the aspartate cycle used to convert fumarate to aspartate in the cytosol are coloured *green*. *ALT*, alanine aminotransferase; *ARG1*, arginase 1 (arginase 2 is extrahepatic and therefore not shown); *ASL*, argininosuccinate lyase; *ASS*, argininosuccinate synthetase; *AST*, aspartate aminotransferase; *CPS1*, carbamoyl phosphate synthetase 1; *CAVA*, carbonic anhydrase Va; *Citrin*, aspartate/glutamate antiporter; *FUM*, fumarase; *GDH*, glutamate dehydrogenase; *GLNase*, glutaminase; *LDH*, lactate dehydrogenase; *MDH*, malate dehydrogenase; *NAGS*, N-acetylglutamate synthase; *OAT*, ornithine ω-aminotransferase; *ORC1*, ornithine/citrulline antiporter; *OTC*, ornithine transcarbamylase; *P5CS*, Δ^1-pyrroline-5-carboxylate synthetase. The encircled plus signs (in *orange*) indicate the allosteric stimulations of CPS1 by N-acetylglutamate (NAG) and of NAGS by arginine. The dotted line from carbamoyl phosphate indicates that several metabolic steps in the cytosol are required for orotic acid (in *brown*) production. In addition to citrin, other mitochondrial carriers exist for glutamate; they have not been specified. Malate can also access the mitochondria in several ways and, again, a specific carrier is not shown for it. The full malate-aspartate shuttle is not shown either

Urea Cycle and Related Enzymes

The urea cycle is the main route for ammonia detoxification (◘ Fig. 19.1). Its defects generally cause hyperammonaemia. The complete cycle is found only in periportal hepatocytes and involves two mitochondrial and three cytosolic enzymes as well as the mitochondrial ornithine/citrulline antiporter and the activating mitochondrial enzyme N-acetylglutamate synthase, which turns the cycle on or off. In addition, mitochondrial carbonic anhydrase Va, the aspartate/glutamate mitochondrial antiporter citrin, and the intestinal enzyme Δ^1-pyrroline-5-carboxylate synthetase supply the cycle with, respectively, bicarbonate, aspartate, and, if needed, with ornithine made *de novo*. In extrahepatic tissues urea cycle enzymes make arginine either from ornithine or from the citrulline produced by nitric oxide synthase. Thus, urea cycle defects can also impact on these other functions.

Urea cycle disorders (UCDs) are loss of function defects of any of the urea cycle (UC) enzymes (carbamoyl phosphate synthetase 1 (CPS1), ornithine transcarbamylase (OTC), argininosuccinate synthetase (ASS), argininosuccinate lyase (ASL) and arginase (ARG1); ► Urea Cycle and related Enzymes), the mitochondrial ornithine/citrulline antiporter (ORC1) and the CPS1-activating enzyme N-acetylglutamate synthase (NAGS). Their frequency is about 1:35,000 births, with 25% presenting in newborns [1]. NAGS, CPS1 and OTC are mitochondrial, and their deficiencies cause hyperammonaemia, while deficiencies of the extramitochondrial enzymes ASS, ASL and ARG1, and of the ORC1 antiporter, also produce specific alterations in the levels of some amino acids that may be important in disease pathogenesis. Deficiencies of carbonic anhydrase Va (CAVA), citrin and Δ^1-pyrroline-5-carboxylate synthetase (P5CS) can cause hyperammonaemia by restricting the supply to the UC of bicarbonate, aspartate and *de novo* made ornithine, respectively. Inheritance of UCDs is autosomal recessive except for OTC deficiency, which is X-linked. P5CS deficiency can have dominant or recessive inheritance.

Acute hyperammonaemia is a clinical emergency caused by most UCDs. It manifests as irritability, food refusal, vomiting, vegetative instability, muscular hypotonia, convulsions, somnolence, lethargy, coma and death or neurological sequelae if untreated. Partial deficiencies can give delayed hyperammonaemia and/or poor appetite, vomiting, failure to thrive, developmental delay, mental retardation, abnormal behaviour or frank neurologic and/or psychiatric manifestations, as well as hepatomegaly and increased liver enzymes or even acute liver failure. ARG1 deficiency rarely causes acute hyperammonaemia. Instead, it produces developmental delay, seizures and spastic diplegia. Increased plasma ammonia and glutamine and low citrulline characterise NAGS, CPS1 and OTC deficiencies but amongst these, a high urinary excretion of orotic acid is only observed in OTC deficiency. ASS, ASL and ARG1 deficiencies present with a high plasma citrulline and characteristic plasma and urinary amino acid profiles.

Treatment of UCDs aims at minimizing ammonia production by using protein restriction and prevention of catabolism, and attempts to rapidly lower ammonia by enhancing residual UC function with arginine or citrulline, by nitrogen scavenging using alternate pathway therapy with benzoate and/or phenylacetate or phenylbutyrate and by employing dialytic measures. Liver transplantation is generally curative. Administration of N-carbamylglutamate can replace the missing N-acetylglutamate in NAGS deficiency, virtually curing this deficiency. Citrin deficiency dramatically differs from other UCDs in that carbohydrates should be limited and high protein given.

19.1 Mitochondrial Urea Cycle Disorders

These comprise CPS1, OTC and NAGS deficiencies. Since the exclusive role of NAGS is to produce the essential activator of CPS1, N-acetyl-L-glutamate (NAG), NAGS deficiency is clinically indistinguishable from CPS1 deficiency. OTC deficiency is the most frequent urea cycle error (about 60% of UCD patients) whereas CPS1 and NAGS deficiency are very rare (respectively, 1:1,300,000 and <1:2,000,000 live births) [1].

19.1.1 Clinical Presentation

The main complication of any UCD in all age groups is acute hyperammonaemia, which clinically presents with encephalopathy. Since CPS1 and OTC catalyse the initial two steps of the UC, in which ammonia is converted to carbamoyl phosphate and then is incorporated into citrulline, these defects, together with NAGS deficiency, tend to produce the most marked hyperammonaemia among all the UCDs [2].

Newborns Newborn patients appear healthy at birth but may already present by day 2 with a rapidly progressing encephalopathy [3][4]. The clinical course is very similar to bacterial sepsis, which can lead to significant delay in the start of specific management for hyperammonaemia. Patients show vomiting, refusal to feed, somnolence/stupor/coma, muscular hypotonia, seizures, hyper- or hypoventilation, and hypo- or hyperthermia. Respiratory alkalosis is a common initial finding. In some patients of CPS1 and OTC deficiency signs of acute liver failure, including coagulopathy, may be found.

Children, adolescents and adults Outside the newborn period, the presentation may be variable. Most patients present during or shortly after an intercurrent infection or any other catabolic situation (e.g. fever, vomiting, diarrhoea, postpar-

tum, surgery, rapid weight loss, treatment with steroids, chemotherapy), or following a high-protein meal (e.g. a barbecue). The symptoms are likewise highly variable, usually nonspecific and may be subtle or only episodic. The most common findings are an unexplained change in consciousness (e.g. reduced vigilance, somnolence) or novel neurological signs (e.g. tremor, irritability, seizures) often mistaken for encephalitis, drug intoxication or brain tumour. Some patients self-select a low-protein diet. Even infants and small children may refuse high-protein containing foods (e.g. meat, fish, dairy products).

Female carriers of OTC deficiency form a particular subgroup. Because of the variable individual inactivation of the X-chromosome hosting the mutant *OTC* gene (X-inactivation or lyonization phenomenon), they have highly variable, sometimes very low, residual OTC function. Thus, some are asymptomatic, others report symptoms over many years that are likely explained by recurrent undiagnosed hyperammonaemia [5], being often detected only after a male offspring is diagnosed, whereas still others present with frank deficiency.

19.1.2 Metabolic Derangements

In NAGS, CPS1 and OTC deficiencies citrulline production is reduced or abolished and this amino acid is consequently low or virtually absent from plasma. The failure to incorporate ammonia into carbamoyl phosphate (CP) explains the hyperammonaemia of CPS1 and NAGS deficiency; in OTC deficiency it may be due to CPS1 inhibition by CP [6] that accumulates in the mitochondria [7]. Some CP leaks out of the mitochondria, leading to excessive pyrimidine biosynthesis and overproduction of orotic acid and uracil. The high urinary excretion of orotic acid and uracil differentiates conditions in which CP accumulates, such as OTC deficiency, from those with normal or low CP, such as CPS1 and NAGS deficiencies [8].

In UCDs excessive ammonia is available for use by glutamine synthetase (present in perivenous hepatocytes, skeletal muscle and glial cells), and thus plasma and tissue glutamine levels increase. This increase can occur before, and can persist after, clinically manifest hyperammonaemia. Other non-essential amino acids that can be made from ammonia, such as glycine, serine, glutamate and, particularly, alanine, are also increased in plasma.

High ammonia levels are neurotoxic. Ammonia enters freely into the brain as NH_3 [9] and is rapidly converted to the more abundant but non permeable NH_4^+, with which it is in equilibrium. The increased brain ammonia causes excessive glial glutamine synthesis and accumulation, leading to astrocyte swelling [10], an important factor in the aetiology of brain oedema that occurs in hyperammonaemia. Recently, evidence has been provided [11] for a direct neurotoxic role of ammonia, by interfering with K^+ buffering of astrocytes, leading to increased extracellular K^+. Many other ammonia-associated neural cell derangements have been postulated [12]. Cortical atrophy, dilated ventricles and demyelination [13], with neurocognitive delay and even cerebral palsy, are classical neurological deficits associated to hyperammonaemia.

19.1.3 Genetics

All genes encoding urea cycle enzymes and transporters are known (see identifiers in ◘ Table 19.1) and are amenable to standard DNA sequencing. Except *OTC*, which is X-linked, all other UCD genes are autosomal. These genes and their intronic regions vary widely in size, potentially contributing to the heterogeneity of possible genetic alterations. Of the few reported *NAGS* mutations, c.1450T>C was found in several independent families, causing the change p.Trp484Arg and possibly a splicing defect, with resultant severe NAGS deficiency [14].

In *CPS1* and *OTC* deficiencies >230 mutations and nearly 500 mutations have been found in the corresponding genes with <10% recurrent [15][16] including the change c.386G>A of *OTC*, known as the mutation of the spf-ash mouse, which, besides causing a benign amino acid change (p.Arg129His), has also a missplicing effect.

The Leiden Open (source) Variation Database (LOVD) is freely available and displays DNA variations for most UCD genes (http://grenada.lumc.nl/LSDB_list/lsdbs).

19.1.4 Diagnostic Tests

(◘ Fig. 19.2)

▪ Biochemical Assays and Enzyme Studies

The hallmark of these UCDs is hyperammonaemia, generally in the absence of hypoglycaemia, lactic acidosis or ketonuria. Plasma ammonia levels at presentation are usually in excess of 400–500 μmol/l. In severe encephalopathy they often exceed 1000 μmol/l (normal <150 μmol/l in newborns and <50 μmol/l outside the newborn period). An introduction to the patient with hyperammonaemia is given in ▶ Chapter 1.

In addition there are characteristic alterations of the plasma amino acid profile in the absence of specific pathologic changes in the urine organic acid or blood acylcarnitine profiles; concentrations of glutamine (often >1000 μmol/l) and alanine (often >600 μmol/l) are increased while citrulline (often <10 μmol/l) and arginine (often <30 μmol/l) are decreased. For NAGS and CPS1 deficiencies there is no additional biochemical marker while in OTC deficiency increased urinary excretions of orotic acid and uracil are frequently found. A test with L-carbamyl-L-glutamate has been proposed to detect NAGS deficiency early [17] but this should not postpone the start of other treatment modalities. Although enzyme studies can confirm a diagnosis, they are rarely used as a liver or intestinal biopsy is needed; diagnosis is now generally confirmed by genetic means [3].

Mutation analysis is the gold standard for diagnosis and prenatal testing. It is crucial for distinguishing between NAGS and CPS1 deficiencies. Although sequencing is straightfor-

Table 19.1 Some traits and database identifiers of urea cycle disorders

Name or abbreviation		Inher-itance[2]	Protein found in	Identifiers[3]	
Disease	Gene and protein[1]			Gene *OMIM GenBank	Phenotype OMIM
NAGS deficiency	*NAGS*	AR	Liver Small intestine	*608300 162417	#237310
CPS deficiency	*CPS1*	AR	Liver Small intestine	*608307 1373	#237300
OTC deficiency	*OTC*	XL *de novo*	Liver Small intestine	*300461 5009	#311250
ASS deficiency (citrullinaemia)	*ASS1* ASS	AR	Liver Fibroblasts	*603470 445	#215700
ASL deficiency (argininosuccinic acidaemia or aciduria)	*ASL*	AR	Liver Red cells Fibroblasts	*608310 435	#207900
ARG1 deficiency (arginase deficiency, argininaemia)	*ARG1*	AR	Liver Red cells	*608313 383	#207800
HHH syndrome	*SLC25A15* *ORNT1* ORC1	AR	Liver Fibroblasts	*603861 10166	#238970
Citrin deficiency (citrullinemia type II)	*SLC25A13* Citrin	AR	Liver Fibroblasts Lymphocytes	*603859 10165	#605814 #603471
Carbonic anhydrase Va (CAVA) deficiency	*CA5A* CAVA	AR	Liver	*114761 763	#615751
Δ^1-Pyrroline-5-carboxylate synthetase (P5CS) deficiency	*ALDH18A1* P5CS	AR AD *de novo*	Small intestine Fibroblasts	*138250 5832	#219150 #616603 #601162 #616586

[1] Gene abbreviations are in italic type. Abbreviated protein names are given only when they differ from the gene abbreviation.
[2] Inheritance: *AR*, autosomal recessive; *AD*, autosomal dominant; *XL*, X-linked
[3] OMIM: Online Mendelian Inheritance in Man (www.omim.org/)
GeneBank: http://www.ncbi.nlm.nih.gov/gene

ward for NAGS deficiency since the gene is small, it is recommended to include the 5'-upstream enhancer region, where one clinically relevant mutation was reported [18]. Sequencing of *CPS1* exons is more difficult given the large number of exons. In addition, several intronic mutations are known that would be missed. cDNA sequencing in fibroblasts and lymphocytes is a less laborious and more sensitive alternative, although special techniques are necessary [19]. Many labs offer DNA sequencing of *OTC*, however, at least partly due to the large intronic regions the mutation detection rate, even in patients with a clear biochemical diagnosis, is only about 80%. Therefore, other methods including multiplex ligation-dependent probe amplification(MLPA) [20], array comparative genomic hybridization (array CGH) or cDNA sequencing (which is difficult, since liver or small intestinal tissue is needed) may be used to improve the diagnostic yield. Next generation sequencing (NGS) is progressively being introduced and it will play an important role in diagnosis in the near future.

Allopurinol testing [5] appears to have limited value [21] and has now been mostly superseded by molecular analysis (see ▶ Chapter 3) but can still be useful in mutation-negative patients. The test is non-invasive and safe and requires 24-hour urine collection after one-dose allopurinol administration (100 mg for children <6 years; 200 mg for children >6 years and adolescents; 300 mg for adults) [5].

Newborn screening is currently not available for mitochondrial UCDs but there is research ongoing to develop algorithms based on ratios of urea cycle metabolites to safely identify patients [22].

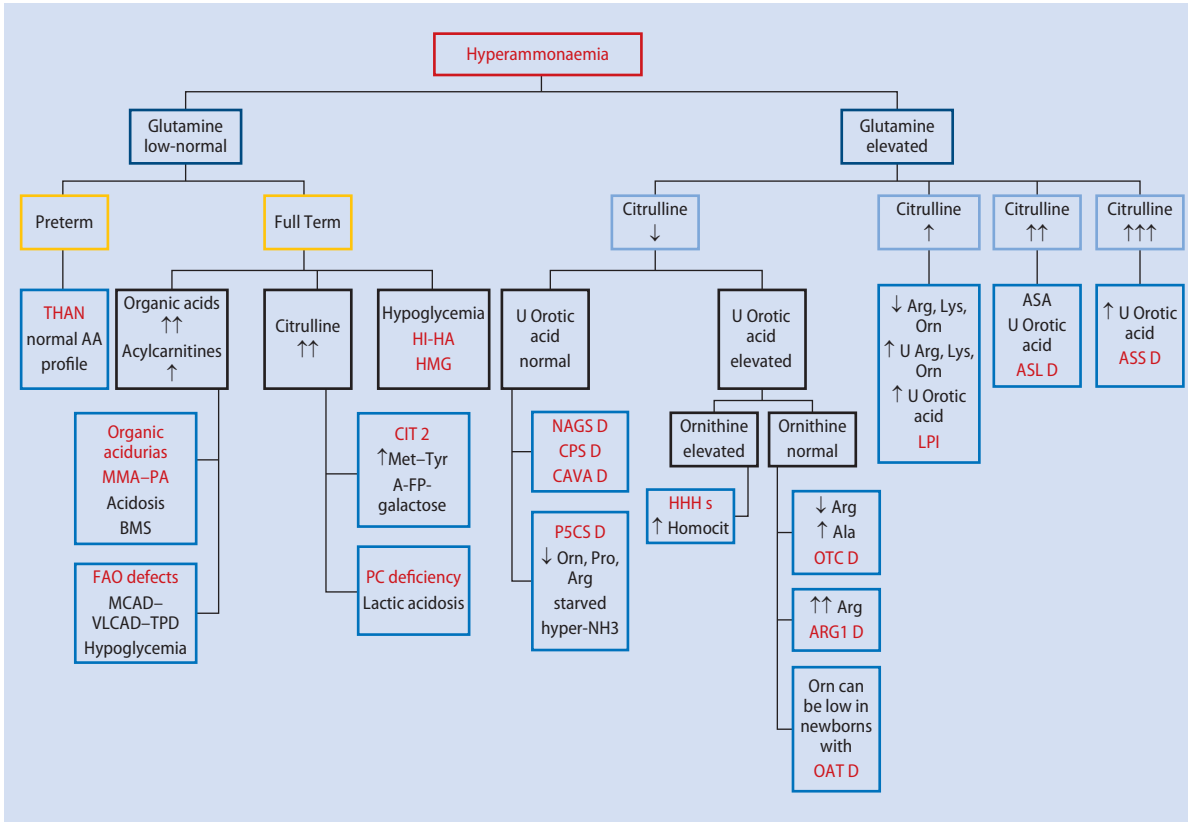

Fig. 19.2 Diagnostic algorithm that can be applied to any hyperammonaemic patient (see also ▶ Chapter 1.4.2) *AA*, amino acids; *A-FP*, alpha-fetoprotein; *Ala*, alanine; *Arg*, arginine; *ARG1 D*, arginase deficiency; *ASA*, argininosuccinic acid; *ASL D*, argininosuccinate lyase deficiency; *ASS D*, argininosuccinate synthetase deficiency; *BMS*, Bone marrow suppression; *CAVA D*, carbonic anhydrase VA deficiency; *CIT2*, citrin deficiency; *CPS1* D, carbamoyl phosphate synthetase 1 deficiency; *FAO*, fatty acid oxidation; *HHHs*, hyperammonaemia, hyperornithinemia, homocitrullinuria syndrome; *HI-HA*, hyperinsulinism-hyperammonaemia syndrome; *HMG*, 3-hydroxy-3-methylglutaryl-CoA lyase deficiency; *Homocit*, homocitrulline; *Hyper-NH3*, hyperammonaemia; *LPI*, lysinuric protein intolerance; *Lys*, lysine; *MCAD*, middle chain acyl-CoA dehydrogenase deficiency; *Met*, methionine; *MMA*, methylmalonic aciduria; *NAGS* D, N-acetylglutamate synthase deficiency; *OAT D*, ornithine aminotransferase deficiency; *Orn*, ornithine; *OTC* D, ornithine transcarbamylase deficiency; *P5CS D*, Δ^1-pyrroline-5-carboxylate synthetase deficiency; *PA*, propionic acidemia; *PC*, pyruvate carboxylase; *Pro*, proline; *THAN*, transient hyperammonaemia of the newborn; *TPD*, trifunctional protein deficiency; *Tyr*, tyrosine; *U*, urine; *VLCAD*, very long chain acyl-CoA dehydrogenase deficiency. (Figure modified from [3])

19.1.5 Treatment and Prognosis

Emergency Management

In acute hyperammonaemia, treatment must be initiated immediately. Even the need to confirm an elevated ammonia level must not delay the start of treatment. The principles of management are [3] (see also ▶ Chapter 4):

- Stop exogenous protein supply.
- Prevent endogenous protein catabolism by ensuring a high energy supply and avoiding deficiency of essential amino acids.
- Reduce ammonia, either with drugs alone, or together with dialysis (depending of the level of hyperammonaemia).

Stopping exogenous protein supply (for maximum 24–48 hours) requires concomitant provision of all the energy needed, to prevent catabolism of endogenous proteins. A severe deficiency in essential amino acids will trigger endogenous protein degradation and hence sustained hyperammonaemia. To achieve a sufficient energy supply, 10% glucose should be given, or if central venous access is available, an infusion of higher concentrations. The aim in newborn patients is to give at least 10 mg/kg/min of glucose (always with sodium and potassium to avoid free water infusion). Parallel to the start of glucose infusions, intravenous boluses of drugs to detoxify ammonia are required.

Sodium benzoate and/or sodium phenylacetate should be given at a dose of 250 mg/kg in 90–120 min followed by a maintenance infusion of these drugs at a dose of 250–500 mg/kg/d. Repeated boluses, if needed at all, should be given with caution to avoid drug toxicity [3][23]. In addition to nitrogen scavenging drugs, L-arginine should be given at a dose of 250 mg/kg in 90–120 min followed by a maintenance dose of

250 mg/kg/d [24]. Ammonia must be determined at least every three hours until the acute situation is successfully managed. If at any time during the crisis it escalates to >500 µmol/l, dialysis should be started as soon as possible. The method will depend on the experience of the local metabolic centre but hemodiafiltration or hemodialysis have been proven most efficient while peritoneal dialysis with currently used solutions is not recommended. Any patient with acute hyperammonaemia should be transferred to a metabolic centre experienced with UCDs and capable of undertaking dialysis.

Maintenance Treatment

NAGS deficiency is the only UCD for which drug treatment is almost curative: N-carbamyl-L-glutamate (also known as carglumic acid; licensed drug Carbaglu), a synthetic analogue of the physiological activator of CPS1, NAG, given orally activates CPS1 and thereby urea cycle function [25]. The recommended maintenance treatment is 100 mg/kg/d in 3 dosages prior to meals, but it may be possible to reduce the dose to as low as 10 mg/kg/d. Most patients will remain stable with this treatment but they should still avoid excess protein; additional nitrogen scavenging drugs may only be needed during periods of catabolism.

Patients with severe CPS1 and OTC deficiencies are prone to recurrent hyperammonaemic crises. Those with complete enzyme deficiency, with very low protein tolerance or frequent metabolic crises despite treatment, should undergo liver transplantation as soon as it is possible and safe (>3 months of age and/or >5 kg body weight) and until then aggressive conservative measures must be employed in order to try to preserve mental function [3].

Conservative treatment requires a low-protein diet in most patients, approaching the safe levels of protein intake in the FAO/WHO recommendations [26], with individual titration of protein tolerance. To avoid dietary deficiencies regular monitoring is required and essential amino acids, vitamins, and trace elements supplemented. In addition, most patients will require nitrogen scavenging drugs. Sodium benzoate (an unlicensed medication) and/or sodium phenylbutyrate (licensed in Europe as Ammonaps or as Pheburane) are recommended, each at a dose of 200–250 mg/kg/d in 3 dosages [3]. Glycerol phenylbutyrate (licensed as Ravicti) is now available in syrup presentation and may represent a more palatable alternative to sodium phenylbutyrate [27]. To support the residual urea cycle function, L-arginine and/or L-citrulline (both chemicals or food supplements and not licensed drugs) are given at a dose of 100–200 mg/kg/d in 3 dosages [3].

Outcome

Patients with mitochondrial UCDs who present during the newborn period have a significant risk of death [28] or, if they survive, of learning difficulties [29]. Both survival and neurocognitive outcome largely depend on the duration and the extent of hyperammonaemia. Several strategies have been proposed to improve outcome, including education of health care professionals, establishment of metabolic centres, and automatic »red flags« in the emergency departments for certain situations [30], in addition to ensuring the availability of routine and rapid determination of ammonia in an emergency setting [3]. Liver transplantation, although preventing further hyperammonaemia, will not restore mental function if lost [31].

Pregnancy and Postpartum Period

Most female OTC patients go through pregnancy without any problems but their increasing extra protein needs (1st trimester 1 g/day; 2nd trimester 10 g/day; 3rd trimester 31 g/day) should be met. The postpartum period also requires special attention for all UCDs, as several case reports on fatal crises highlight the particular risk during this period of severe protein catabolism [32][33].

19.2 Cytosolic Urea Cycle Disorders

This group of disorders is the second most frequent among the UCDs and includes ASS and ASL deficiency, each representing about 15% of the UCD patients, and ARG1 deficiency, representing 3% [1].

19.2.1 Clinical Presentation

Newborns Newborn presentations of ASS and ASL deficiencies closely resemble those of mitochondrial UCDs (▶ Section 19.1.1), with hyperammonaemic encephalopathy of similar severity, although peak plasma ammonia may not be as high and the onset delayed to day 6–7 of life or even later [3][4]. At the other end of the clinical spectrum are patients who may have been detected by newborn screening but who are entirely asymptomatic [34][35]. ARG1 deficiency only rarely presents in the newborn period, either with neonatal hyperammonaemia and/or cholestasis [36][37][38].

Children, adolescents and adults Outside the newborn period, the symptoms in patients with ASS and ASL deficiencies are similar to those of mitochondrial UCDs (▶ Section 19.1.1). ASS deficiency has been reported presenting with acute liver failure [39], treated in some patients with liver transplantation, although others recovered with conservative management. ASS deficient patients are at particular risk of developing acute hyperammonaemia in the post-partum period and in other severe catabolic circumstances. In contrast, ASL deficient patients are less prone to recurrent hyperammonaemic decompensation but can still develop intellectual disability, seizures and chronic hepatopathy [28]. Marked hepatomegaly can be a presenting sign mimicking glycogenosis type 3. Arterial hypertension is also frequent in adolescents and adults with ASL deficiency [40]. Brittle hair due to trichorrhexis nodosa is almost pathognomonic for ASL deficiency, resulting from arginine deficiency and responding well to arginine administration. The clinical picture of patients with ARG1 deficiency is entirely different, being characterised primarily by developmental delay with neurological and intellectual im-

pairment, growth retardation and spastic tetra- or diplegia [41]. This last manifestation starts in late infancy and is progressive if plasma arginine levels remain elevated. Many patients with ARG1 deficiency have seizures and may even develop status epilepticus.

19.2.2 Metabolic Derangements

The impairment of the UC at the level of ASS, ASL and ARG1 explains the elevated plasma and urinary levels of citrulline, argininosuccinate and arginine in deficiencies of ASS, ASL, and ARG1, respectively, and the respective low plasma levels of argininosuccinate and arginine in ASS deficiency and of arginine in ASL deficiency (prior to treatment with L-arginine). Inhibition of ASS by argininosuccinate or by arginine [42] accounts for the increased citrulline levels (although not as high as in ASS deficiency) in ASL deficiency and ARG1 deficiency. These high citrulline levels differentiate extramitochondrial from mitochondrial UCDs. The high renal clearance of argininosuccinate explains the more modest relative elevation of this amino acid in plasma in ASL deficiency than of citrulline (typically 1000-fold increase) in ASS deficiency. In ARG1 deficiency, the induction in extrahepatic tissues of a second arginase (ARG2) may explain the relatively modest increase (about 15-fold) of plasma arginine, the normal or near-normal plasma ornithine, and the presence of urea (but which, nevertheless, is generally decreased) [41].

Citrulline and argininosuccinate include in their molecular structure one molecule of ornithine and, respectively, one and two atoms of waste nitrogen. Consequently, the abundant urinary excretion of these intermediates in ASS and ASL deficiencies effectively removes waste nitrogen, although with simultaneous loss of two (ASS deficiency) or one (ASL deficiency) ornithine molecules per urea equivalent. This renders the supply of ornithine an essential determinant of how much waste nitrogen is excreted in ASS and ASL deficiencies, justifying the administration of arginine (converted to ornithine upon cleavage by arginase) [24]. In line with the poorer waste nitrogen-carrying capacity of citrulline than that of argininosuccinate, hyperammonaemic crises are more frequent in ASS deficiency than in ASL deficiency and appear to be due to a secondary impairment of OTC because of the poor availability of ornithine (in keeping with the increased orotic acid excretion found during these crises). Interestingly, orotic acid excretion is also frequently elevated in ARG1 deficiency [41], possibly reflecting increased CP production because of overactivation of the NAGS-CPS1 axis (arginine is a NAGS activator [43]), compounded with decreased ornithine availability for the OTC reaction in the liver.

ASS and ASL have a paramount role in the recycling to arginine of the citrulline produced when nitric oxide (NO) is made by NO synthase. ASL also belongs to an intracellular membrane-bound protein complex that channels exogenous arginine to NO synthase, and its mutations can prevent such channelling [44]. Inadequate NO synthesis and other toxic factors, perhaps argininosuccinate or its derivative guanidinosuccinate, may be involved in the pathogenesis of ASL deficiency and explain the more important neurocognitive alterations than in other UCDs [45]. Animal studies and preliminary data in humans suggest that drugs that supply NO might be beneficial in ASL deficiency [46].

In ARG1 deficiency, the mild and sporadic hyperammonaemia does not account for the spastic diplegia and the seizures, suggesting that central nervous system toxicity of increased arginine or its metabolites (polyamines, guanidino compounds, NO, agmatine) is a crucial pathogenic factor.

19.2.3 Genetics

DNA is generally used for genetic analysis of these disorders (◘ Table 19.1 for gene identifiers). The existence of pseudogenes restricts cDNA studies of *ASS1* to fibroblasts and the liver. One common mutation has been described in classical ASS deficiency (c.1168G>A, p.Gly390Arg) in patients from all ethnic backgrounds [47]. Common mutations in mild ASS deficiency (most frequent, c.535T>C, p.Trp179Arg and c.1085G>T, p.Gly362Val) have mainly been found in Turkish patients [47]. There are few recurrent *ASL* mutations associated with a severe phenotype (c.857A>G, p.Gln286Arg is the most frequent) or with milder clinical course (e.g. c.532G>A, p.Val178Met) [48]. Intragenic complementation [49] complicates determination of disease-causality of individual mutations. Mutations in *ARG1* (approximately 40 reported) are mainly private, with few being recurrent. See http://grenada.lumc.nl/LSDB_list/lsdbs for listing of mutations.

19.2.4 Diagnostic Tests

Biochemical assays The mainstay of biochemical diagnosis of cytosolic UCDs is the plasma amino acid profile. Markedly elevated citrulline levels are highly suggestive of ASS deficiency, with few alternatives to consider in the differential diagnosis, including the deficiencies of ASL, citrin and pyruvate carboxylase. Plasma citrulline levels >500 μmol/l are pathognomonic for ASS deficiency. Similarly, the presence of argininosuccinate (and/or its two anhydrides) in plasma and urine is pathognomonic for ASL deficiency. In ARG1 deficiency elevation of arginine in plasma is characteristic. Levels are often not very high in newborns but they increase during infancy and often reach >500 μmol/l in untreated patients.

Enzyme studies In ASS deficiency, ASS activity is rarely assayed since it requires liver biopsy or indirect measurement of ^{14}C-citrulline incorporation in cultured fibroblasts (available in research laboratories). In ASL and ARG1 deficiencies red blood cells are an easily accessible source for direct enzyme assays but conflicting results for ASL have been reported [50]. Overall, enzyme studies are currently not standard for confirmation of the diagnosis of a cytosolic UCD but are a valuable tool if mutation analysis fails.

Mutation analysis Mutation analysis is now used to confirm the diagnosis and to offer future prenatal testing. In ASS deficiency, it is performed with a high success rate by sequencing the 14 coding *ASS1* exons and flanking intronic regions. To improve the detection rate, RNA from fibroblasts (but not from blood cells due to the expression of pseudogenes) can be used. The same approach with a similar excellent detection rate can be applied in ASL deficiency and in ARG1 deficiency, where RNA studies using lymphocytes further improve the diagnostic yield.

Newborn screening Newborn screening for ASS and ASL deficiencies can be incorporated in the amino acid profile determined routinely by tandem-MS/MS. Elevation of citrulline and the presence of argininosuccinate respectively suggest ASS and ASL deficiency. Based on the experience in some newborn screening programs, there are concerns that patients with mild or asymptomatic disease might be subjected to unnecessary treatment [34][50], rendering further studies essential. In ARG1 deficiency, elevations of arginine are suggestive but not present in all patients and thus sensitivity is possibly not high.

19.2.5 Treatment and Prognosis

Emergency management follows the same principles as for mitochondrial UCDs, with identical dosages of infusions and medications (▶ Section 19.1.5), except for ASL deficiency, for which a bolus of L-arginine is given at up to 400 mg/kg over 90–120 min, followed by maintenance infusion of up to 400 mg/kg/day, since the response of some patients is very rapid and renders additional drugs unnecessary.

Maintenance treatment for ASS and ASL deficiencies is as for CPS1 and OTC deficiencies (▶ Section 19.1.5), although, particularly in patients with ASL deficiency, there is a lower risk of recurrent metabolic crises. Nevertheless, a low-protein diet is required in most patients including its supplementation with essential amino acids, vitamins, and trace elements. Nitrogen scavenging drugs are usually needed for metabolic stability: 200–250 mg/kg/day sodium benzoate and/or sodium phenylbutyrate or glycerol phenylbutyrate), distributed in 3 equal doses. To enhance partial urea cycle function, L-arginine (but not L-citrulline) should be given at a dose of 100–300 mg/kg/day in 3 dosages. To minimize potential argininosuccinate toxicity in ASL deficiency a reduced dose of arginine is recommended (100 mg/kg/day) [3].

Liver transplantation prevents hyperammonaemia but it does not reverse neurological damage. It should be considered for patients with poor metabolic control despite compliance with conservative therapy, or those with liver failure. In ARG1 deficiency L-arginine and L-citrulline must not be given, and the aim is to lower plasma arginine to <200 µmol/l, although this goal is difficult to achieve because of the strict protein restriction required. The existence of recombinant pegylated human arginase raised hopes of future substitutive treatment for ARG1 deficiency, although initial trials in a rodent model for ARG1 deficiency were not encouraging [51]. Although the experience is limited, liver transplantation in ARG1 deficiency appears not only to normalize urea cycle function but also to prevent further progression of neurological disease [52].

Outcome Survival is better than for mitochondrial UCDs, however, neurocognitive morbidity in ASL deficiency is similarly poor [2] and patients with ARG1 deficiency have an important risk of progressive spastic paraplegia [41].

19.3 Urea Cycle Mitochondrial Transporter Defects

Two mitochondrial transporters defects can cause disruption of the urea cycle.

19.3.1 Hyperornithinemia, Hyperammonaemia and Homocitrullinuria (HHH) Syndrome

Deficiency of the ornithine/citrulline mitochondrial antiporter ORC1 causes a rare (<1:2,000,000 live births) [1] syndrome characterized by hyperornithinemia, hyperammonaemia and homocitrullinuria, described in ▶ Chapter 21. Hyperammonaemia occurs because of an insufficient ornithine supply to OTC.

19.3.2 Citrin Deficiency

Citrin deficiency (also known as AGC2 deficiency) is due to lack of function of the hepatic mitochondrial aspartate/glutamate antiporter citrin, which can supply cytosolic aspartate for the ASS reaction. Citrin deficiency, initially identified in Japan as citrullinemia type II (CTLN2) [53], is largely a Far East disease although patients have been found in other populations (see for example [54]), at much lower frequency (<1:2,000,000 live births in the West) [1].

■ Clinical Presentation

There are two main age dependent clinical presentations: Neonatal Intrahepatic Cholestasis Caused by Citrin Deficiency (NICCD) and citrullinemia type II (CTLN2) which occurs in adolescents and adults. A third, less common, clinical phenotype, Failure To Thrive and Dyslipidemia Caused by Citrin Deficiency (FTTDCD) may also occur in childhood.

Newborn A large proportion of newborn infants with citrin deficiency develop neonatal intrahepatic cholestasis with persistent jaundice, failure to thrive, hepatomegaly and cholestasis [55]. Anaemia, low plasma albumin and total protein, impaired coagulation with prolonged prothrombin time, raised serum AST, hypoglycaemia, galactosaemia and galactosuria are frequent findings. Plasma citrulline is moderately increased (levels are often in the range of 100–500 µmol/l) and

other amino acids are also raised (methionine, threonine, tyrosine, serine and/or phenylalanine). These may be detected by newborn screening, although some patients with NICCD have had negative screening results. For most patients liver disease and biochemical abnormalities resolve by 12 months of age.

Childhood Many children remain well during childhood and have normal plasma amino acids and liver function. Sometime after 1 year of age, most develop a characteristic craving for protein-rich/fat-rich foods and avoidance of carbohydrate-rich foods and sugars. A proportion of those over a year of age develop FTTDCD. Poor appetite, fatigue, growth retardation, reduced quality of life scores, hypercholesterolemia affecting both HDL- and LDL-cholesterol, hypoglycaemia, and modest increases of plasma citrulline and ornithine are the main manifestations. Some patients develop recurrent pancreatitis or hepatoma.

Adolescents and adults CTLN2 can follow a period of normal health lasting several decades (age of onset 11–79 years; most between 20–50 years), and predominantly affects males (2.4:1 male/female ratio in Japan). It is characterised by neuropsychiatric symptoms of sudden onset, recurrent ammonia intoxication that can lead to coma and death, and high plasma citrulline levels (100–500 μmol/l). Alcohol, sugars (carbohydrate toxicity) or catabolic insults such as surgery or infection may be triggering events. Glycerol administration for cerebral oedema leads to further deterioration. Steatohepatitis and some liver fibrosis are frequent, while hypertriglyceridemia, chronic pancreatitis and hepatoma are potential complications. Food preferences are as in FTTDCD.

Metabolic Derangements

The exchange of mitochondrial aspartate for cytosolic glutamate and the malate/aspartate shuttle (Fig. 19.1), of which citrin is a part, are both affected [56][57]. There is insufficient supply of mitochondrial aspartate for ASS within hepatocytes, and the conversion of the fumarate released by ASL, to form aspartate within the cytosol, is impaired, due to the low cytosolic NAD resulting from lack of malate-aspartate shuttle operation (this shuttle transfers reducing equivalents from cytosolic NADH to the mitochondria, regenerating NAD in the cytosol). The low cytosolic aspartate decreases liver ASS activity, resulting in citrulline accumulation, and also impairs protein and pyrimidines synthesis in liver cells (both processes are cytosolic and use aspartate), explaining the hypoalbuminemia and hypoproteinemia of NICCD and the lack of urinary orotic acid that differentiates citrin deficiency from classical ASS deficiency [56]. The high cytosolic NADH/NAD ratios in the liver explain the hypoglycaemia and the galactosaemia that are frequently observed in NICCD, since cytosolic NAD is needed both for gluconeogenesis from lactate and for UDP-galactose to UDP-glucose conversion. Sugars, glycerol and alcohol are toxic in citrin deficiency because their metabolism in liver cells increases the cytosolic NADH/NAD ratio, magnifying the cytosolic NAD deficiency [57].

Hyperammonaemia develops in CTLN2 but not in NICCD because liver ASS is significantly decreased in CTLN2, whereas it is normal in NICCD [53][56]. This decrease in ASS, combined with the poor aspartate supply, leads to dysfunction of the UC. Arginine levels are normal or even elevated in CTLN2 and NICCD, probably reflecting normal extrahepatic ASS activity and a supply of aspartate by another mitochondrial carrier that catalyzes the aspartate/glutamate exchange (aralar or AGC1) and that is not expressed in the liver [58].

Pyruvate administration has been reported to be effective in citrin deficiency [57]. Its efficacy can stem from the ability of pyruvate to decrease the NADH/NAD ratio (consequently increasing cytosolic aspartate production from fumarate) and to be an energy source that, in contrast to glucose, does not need cytosolic NAD for being utilised. Arginine also appears beneficial in CTLN2. Perhaps the ornithine produced in the liver from arginine speeds the OTC reaction, increasing the intrahepatic citrulline level, secondarily lowering the apparent K_m for aspartate of human ASS [59], thus improving ASS activity at suboptimal aspartate concentrations.

Genetics

Citrin deficiency is due to mutations in *SLC25A13* (Table 19.1) and shows recessive inheritance. Many of the >80 disease-associated *SLC25A13* variants reported (http://chromium.lovd.nl/LOVD2/home.php?select_db=SLC25A13) are splicing mutations, frameshifts and premature stops that truncate or ablate parts of the citrin molecule [53]. A few mutant alleles predominate in given populations such as c.1177+1G>A and c.851-854del in Japan (70% of the disease alleles) or c.851-854del, c.615+5G>A, IVS16ins3kb (c.1750+72_1751-4dup17ins), and c.1638_1660dup23 among Chinese (>80% of the alleles in a large patient cohort). A number of alleles are shared by different Far East populations, while the p.Arg360* mutation appears to be widespread [54].

A frequency of 1/17,000 homozygotes or compound heterozygotes for disease-causing alleles has been estimated for Japan, similar to the NICCD frequency in that country, indicating full penetrance for this clinical form, but much more frequent than CTLN2 (1/100,000-1/230,000 in Japan) [53]. A lower penetrance among females than among males can account for the lower frequency of CTLN2 in women than in men.

Diagnosis

Biochemical assays In newborns with intrahepatic cholestasis the finding of increased plasma citrulline without significant hyperammonaemia, with normal or elevated levels of arginine and without urinary orotic acid, particularly with a high plasma level of alpha-fetoprotein and/or increased galactose in blood and urine, is strongly suggestive of NICCD [54]. In patients identified by neonatal screening with increased blood citrulline, it is important to first exclude ASS deficiency. Plasma ammonia and glutamine and urinary orotic acid are high in severe ASS deficiency but not in NICCD, and the reverse is true for arginine. Alpha-fetoprotein is increased in NICCD only. If tyrosine and alpha-fetoprotein are elevated, succinylacetone should be assayed to exclude tyrosinemia type 1.

A specific diagnosis of FTTDCD is difficult to make unless NICCD had been diagnosed previously. The presence of dyslipidemia is the paramount chemical indicator of FTTDCD, although increased citrulline levels and high lactate/pyruvate ratios can also be suggestive biomarkers.

CTLN2 can be differentiated from classical ASS deficiency by the normal or somewhat increased arginine level in citrin deficiency, this level being low in ASS deficiency, and by the absence of urinary orotic acid in CTLN2.

Protein studies Western blots of lymphocytes or cultured fibroblasts using antibodies that recognize the N-terminal moiety of citrin generally detect little or no cross-reactive immune material in most patients with citrin deficiency (but see [60]).

Mutation analysis Mutation detection in both copies of the *SLC25A13* gene is the gold standard for diagnosis. In populations in which a few alleles explain most of the cases these alleles are searched first on genomic DNA by multiple mutation detection approaches such as using long and accurate PCR or PCR-restriction fragment length polymorphism before more extensive sequencing. RNA analysis is possible in cultured fibroblasts and peripheral blood lymphocytes [61].

▪ Treatment and Prognosis

Emergency management of hyperammonaemic episodes in CTLN2 should avoid carbohydrate or glycerol infusions, because they worsen the hyperammonaemia [53][57]. Mannitol infusion to combat brain oedema appears safe. Measures to rapidly decrease ammonia such as haemodialysis can be used, as well as administration of intravenous sodium benzoate and sodium phenylacetate. Arginine appears beneficial and should be administered. Energy should be provided while restricting the carbohydrate supply by using triglycerides and amino acids. In NICCD the initial treatment may require packed red blood cells [54] and albumin for anaemia and hypoproteinaemia, respectively, and supportive measures for coagulopathy and liver insufficiency when present and severe. It is also essential to provide a lactose-free formula particularly if galactosaemia is observed, with some reduction in the fraction of calories provided by sugars in favour of medium-chain triglycerides and protein. Levels of fat-soluble vitamins and Zn should be monitored and supplemented when needed.

Maintenance treatment of NICCD involves the use of lactose-free or medium-chain triglyceride-enriched formula, relaxing this use with clinical improvement or at the end of the first year. When introduced, other foods should be protein-rich and fat-rich, such as eggs or fish. In CTLN2, the diet should follow the patient preferences for high protein/fat and low carbohydrate. Arginine (5–15 g/day reported) and sodium pyruvate (3–6 g in three 2 g-dosages reported) should be given [53]. Alcohol must be prohibited. Vigilance for hepatocellular carcinoma is necessary. If steatohepatitis and neuropsychiatric manifestations do not improve, liver transplantation provides a permanent cure. For FTTDCD a protein and medium-chain triglyceride-rich and carbohydrate-poor diet with no lactose is recommended. Sodium pyruvate may improve growth.

Arginine can be obtained as a chemical or a food supplement. Sodium pyruvate is a chemical, although calcium pyruvate is sold as a food supplement.

Outcome of NICCD is generally good with little intervention. The prognosis for CTLN2 has been poor without liver transplantation. Presently the awareness of avoiding glucose or glycerol infusions and the introduction of arginine and pyruvate appear to have improved the outcome of conservative treatment.

19.4 Urea Cycle Defects due to Deficiencies of Ancillary Enzymes

19.4.1 Δ^1-Pyrroline-5-Carboxylate Synthetase (P5CS) Deficiency

P5CS catalyses the first common step of *de novo* ornithine and proline synthesis. P5CS deficiency, due to *ALDH18A1* mutations (Table 19.1), has been reported to cause fasting hyperammonaemia, cutis laxa syndrome (De Barsy syndrome) and spastic paraplegia with the peculiarity of having dominant or recessive inheritance and frequent *de novo* mutations [62][63][64][65]. This disorder is described in ▶ Chapter 21.

19.4.2 Carbonic Anhydrase Va (CAVA) Deficiency

▪ Clinical Presentation

All known patients have developed neonatal symptoms identical to those with neonatal onset UCDs, presenting with hyperammonaemic encephalopathy within the first days after birth. In contrast to other neonatally presenting UCDs, all patients survived, including those requiring haemodialysis. The majority of patients have had only a single hyperammonaemic crisis; those who had a second one had a milder episode [66][67]. No symptoms have yet been described in children, adolescents or adults.

▪ Metabolic Derangement

Bicarbonate cannot cross the mitochondrial membrane, and the spontaneous conversion of CO_2 to bicarbonate is too slow for the needs of urea synthesis. CAVA accelerates this conversion within liver mitochondria, supplying the bicarbonate used intramitochondrially by CPS1, pyruvate carboxylase, propionyl CoA carboxylase and 3-methylcrotonyl CoA carboxylase. Therefore CAVA deficiency impairs the urea cycle, gluconeogenesis and branched-chain amino acid metabolism, yielding an unusual combination of biochemical findings [68]. These include hyperammonaemia, decreased plasma citrulline and absence of urinary orotic acid, hypoglycaemia, metabolic acidosis, high plasma lactate and urinary ketone bodies, and a urinary profile of organic acids containing carboxylase-related metabolites [68] (▶ Chapter 26).

Attempts to maximally activate CPS1 with the NAG analogue N-carbamylglutamate (carglumic acid; Carbaglu) are justified, since the affinity of CPS1 for NAG should decrease when bicarbonate levels are low [69], as in CAVA deficiency.

Genetics

CAVA is caused by mutations in *CA5A*. No common mutations have been identified as yet. A deletion of exon 6 (c.619-3421_774+502del) may be prevalent in patients from the Indian subcontinent [66][68]. A locus specific homepage can be found at: http://databases.lovd.nl/shared/genes/CA5A.

Diagnostic Tests

Biochemical assays should include plasma ammonia, blood lactate and urine ketone bodies, in addition to blood glucose and plasma amino acid profiles. Organic acids in the urine should be analysed to search for carboxylase metabolites. Blood acylcarnitine profiles are normal in this disorder [68].

Enzyme studies would require analysis of liver tissue and thus mutation analysis is used to confirm CAVA deficiency.

Mutation analysis. The first mutations were reported in 2014 [67]. The *CA5A* gene is small (only seven coding exons) but requires careful design of oligonucleotide primers because of highly homologous sequences of pseudogenes. As the gene is mainly expressed in the liver, use of RNA for analysis would require invasive sampling and therefore has not been reported.

Treatment and Prognosis

Emergency management for CAVA deficiency is mainly symptomatic, focusing on treating hyperammonaemia as for intramitochondrial UCDs (▶ Chapter 19.1.5). Carglumic acid (100 mg/kg as a single oral dose, possibly repeated after 4–6 hours) can be considered as some patients apparently responded well [67]. Otherwise, management is standard, including sufficient fluid and energy substitution, balance of acid-base status and of glucose homeostasis, and protein restriction during hyperammonaemia.

Maintenance treatment was not required in the patients reported in the literature as all interventions could be reduced soon after recovery from the metabolic crisis. Nevertheless, an emergency plan should be provided along with appropriate family counselling.

Outcome in CAVA deficiency appears excellent but observations in more patients are needed.

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Disorders of Sulfur Amino Acid Metabolism

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Sulfur Amino Acid Metabolism

The essential amino acid methionine is converted by two methionine adenosyltransferases (MAT I/III and MATII) to S-adenosylmethionine (SAM) (Fig. 20.1). The methyl group of SAM is used in numerous biologically important methylation reactions, yielding S-adenosylhomocysteine (SAH); excess SAM is removed from the cycle by glycine N-methyltransferase (GNMT). SAH is cleaved by S-adenosylhomocysteine hydrolase (SAHH) to homocysteine and adenosine, which is phosphorylated by adenosine kinase (ADK). Homocysteine can be converted back to methionine by the remethylation pathway or using betaine as a methyl-group donor, in patients treated with this drug. Alternatively, homocysteine is irreversibly metabolized to sulfate by the transsulfuration pathway. Homocysteine is condensed with serine to form cystathionine, which is subsequently cleaved to form cysteine and α-ketobutyrate; these reactions are catalysed by cystathionine.

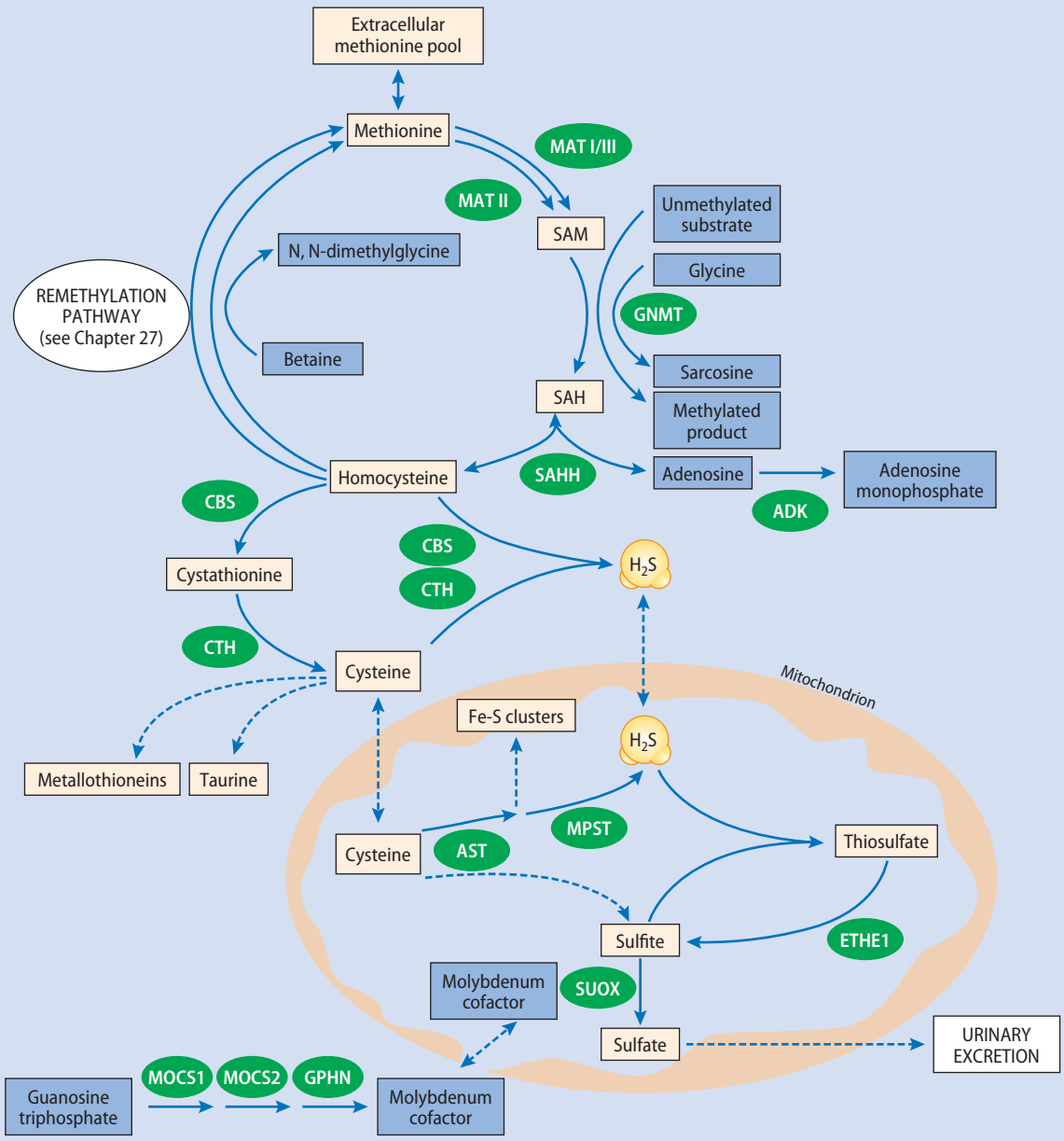

Fig. 20.1 Sulfur Amino Acid Metabolism. See text below for abbreviations

β-Synthase (CBS) and cystathionine γ-lyase (CTH), respectively, which can also use cysteine and/or homocysteine to synthesize hydrogen sulfide. Cysteine can be further converted in a series of reactions to taurine, or via the mitochondrial enzymes, aspartate aminotransferase (AST) and 3-mercaptopyruvate sulfurtransferase (MPST), to pyruvate and hydrogen sulfide. Mitochondrial oxidation of hydrogen sulfide and of cysteine involves several steps yielding thiosulfate, sulfite and finally sulfate; the figure only shows sulfur dioxygenase (ETHE1) and sulfite oxidase (SUOX), which requires the molybdenum cofactor, produced by enzymes encoded by molybdenum cofactor synthesis 1 and 2 genes (*MOCS1* and *2*) and by gephyrin (*GPHN*). Inorganic sulfur released from cysteine residues by a series of reactions is used in the formation of mitochondrial iron-sulfur (FeS) cluster cofactors (► Chapter 14 for details). The availability of cysteine in the neonatal period is limited because its endogenous synthesis from methionine by the transsulfuration pathway is markedly attenuated. The activity of the rate limiting enzyme in the pathway, cystathionase, is very low at birth and increases slowly during the first few months of life. For this reason, cysteine is considered a conditionally essential amino acid, at least in preterm infants.

Disorders in sulfur amino acid metabolism exhibit altered methionine, S-adenosylmethionine, sarcosine, S-adenosylhomocysteine, total homocysteine or cystathionine concentrations in blood, and/or adenosine or thiosulfate excretion in urine (► Sulfur Amino Acid Metabolism).
Methionine demethylation disorders can present with liver disease (GNMT deficiency), neurodevelopmental delay, dysmyelination and other neurological complications (some patients with MAT I/III deficiency), sometimes accompanied by myopathy (SAHH and ADK deficiencies). Methionine restriction may be beneficial in some patients with MAT I/III or ADK deficiency. SAHH deficiency is not treatable and GNMT deficiency is probably benign.
Disorders of homocysteine remethylation are considered in ► Chapter 27.
CBS deficiency – classical homocystinuria – is the commonest disease in this group. Its severity varies from multisystemic childhood condition (with lens dislocation, osteoporosis, marfanoid features, central nervous system and vascular complications) to an isolated thromboembolic disease in adults. Treatment is with vitamin B_6 in pyridoxine-responsive patients and a low-methionine diet or betaine in pyridoxine non-responders. Treatment can prevent most complications if started early in life following neonatal screening.
CTH deficiency appears to be a biochemical trait with no major clinical sequelae.
Disorders of cysteine and hydrogen sulfide oxidation pathway include ethylmalonic encephalopathy, isolated sulfite oxidase deficiency and combined sulfite oxidase deficiency due to impaired molybdenum cofactor synthesis. These are severe disorders with early-onset seizures and other neurological complications; other signs include orthostatic acrocyanosis, lens dislocation or urolithiasis; only molybdenum cofactor deficiency type A can be treated successfully, with a synthetic cofactor.

20.1 Methionine S-Adenosyltransferase Deficiency (Mudd's Disease)

20.1.1 Clinical Presentation

A few patients have presented with neurodevelopmental problems or malodorous breath but most patients have been detected by newborn screening programs for cystathionine β-synthase (CBS) deficiency using methionine as a marker. Some of these patients have developed neurological symptoms, such as learning difficulties or dystonia, or hypomyelination on neuroimaging. Neurological abnormalities have occurred in most patients with plasma methionine concentrations above 800 μmol/l, whereas they have been rare in subjects with lower levels [1].

20.1.2 Metabolic Derangement

Methionine S-adenosyltransferase converts methionine to S-adenosylmethionine (SAM) using ATP. MAT exists in 3 forms. MAT I and III are encoded by the same gene; they are tetrameric and dimeric forms, respectively, and are both liver-specific. MAT II is encoded by a different gene and converts methionine to SAM outside the liver, explaining why MAT I/III deficiency is relatively benign.

20.1.3 Genetics

MAT I/III deficiency is inherited as an autosomal recessive trait. Several mutations (e.g. p.R264H) exhibit a dominant negative effect; hypermethioninemia in these cases is inherited as an autosomal dominant trait and is benign.

20.1.4 Diagnostic Tests

In MAT I/III deficiency, the plasma methionine concentration ranges from 50 to >2000 μmol/l. Other causes of hypermethioninaemia include liver disease, prematurity hydrolase (SAHH) and, less often, CBS, S-adenosylhomocysteine hydrolase and adenosine kinase (ADK) deficiencies or an excessive intake of methionine (for details see Table 20.1). CBS deficiency can usually be distinguished by measuring the plasma total homocysteine (tHcy) though, surprisingly, tHcy is often

Table 20.1 Biochemical findings in inborn errors of sulfur amino acid metabolism.

Condition-enzyme deficiency	Concentrations in plasma/serum					Other tests
	Methionine µmol/l	tHcy µmol/l	SAM nmol/l	SAH nmol/l	Cystathionine* nmol/l	
Typical Low and High limits of reference ranges	L: 12–15 H: 40–45	L: 5–7 H: 10–15	L: 50–80 H: 120–170	L: 10–20 H: 40	L: 50–80 H: 350–500 (1,000 in newborns)	
Defects of methionine demethylation						
MAT I/III	↑↑	n–↑	n	n	n–↑	
GNMT	n–↑↑	n–↑	↑↑	n	↑	Sarcosine normal
SAHH	n–↑↑	n–↑	↑↑	↑↑	n	↑ Creatine kinase in serum, sarcosine ↑ Folates in serum n-↓
ADK	n–↑↑	n–↑	↑–↑↑	↑–↑↑	N/A	↑ Adenosine in urine
Defects of transsulfuration						
CBS	n–↑↑	↑–↑↑	↑–↑↑	↑–↑↑	n–↓	↑ Met/Cystathionine ratio, sarcosine ↑
CTH	n	n-↑	N/A	N/A	↑↑	
Defects of cysteine and H_2S oxidation						
SUOX	N/A	↓	N/A	N/A	N/A	↓ P-tCys; ↑sulfite, S-sulfocysteine and thiosulfate in urine
MOCS1, MOCS2, GPHN	N/A	↓	N/A	N/A	N/A	↓ P-tCys; ↑sulfite, S-sulfocysteine and thiosulfate in urine; ↓↓urate in serum
ETHE1	n	N/A	N/A	N/A	N/A	↑ Sulfite and thiosulfate in urine; ↑ C4 and C5 acylcarnitine in blood
Inborn errors in the remethylation pathway (for details ▶ Chapter 27)						
MTHFR	n–↓	↑–↑↑	n–↓	↑–↑↑	↑–↑↑	Folates in serum n-↓
cblE, cblG, cblD- var.1	n–↓	↑–↑↑	n–↓	↑–↑↑	↑–↑↑	
Combined cbl defects cblC, cblD, cblF, cblJ	n–↓	↑–↑↑	n–↓	↑–↑↑	↑–↑↑	↑–↑↑ C3 acylcarnitine in blood and MMA in serum/urine, vitamin B_{12} n-↓
Secondary disorders of sulfur amino acid metabolism						
Vitamin B_{12} deficiency	n–↓	↑–↑↑	n–↓	↑	↑–↑↑	↓-↓↓ Serum vitamin B_{12} and/or holo-TCII; ↑–↑↑ C3 acylcarnitine in blood and MMA in serum/urine
Folate deficiency	n–↓	↑–↑↑	n-↓	↑	↑–↑↑	↓-↓↓ Serum folate, sarcosine n-↑↑
Liver disease	n–↑↑	n–↑	n–↑	n–↑	n–↑	
Renal failure	n	↑–↑↑	↑–↑↑	↑↑	↑–↑↑	Serum 2-methylcitrate ↑↑ > ↑ MMA

N/A, data not available; *n* normal (within reference range); ↓ metabolite decreased; ↑ and ↑↑ metabolite increased and grossly elevated; *,↓ and ↑ cystathionine concentrations detectable only by sensitive LC-MS or GC-MS methods, ↑↑ elevated cystathionine levels detectable also by amino acid analyzer

MTHFR, methylenetetrahydrofolate reductase; *cbl*, cobalamin complementation group; *cblD-var.1*, remethylation defect cblD-variant without methylmalonic aciduria; *tHcy*, total homocysteine; *tCys*, total cysteine; *holoTCII*, holotranscobalamin II (active vitamin B_{12}); *MMA*, methylmalonic acid; see **Sulfur Amino Acid Metabolism** for other abbreviations

slightly increased in MAT I/III, SAHH and ADK deficiencies. Plasma SAM and SAH levels are normal in MAT I/III deficiency, whereas at least one of these metabolites is increased in SAHH, ADK and CBS deficiencies. The diagnosis is generally confirmed by mutation analysis because the enzyme assay requires a liver biopsy.

20.1.5 Treatment and Prognosis

A methionine- or protein-restricted diet is recommended in patients with plasma methionine levels above 800 µmol/l, aiming to achieve methionine levels around 500–600 µmol/l. As yet, however, there is limited clinical evidence of benefit. Improved myelination has also been reported after treatment with oral SAM [1].

20.2 Glycine N-Methyltransferase Deficiency

20.2.1 Clinical Presentation

The four published patients with glycine N-methyltransferase (GNMT) deficiency [2][3] showed mild to moderate elevation of plasma aminotransferases, although values fluctuated and were sometimes within the reference range. Two siblings had mild hepatomegaly and a liver biopsy in one of them showed mild centrilobular fibrosis. Liver histology was normal in a patient without hepatomegaly. There were no other consistent symptoms, although one patient showed failure to thrive.

20.2.2 Metabolic Derangement

GNMT is a liver enzyme involved in methionine degradation, using the methyl group from SAM to convert glycine to sarcosine. Its deficiency causes the accumulation of methionine and SAM while plasma sarcosine is normal.

20.2.3 Genetics

GNMT deficiency is inherited as an autosomal recessive trait.

20.2.4 Diagnostic Tests

GNMT deficiency can be distinguished from other causes of hypermethioninaemia by demonstrating grossly increased plasma SAM levels with normal sarcosine and SAH and a raised SAM/SAH ratio. Assay of GNMT activity is complicated by its liver specificity. Molecular genetic analysis of the *GNMT* gene is recommended to confirm the diagnosis.

20.2.5 Treatment and Prognosis

The biochemical abnormalities in one patient resolved on a low methionine diet (300 mg/d) [2] but the three other patients are healthy on a normal diet, 14, 16 and 17 years after diagnosis [personal communications R.Cerone and P.Augoustides-Savvopoulou]. Thus, the human disorder appears to be benign and not to warrant dietary treatment.

The GNMT knockout mouse develops steatosis that progresses to steatohepatitis, cirrhosis and hepatocellular carcinoma. Nicotinamide is an acceptor for the methyl-group of SAM; its administration lowered SAM levels and prevented steatosis and liver fibrosis in these mice. Patients with GNMT deficiency should, therefore, have follow-up with monitoring of liver function. Treatment with nicotinamide may be considered if they develop complications.

20.3 S-Adenosylhomocysteine Hydrolase Deficiency

20.3.1 Clinical Presentation

S-Adenosylhomocysteine hydrolase (SAHH) deficiency has been reported in eight patients [4][5][6]. All had a severe myopathy, with hypotonia and raised plasma creatine kinase; several patients died of respiratory failure in infancy. The survivors all had delayed psychomotor development and some had strabismus; neuroimaging usually showed hypomyelination. Most patients had hepatomegaly and/or liver disease with coagulopathy, and this was probably responsible for fetal hydrops in two siblings.

20.3.2 Metabolic Derangement

SAHH activity is present in all cells and converts SAH to homocysteine and adenosine. In SAHH deficiency, SAH accumulates and inhibits a number of essential methyltransferase reactions.

20.3.3 Genetics

SAHH deficiency is inherited as an autosomal recessive trait.

20.3.4 Diagnostic Tests

Patients with SAHH deficiency have increased plasma SAM and grossly elevated SAH levels. Plasma methionine concentrations are also usually raised, with slightly increased tHcy. Creatine kinase is markedly increased. The diagnosis can be confirmed by enzyme analysis in red blood cells or fibroblasts or by molecular genetic analysis of the *AHCY* gene.

20.3.5 Treatment and Prognosis

A methionine- or protein-restricted diet will decrease and sometimes even normalize plasma SAM and SAH [4][5][6] and may be beneficial if started early in life. Phosphatidylcholine and creatine supplements have been given because SAH is formed during the synthesis of these molecules. Successful treatment with liver transplantation has also been reported [6].

20.4 Adenosine Kinase Deficiency

Adenosine kinase (ADK) deficiency has been reported in seventeen patients [7][8]. ADK converts adenosine to AMP (adenosine monophosphate). In ADK deficiency, adenosine accumulation leads to increased urinary adenosine excretion, raised plasma SAM and SAH concentrations, usually accompanied by increased plasma methionine and slightly increased tHcy levels. Raised SAH levels are likely to inhibit essential methyltransferase reactions and AMP deficiency will impair ADP and ATP synthesis (for details see ▶ Chapter 35).

20.5 Cystathionine β-Synthase Deficiency

20.5.1 Clinical Presentation

Patients with cystathionine β-synthase (CBS) deficiency show a wide spectrum of severity and age at presentation. Some patients are asymptomatic into adulthood, whilst others have a severe multisystem disease. The clinical features predominantly involve four organ systems:

- **Eye**: Dislocation of the lens (ectopia lentis) is the most characteristic finding. In untreated patients, it usually occurs between 2 and 12 years of age [9]. It may be preceded by severe (>5 dioptres), progressive lenticular myopia, which is unusual outside this condition [10]. Following dislocation of the lens, movement of the eye may cause the iris to tremble (iridodonesis) and there is a risk of glaucoma.
- **Skeleton**: Many patients have excessive growth, particularly around puberty, with elongation and thinning of long bones, enlarged epiphyses (especially at the knee) and arachnodactyly. This is often called a ›marfanoid‹ habitus but the joints are stiff, in contrast to Marfan syndrome. Adults have premature osteoporosis, which may lead to scoliosis and pathological fractures. Other deformities include genu valgum, pes cavus, and pectus excavatum or carinatum.
- **Brain**: Approximately half the patients have learning difficulties. Problems usually become apparent in early to mid-childhood. Patients are often clumsy and some have progressive dystonia. Seizures and psychiatric problems are common in untreated adults [11].
- **Vascular system**: Thromboembolism is rare in the first decade but may occur at any later age; it is a common presentation in mildly affected adults. Deep venous thrombosis is commonest and may lead to pulmonary embolism [9]. Sagittal sinus thrombosis occurs in children as well as adults. In adults, there is also an increased risk of carotid and renal artery thrombosis. The risk of coronary heart disease is increased to a lesser degree.

Rarer complications include spontaneous pneumothorax and pancreatitis. The skin and hair may show hypopigmentation that is reversed by treatment.

The ages of onset for the main clinical features were reviewed by Mudd and colleagues [9]. Patients who respond to pyridoxine are less likely to suffer complications and these occur at an older age than in pyridoxine-unresponsive patients. Patients who are homozygous for the c.833T>C (p.I278T) mutation generally present as adults with vascular problems but it is likely that a number of these patients remain asymptomatic, particularly if they have a relatively high pyridoxine intake [12]. These patients were under-represented in Mudd's review of the natural history of CBS deficiency.

20.5.2 Metabolic Derangement

CBS – a cytosolic tetrameric enzyme – is expressed predominantly in liver, pancreas, kidney and brain. Its activity can also be determined in cultured fibroblasts and in plasma due to its release from the liver [13]. Its catalytic domain binds heme and the cofactor pyridoxal 5´phosphate (PLP) in addition to its substrates; the regulatory domain binds the allosteric activator SAM.

The pathophysiology is not fully understood. CBS deficiency leads to the accumulation of SAH and homocysteine, enhanced remethylation to methionine, and depletion of cystathionine and cysteine. Accumulated homocysteine results in endoplasmic reticulum stress, alters intracellular signaling and modifies sulfhydryl groups on proteins; increased SAH impairs methylation reactions; and decreased cystathionine and cysteine are associated with apoptosis, oxidative stress and alterations of structural proteins. Altered synthesis of hydrogen sulfide may also contribute to the pathophysiology.

The elevated homocysteine is thought to be responsible for thromboembolism and vascular disease. The connective tissue abnormalities, which are rare in disorders of homocysteine remethylation, may be due to raised homocysteine concentrations combined with decreased cysteine [14]. Animal models with *CBS* gene deletions are lethal due to severe hepatopathy but the knock-in models recapitulate in part the human phenotype, including connective tissue involvement.

20.5.3 Genetics

CBS deficiency is inherited as an autosomal recessive disease and occurs worldwide. The true prevalence is unknown and ranges between 1:1,800 and 1:900,000. More than 160 mutations are included in the CBS mutation database (http://cbs.lf1.cuni.cz) [15]. Mutations associated with a severe pyridox-

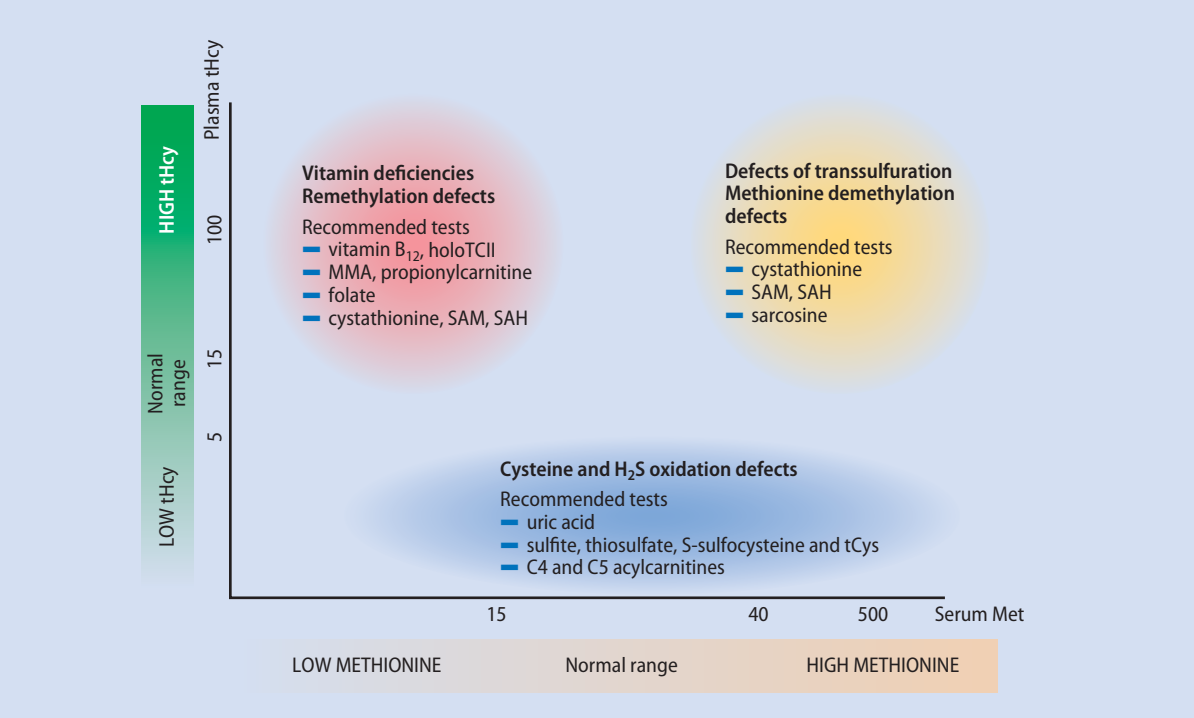

Fig. 20.2 Disorders of sulfur amino acid metabolism. Graph showing the most likely diagnoses as a function of plasma total homocysteine and serum methionine concentrations. See Table 20.1 for lists of the individual disorders and results of the recommended tests. *MMA*, methylmalonic acid; *Met*, methionine; *SAM*, S-adenosylmethionine; *SAH*, S-adenosylhomocysteine; *tHcy*, total homocysteine; *tCys*, total cysteine; *holoTCII*, holotranscobalamin II

ine unresponsive phenotype include c.572T>C (p.T191M) in patients from Iberian Peninsula and South America, c.919G>A (p.G307S) in patients of Irish and British ancestry, c.1006C>T (p.R336C) in Qatari patients, c.1224-2A>C, (p.W409_G453del or deletion of exon 12) in Central Europe and Turkey, and c.969G>A (p.W323X) in Saudi Arabs. Mutations associated with pyridoxine responsiveness include the c.833T>C (p.I278T), which is widespread in populations of European origin, as well as c.341C>T (p.A114V) and the extremely pyridoxine sensitive variant c.146C>T (p.P49L) [16].

CBS mutations affect *in vivo* activity by various molecular mechanisms including decreased stability of mutant enzymes due to misfolding and dysregulated activation by SAM. The molecular mechanism of pyridoxine responsiveness remains unproven, although stabilization of the mutant enzyme by PLP seems plausible. Other small osmolytes and ligands, including heme, have chaperone activity which may be used in developing novel forms of treatment [17][18].

20.5.4 Diagnostic Tests

The principal test for CBS deficiency is determination of the plasma total homocysteine (tHcy) concentration. Typical values are >100 µmol/l, although values between 50 and 100 µmol/l have been reported, especially in adult presentations. Plasma should ideally be separated from whole blood within one hour of venepuncture [19]. Due to low sensitivity and the complicated pre-analytical requirements, measurement of free homocystine is not recommended. To avoid misdiagnosis in pyridoxine responsive patients, pyridoxine supplements including multivitamins should be avoided for at least 2 weeks prior to testing. The diagnosis of CBS deficiency is very likely if the plasma methionine is high or borderline high and further supported by low to low-normal plasma cystathionine levels determined by sensitive methods, with an increased methionine-to-cystathionine ratio [16]. Other causes of hyperhomocysteinemia include inborn errors of homocysteine remethylation, vitamin deficiencies, renal insufficiency and drugs (Fig. 20.2; Table 20.1 for biochemical findings in other conditions) [19]. CBS deficiency can be confirmed by enzyme assay in cultured fibroblasts or plasma, and/or mutation analysis of the *CBS* gene, however, in some patients one of these two techniques may yield normal results.

Newborn screening for homocystinuria generally uses methionine or the methionine-to-phenylalanine ratio as primary markers [20]. Sensitivity is low, however, for pyridoxine responsive patients and limited for pyridoxine non-responsive homocystinuria, with an inverse relationship to the cut-off used. Use of tHcy in dried blood spots as a second tier marker increases the specificity of newborn screening but its use as a primary marker has seldom been reported [20].

Prenatal testing is best done by molecular genetic analysis, if the mutations in both parents are known. Preimplantation diagnosis is also feasible. If needed, enzymatic analysis can be done in cultured amniocytes.

20.5.5 Treatment and Prognosis

The main forms of treatment are pyridoxine, betaine and a methionine-restricted diet. Treatment aims to prevent complications by lowering the plasma tHcy concentration, whilst maintaining normal growth and avoiding abnormally low methionine concentrations. Plasma tHcy levels can often be brought below 50 µmol/l in pyridoxine-responsive patients. In pyridoxine-unresponsive patients, good outcomes have been achieved by keeping the lifetime median free homocystine concentration below 11 µmol/l [21], suggesting that tHcy levels below 100 µmol/l are acceptable.

Approximately 50% patients with CBS deficiency respond to pharmacological doses of pyridoxine. Vitamin B_{12} and folate deficiencies are common in CBS deficiency and must be corrected before assessing the response. After infancy, we recommend giving 10 mg/kg/day pyridoxine up to a maximum of 500 mg/day for 6 weeks; the plasma tHcy concentration should be measured before treatment and two to three times while on treatment. The test should be done on a normal diet and should be deferred if the patient is catabolic. If tHcy falls by <20%, the patient is likely to be unresponsive. Patients detected by newborn screening are seldom responsive but we recommend a trial of 100 mg pyridoxine daily for 2 weeks.

For long-term treatment, the pyridoxine dose should be the lowest that achieves maximum lowering of homocysteine. It should not exceed 10 mg/kg/day (maximum 500 mg/day) as peripheral neuropathy has been reported after long-term high doses (generally >900 mg/day) [22].

Dietary treatment should be considered in all patients who do not achieve satisfactory tHcy levels with pyridoxine alone. Most patients require a low-methionine diet with supplements of a methionine-free amino acid mixture, analogous to that used in PKU. This can achieve excellent tHcy levels but the diet is difficult, particularly if started after infancy. Moreover, life-long treatment is needed and compliance often deteriorates during adolescence. In patients with severe CBS deficiency, the methionine intake must be markedly restricted to achieve plasma tHcy concentrations below 100 µmol/l; other patients tolerate more methionine, presumably because they have some residual CBS activity. The quantity of methionine that can be taken is relatively constant throughout life. Anthropometric measurements and nutritional status should be assessed regularly. Babies are given a combination of breast milk or normal infant formula and a special methionine-free infant formula; the proportions are adjusted according to the plasma methionine and tHcy concentrations. Older patients are given small, measured amounts of foods containing methionine each day, along with low-protein foods and supplements of a methionine-free amino acid mixture, vitamins and minerals [23]. Cysteine is a conditionally essential amino acid in CBS deficiency but it is uncertain how much is needed.

Late-diagnosed patients who cannot manage a low-methionine diet may profit from a milder protein restriction that is above the minimum safe intake, without supplements of an amino acid mixture. This may lead to acceptable tHcy levels in some partially pyridoxine responsive patients.

Betaine acts as a methyl donor for the remethylation of homocysteine to methionine, catalyzed by the enzyme, betaine homocysteine methyltransferase. Treatment with betaine leads to a fall in plasma tHcy accompanied by a rise in methionine concentrations. The starting dose is 100 mg/kg/day in children and 6 g/day in adults, divided into two daily doses as the half-life is >14 hours. The dose is adjusted according to response but doses above 150–200 mg/kg/day are unlikely to confer additional benefit [24]. Betaine is generally well tolerated but some patients dislike the taste and high doses are associated with a fishy odour. There have been a few reports of cerebral oedema in patients taking betaine, who had plasma methionine concentrations above 1000 µmol/l [25]. Betaine is best used as adjunctive treatment in patients who are partially pyridoxine-responsive or who cannot comply adequately with dietary treatment. If used alone, betaine seldom achieves target tHcy levels and is likely to cause high methionine concentrations. As well as promoting remethylation of homocysteine, betaine may act as a chaperone for the mutant CBS enzyme [17].

Vitamin B_{12} and folate deficiencies may occur in patients with CBS deficiency, probably due to increased flux through the remethylation pathway and inhibition of MTHFR by high SAM concentrations. Vitamin B_{12} deficiency should be corrected and all patients should be given folate supplements.

Thromboembolism is a major cause of death. Anti-thrombotic drugs, such as aspirin or dipyridamole, may be justified in poorly controlled patients; vitamin C may improve endothelial function. Dehydration should be avoided. Biochemical control should be optimized before elective surgery and standard post-operative precautions should be taken, including low molecular weight heparin in cases of prolonged immobilisation.

Maternal homocystinuria does not appear to cause fetal malformations [26]. It is essential to continue treatment, including betaine, during pregnancy with regular biochemical and dietary monitoring. There is a high risk of thromboembolism and low molecular weight heparin should be given at least during the third trimester and for 6 weeks post-partum [27].

Without treatment, approximately 20% pyridoxine-unresponsive patients die by 20 years of age [9]; fewer than 5% pyridoxine-responsive patients die by this age but the precise figure is unknown due to their under-ascertainment in the original series. Treatment greatly improves the prognosis: all the major complications can be prevented if patients are diagnosed by newborn screening and comply with treatment [21] [28]. Even with imperfect control, treatment greatly reduces the vascular risk [29].

20.6 Cystathionine γ-Lyase Deficiency

20.6.1 Clinical Presentation

Cystathioninuria was originally described in patients with cognitive impairment, however further studies have shown that there was an ascertainment bias. The available data do not provide convincing evidence that cystathionine γ-lyase (CTH) deficiency is associated with adverse clinical outcomes [30].

20.6.2 Metabolic Derangement

Cystathionine and N-acetylcystathionine accumulate in plasma and grossly increased amounts are excreted in urine. Hypertension has been reported in a mouse model and attributed to impaired hydrogen sulfide synthesis but it has not been found in human subjects.

20.6.3 Genetics

CTH deficiency is inherited as an autosomal recessive trait.

20.6.4 Diagnostic Tests

Physiological concentrations of cystathionine in plasma are in the submicromolar range; detection is impossible with conventional amino acid analysis and requires sensitive methods using LC-MS/MS or GC/MS. In CTH deficiency, however, plasma and urinary cystathionine are grossly elevated, accompanied by a slight increase in plasma tHcy. The differential diagnosis for elevated cystathionine includes prematurity in newborns, vitamin B_6 deficiency, defects of homocysteine remethylation and neuroblastoma. The diagnosis is confirmed by *CTH* mutation analysis since enzyme assay in cultured fibroblasts is not reliable.

20.6.5 Treatment and Prognosis

Some individuals may respond biochemically to pyridoxine administration, however, treatment appears unnecessary.

20.7 Molybdenum Cofactor Deficiency

20.7.1 Clinical Presentation

Patients usually present soon after birth with poor feeding, hypotonia, exaggerated startle reactions and intractable seizures, resembling hypoxic ischaemic encephalopathy. Neuroimaging shows cerebral oedema and restricted diffusion in the cortex, followed by subcortical necrosis. Within weeks, this leads to multicystic leukoencephalopathy with microcephaly, profound psychomotor retardation and severe spasticity. Dislocation of the ocular lens occurs during infancy and xanthine renal stones can develop later. There may be mild dysmorphism, with puffy cheeks, a long philtrum and a small nose; cerebral malformations occur rarely. A few patients present later in childhood with neurological problems and dislocated lenses [31].

20.7.2 Metabolic Derangement

Molybdenum cofactor (MoCo) synthesis involves three steps. MoCo deficiency type A affects the conversion of GTP to cyclic pyranopterin monophosphate (cPMP). Patients with MoCo deficiency type B cannot convert cPMP to molybdopterin. MoCo deficiency type C affects gephyrin, which catalyses adenylation of molybdopterin and insertion of molybdenum to form the cofactor.

The molybdenum cofactor is needed for sulfite oxidase, aldehyde oxidase, the mitochondrial amidoxime reducing component and xanthine dehydrogenase. Deficiency of the latter causes raised xanthine and low urate concentrations (see also ▶ Chapter 35). Sulfite accumulation is responsible for the neurotoxicity and lens dislocation (▶ Section 20.8).

20.7.3 Genetics

All forms of MoCo deficiency are inherited as autosomal recessive traits. Type A is commonest with mutations in *MOCS1*, which encodes two proteins that catalyse the conversion of GTP to cPMP [32]. Most other patients have Type B, caused by mutations in *MOCS2*, which also encodes two proteins. Few mutations have been found in *GPHN*, which encodes Gephyrin.

20.7.4 Diagnostic Tests

The plasma urate concentration is initially normal but decreases after a few days and remains low (<0.06 mmol/L) while xanthine is elevated in urine. Sulfite can be detected in fresh urine using dipsticks but false positive and negative results occur. S-Sulfocysteine is a more reliable indicator and can be detected in urine or blood. S-Sulfocysteine accumulation can lead to inhibition of antiquitin with secondary elevation of pipecolic acid (see ▶ Chapter 28).Taurine and thiosulfate concentrations are increased and plasma total cysteine and tHcy are low. The diagnosis is confirmed by mutation analysis, which is generally used for prenatal diagnosis, though sulfite oxidase activity can be assayed in chorionic villi.

20.7.5 Treatment and Prognosis

Without treatment, patients have profound handicap and die early. Recently, several patients with MoCo deficiency type A

have been treated with daily intravenous infusions of cPMP [33]. Three patients are seizure-free with near-normal development after treatment for up to 6 years. To avoid irreversible damage, treatment generally needs to be started within 24 hours of birth. There is no effective treatment for other types of MoCo deficiency.

20.8 Isolated Sulfite Oxidase Deficiency

20.8.1 Clinical Presentation

The clinical features usually resemble those of MoCo deficiency, with poor feeding, hypotonia and seizures soon after birth, followed by spasticity and severe developmental impairment [34]. A number of patients present later in childhood with a movement disorder, stroke or developmental regression, often after an infection [35]. Most patients have dislocated lenses.

20.8.2 Metabolic Derangement

Sulfite derived from cysteine is normally oxidised to form sulfate. In sulfite oxidase deficiency, accumulating sulfite damages the brain; this is partly due to the production of sulfocysteine, which mediates excitotoxicity. Sulfite probably causes lens dislocation by disrupting cystine cross-linkages in the suspensory ligament.

20.8.3 Genetics

Sulfite oxidase deficiency is an autosomal recessive disorder caused by mutations in *SUOX*.

20.8.4 Diagnostic Tests

Sulfite can be detected in fresh urine using dipsticks, though these are not entirely reliable. Elevated S-sulfocysteine can be demonstrated by standard amino acid analysis or by LC-MS/MS. Plasma taurine concentrations are raised and plasma total cysteine and tHcy are abnormally low. Urate and xanthine concentrations are normal. The diagnosis is confirmed by mutation analysis; the enzyme can be assayed in fibroblasts or chorionic villi but this is now seldom needed.

20.8.5 Treatment and Prognosis

The prognosis for neonatal-onset cases is poor. Treatment with a diet low in cysteine and methionine may help patients with a mild form of sulfite oxidase deficiency [35].

20.9 Ethylmalonic Encephalopathy

20.9.1 Clinical Presentation

Ethylmalonic encephalopathy (EE) is a progressive multisystem disease. It presents in the first months of life with hypotonia, chronic diarrhoea, orthostatic acrocyanosis, a recurrent petechial rash and bruising (with normal platelets). Other features include developmental regression, microcephaly, seizures, episodes of coma, poor growth and hyperlactataemia. Most patients die in early childhood, though some have a milder course. Cerebral imaging shows necrotic lesions in the putamen, caudate nuclei and periaqueductal region, and sometimes abnormalities in the subcortical white matter and brainstem. Neurological malformations have also been reported.

20.9.2 Metabolic Derangement

EE is caused by deficiency of a mitochondrial sulfur dioxygenase necessary for the detoxification of sulfide [36]. Hydrogen sulfide (H_2S) is synthesized endogenously by CBS, CTH and 3-mercaptosulfurtransferase, and also formed by bacterial anaerobes in the large intestine. In EE, the accumulating H_2S inhibits cytochrome c oxidase and short-chain fatty acid oxidation; the latter results in ethylmalonic aciduria, and raised C4- and C5-acylcarnitines in blood. H_2S also has vasoactive and vasotoxic effects; damage to small blood vessels causes bleeding into the skin. Production of H_2S by gut bacteria causes the severe, persistent diarrhea.

20.9.3 Genetics

EE is a rare autosomal recessive disorder caused by mutations in *ETHE1*. No genotype-phenotype correlation has been established [37].

20.9.4 Diagnostic Tests

Ethylmalonic acid and C4- and C5-acylglycines are consistently present in urine, with raised C4- and C5-acylcarnitines in blood. Urinary thiosulfate is also markedly elevated. The diagnosis is confirmed by mutation analysis of *ETHE1*.

20.9.5 Treatment and Prognosis

Treatment has been undertaken with metronidazole (to reduce bacterial H_2S production) and N-acetylcysteine (a precursor of glutathione, which can accept the sulfur atom of H_2S). Though this leads to some clinical and biochemical improvement [38], the prognosis remains poor. Recently, a better outcome has been reported following liver transplantation [39].

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Disorders of Ornithine and Proline Metabolism

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Ornithine and Proline Metabolism

Ornithine is an intermediate in metabolic pathways involving the urea cycle, proline metabolism and the biosynthesis of creatine and polyamines. Ornithine-δ-aminotransferase (OAT) is a pyridoxal phosphate-requiring, mitochondrial matrix enzyme that plays a pivotal role in these pathways. The OAT reaction is freely reversible: during the neonatal period the net flux is in the direction of ornithine and, via the urea cycle, arginine biosynthesis, while after a few months of age the net flux reverses to favour arginine disposal via the synthesis of Δ1-pyrroline-5-carboxylate (P5C), an intermediate in proline and glutamate synthesis. Ornithine also plays an essential role, serving as the substrate upon which urea is assembled (Fig. 21.1). Since both OAT and ornithine transcarbamoylase (OTC) are mitochondrial matrix enzymes, ornithine produced in the cytoplasm from arginine must be transported into the mitochondrial matrix by a specific energy-requiring transport system involving ORNT1 (SLC25A15), an antiporter in the inner mitochondrial membrane, which exchanges cytosolic ornithine with mitochondrial citrulline. In the cytoplasm ornithine is decarboxylated to putrescine which is then converted to spermine. Proline, unlike all other amino acids (except hydroxyproline), has no primary amino group (it is termed as an imino acid) and uses, as a consequence, a specific set of enzymes for its metabolism. P5C, the product or precursor of the OAT reaction, is both the immediate precursor and the degradation product of proline. P5C synthetase, a bifunctional ATP- and NADPH-dependent mitochondrial enzyme that is highly active in the gut and also expressed in brain catalyses the reduction of glutamate to P5C. The P5C/proline cycle transfers reducing/oxidizing potential between cellular organelles. Owing to its pyridinoline ring, proline (together with hydroxyproline) contributes to the structural stability of proteins, particularly collagen.

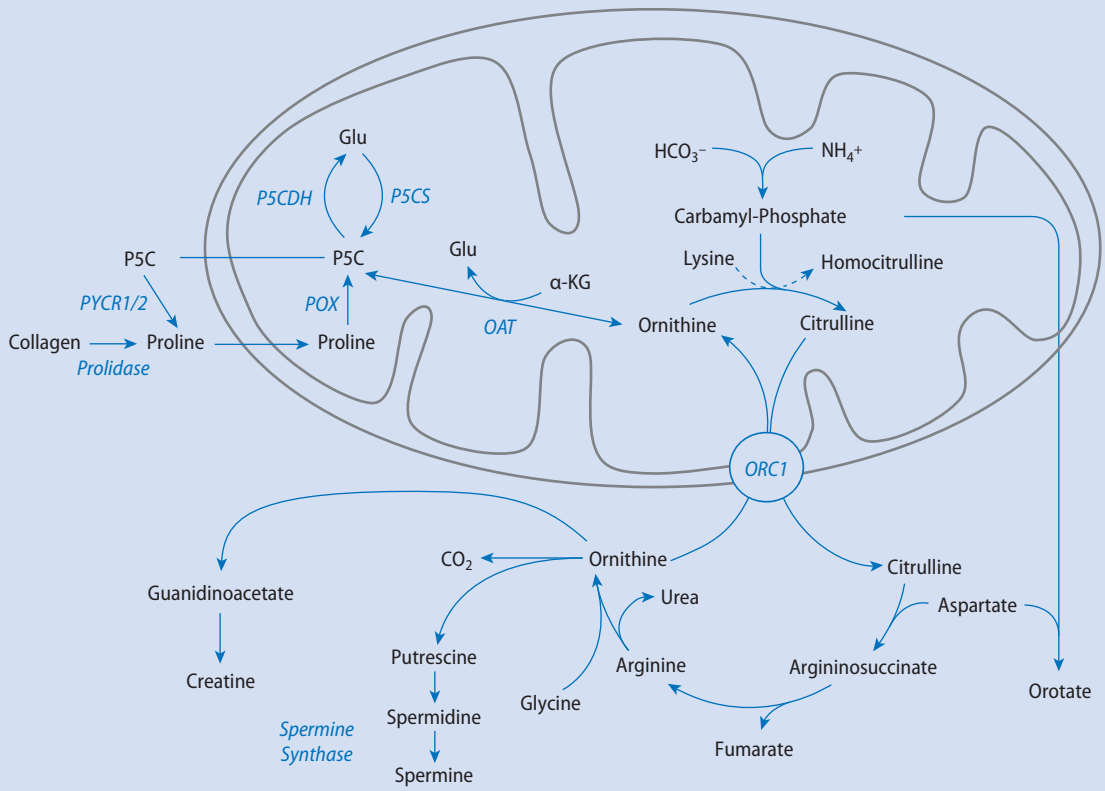


Fig. 21.1 Ornithine and proline metabolic pathways. *P5C*, Δ1-pyrroline 5-carboxylate; *Glu*, glutamate; α-*KG*, α-ketoglutarate; *OAT*, ornithine-δ-aminotransferase; *ORC1*, ornithine/citrulline antiporter; *P5CS*, Δ1-pyrroline-5-carboxylate synthetase; *P5CDH*, Δ1-pyrroline-5-carboxylate dehydrogenase; *PYCR1/2*, Δ1-pyrroline-5-carboxylate reductase; *POX*, proline oxidase; the step indicated by the broken line, lysine transcarbamylase, is not well defined

Hyperornithinaemia due to ornithine aminotransferase (OAT) deficiency results in gyrate atrophy of the choroid and retina (GA) and leads to progressive visual loss. Treatment includes an arginine-restricted diet and a trial of pyridoxine (vitamin B6) which, in some patients, can slow visual loss and chorioretinal degeneration. Rarely, neonates with OAT-deficiency present with hyperammonaemia and require treatment with arginine supplementation.
In the *hyperornithinaemia, hyperammonaemia, and homocitrullinuria (HHH) syndrome* clinical manifestations are variable and may be related to intermittent episodes of hyperammonaemia. Progressive spastic paraparesis is often a late complication. Deficient transport of ornithine into the mitochondria impairs the urea cycle and results in episodic hyperammonaemia, hyperornithinaemia and increased urinary excretion of homocitrulline and orotic acid. Treatment includes protein restriction, citrulline or arginine supplementation and in some cases ammonia scavengers.
P5C synthetase (P5CS) deficiency is a rare recessive neurocutaneous syndrome with cutis laxa, developmental delay, joint laxity and cataracts, but mutations affecting specific residues may also cause autosomal dominant cutis laxa as well as adult onset autosomal dominant spastic paraplegia. The metabolic phenotype includes mild hyperammonaemia, hypoornithinaemia, hypocitrullinaemia, hypoargininaemia and hypoprolinaemia.
Deficiency of P5C reductase (P5CR) associated to mutations in *PYCR1* causes autosomal recessive cutis laxa with progeroid features, while mutations in *PYCR2,* a paralog of *PYCR1,* cause microcephaly and hypomyelination. Both disorders show no apparent metabolic phenotype.
The phenotypic consequences of *Hyperprolinaemia type I* are uncertain, while *Hyperprolinaemia type II* appears to be associated with a disposition to recurrent seizures.
Prolidase deficiency causes skin lesions and recalcitrant ulceration in addition to other features, such as impaired psychomotor development and recurrent infections. The severity of clinical expression is highly variable (► Ornithine and Proline Metabolism).

21.1 Hyperornithinaemia Due to Ornithine Aminotransferase Deficiency (Gyrate Atrophy of the Choroid and Retina)

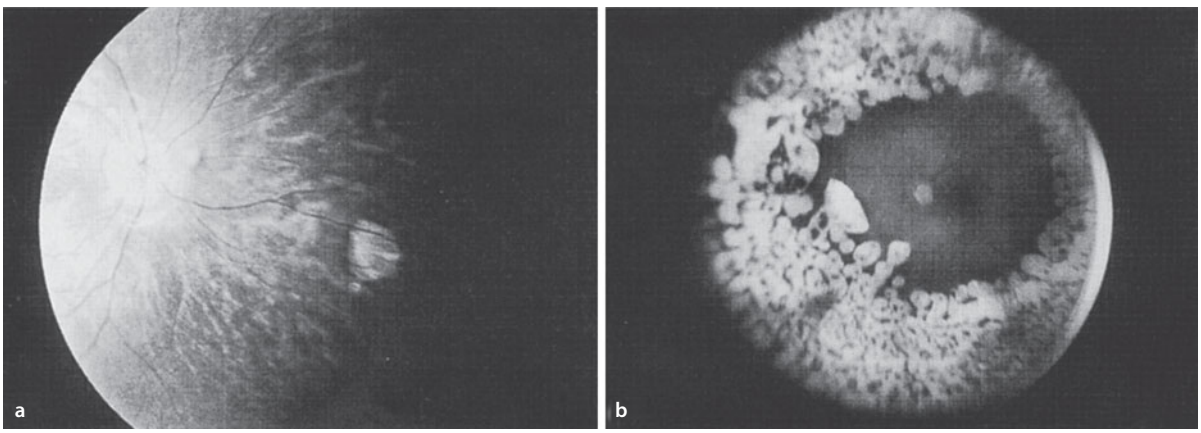
Clinical Presentation

The initial visual symptoms include myopia followed by night blindness and usually begin in early to mid-childhood [1]. Additional ophthalmological findings include constricted visual fields, posterior subcapsular cataracts with onset in the late teens, elevated dark adaptation thresholds and reduced or nondetectable electroretinographic (ERG) responses. Retinopathy can be detected before the patient notes visual disturbances. The fundoscopic appearance of the chorioretinal atrophy in gyrate atrophy is highly specific and is illustrated in ◘ Fig. 21.2.

The chorioretinal degeneration in gyrate atrophy is progressive, and most patients become virtually blind between the ages of 45 and 65. A few patients demonstrate a significant reduction in plasma ornithine levels in response to pharmacological doses of vitamin B6 and usually have a milder course and maintain central visual function at older ages. In general, intrafamilial variation in the extent and progress of the chorioretinal degeneration is much less than interfamilial variation. Vitreous haemorrhage causing sudden loss of vision is a rare complication. Most patients have normal intelligence consistent with other family members, although one report suggests an increased incidence of intellectual disability [1][2].

A few patients have presented in the neonatal period with poor feeding, failure to thrive, symptomatic hyperammonaemia and orotic aciduria mimicking OTC deficiency (► Chapter 19) [3][4].

Post-mortem histopathological study of the retina in a pyridoxine-responsive patient showed focal areas of photoreceptor atrophy with adjacent retinal pigment epithelial hyperplasia [1]. Electron microscope studies revealed abnormal mitochondria in the corneal endothelium and the non-pigmented ciliary epithelium and similar, but less severe, ab-



◘ **Fig. 21.2a, b** Fundoscopic appearances of the chorioretinal atrophy showing **a** early and **b** advanced changes

normalities in the photoreceptors. In addition to the ocular findings, systemic abnormalities have been reported in some patients. These include diffuse slowing on EEG, abnormal muscle histopathology, muscle weakness, abnormal ultrastructure of hepatic mitochondria and peculiar fine, sparse, straight hair with microscopic abnormalities [1]. Early degenerative and atrophic brain changes that were not age related were found by magnetic resonance imaging (MRI) of the brain, and evidence of peripheral nervous system involvement was noted in half the patients studied [5].

Metabolic Derangement

Beyond the neonatal period, patients develop hyperornithinaemia (fasting plasma ornithine in the range of 400–1200 μM) due to a deficiency of OAT activity. The enzyme deficiency has been demonstrated in liver, muscle, hair roots, cultured skin fibroblasts and lymphoblasts. The pathophysiological mechanism of the retinal degeneration is unclear. OAT requires pyridoxal phosphate (PLP) as a cofactor. In a few patients (<10%), fibroblast OAT activity increases significantly when assayed in the presence of high concentrations of PLP. Most of these patients also show a partial reduction (>30% of baseline fasting values on a constant protein intake diet) of plasma ornithine when given pharmacological doses of pyridoxine (vitamin B6).

Neonates who have presented with increased blood ammonia have low levels of plasma ornithine, citrulline and arginine and orotic aciduria in their first weeks of life, with hyperornithinaemia developing later in life [3][4]. One infant with OAT deficiency, diagnosed prenatally, had normal plasma ornithine and arginine in cord blood but developed reduced levels of these amino acids at 2–4 months of age on a normal diet, with concomitant increases in plasma ammonia and glutamine. Arginine administration corrected the low plasma arginine and hyperammonaemia, but produced hyperornithinaemia. This human phenotype is similar to, but less severe than, that of mice homozygous for targeted disruption of the *OAT* gene, which require arginine supplementation to survive the neonatal period [4]. These observations indicate that the net flux in the OAT reaction in the newborn period is in the direction of ornithine synthesis rather than ornithine degradation. Disruption of the anapleurotic function of the OAT reaction for the urea cycle, especially in patients whose dietary arginine is less than that required for growth, can lead to insufficient levels of citrulline and arginine, inadequate ureagenesis and consequent hyperammonaemia.

Children and adults with OAT deficiency have reduced levels of creatine in blood, urine, muscle and brain [5] as a result of ornithine inhibition of glycine transamidinase and the subsequent reduction of creatine biosynthesis (Fig. 21.1). Subnormal levels of serum creatinine reflect the reduction in total body creatine. In contrast to other series, which find normal intellect in adults [1], a series of seven French paediatric patients revealed a high prevalence of neurological impairment [2]. The authors speculated that these phenotypic features could be related to secondary brain creatine deficiency. This possibility should be carefully evaluated in future studies to consider possible complications of neonatal hyperammonaemia as an alternative explanation.

Genetics

OAT deficiency is an autosomal recessive disorder and has been described in patients from various ethnic backgrounds, but its incidence is highest in the Finnish population [1]. Intermediate levels of OAT activity are observed in skin fibroblasts from obligate heterozygotes for both pyridoxine-nonresponsive and pyridoxine-responsive variants.

More than 70 mutations have been defined in patients of various ethnic origins [1]. In Finns, one mutant allele, *OAT-L402P*, accounts for >85% of all *OAT* alleles and has only been described in individuals of Finnish origin [1]. Several other *OAT* alleles have been shown to be characteristic of specific populations.

Diagnostic Tests

The most prominent biochemical abnormality in those ingesting an unrestricted diet is a 5- to 20-fold elevation of plasma ornithine. Patients with the pyridoxine-responsive variant tend to have lower levels than those with the pyridoxine-nonresponsive variant, although this distinction is unreliable. Urinary excretion of ornithine and that of lysine, arginine and cystine is increased when plasma ornithine is 400 μmol/l or greater. These changes are secondary to competitive inhibition by ornithine of the common renal transport shared by these amino acids. Plasma ornithine levels are usually higher than those in the HHH syndrome, and the characteristic presence of homocitrulline in the urine in HHH differentiates these two hyperornithinaemic conditions. Neonatal OAT deficiency can be difficult to distinguish from OTC deficiency, as plasma levels of ornithine, arginine, and citrulline are reduced in both disorders and orotic acid is also increased. Since hyperornithinaemia is not present in all (or perhaps any) neonates with GA, newborn screening using this as a marker will be unreliable.

For confirmation of the diagnosis, molecular genetic analysis of *OAT* or direct assay of OAT activity can be performed in extracts of cultured skin fibroblasts or lymphoblasts. When the mutation is known, molecular analysis is appropriate for prenatal diagnosis and carrier detection.

Treatment and Prognosis

The goal of treatment has been to reduce plasma ornithine levels to less than 200 μM. Reduction of plasma ornithine can be achieved by dietary restriction of arginine (the precursor of ornithine in foods) [1]. On average, food proteins contain 4–6% arginine (nuts and seeds have higher arginine content). To limit arginine intake sufficiently to reduce ornithine accumulation, it is usually necessary to limit natural protein severely and supplement the diet with a mixture of essential amino acids to provide adequate nutrition. Care must be taken to avoid excessive arginine restriction, which will result in hypoargininaemia with associated poor growth and skin rash, and even hyperammonaemia, especially if total nitrogen intake is high. Thus, successful management of an arginine-restricted diet requires careful monitoring of growth,

physical examinations, nutritional status and plasma amino acid levels.

Arginine is an essential amino acid in patients with OAT deficiency. Infants with symptomatic hyperammonaemia or evidence of impaired waste nitrogen metabolism (hyperglutaminaemia, orotic aciduria) should be supplemented with arginine. Arginine intake in patients less than 3–4 months of age should not be restricted until plasma ornithine begins to increase.

Pharmacological dosage of pyridoxine HCl has resulted in plasma ornithine reduction in a small number of patients where doses between 200 and 500 mg a day lowered levels by between 25–60% [1]. A 2- to 4-week trial of pyridoxine treatment (300–500 mg/day) with no change in dietary protein intake and comparison of fasting plasma ornithine levels pre- and post-pyridoxine is recommended for all newly diagnosed patients, to determine their responsiveness.

Over 30 patients have been given a low-arginine diet in the long term, some in combination with pharmacological doses of pyridoxine. Compliance with diet restriction and long-term commitment and motivation are important factors influencing the outcome. A series of 17 patients on an arginine-restricted diet had plasma ornithine levels in the range of 400–500 µmol/l and showed slower loss of visual function after 13.9 years than 10 patients not on the diet [6]. Long-term substantial reduction of plasma ornithine levels started at an early age may be beneficial in slowing the progression of chorioretinal lesions and loss of retinal function. In a study of two sets of siblings with GA who were treated with an arginine-restricted diet for 16 to 17 years, each younger sibling, who was prescribed the diet at an earlier age, demonstrated a dramatic reduction in progression of lesions compared with the older sibling [7]. One patient was unable to tolerate the semisynthetic low-arginine diet and was treated with a natural food low-protein diet (0.8 g/kg/day) for 26 years, with moderate reduction of plasma ornithine levels and delayed progression of chorioretinal degeneration [8].

The effects of the above therapeutic measures on vision late in life have yet to be assessed. A study of a knockout mouse model for OAT deficiency has shown that a trial of dietary arginine restriction completely prevented the appearance of retinopathy at the age when untreated mice developed GA [4]. This observation validates the efficacy of reduction in ornithine accumulation by arginine restriction and emphasizes the importance of early diagnosis and early treatment.

Other therapeutic approaches applied in small numbers of patients have included supplementation of proline [6], creatine [9] and lysine [1]. Creatine supplementation corrected the muscle histopathology and phosphocreatine deficiency as measured by NMR, but did not have an obvious symptomatic effect; nor did it halt the progression of retinal degeneration.

Children born to women with OAT deficiency on an unrestricted diet appear to have no adverse effects of exposure to hyperornithinaemia. In multiple instances, it has been possible to manage an arginine-restricted diet successfully over a pregnancy in affected women [6]. As in other disorders with amino acid accumulation, these women will have an increasing requirement for the restricted amino acid (arginine) in the last trimester and must be followed carefully with weight checks, nutritional measures and plasma amino acid levels. Hyperammonaemia in neonates with OAT deficiency responds to standard treatment, and particularly to arginine supplementation.

21.2 Hyperornithinaemia, Hyperammonaemia and Homocitrullinuria (HHH) Syndrome

Clinical Presentation

The clinical manifestations in the HHH syndrome cover a broad spectrum, with some related to episodic hyperammonaemia (Table 21.1) [10]. Intolerance to protein feeding, vomiting, seizures and developmental delay from infancy are common complaints. Neonatal onset of lethargy, hypotonia and seizures, with progression to coma and death has been observed in the most severe form [10]. Persistent or recurrent liver dysfunction was the presenting symptom in over a third of patients in a series of French-Canadian patients [11]. Also, severe but reversible hepatocellular necrosis and acute hepatitis-like episodes and coagulopathy, especially factor VII and X deficiencies, have been reported [10][11], sometimes in the absence of overt hyperammonemia, suggesting that HHH syndrome should be added to the list of metabolic disorders causing liver failure. HHH syndrome may also present a more chronic and slowly progressive course, characterized by an aversion to protein rich foods, variable intellectual impairment or mental regression and signs of motor dysfunction with no obvious relationship to compliance with treatment [10]. Regardless of the age and type of onset, most patients present with progressive neurological dysfunction, mainly characterized by pyramidal tract signs with spastic gait, associated with cerebellar symptoms; seizures, mainly myoclonic, are also frequently observed [10]. Retinal abnormalities and abnormal neuroimaging studies, including stroke-like lesions, have also been described [12]. Mildly affected adult patients may have apparently normal intelligence but may display behavioural or psychiatric disturbances with protein intolerance.

Metabolic Derangement

Patients with the HHH syndrome have a marked elevation of plasma ornithine associated with hyperammonaemia and increased urinary excretion of homocitrulline. The HHH syndrome is a disorder of metabolic compartmentation, with impaired importation of ornithine into the mitochondria (Fig. 21.1), resulting in a functional deficiency of both OTC and OAT activities (Table 21.1). The intramitochondrial deficiency of ornithine leads to utilisation of carbamoylphosphate by pathways other than that catalysed by OTC, including formation of homocitrulline from lysine (Fig. 21.11) and formation of orotic acid secondary to excess flux down the pyrimidine biosynthetic pathway (▶ Chapter 35, ▶ Fig. 35.3).

Table 21.1 Differential diagnosis of disorders involving ornithine and proline metabolism

	OAT	HHH	P5CS	PYCR1	PYCR2	POX	P5CDH	Prolidase
Inheritance	AR	AR	AR/AD	AR	AR	AR	AR	AR
Retina degeneration	+ gyrate atrophy	+/-						
Episodic lethargy/coma	neonates only	+						
Liver dysfunction		+						
Developmental delay/MR	+/-	+	+	+	+	+/-	+/-	+/-
Seizures		+				+/-	+	-
Cerebellar ataxia		+						
Hypomyelination					+			
Thin corpus callosum			+/-	+/-	+			
Pyramidal signs/spastic paraparesis		+	+/-	+/-	+/-			
Dysmorphisms			+	+	+/-	+/-		+
Progeroid appearance			+	+				
Microcephaly			+/-	+/-	+			
Cataract			+	+				
Lax and wrinkled skin			+	+				
Joint laxity			+	+	+/-			
Visible veins			+	+				
Skin ulceration								+
Fever/infections								+/-
Immune dysfunction								+/-
Plasma ammonia	↑ neonates only	↑ +/-	↑ +/-					
Plasma ornithine	↑ +	↑ +	↓ (+/-)					
Plasma proline			↓ +/-			↑ +	↑ +	
Plasma citrulline/arginine		↓ +/-	↓ (+/-)					
Homocitrullinuria	+/-	+						
Orotic aciduria	neonates only	+/-						
Iminopeptiduria								+

OAT, ornithine aminotransferase deficiency; *HHH*, hyperornithinaemia, hyperammonaemia, homocitrullinuria syndrome; *PC5S*, Δ1-pyrroline-5-carboxylate synthase deficiency; *PYCR1*, Δ1-pyrroline-5-carboxylate reductase 1 deficiency; *PYCR2*, Δ1-pyrroline-5-carboxylate reductase 2 deficiency; *POX*, proline oxidase deficiency; *P5CDH*, Δ1-pyrroline 5-carboxylate dehydrogenase deficiency; *MR*, mental retardation. +, characteristic finiding; +/-, frequently seen; (+/-), less frequently seen; ↑, elevated; ↓, reduced.

Genetics

The HHH syndrome is a panethnic disease with over 100 patients reported [24]. The disease has been reported to be more frequent in Canada, as a result of a founder mutation in Quebec [13], in Italy and in Japan. Inheritance is autosomal recessive. The gene (*ORNT1* or *SLC25A15*) encoding the transporter protein ORC1 is located at 13q14. The common mutant allele in patients of French-Canadian origin is F188Δ, a 3-bp inframe deletion [13]. Even in homozygotes for this deletion there is considerable phenotypic variability, and also for other patients there is no clear-cut genotype-phenotype correlation [11][14]. The R197X mutation has been reported in multiple Japanese patients [15]. Obligate heterozygotes are clinically normal and cannot be identified by biochemical studies.

Diagnostic Tests

The HHH syndrome can be differentiated from other hyperammonaemic syndromes by laboratory findings (Table 21.1). The triad of hyperornithinaemia, hyperammonaemia and homocitrullinuria is pathognomonic. Homocitrulline can be mistaken for methionine in some amino acid analysers (Table 21.1). The plasma ornithine concentration is elevated to 3 to 10 times normal and tends to be somewhat lower than that seen in OAT deficiency. Despite a functional deficiency of OTC activity, plasma citrulline reduction is less pronounced than in OTC deficiency.

In addition to homocitrullinuria, urine amino acid screening shows increased ornithine and hyperdibasic amino aciduria when the plasma ornithine concentration is above 400 μmol/l. At lower plasma ornithine concentrations, homocitrullinuria may be the only urine amino acid abnormality. Furthermore, excessive homocitrulline excretion is observed in infants ingesting certain artificial formulas and may also be formed during heating of milk [16]. Persistent homocitrullinuria without a dietary source is abnormal and has also been detected in hyperlysinaemia. Orotic aciduria is common in HHH and can be induced by allopurinol challenge, as in patients with primary OTC deficiency (▶ Chapter 19).

In a few countries, HHH syndrome is part of the expanded newborn screening program. However, it may be missed because some affected neonates may not show elevated plasma ornithine levels in the first days of life [17].

The metabolic defect can be detected with an assay measuring ^{14}C-L-ornithine incorporation into protein using fibroblast monolayers [18]. Fibroblasts from patients with either OAT deficiency or HHH syndrome fail to incorporate ^{14}C derived from ornithine into protein, but OAT activity distinguishes the two disorders. The method of choice for prenatal diagnosis in couples of known genotype is mutation analysis.

Treatment and Prognosis

Treatment is aimed at preventing ammonia toxicity and, during episodes with hyperammonaemia, follows the principles outlined for the urea cycle disorders (▶ Chapter 19). In general, a low-protein diet combined with citrulline or arginine supplementation have been effective in achieving biochemical control for most patients. In some patients, ammonia scavengers such as sodium benzoate and/or sodium phenylbutyrate are used to improve metabolic control. Treatment may not prevent the late development of spastic gait, although the authors' personal experience includes multiple patients who have been treated with citrulline supplementation and mild dietary protein restriction for more than 20 years with no progression of neurological abnormalities.

Prognosis is variable, ranging from mild neurological involvement to a severely disabling disease; mortality is relatively low and treated patients are usually metabolically stable and do not experience relapses of hyperammonaemia [10].

Successful pregnancies have been reported in HHH syndrome [10]. However, hyperammonaemia during pregnancy and post partum is a potential risk in women with the HHH syndrome. It is thus advisable to exercise caution in the postpartum dietary management of HHH patients. Offspring of both women and men with HHH syndrome have been apparently normal.

21.3 Δ1-Pyrroline-5-Carboxylate Synthetase Deficiency

Clinical Presentation

The first two patients aroused clinical attention in early infancy because of developmental delay, lax skin and joints, muscular hypotonia and failure to thrive [19]. Bilateral subcapsular cataracts were noted at 4 years in the boy and at 20 months in his younger sister. Both siblings showed progressive deterioration in mental and motor skills after the age of 5 years, resulting in severe mental retardation. The patients had severe hypotonia, dystonia of hands and feet, muscular wasting of limbs, pyramidal syndrome and peripheral, predominantly axonal, neuropathy with progressively decreasing motor nerve conduction velocity, which left them unable to walk before reaching the ages of 15 and 21. In the last decade 19 patients from 10 additional families with the autosomal recessive neurocutaneous form of the disease have been described. Typical features included failure to thrive, hypotonia and severe developmental delay with cognitive impairment, associated with progeroid features, cutis laxa, joint hyperlaxicity, hip dislocation, adducted thumbs, cataracts, short stature, spasticity and often microcephaly [20][21][22][23]. Cutis laxa is not an obligate sign and may improve or disappear with age [20]. Other less consistent features included intrauterine growth retardation, corneal clouding, retinitis pigmentosa, visible skin veins, kinky tortuosity of brain vessels, abnormal fat pads and cerebellar atrophy. Recently, recurrent de novo mutations affecting residue Arg138 of P5CS were shown to cause a progeroid form of autosomal-dominant cutis laxa [24].

Finally, an adult onset dominant form of the disease, characterized by hereditary spastic paraplegia (HSP), was described in 3 families and 2 sporadic cases [22]. All of the 15 reported patients presented with pure or complex HSP with onset in late adolescence or adulthood. These findings were corroborated in a further two families with SPG9, a rare form of autosomal dominant HSP complicated with vomiting and congenital cataracts [25], one family segregating the same mutation as found in the original paper [22].

Metabolic Derangement

The metabolic phenotype described in many but not in all patients includes hypoornithinaemia, hypocitrullinaemia, hypoargininaemia, hypoprolinaemia and mild hyperammonaemia (Table 21.1), a pattern of metabolic abnormalities consistent with impaired proline and ornithine synthesis due to deficiency of Δ^1-pyrroline-5-carboxylate synthetase (P5CS). This enzyme catalyses an essential step in the pathways by which proline, ornithine and arginine are synthesised from glutamate (Fig. 21.1). Abnormal profiles were only reported in patients with mutations affecting the glutamate-

5-kinase domain or with a complete abolition of protein expression. In autosomal dominant families the most striking anomaly were very low citrulline levels regardless of the domain affected by the mutation. As in the original patients glutamine loading tests confirmed a metabolic block at the level of P5CS *in vivo* in fibroblast cultures from two related subjects with the dominant form of the disease [19][22]. The cellular phenotype is characterized by diminished production of collagen type I and III, altered elastin structure and diminished cell proliferation of cultured fibroblasts [23]. In connective tissue there is a high proline requirement for collagen synthesis. Deficient proline synthesis may impair protein synthesis in the lens epithelium and/or fibrocytes, and it is also possible that P5C metabolism contributes to the antioxidant defence of the lens. P5CS activity is present in the brain, and proline is thought to act as an inhibitory neurotransmitter in the CNS. Thus, impaired synthesis of proline is consistent with many of the clinical abnormalities in these patients, such as lax joints and skin, cataracts and neurodegeneration [26]. Regarding the pyramidal signs that appear in the course of the autosomal recessive disease and are the hallmark of the dominant form, it has been speculated that a decrease in the mitochondrial pool of ornithine may be responsible for motor neuron degeneration [22]. This pathomechanism may be shared with HHH syndrome and arginase deficiency, both of which are also associated with low mitochondrial ornithine and spastic paraplegia in adults.

The paradoxical fasting hyperammonaemia reported in one of the original patients is consistent with a relative deficiency of ornithine limiting ureagenesis and ammonia detoxification in the liver. Following a meal, arginine derived from dietary protein temporarily corrects this deficit by producing ornithine through arginase and thus enhancing urea cycle function, with the result that plasma ammonia decreases despite the nitrogen load in the meal. Notably, in this special situation, arginine becomes an essential amino acid. Ammonia was either normal or not measured in the other patients.

Genetics

Biallelic mutations in *ALDH18A1* encoding P5CS cause the early onset autosomal recessive neurocutaneous form. Most patients carry missense mutations that are scattered over both, the γ-glutamyl kinase and the γ-glutamyl phosphate reductase domain of the protein [22]. Recurrent de novo mutations of Arg 138 cause autosomal dominant cutis laxa while monoallelic mutations affecting specific residues are associated with the autosomal dominant HSP form [22][24][25].

Diagnostic Tests

Since the abnormal metabolite profile is corrected in the fed state, the metabolic phenotype of P5CS deficiency is easily missed. The combination of low fasting levels of ornithine, citrulline, arginine and proline plus a tendency to paradoxical fasting hyperammonaemia or one of the above together with a clinical phenotype of mental retardation, connective tissue manifestations and/or cataracts should suggest this disorder (Table 21.1). In the autosomal dominant adult form of the disease, citrulline may be a potential trait biomarker as the associated phenotype is not distinct from other causative genes.

P5CS activity is undetectable in control fibroblasts. Ornithine loading tests in the reported siblings resulted in transient partial correction of proline, citrulline and arginine concentrations, and indirect enzyme studies showed reduced proline biosynthesis in fibroblasts [19][22], corroborating the biological significance of the metabolic block at the level of P5CS in vivo.

Treatment and Prognosis

Supplementation of the deficient amino acids seems to be a reasonable therapeutic approach. However, administration of ornithine in the two reported siblings at a late stage of the disease did not result in any clinical improvement. Early recognition would allow the opportunity for a therapeutic trial with a combination of amino acids, such as citrulline, arginine, ornithine and proline.

21.4 Δ1-Pyrroline-5-Carboxylate Reductase Deficiency 1 (PYCR1) and 2 (PYCR2)

Mutations in the gene encoding P5C reductase (*PYCR1*) cause autosomal recessive cutis laxa with progeroid features and psychomotor retardation supporting the assumption of a significant role for proline biosynthesis in connective tissue and in normal intellectual development [27][28]. Clinical features include lax and wrinkled skin apparent at birth, joint hyperlaxity, distal arthrogryposis, characteristic facial features including a triangular face, short nose, long philtrum, and large ears, mild to moderate microcephaly, and mental retardation [29]. Cells and tissues from these individuals display increased apoptosis in response to oxidative stress [27]. Serum proline levels in these patients were normal.

Interestingly, the clinical manifestations observed in patients with *PYCR1* mutations are similar to those seen in P5CS deficient patients and often fit the diagnosis of De Barsy syndrome (MIM 219150) [30].

Recently, mutations in *PYCR2,* encoding the isoenzyme of PYCR1 have been described in children with microcephaly and hypomyelination [31].

21.5 Proline Oxidase Deficiency (Hyperprolinaemia Type I)

Clinical Presentation

Hyperprolinaemia type I is a rare disorder which appears to be well tolerated in some individuals, but in others may contribute to risk for schizophrenia or other psychiatric, cognitive or behavioural abnormalities [32][33][34].

Metabolic Derangement

Hyperprolinaemia type I is caused by a deficiency of proline oxidase (a mitochondrial inner-membrane enzyme), which

catalyses the conversion of proline into P5C (Fig. 21.1). Hence, in hyperprolinaemia type I, there are increased levels of proline in plasma (usually not above 2000 μM; normal range 100–450 μM), urine and cerebrospinal fluid (CSF). Hyperprolinaemia (as high as 1000 μM) is also observed as a secondary phenomenon in hyperlactataemia, possibly because proline oxidase is inhibited by lactic acid. Remarkably, and in contrast to hyperprolinaemia type II, heterozygotes may have mild hyperprolinaemia.

Of note, neonatal hyperprolinaemia mimicking hyperprolinaemia type I has recently been found in mild forms of glutaric aciduria type 2 [35]. Hyperprolinaemia was also a feature in 3 cases with *SLC25A22* mutations (▶ Chapter 29) [36].

Genetics

The mode of inheritance is autosomal recessive. *PRODH*, the gene encoding proline oxidase, maps to 22q11, in the region deleted in the velocardiofacial syndrome/DiGeorge syndrome. Numerous missense mutations have been identified, not all of which are associated with enzyme deficiency [33][37].

Diagnostic Tests

The diagnosis is made by amino acid analysis. Direct enzyme assay is not possible, since the enzyme is not expressed in leukocytes or skin fibroblasts. Mutation analysis is thus necessary to confirm the diagnosis [37].

Treatment and Prognosis

Since the prognosis is generally excellent, dietary treatment is not indicated.

21.6 Δ1-Pyrroline-5-Carboxylate Dehydrogenase Deficiency (Hyperprolinaemia Type II)

Clinical Presentation

This is a relatively benign disorder, though a predisposition to recurrent seizures is highly likely [38].

Metabolic Derangement

Hyperprolinaemia type II is caused by a deficiency of pyrroline 5-carboxylate (P5C) dehydrogenase, a mitochondrial inner-membrane enzyme involved in the conversion of proline into glutamate (Fig. 21.1). Hence, in hyperprolinaemia type II there are increased levels of proline in plasma (usually exceeding 2000 μM; normal range 100–450 μM), urine and CSF, as well as of P5C. Heterozygotes do not have hyperprolinaemia. Evidence has been presented that the accumulating P5C is a vitamin B6 antagonist (owing to adduct formation) and that the seizures in this disorder may be due at least in part to vitamin B6 inactivation [39][40].

Genetics

This is an autosomal recessive disease. *ALDH4A1* mutations have been reported in at least four patients [32][41].

Diagnostic Tests

The accumulation of P5C in physiological fluids is used to differentiate between type II and type I hyperprolinaemia. This compound can be qualitatively identified by its reactivity with ortho-aminobenzaldehyde and can be quantitatively measured by several specific assays [32]. P5C dehydrogenase activity can be measured in skin fibroblasts and leukocytes.

Treatment and Prognosis

The benign character of the disorder does not justify dietary treatment (which, in any case, would be very difficult). Seizures are B_6 responsive.

21.7 Prolidase Deficiency

Clinical Presentation

Some 90 individuals with prolidase deficiency, also called iminopeptiduria, have been reported since 1968 [42][43][44]. The onset of clinical symptoms, which initially may not be dermatological, has been reported to occur anywhere between the first days of life and early adulthood. All patients showed skin lesions, either mild (face, palms, soles) or severe, and had recalcitrant ulceration, particularly on the lower legs. Eczematous lesions, hyperkeratosis, photosensitivity, telangiectasia, and purpura have also been reported. Other features included a characteristic face, impaired motor or cognitive development, recurrent infections, hepato- and splenomegaly, wasting of the small muscles of the hand, and chronic lung disease resembling cystic fibrosis [43]. Immunological abnormalities are common and include high serum concentrations of immunoglobulins, hyperimmunoglobulinaemia E, increased erythrocyte sedimentation rate, elevated transaminases, complement consumption, abnormal neutrophil chemotaxis, and presence of autoantibodies. Prolidase deficiency seems to be a risk factor for the development of systemic lupus erythematosus; patients with systemic lupus erythematosus should, where there is a family history or presentation in childhood, be specifically investigated for prolidase deficiency [43][45].

Metabolic Derangement

The hallmark biochemical finding is massive hyperexcretion of a large number of imidodipeptides (dipeptides with an N-terminal proline or hydroxyproline, particularly glycylproline). This is due to a deficiency of the exopeptidase prolidase (or peptidase D).

Genetics

Inheritance is autosomal recessive. At least 22 different pathogenic mutations have been identified [44].

Diagnostic Tests

The hyperimidodipeptiduria can be detected and quantified by partition and elution chromatography and by direct chemical ionisation mass spectrometry. The finding of low or absent prolidase activity in haemolysates or in homogenates of leukocytes or fibroblasts confirms the diagnosis.

Treatment and Prognosis

Owing to the rarity of the disease, experience with treatment is scarce. The skin ulcers improved with oral ascorbate, manganese (cofactor of prolidase), with an inhibitor of collagenase in one patient, and with local applications of L-proline- and glycine-containing ointments in others. Skin grafts have been unsuccessful [46]. As to prognosis, the age at onset and the severity of clinical expression are highly variable.

21.8 Spermine Synthase Deficiency (Snyder Robinson Syndrome)

Snyder Robinson syndrome is an X-linked disorder characterised by moderate to severe intellectual disability, unsteady gait, hypotonia and variable dysmorphism with osteoporosis. It is due to deficiency of spermine synthase, an enzyme in the ornithine decarboxylase pathway leading from putrescine to spermine (Fig. 21.1). An evocative metabolic signature including elevation of spermidine, isoputreanine, ornithine and N8-acetylspermidine, a novel potential biomarker, has recently been identified by metabolomics [47].

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Cerebral Organic Acid Disorders and Other Disorders of Lysine Catabolism

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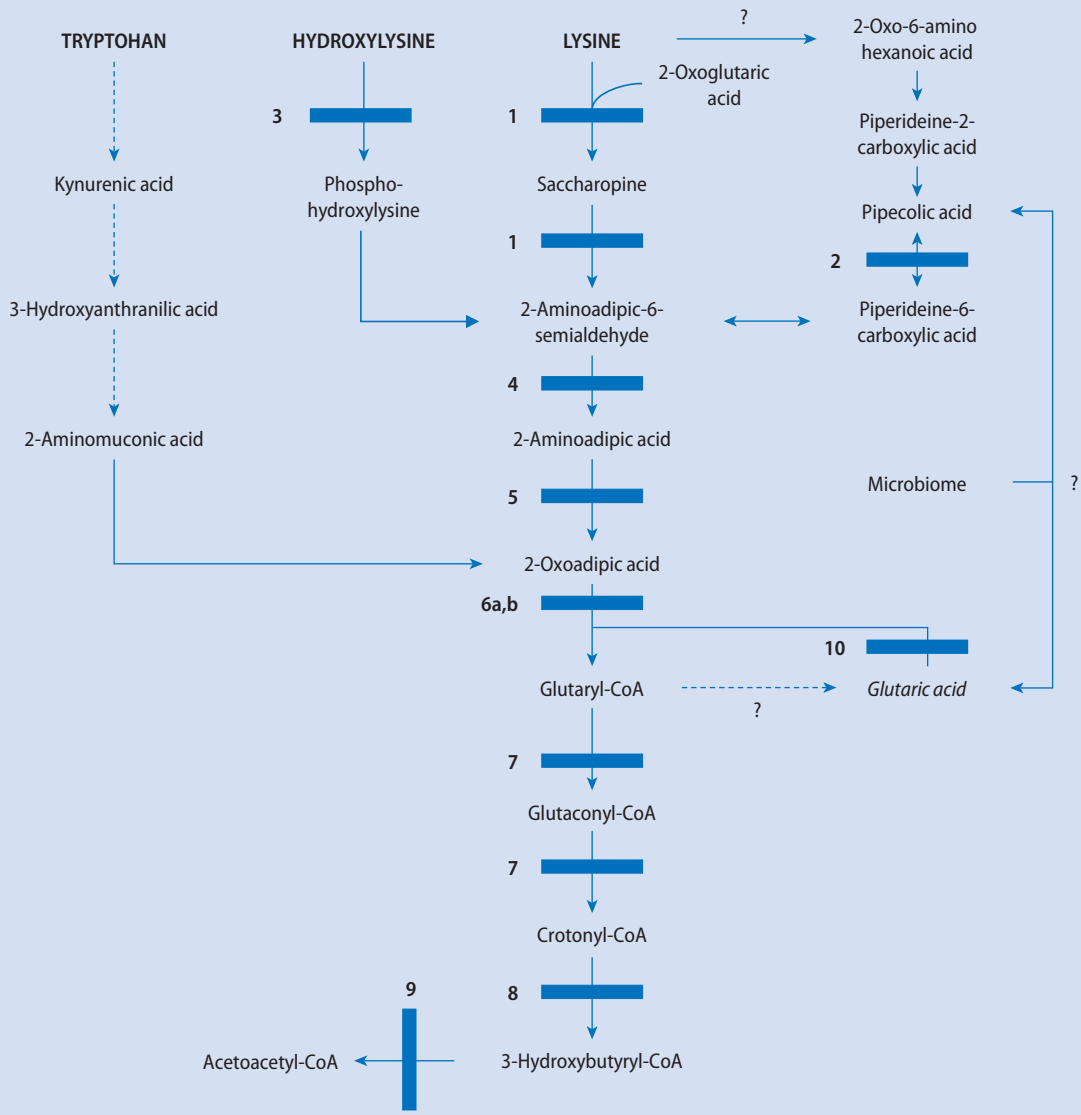
Catabolism of Lysine, Hydroxylysine, and Tryptophan

Species-, organ- and organelle-specific differences in the enzymes involved in the catabolism of lysine, hydroxylysine and tryptophan are not yet completely unravelled, and this synopsis is therefore partially hypothetical as far as human metabolism is concerned. Lysine, hydroxylysine and tryptophan are thought to be degraded within the mitochondrion, initially via separate pathways, which converge into a common pathway at the point of 2-aminoadipic-6-semialdehyde (hydroxylysine catabolism and pipecolic acid pathway of lysine catabolism) and at the point of 2-oxoadipic acid (tryptophan catabolism; ◘ Fig. 22.1). The major route of lysine catabolism in most tissues is via the bifunctional enzyme, 2-aminoadipic-6-semialdehyde synthase (enzyme 1). A small amount of lysine is catabolised via pipecolic acid and the peroxisomal key



◘ **Fig. 22.1 Tryptophan, hydroxylysine and lysine catabolic pathways.** *1*, 2-aminoadipic-6-semialdehyde synthase; *2*, pipecolic acid oxidase; *3*, hydroxylysine kinase; *4*, 2-aminoadipic-6-semialdehyde dehydrogenase (antiquitin); *5*, 2-aminoadipate aminotransferase; *6a*, 2-oxoglutarate dehydrogenase-like complex using DHTKD1 as E1 subunit; *6b*, alternatively, 2-oxoglutarate dehydrogenase complex, an enzyme of the Krebs cycle, has also substrate affinity for 2-oxoadipic acid; *7*, glutaryl-CoA dehydrogenase; *8*, short-chain enoyl-CoA hydratase 1 (crotonase); *9*, 3-hydroxyacyl-CoA dehydrogenase; *10*, succinate-hydroxymethylglutarate CoA-transferase. Enzyme deficiencies are indicated by solid bars across the arrows. Question marks indicate current uncertainties in the human lysine catabolic pathway

enzyme, pipecolic acid oxidase (enzyme 2); this pathway, however, is regarded as the major route of lysine catabolism in the brain. Unlike in bacteria, however, the human origin of pipecolic acid is not yet fully understood. An orthograde production of pipecolic acid from lysine but also retrograde production from 2-aminoadipic-6-semialdehyde was shown. In addition, the microbiome should be considered as an alternative source. Hydroxylysine enters the pathway after phosphorylation by hydroxylysine kinase (enzyme 3). 2-Aminoadipic-6-semialdehyde is converted into 2-aminoadipic acid by 2-aminoadipic-6-semialdehyde dehydrogenase (antiquitin, enzyme 4), which is then converted to 2-oxoadipic acid by 2-aminoadipate aminotransferase (enzyme 5). 2-Oxoadipic acid is primarily converted to glutaryl-CoA by the 2-oxoglutarate dehydrogenase-like complex (enzyme 6a) since its E1 subunit (DHTKD1) has a higher substrate affinity for 2-oxoadipic acid than the 2-oxoglutarate dehydrogenase complex in the Krebs cycle (enzyme 6b). 2-Oxoglutarate dehydrogenase complex, however, may serve as an alternative route. 2-Oxoadipic acid is dehydrogenated and decarboxylated to crotonyl-CoA by glutaryl-CoA dehydrogenase (enzyme 7). This enzyme tranfers electrons to flavin adenine dinucleotide (FAD) and hence to the respiratory chain via electron transfer protein (ETF)/ETF-dehydrogenase (ETF-DH). Crotonyl-CoA is subsequently converted to 3-hydroxybutyryl-CoA by short-chain enoyl-CoA hydratase 1 (ECHS1, (crotonase, enzyme 8, ▶ Chapter 18). This enzyme is multispecific and also acts as a crotonase in the degradative pathways of valine, isoleucine and short-chain fatty acids. 3-Hydroxybutyryl-CoA is then converted to acetoacetyl-CoA by 3-hydroxyacyl-CoA dehydrogenase (enzyme 9, ▶ Chapter 12).Glutaric acid which may derive from the intestinal microbiome, spontaneous disintegration of glutaryl-CoA or other sources, is normally reactivated by succinyl-CoA-dependent conversion of succinate-hydroxymethylglutarate CoA transferase to glutaryl-CoA (enzyme 10). From the six distinct enzyme deficiencies identified in the degradation of lysine, only enzymes 4, 6b, 7, and 8 have proven relevance as neurometabolic disorders. Glutaric aciduria type I is caused by deficient glutaryl-CoA dehydrogenase (enzyme 7). Glutaric aciduria type II, caused by ETF/ETF-DH deficiencies, is discussed in ▶ Chapter 12. Pipecolic acid oxidase (enzyme 2) is discussed with peroxisomal disorders in ▶ Chapter 40, 2-aminoadipic-6-semialdehyde dehydrogenase (antiquitin, enzyme 4) deficiency in ▶ Chapter 28, 2-oxoglutarate dehydrogenase deficiency (enzyme 6b) in ▶ Chapter 11, and ECHS1 deficiency (enzyme 8) in ▶ Chapter 18, since its major pathogenic effect is located in the valine catabolic pathway. Finally, several recent findings point to new functions for different lysine acetylating and deacylating enzymes and also highlight the mechanisms by which acetylation and other posttranslational modifications of lysine residues regulate various cellular processes. Human inborn errors of these processes are yet to be discovered.

L-2- and D-2-Hydroxyglutaric aciduria type I are caused by deficiencies of specific FAD-dependent dehydrogenases, whereas D-2-hydroxyglutaric aciduria type II is caused by deficient mitochondrial isocitrate dehydrogenase 2. Recently, D-2-/L-2-hydroxyglutaric acidurias was shown to be caused by inherited deficiency of the mitochondrial citrate carrier which mediates transport of dicarboxylic metabolites between the mitochondrion and the cytosol. Aspartoacylase (aminoacylase 2) irreversibly splits *N*-acetylaspartic acid (NAA), a brain-specific compound where its concentration reaches approximately 20 mM, into acetate and aspartate in oligodendrocytes (not illustrated). Deficiency of this enzyme causes *N*-acetylaspartic aciduria (Canavan disease). Recently, the molecular identity of the *N*-acetyltransferase (NAT) that catalyses NAA synthesis has been identified and found to be mutated in a patient with hypoacetylaspartia.

Thirteen inborn errors of metabolism are described in this chapter. Glutaric aciduria type I, L-2-hydroxyglutaric aciduria, D-2-hydroxyglutaric aciduria (type I and II), D-2-/L-2-hydroxyglutaric aciduria, N-acetylaspartic aciduria, hypoacetylaspartia, and aspartate-glutamate carrier 1 deficiency are all associated with neurological disease of varying severity whereas hyperlysinaemia/saccharopinuria, hydroxylysinuria, 2-amino-/2-oxoadipic aciduria, aminoacylase 1 deficiency and glutaric aciduria type III are likely non diseases or have an unclear clinical significance, although some patients are retarded and show variable neurological abnormalities (▶ Catabolism of Lysine, Hydroxylysine, and Tryptophan).

22.1 Introduction

A group of organic acid disorders presents exclusively with progressive neurological symptoms of ataxia, epilepsy, myoclonus, extrapyramidal symptoms, metabolic stroke, and macrocephaly [1]. The core cerebral organic acid disorders are glutaric aciduria type I, D-2-hydroxyglutaric aciduria (types I and II), L-2-hydroxyglutaric aciduria, D-/L-2-hydroxyglutaric aciduria, 4-hydroxybutyric aciduria (▶ Chapter 29: Neurotransmitters) and *N*-acetylaspartic aciduria. Strikingly, in all these disorders the pathological compounds that accumulate either are odd-chain dicarboxylic acids (D-2-, L-2-, 3-hydroxyglutarate, glutarate) sharing the same carbon backbone with the excitatory amino acid glutamate (2-amino-glutarate), or have been suggested to be neurotransmitters/-modulators (γ-hydroxybutyrate, *N*-acetylaspartylglutamate). Evidence is accumulating from *in vitro* and *in vivo* studies showing that these acids indeed interfere with important pathways of cerebral metabolism, including glutamatergic or gamma amino butyric acid (GABA)-ergic neurotransmission, cerebral energy metabolism, myelin metabolism and/or metabolic water homeostasis. Delayed myelination or progressive white matter disease, basal ganglia injury and cerebellum pathology, the main pathologies in cerebral organic acid disorders, are also characteristic of mitochondrial disorders, suggesting at least partial common pathological mechanisms.

Patients with cerebral organic acid disorders often suffer a diagnostic odyssey and may even remain undiagnosed.

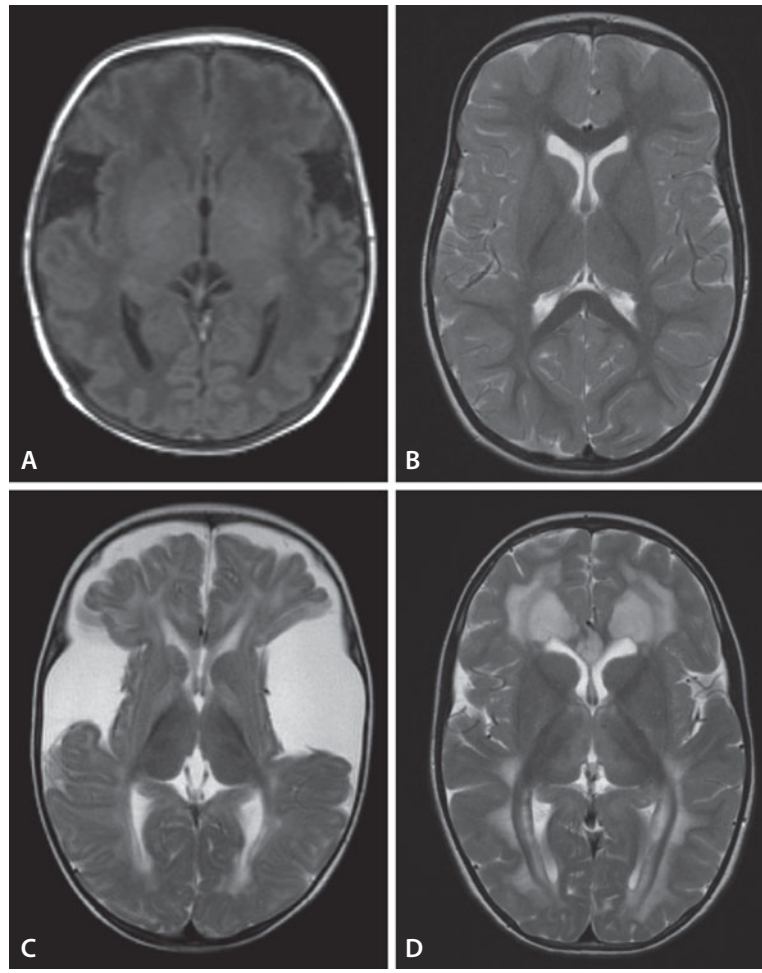


Fig. 22.2 A–D, MRI findings in patients with glutaric aciduria type I. A, T1-weighted axial MRI of an asymptomatic male newborn with glutaric aciduria type I, showing enlargement of temporopolar and frontopolar CSF spaces and an immature gyration pattern. **B,** T2-weighted axial MRI of an asymptomatic 2-year-old girl identified by newborn screening. Previously dilated external CSF spaces and temporal hypoplasia have normalised. There is no pathology of the basal ganglia. **C,** T2-weighted axial MRI at age 7.5 months showing striatal atrophy and markedly dilated temporopolar and frontopolar CSF spaces. Signal abnormalities of globus pallidus, thalamus, and supratentorial white matter are also found. This child presented with moderate axial hypotonia, which progressed after a delay in the start of emergency treatment during an infectious disease. After a further 4 weeks, the child also developed dystonia of all extremities. **D,** T2-weighted axial MRI of a girl at age 11 years with suspected late-onset disease variant showing marked hyperintensity of the supratentorial white matter sparing the U fibres and mild to moderate signal changes of the caudate, thalamus, and dentate nuclei (not shown). The girl presented with nausea and vertigo at 10 years of age, which has improved following the start of carnitine supplementation and a protein-controlled diet. Motor and cognitive function is normal. (By courtesy of Dr Inga Harting and Dr Angelika Seitz)

Among this disease group, only glutaric aciduria type I forms characteristic acylcarnitines (i.e. glutarylcarnitine), which can be used for mass screening of newborns by tandem mass spectrometry. Metabolic hallmarks such as hypoglycaemia, metabolic acidosis, lactic acidaemia or hyperammonaemia, the usual concomitants of ›classic‹ organic acid disorders (▶ Chapter 18), are generally absent. Furthermore, elevations of diagnostic metabolites may be small and therefore missed on ›routine‹ organic acid analysis. The correct diagnosis requires an increased awareness of these disorders by the referring physician as well as the biochemist in the metabolic laboratory. Diagnostic clues can be derived from neuroimaging findings (Fig. 22.2, Fig. 22.3). Progressive disturbances of myelination, cerebellar atrophy, cortical atrophy, signal changes and/or atrophy of the basal ganglia and any symmetrical (fluctuating) pathology apparently independent of defined regions of vascular supply are suggestive of cerebral organic acid disorders.

In contrast to the cerebral organic acid disorders, the other known defects of lysine and hydroxylysine degradation all appear to be rare biochemical variants of human metabolism without clinical significance.

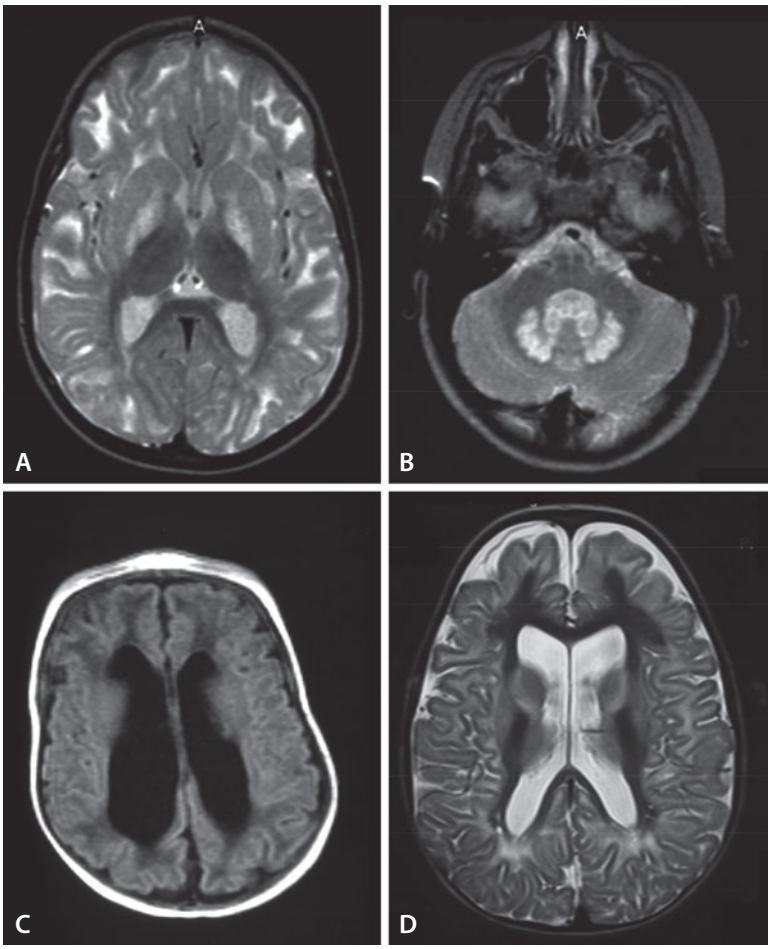


Fig. 22.3 A–D, MRI findings in patients with other cerebral organic acidurias. A & B, Axial T2-weighted MRI of a 8.5-year-old boy with L-2-hydroxyglutaric aciduria, illustrating characteristic involvement of subcortical white matter (also affecting the U fibres) and globus pallidus (A), and symmetrical involvement of the dentate nuclei (B). **C,** Axial MRI of a 2-month-old girl with D-2-hydroxyglutaric aciduria. Note the delayed myelination and considerable occipitally pronounced enlargement of lateral ventricles. **D,** Axial fast spin echo image of a 6.5-year-old girl suffering from N-acetylaspartic aciduria. Note the marked discrepancy between the severely affected subcortical white matter and the relatively spared central white matter, at least frontally

Increasing evidence points to a close link between metabolism and cell signalling via acetylation, succinylation, malonylation, glutarylation (among other biochemical modifications) of lysine residues of proteins. Lysine acylation preferentially targets large macromolecular complexes involved in cellular processes such as chromatin remodelling, cell cycle, splicing, nuclear transport, and actin nucleation. This interaction modifies mitochondrial function, enzyme activity and enables concerted adaptation to environmental changes. Therefore, lysine acylation is broad and similar to other major posttranslational modifications.

22.2 Hyperlysinaemia/Saccharopinuria

22.2.1 Clinical Presentation

Hyperlysinaemia/saccharopinuria appears to be a rare ›non-disease‹. About half of the identified individuals were detected incidentally and are healthy [2]. Symptoms have included psychomotor retardation, epilepsy, spasticity, ataxia and short stature. Individual patients have been described with joint laxity and spherophakia, respectively.

22.2.2 Metabolic Derangement

Hyperlysinaemia/saccharopinuria is caused by deficiency of the bifunctional protein 2-aminoadipic semialdehyde synthase (enzyme 1 in Fig. 22.1). This is the first enzyme of the

mitochondrial saccharopine pathway, which is the main route of lysine degradation in most tissues but not in the brain [3]. The minor relevance of this pathway in the brain may prevent humans from developing a neurological disease if 2-aminoadipic semialdehyde synthase is deficient. The two functions of this enzyme, lysine:2-oxoglutarate reductase and saccharopine dehydrogenase, may be affected differently by mutations. Most often, both activities are severely reduced, resulting predominantly in hyperlysinaemia and hyperlysinuria, accompanied by relatively mild saccharopinuria (hyperlysinaemia I). In hyperlysinaemia II/saccharopinuria, there is a relatively more pronounced decrease in saccharopine dehydrogenase activity, with residual activity of lysine:2-oxoglutarate reductase causing a predominant excretion of saccharopine.

Failure to remove the ε-amino group is thought to result in an overflow of the minor lysine degradation pathway, with removal of the α-amino group by oxidative deamination. The oxoacid cyclises and is reduced to pipecolic acid. As a consequence, hyperpipecolataemia is regularly observed in hyperlysinaemia. Unlike in bacteria, however, this orthograde production of pipecolic acid from lysine is not generally accepted, since the enzyme initiating this pathway has not yet been identified in man.

Hyperlysinuria can also result from impaired renal tubular transport, often as part of a genetic transport defect of dibasic amino acids (▶ Chapter 25). In this situation it occurs without hyperlysinaemia.

22.2.3 Genetics

Hyperlysinaemia/saccharopinuria follows an autosomal recessive inheritance. The gene has been characterised and a homozygous out-of-frame 9-bp deletion identified in an affected boy [3].

22.2.4 Diagnostic Tests

The initial observation in patients with hyperlysinaemia/saccharopinuria is an impressive lysinuria with up to 15,000 mmol/mol creatinine (controls <70). Detailed amino acid analysis reveals additional accumulation of saccharopine, homoarginine, 2-amino adipic acid and pipecolic acid [4]. Elevations of the same metabolites can be documented in other body fluids, such as plasma and cerebrospinal fluid (CSF), with high lysine as the predominant abnormality (up to 1700 μmol/l in plasma, controls <200, and up to 270 μmol/l in CSF, controls <28). Differential diagnosis includes hyperlysinaemias secondary to low 2-oxoglutarate availability as observed in urea cycle disorders (OTC deficiency: lysine up to 1400 μmol/l), pyruvate carboxylase deficiency (lysine up to 800 μmol/l), and also methylmalonic and propionic acidaemias [5].

The deficiency of 2-aminoadipic semialdehyde synthase can be ascertained in fibroblasts and tissue biopsies by determining the overall degradation of [1-^{14}C] lysine to $^{14}CO_2$. Specific assays for lysine:2-oxoglutarate reductase and saccharopine dehydrogenase have been described. Molecular diagnosis is available [3].

22.2.5 Treatment and Prognosis

Long-term dietary restriction of lysine has no benefit. As affected individuals do not suffer from metabolic decompensations, specific interventions during intercurrent illnesses are unnecessary. As hyperlysinaemia/saccharopinuria is a benign condition it is not associated with any increase in morbidity or mortality.

22.3 Hydroxylysinuria

Hydroxylysinuria and concomitant hydroxylysinaemia has been identified in a few patients, all of whom showed some degree of mental retardation [6]. No further clinical and/or biochemical studies were reported. The abnormality can be assumed to be caused by a defect of hydroxylysine kinase (enzyme 3 in ◘ Fig. 22.1).

22.4 2-Amino-/2-Oxoadipic Aciduria

22.4.1 Clinical Presentation

2-Amino-/2-oxoadipic aciduria is probably of no clinical significance. Over 20 individuals are known, more than half of whom are asymptomatic [7]. Symptoms include psychomotor retardation, muscular hypotonia, epilepsy, ataxia and failure to thrive, but it is likely that these are coincidental findings. Recently, nonsense mutation in *DHTKD1* have been associated with Charcot-Marie-Tooth disease type but this remains controversial and whether or not the mutation is causative remains to be confirmed [8].

22.4.2 Metabolic Derangement

The metabolic profile is heterogeneous, with most patients showing elevations of 2-aminoadipic, 2-oxoadipic and 2-hydroxyadipic acid, whereas some excrete only 2-aminoadipic acid. Isolated excretion of 2-aminoadipic acid may be caused by vigabatrin treatment via inhibition of 2-aminoadipate aminotransferase. 2-Aminoadipic acid is deaminated to 2-oxoadipic acid by a mitochondrial 2-aminoadipate aminotransferase. 2-Oxoadipic acid is also thought to be formed from the degradation of tryptophan, but this is not yet fully understood in humans. 2-Oxoadipic acid is further metabolised to glutaryl-CoA via two distinct enzyme complexes: The major pathway involves 2-oxoglutarate dehydrogenase-like (OGDHL) complex which contains dehydrogenase E1 and transketolase domain-containing protein 1 (DHTKD1) as E1 subunit. DHTKD1 has a high affinity for 2-oxoadipic acid. Alternatively, 2-oxoadipic acid can be handled by the oxoglutarate dehy-

drogenase complex (OGDHc). However, OGDHc has a higher affinity for 2-oxoglutaric acid than for 2-oxoadipic acid and a lower affinitiy for 2-oxoadipic acid than OGDHL.

22.4.3 Genetics

Autosomal recessive inheritance is implied by the pedigrees and by the finding that parents cannot be biochemically differentiated from controls. In 2012, pathogenic mutations in the *DHTKD1* gene localized on 10p14 were identified as molecular cause of 2-aminoadipic/2-oxoadipic aciduria [9].

22.4.4 Diagnostic Tests

Affected individuals are diagnosed by demonstrating variable elevations of 2-aminoadipic acid on amino acid chromatography and/or of 2-oxoadipic and 2-hydroxyadipic acids on urinary organic acid analysis. Plasma lysine may be twofold elevated and urinary glutaric acid up to 50 mmol/mol of creatinine (controls <9) [4].

22.4.5 Treatment and Prognosis

As 2-amino-/2-oxoadipic aciduria is likely to be a non-disease; it does not determine morbidity or mortality. Patients do not suffer from metabolic decompensations, and specific interventions during intercurrent illnesses do not appear necessary. Administration of pharmacological doses of vitamins B_1 and B_6 had no effect on the levels of pathological metabolites. Dietary restriction of lysine also failed to correct the biochemical abnormalities in some patients and has no proven long-term benefit.

22.5 Glutaric Aciduria Type I

22.5.1 Clinical Presentation

Glutaric aciduria type I should be seriously considered in the differential diagnosis of any infant who has macrocephaly combined with progressive atrophic changes on magnetic resonance imaging (MRI) or computerised tomography (CT; ◘ Fig. 22.2) and/or a complex extrapyramidal syndrome of predominantly dystonia, orofacial dyskinesia and dysarthria superimposed on axial hypotonia. Choreic movements may also be observed [10][11]. In many patients macrocephaly is present at or shortly after birth and precedes the severe neurological disease. An important clue to early diagnosis is the observation of pathologically increased head growth crossing the percentiles and peaking at the age of 3–6 months. Furthermore, affected babies often present additional ›soft‹ neurological symptoms of hypotonia with prominent head lag, irritability, jitteriness and feeding difficulties. Neonatal posture and tone may persist until 6 months of age. During febrile illnesses or after immunisations, muscular hypotonia is often aggravated and unusual hand movements and postures appear. All these signs are usually reversible and of little prognostic significance. Neuroimaging studies have been performed in a number of asymptomatic newborns and infants, revealing the characteristic findings of temporal hypoplasia (95% of all patients; ◘ Fig. 22.2), wide anterior temporal and sylvian CSF spaces, an immature gyration pattern, delayed myelination, and isolated T_2 hyperintensity in the globus pallidus [12]. These extrastriatal MRI abnormalities may completely resolve if treatment is started in the newborn period (◘ Fig. 22.2). The clinical significance of enlarged subdural fluid spaces in infants with glutaric aciduria type I is the unprotected crossing of these spaces by bridging veins. Such infants are prone to suffer acute subdural haemorrhages including retinal haemorrhages after minor head trauma, particularly around the first birthday when starting to walk. Parents of children with glutaric aciduria type I have been wrongly charged with child abuse because of chronic or acute subdurals and/or retinal haemorrhages [13]. Alternatively, vascular abnormalities in glutaric aciduria type I have been explained by altered haemodynamics [14].

At an average age of around 9 months the majority of untreated patients suffer an acute brain injury, usually associated with an upper respiratory and/or gastrointestinal infection, but this encephalopathic crisis may also develop in association with fasts required for surgery, or after routine immunisations [11]. MRI reveals striatal injury spreading in a dorsoventral direction (◘ Fig. 22.2). Almost all reported encephalopathic crises have occurred by 36 months of age. They have not yet been described at school age, during adolescence or in adulthood. Neurological functions are often acutely lost, including the ability to sit and to pull up to standing, head control, and suck and swallow reflexes. The infants appear alert with profound hypotonia of the neck and trunk, stiff arms and legs and twisting (athetoid) movements of hands and feet. There may also be generalised seizures. Usually there are no metabolic derangements. Then a severe dys-/hypotonic movement disorder develops. At this point the distinctive clinical picture of a dystonic-dyskinetic syndrome in an alert-looking child with relatively well-preserved intellectual functions and a prominent forehead may be recognised. If the underlying metabolic disorder remains undiagnosed, additional cerebral systems are slowly but progressively affected. Impaired chewing and swallowing, vomiting and aspiration, plus increased energy demand due to increased muscle tone frequently results in failure to thrive and malnutrition. Kyphoscoliosis and chest wall dystonia can cause restrictive lung disease. Early death (40–50% of symptomatic patients by the age of 20 years) may occur in the course of intercurrent pneumonia and respiratory failure, during hyperpyrexic crises or suddenly without warning.

Although the majority of patients present with characteristic symptoms and disease course, the natural history of glutaric aciduria type I can be variable even within families. A minority of patients have developmental delay from birth and (progressive) dystonic cerebral palsy. This so-called insidious-onset variant may thus reflect an intrauterine or perinatal neu-

ropathology that can become clinically apparent with a delay of weeks or months [12][13][15]. A few individuals, mainly diagnosed in adolescence or adulthood during family studies, have not developed neurological disease despite never having been treated. Finally, late-onset-type glutaric aciduria type I has been described in previously unaffected adolescent/adult patients presenting with signal changes in the white matter but unaffected basal ganglia [12], suggesting an additional disease variant (Fig. 22.2). Recent MRI studies, however, demonstrate that white matter changes are a common finding in glutaric aciduria type I. They progress with age, are more often found in high than in low excretors and of yet unclear clinical relevance [13][16].

22.5.2 Metabolic Derangement

Glutaric aciduria type I is caused by a deficiency of glutaryl-CoA dehydrogenase, a mitochondrial flavin adenine dinucleotide-requiring enzyme, which catalyses the dehydrogenation of glutaryl-CoA as well as the subsequent decarboxylation of glutaconyl-CoA to crotonyl-CoA (▶ enzyme 7 in Fig. 22.1). In glutaric aciduria type I, part of the accumulating glutaryl-CoA is esterified with carnitine to glutarylcarnitine by carnitine acyltransferase, leading to an increased ratio of acylcarnitines to free carnitine in plasma and urine. Glutarylcarnitine is excreted, contributing to secondary carnitine deficiency. Patients with glutaric aciduria type I often show increased urinary excretion of dicarboxylic acids, 2-oxoglutarate and succinate indicating of disturbed mitochondrial function.

The mechanisms of age-specific destruction of specific cerebral structures in glutaric aciduria type I have been a subject of intense debate and generated different hypotheses. The most substantiated evidence points to impaired brain energy metabolism induced by accumulating glutaric acid, 3-hydroxyglutaric acid and glutaryl-CoA: glutaryl-CoA inhibits the 2-oxoglutarate dehydrogenase complex, glutaric acid impairs the dicarboxylic acid shuttle between astrocytes and neurons, and 3-hydroxyglutaric acid is thought to weakly activate glutamatergic neurotransmission [17][18]. Accumulation of these putatively dicarboxylic neurotoxins in the brain is facilitated by the weak permeability of the blood-brain barrier for dicarboxylic acids, causing ›trapping‹ of these metabolites in the brain compartment of patients [18]. It has been suggested that disturbed cerebral haemodynamics, such as disturbed autoregulation and regional perfusion pressure gradients, adds to the metabolic toxicity of this disease [14].

22.5.3 Genetics

Glutaric aciduria type I is an autosomal recessive disorder caused by pathogenic mutations in *GCDH*. Results of newborn screening programmes in various regions and cohorts worldwide give an overall mean frequency of 1:100,000 [15]. The disease is much more frequent in certain communities, such as the Amish people in Pennsylvania (homozygous for p.Ala421Val, incidence of 1 in 300–400 newborns), the Oji-Cree First Nations in Canada (homozygous for the splice site mutation IVS-1+5 g>t, incidence of 1 in 300 newborns) and the Irish travellers (homozygous for p.Glu365Lys).

More than 200 different disease-causing mutations in the *GCDH* gene have been identified so far [19]. There is a correlation between genotype and biochemical phenotype in that specific mutations with significant residual enzyme activity may be associated with low excretions of metabolites. However, no correlation between genotype and clinical phenotype has yet been found [11]. Single common mutations are found in genetically homogenous communities (see above), but glutaric aciduria type I is in general genetically quite heterogeneous: the most frequent mutation in Caucasians, p.Arg402Trp, has been identified on 10–20% of alleles [19].

22.5.4 Diagnostic Tests

Patients with glutaric aciduria type I have generally been diagnosed by urinary organic acid analysis [20]. Repeated and quantitative urinary organic acid analyses may be necessary. Additional diagnostic hints can be obtained by finding carnitine deficiency in plasma and/or a pathologically increased ratio of acylcarnitines to free carnitine in plasma and urine. Elevations of glutarylcarnitine in body fluids of patients can be detected through acylcarnitine analysis [21]. Application to analyses of blood spots (Guthrie cards) has enabled the inclusion of glutaric aciduria type I into the newborn screening programmes of a growing number of countries. However, individuals with deficiency of glutaryl-CoA dehydrogenase and severe characteristic neurological disease but with only slight or inconsistent elevations of glutaric acid or glutarylcarnitine have been diagnosed in increasing numbers. Patients with this phenotype have been referred to as low excretors [20]. Furthermore, elevated urinary excretion of glutaric acid can also be found in a number of other disease states, mostly related to mitochondrial dysfunction and renal failure. Quantitative analysis of 3-hydroxyglutaric acid in urine has a high sensitivity including patients with the low-excretor phenotype and those having secondary carnitine depletion [20]. However, it is known that elevated 3-hydroxyglutaric acid is not absolutely specific for glutaric aciduria type I but is also found in patients with short-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (▶ Chapter 12) and in patients with severe ketosis.

Loading tests, e.g. with lysine, or prolonged fasting tests provoking catabolism may be extremely harmful and should be avoided. Ultimately, demonstration of two known pathogenic *GCDH* gene mutations or enzyme analysis of glutaryl-CoA dehydrogenase is the only method that can establish the diagnosis of glutaric aciduria type I with certainty in diagnostically problematic cases. Glutaryl-CoA dehydrogenase activity can be determined in tissues, cultured fibroblasts, peripheral leukocytes, amniocytes and chorionic villi cells [22].

Carrier detection is possible by enzyme assay, though the results are not always unequivocal [22], and by molecular

means, especially in families in which the mutations are already known [19]. Reliable *prenatal diagnosis* can be offered by enzyme assay [23], determination of glutaric acid by stable isotope dilution gas chromatography–mass spectrometry (GC-MS) assay in amniotic fluid [20], and by molecular analysis [19]. Evidence-based recommendations for the diagnosis of glutaric aciduria type I have been published [23].

22.5.5 Treatment and Prognosis

Four decades after the first description of glutaric aciduria type I, more than 500 patients have been identified worldwide and major progress has been achieved in the prevention of acute striatal necrosis and neurological sequelae. A prerequisite is that the diagnosis is made early and treatment is started before manifestations of an acute encephalopathic crisis. Early diagnosis and treatment of the asymptomatic child is essential, as current therapy has little effect once brain injury has occurred. Following acute or insidious onset of striatal damage, the majority of patients remained severely handicapped and have a reduced life expectancy. However, even with early diagnosis up to 10–35% of patients do not benefit, or benefit only incompletely, from the current management [10][15][24]. Differences in the neurological outcome are mostly related to differences in the therapeutic management.

The following therapeutic measures are generally employed and are recommended by a recent guideline [23].

1. **Emergency treatment**. Emergency treatment during every intercurrent illness must start immediately and before the onset of neurological signs. Gastrointestinal infections are especially dangerous. Treatment should consist of frequent high carbohydrate feeds and increased carnitine supplementation. If feeds are not tolerated high-dose intravenous glucose and carnitine must be given [23]. If mixtures of free amino acids devoid of lysine are used, these are offered orally, in addition. If the temperature rises above 38.5°C (101°F) antipyretics should be administered. All patients should be supplied with an emergency card. Frequent visits and regular information and training of parents may help to prevent lapses or mistakes. This concept must be strictly followed for the first 6 years of life. After this age emergency treatment is individually adjusted. Emergency treatment is thought to be the most effective component of current treatment strategies to prevent acute striatal injury [15][25].
2. **Oral supplementations with carnitine and riboflavin**. Carnitine should be supplemented lifelong to prevent secondary carnitine depletion. Furthermore, lack of carnitine supplementation has been associated with high mortality [11]. Riboflavin responsiveness appears to be extremely rare, if it exists at all, and the therapeutic benefit of riboflavin is unproven. Furthermore, there is no standardised protocol to test for riboflavin responsiveness, but significant and permanent reduction of pathological metabolites should be expected.
3. **Dietary treatment**. Application of a low-lysine diet aims to reduce the quantitatively most relevant precursor amino acid of the putatively neurotoxic glutaric and 3-hydroxyglutaric acids. Dietary treatment involves reduced intake of natural protein, with or without supplementation with lysine-free amino acid mixtures (Table 22.1). Application of lysine-free, tryptophan-reduced and arginine-enriched amino acid mixtures aims to minimise the risk of malnutrition. A low-lysine diet is recommended during the vulnerable period for acute encephalopathic crises, i.e. the first 6 years of life [15][23]. There are only anecdotal data about the value of protein restriction beyond 6 years of age. However, protein excesses should be avoided. Special efforts to supply adequate calories are often necessary in patients with motor dysfunction and swallowing difficulties, since an improved nutritional status is often paralleled by a reduction of the dystonic/dyskinetic syndrome. This may require nasogastric or gastrostomy feeding.
4. **Treatment of the complex movement disorder**. The complex movement disorder in symptomatic patients is difficult to treat, and the efficacy of a drug cannot be predicted precisely for an individual patient [23]. Baclofen (1–2 mg/kg daily) and/or diazepam (0.1–1 mg/kg daily) are commonly used to reduce involuntary movements and improve motor function, mostly through muscle relaxation. In some patients their use and dosage are limited by worsening of axial hypotonia. Trihexiphenidyl should be considered as a second-line treatment for dystonia, in particular for adolescent and adult patients. Antiepileptics, L-DOPA and amantadine are not useful unless there are seizures or even contraindicated (i.e. valproic acid). There are anecdotal reports of sustained improvement with experimental therapies, including botulinum toxin A injections and a baclofen pump. The long-term benefit to patients with glutaric aciduria type I of neurosurgical interventions in the form of subdural effusions, pallidotomy and deep brain stimulation (globus pallidus internus) is uncertain and, since they involve a significant risk of neurological deterioration, these interventions should be decided upon very cautiously.
5. **Nonspecific multiprofessional support**. In all patients with glutaric aciduria type I, expert neurological evaluation should be performed by a neuropaediatrician and later on by a neurologist for clear identification of the type of movement disorder. In addition, dietitians, physiotherapists, occupational therapists, orthopaedists, and speech specialists, and providers of communication aids should be consulted to provide multi-professional support for children with movement disorders. The involvement of a specialised team clearly reduces the risk of neurological impairment [15].

The long-term outcome of patients with glutaric aciduria type I is still uncertain. However, early diagnosis by newborn screening in combination with metabolic treatment has significantly

Table 22.1 Maintenance therapy in patients with glutaric aciduria type I. (After [22])

		Patient age				
Treatment		0–6 mo	7–12 mo	1–3 y	4–6 y	>6 y
1. Low-lysine diet						
- Natural protein[1]	g/kg/d	1.4–1.3	1.5–1.3	1.4–1.3	1.3–1.1	Avoid excessive intake of natural protein; favor natural protein with a low lysine content
- Amino acid supplements[2]	g/kg/d	1.3–0.8	1.0–0.8	0.8	0.8	
- Lysine	mg/kg/d	100	90	80–60	60–50	
- Tryptophan	mg/kg/d	20	17	17–13	13	
- Energy	kcal/kg/d	115–82	95–80	95–82	90–78	
2. Micronutrients	%	≥100	≥100	≥100	≥100	>100
3. Carnitine	mg/kg/d	100	100	100	100–50	30–50

[1]Food with a low lysine content. [2]Lysine-free, tryptophan-reduced. Consider an individualisation of treatment if normal growth is not achieved

improved the outcome of affected individuals by decreasing the frequency of children with striatal injury who would have developed a complex movement disorder [15][24][25].

22.6 Glutaric Aciduria Type III

22.6.1 Clinical Presentation

Glutaric aciduria type III is an autosomal recessive metabolic abnormality with unknown incidence. It is a clinically benign condition [26].

22.6.2 Metabolic Derangement

Individuals with glutaric aciduria type III present with isolated glutaric acid accumulation, without the elevated levels of 3-hydroxyglutaric acid and glutarylcarnitine that are found in glutaric aciduria type I (▶ Section 23.5). This indicates absence of elevated glutaryl-CoA. Deficiency of succinate hydroxymethylglutarate CoA-transferase causes glutaric aciduria type III (enzyme 10 in Fig. 22.1). This enzyme converts glutaric acid to glutaryl-CoA using succinyl-CoA as a coenzyme donor [27]. The origin of glutaric acid as substrate for this enzyme remains to be elucidated. Bacterial production in the intestine and spontaneous breakdown of glutaryl-CoA might be considered as a source.

22.6.3 Genetics

Pathogenic mutations in the *C7orf10* have been identified as causative for glutaric aciduria type III [28].

22.6.4 Diagnostic Tests

Patients with glutaric aciduria type III are diagnosed by urinary organic acid analysis; mutation analysis of the *C7orf10* gene can confirm the diagnosis.

22.6.5 Treatment and Prognosis

Since this is a biochemical abnormality with minor or even no clinical significance, there is no indication for treatment. The prognosis of affected individuals is likely to be favourable.

22.7 L-2-Hydroxyglutaric Aciduria

22.7.1 Clinical Presentation

Most patients with L-2-hydroxyglutaric aciduria follow a characteristic disease course [29][30][31]. In infancy and early childhood mental and psychomotor development appears normal or only slightly retarded. Thereafter seizures, progressive ataxia, pyramidal tract signs, slight extrapyramidal signs and progressive mental retardation become the most obvious clinical findings. Progressive macrocephaly is present in about half of the patients. The IQ in teenagers is about 40–50. Sometimes mental deterioration is rapidly progressive, and a single patient with fatal neonatal outcome has been described [31].

In L-2-hydroxyglutaric aciduria the neuroimaging findings are very specific [29][30]. The subcortical white matter appears mildly swollen with some effacement of gyri. The progressive loss of arcuate fibres is combined with severe cerebellar atrophy and increased signal densities of dentate nuclei and globi pallidi (Fig. 22.2) on T_2-weighted images. In some patients different types of malignant brain tumours, such as me-

dulloblastoma, glioblastoma multiforme, astrocytoma, and primitive neuroectodermal tumour, have been reported [32].

22.7.2 Metabolic Derangement

The disorder is caused by an inherited deficiency of FAD-linked 2-hydroxyglutarate dehydrogenase, a mitochondrial enzyme converting L-2-hydroxyglutarate to 2-oxoglutarate [33].There is an increase in L-2-hydroxyglutarate in CSF, plasma and urine [34]. In addition, a number of hydroxydicarboxylic acids (glycolate, glycerate, 2,4-dihydroxybutyrate, citrate and isocitrate) are elevated in CSF. Another consistent biochemical finding is an increase of lysine in blood and CSF.

L-2-Hydroxyglutarate has no known functions, but its formation is the result of the fact that L-malate dehydrogenase is not absolutely specific for oxaloacetate. L-Malate dehydrogenase thus slowly catalyses the reduction of 2-oxoglutarate, the structural homologue of oxaloacetate, to L-2-hydroxyglutarate. The latter is reoxidised to 2-oxoglutarate by L-2-hydroxyglutarate dehydrogenase [35]. Among many other functions, 2-oxoglutarate is also used for the first step of mitochondrial lysine oxidation, i.e. the formation of saccharopine, which explains elevated lysine concentrations in this disease. L-2-Hydroxyglutaric aciduria is now considered a disease of deficient correction of a nonsense metabolite, or ›metabolite repair‹.

22.7.3 Genetics

L-2-Hydroxyglutaric aciduria is an autosomal recessive disorder. Mutations in the *L2HGDH (C14orf160/duranin)* have been identified as causative for this disease [36].

22.7.4 Diagnostic Tests

L-2-Hydroxyglutarate is found elevated in all body fluids [29] [37]. In addition, lysine is slightly increased in CSF, as is protein, the latter occurring in the absence of pleocytosis. Differentiation between the two isomers of 2-hydroxyglutarate, L-2- and D-2-hydroxyglutarate, is necessary for the correct diagnosis. *Prenatal diagnosis* is possible by accurate determination of L-2-hydroxyglutarate using a stable isotope dilution GC-MS assay in amniotic fluid [30][37] and by molecular diagnosis.

22.7.5 Treatment and Prognosis

Riboflavin has been reported to have led to a partial improvement of neurological symptoms in a few patients and reduced urinary excretion of L-2-hydroxyglutarate in some [38] but not in others (G.F. Hoffmann, personal observation). Epilepsy can generally be controlled by standard medications. The oldest known patients are over 30 years of age. They are bedridden and severely retarded.

22.8 D-2-Hydroxyglutaric Aciduria

22.8.1 Clinical Presentation

Patients with D-2-hydroxyglutaric aciduria exhibit a more variable phenotype than patients with L-2-hydroxyglutaric aciduria. The clinical spectrum varies from neonatal onset, severe seizures, lack of psychomotor development and early death to mild developmental delay to no symptoms at all. An international survey revealed a continuous spectrum between these extremes, with most patients suffering from a severe early-onset epileptic encephalopathy, while a substantial subgroup showed mild symptoms or were even asymptomatic [39]. Clinical and neuroradiological symptoms and signs in the severely affected patients were quite uniform. Severe, often intractable seizures started in early infancy. The babies were extremely hypotonic. Conscious levels varied from irritability to stupor. Cortical blindness was uniformly present, and psychomotor development almost absent. Cardiomyopathy (primarily dilated, but in one case hypertrophic) occurred in about a third of the severely affected patients, exclusively in those suffering from the cytosolic form of the disease (type II disease, see below). Less severely affected patients exhibited mostly mild neurological symptoms, including slight developmental delay, delayed speech and febrile convulsions.

In the severely affected patients neuroimaging uniformly revealed disturbed and delayed gyration, myelination and opercularisation, ventriculomegaly, more pronounced of the occipital horns, and cysts over the head of the caudate nucleus (◘ Fig. 22.3).

22.8.2 Metabolic Derangement

Patients show moderately to highly elevated levels of D-2-hydroxyglutarate in all body fluids. In addition, Krebs cycle intermediates are found to be elevated in the urine of some patients, as well as GABA in CSF [39]. Molecular studies have differentiated two groups of patients, D-2-hydroxyglutaric aciduria types I and II. In half of the patients, type I, following an autosomal recessive mode of inheritance, D-2-hydroxyglutaric aciduria has been related to deficient D-2-hydroxyglutarate dehydrogenase, an enzyme that converts D-2-hydroxyglutarate to 2-oxoglutarate [40]. The enzyme is homologous to FAD-dependent D-lactate dehydrogenase. Recently, specific autosomal dominant mutations in mitochondrial isocitrate dehydrogenase 2 have been identified in the other half of patients with D-2-hydroxyglutaric aciduria, type II [41]. These mutations disable the enzyme's normal function to convert D-isocitrate into 2-oxoglutarate but confer the new ability to convert 2-oxoglutarate into D-2-hydroxyglutarate.

A similar mechanism explains the D-hydroxyglutaric aciduria observed in some patients with malignant gliomas and acute myeloid leukaemia and mutated isocitrate dehydrogenase 1 (cytosolic) or 2 (mitochondrial). Based on these findings it has been hypothesised that D-2-hydroxyglutaric acid is

an ›onco-metabolite‹ that contributes to the formation of gliomas. However, the absence of cancer diagnoses in the patients with inborn D-2-hydroxyglutaric aciduria is not consistent with this role.

22.8.3 Genetics

Autosomal recessively inherited mutations in human *D2HGDH* have been identified as the molecular cause of D-2-hydroxyglutaric aciduria type I [40]. However, presumed pathogenic mutations in the *D2HGDH* gene were only found in 24 of 50 patients with this disease, suggesting genetic heterogeneity for D-2-hydroxyglutaric aciduria [42]. Significantly lower D-2-hydroxyglutarate concentrations in body fluids were observed in mutation-positive than in mutation-negative patients. Autosomal dominant germline mutations of the *IDH2* gene [41] have been identified as a second molecular cause of D-2-hydroxyglutaric aciduria, type II, with a high frequency of de novo mutations.

22.8.4 Diagnostic Tests

L-2- and D-2-Hydroxyglutaric acid cannot be differentiated by conventional GC-MS analysis. The chromatographic separation of these enantiomers can be performed using derivatisation with a chiral reagent or a chiral stationary phase. D-2-hydroxyglutaric acid is found elevated in urine, plasma and CSF [37][39][42]. In addition, GABA was found to be elevated in CSF, and intermediates of energy metabolism in urine (lactic, succinic, malic, and 2-oxoglutaric acids) in some patients. Differentiation between the two isomers of 2-hydroxyglutarate is essential for diagnosis. Prenatal diagnosis has been successfully performed by accurate determination of D-2-hydroxyglutarate by stable isotope dilution GC-MS assay in amniotic fluid as well as by molecular diagnosis [37].

D-2-Hydroxyglutaric acid can also be elevated in multiple acyl-CoA dehydrogenase deficiency (glutaric aciduria type II), and – rarely – in patients with glutaric aciduria type I and succinic semialdehyde dehydrogenase deficiency, but these can be readily distinguished by the urine organic acid profile (▶ Chapter 12).

22.8.5 Treatment and Prognosis

To date there is no rational therapy for D-2-hydroxyglutaric aciduria; riboflavin and L-carnitine supplementation has not been of benefit. Seizures can be very difficult to control, and patients have died early with profound developmental delay. The clinical course in type I disease does not appear to be progressive, if affected children do not develop an early onset epileptic encephalopathy. The course of type II disease is primarily progressive with early death in childhood in about 50%.

22.9 D-2-/L-2-Hydroxyglutaric Aciduria

22.9.1 Clinical Presentation

D-2-/L-2-Hydroxyglutaric aciduria has been described in less than 10 patients. Affected individuals display a severe clinical phenotype with neonatal onset metabolic encephalopathy, infantile epilepsy refractory to antiepileptic drug therapy, severe global developmental retardation, muscular hypotonia and early death [43].

22.9.2 Metabolic Derangement

The major biochemical finding is accumulation of D-2- and L-2-hydroxyglutaric acids in body fluids with a predominance of D-2-hydroxyglutaric acid. Krebs cycle intermediates including 2-oxoglutarate, malate, fumarate and succinate are also elevated accompanied by decreased concentrations of citrate and isocitrate [44]. Impaired dicarboxylic acid transport disrupts the export of citrate and isocitrate into the cytosol resulting in increased mitochondrial concentrations of Krebs cycle intermediates downstream of isocitrate.

22.9.3 Genetics

Recently, pathogenic mutations in *SLC25A1*, encoding the mitochondrial citrate carrier, were identified as the molecular cause of this disease [44].

22.9.4 Diagnostic Tests

The metabolic work-up is performed in analogy to D-2- and L-2-hydroxyglutaric acidurias (see above).

22.9.5 Treatment and Prognosis

The prognosis in the hitherto described patients was poor. Treatment with citrate has recently been demonstrated in a single patient resulting in biochemical and clinical improvement to some extent [45].

22.10 N-Acetylaspartic Aciduria (Canavan Disease)

22.10.1 Clinical Presentation

N-Acetylaspartic (NAA) aciduria mostly manifests at 2–4 months of age with head lag, hypotonia and macrocephaly, progressing to marked developmental delay, seizures, optic nerve atrophy, progressive spasticity and opisthotonic posturing [46]. At birth the head circumference may not be remarkably increased; however, in the majority of cases it increases

pathologically after 6 months of age, crossing the percentiles with obvious macrocephaly by 1 year. In the 2nd year of life seizures often develop, together with irritability and sleep disturbance. Muscular hypotonia gives way to spasticity reminiscent of cerebral palsy. Impaired chewing and swallowing, problems with gastro-oesophageal reflux, vomiting and aspiration can result in recurrent infections and failure to thrive.

The most consistent findings on MRI studies are diffuse abnormalities of white matter [47]. Although not always present and not uniform, MRI usually shows symmetric diffuse low signal intensity on T_1-weighted images and high signal intensity on T_2-weighted images (Fig. 22.3).

The neuropathology of Canavan disease is characterised by a progressive loss of myelinated arcuate fibres [47]. Detailed histopathological descriptions at autopsy have elucidated that white matter is characteristically soft and gelatinous. The spongy or vacuolisation changes are clearly seen in the lower layers of the grey matter and in the subcortical white matter, with the more central white matter relatively spared.

Most patients follow the disease course described above, which is also termed the infantile form. Rare clinical variants with different disease courses have been described as congenital, i.e. presenting at or shortly after birth, or as juvenile forms, i.e. presenting after 5 years of age.

22.10.2 Metabolic Derangement

The disease is caused by aspartoacylase (aminoacylase 2) deficiency leading to the accumulation of NAA in brain, CSF, plasma, and urine. In the brain, aspartoacylase is exclusively located in oligodendrocytes hydrolysing its natural substrate NAA, which is formed in neurons from L-aspartate and L-acetate. Defective NAA catabolism is thought to result in reduced brain acetate levels and myelin lipid synthesis. This has been demonstrated in aspartoacylase-deficient mice showing a 30% decrease in total myelin lipids at the time of peak postnatal myelination in the brain [48]. Besides acetate depletion, it has been hypothesised that NAA may act as an efflux molecular water pump between neurons and oligodendrocytes enabling the removal of neuronal metabolic water produced by glucose oxidation; if this is the case then decreased NAA catabolism might also result in osmotic dysregulation of the brain and, subsequently, spongiform leukodystrophy [49].

22.10.3 Genetics

N-Acetylaspartic aciduria is an autosomal recessive disease caused by pathogenic mutations in *ASPA*. It is a pan-ethnic disease with a higher frequency among Askenazi Jews, most of whom carry two specific mutations, a missense mutation, p.Glu285Ala, accounting for 84% of mutant alleles, and a nonsense mutation, p.Tyr231Xaa, accounting for 13% [50]; the frequency of these two mutations makes carrier screening possible [51]. In non-Jewish patients the mutations are diverse and mostly private.

22.10.4 Diagnostic Tests

The diagnosis is best established by determining NAA in the urine by organic acid analysis. Hundredfold elevations are pathognomonic but the disorder should be confirmed by demonstrating the enzyme deficiency in fibroblasts and/or mutation analysis. Borderline elevated levels of NAA are sometimes found in different forms of white matter disease and can cause diagnostic confusion. Prenatal diagnosis can be problematic, as the assay of aspartoacylase in amniocytes is not reliable [52]. A combination of mutation analysis together with the exact quantitation of NAA in the amniotic fluid gives double certainty.

22.10.5 Treatment and Prognosis

No effective treatment exists for *N*-acetylaspartic aciduria. Lithium citrate, which induces a mild decrease in brain NAA levels of affected children, is safe. It remains to be elucidated, however, whether this treatment is beneficial [53]. Because acetate in the form of acetyl-CoA is a building block for lipids, it has been proposed that dietary acetate supplementation with glyceryl triacetate might be a therapeutic option. The result of a low-dose safety study has been published recently; however, there is still no proof for the therapeutic efficacy of glyceryl triacetate [54]. Adenoviral transfer of the *ASPA* gene to the brains of patients has been initiated [55]. However, no follow-up study showing significant myelination or motor improvements in these children has been published to date. A potential problem of this approach is that due to the neurotrophic viral vector (AAV-2) the majority of cells expressing *ASPA* were neurons, so that the entire oligodendroglial defect remains uncorrected, as has been demonstrated in the tremor rat model of Canavan disease [56].

The prognosis for most affected individuals remains very poor, with death usually occurring in the first decade of life although there may be survival into the second decade in a vegetative or near-vegetative state.

22.11 Aminoacylase 1 Deficiency

Aminoacylase 1 deficiency is a rare disease with less than 20 patients reported. The clinical relevance of this disorder has not yet been fully elucidated. Although initially considered a non-disease, some patients present with a heterogeneous clinical spectrum including intellectual and motor disability, delayed speech development, muscular hypotonia and autistic features. However, since detection of aminoacylase 1 deficiency was part of selective screening in symptomatic patients with suspected metabolic disease, a strong selection bias of this cohort is likely.

N-Acetylation of a protein extends its half life; 50-80% of proteins show formylated or acetylated N-termini. Free amino acids can be recycled after protein breakdown by hydrolysis of N-acetylated amino acids using aminoacylases. Aminoacylase

1 catalyzes the release of free amino acids from a variety of N-acetylated precursors – except for N-acetylaspartate (▶ Section 22.10). It has a high tissue-specific activity in kidney and brain. Enzyme deficiency results in increased formation and urinary excretion of acetylated amino acids [57].

Aminoacylase 1 deficiency is an autosomal recessive disorder caused by homozygous or compound heterozygous mutations in *ACY1* [57].

22.11.1 Diagnostic Tests

Elevated urinary exretion of N-acetylated amino acids including derivatives of methionine, glutamine, alanine, leucine, glycine, valine, and isoleucine can be detected by gas chromatography/mass spectrometry or NMR spectroscopy [57]. Decreased aminoacylase 1 activity in lymphoblasts confirms the diagnosis.

22.11.2 Treatment and Prognosis

Since the clinical significanc of aminoacylase 1 deficiency remains unclear, there is no clear-cut indication for treatment. Studies systemically evaluating the effect of metabolic treatment are pending. The prognosis of affected individuals is likely to be favourable.

22.12 Hypoacetylaspartia and Aspartate-Glutamate Carrier 1 Deficiency

A single patient with hypoacetylaspartia has been described with marked developmental delay and secondary microcephaly with truncal ataxia, seizures, and behaviour abnormalities on follow-up, in whom 1H-MRS had revealed the absence of NAA signal [58]. A defect of l-aspartate N-acetyltransferase, the enzyme that synthesis NAA, was suspected, and a neuron-specific protein, NAT8L, was found to be responsible for NAA synthesis, and was mutated in the patient [59].

Inherited deficiency of the mitochondrial aspartate-glutamate carrier isoform 1 (ACG1) has been reported in few patients and is associated with global cerebral hypomyelination, arrested psychomotor development, muscular hypotonia and infantile-onset epilepsy. It is caused by biallelic mutations in the *SLC25A12* gene [60]. ACG1 is one of two enzymatic isoforms (ACG1 and 2) catalyzing an exchange between intramitochondrial aspartate and cytosolic glutamate plus a proton. Deficiency of ACG2 cause citrin deficiency (citrullinaemia type II, see ▶ Chapter 19). ACG1 is the only of the two isoforms expressed in brain and skeletal muscle. Global hypomyelination and neurological presentation is thought to result from impaired efflux of aspartate from neuronal mitochondria preventing normal myelination. ACG1 is part of the malate-asparte shuttle and thus has also a role in transporting reducing equivalents from NADH from the cytosol to mitochondria [60]. Standard metabolic investigations are normal in this disorder. There is no known specific treatment. Whether there is an association between genetic variants in *SLC25A12* and the risk of autism spectrum remains controversial.

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Nonketotic Hyperglycinemia (Glycine Encephalopathy) and Lipoate Deficiency Disorders

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23

Glycine Metabolic Scheme

Fig. 23.1 and Fig. 23.2

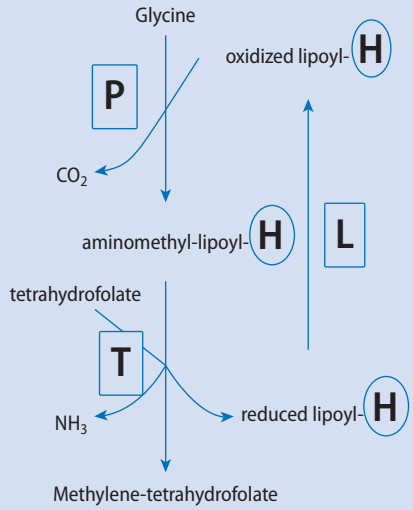

Fig. 23.1 The glycine cleavage enzyme is a four protein complex with the H-protein carrying a lipoyl-group as the central core. The P-protein contains pyridoxal-5′-phosphate and decarboxylates glycine with transfer of the aminomethyl-group onto lipoate of the H-protein. The T-protein releases ammonia and transfers the remaining methyl-group onto tetrahydrofolate making 5,10-methylenetetrahydrofolate. The resulting reduced lipoate on the H-protein is reoxidized by the L-protein

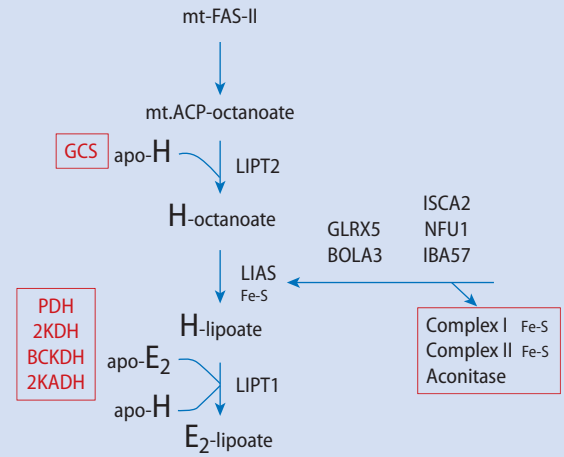

Fig. 23.2 Octanoate is synthesized by intramitochondrial fatty acid synthesis (mt.-FAS II) on the mitochondrial acylcarrier protein (mt.ACP) and transferred to apo-H protein by lipoyltransferase 2 (*LIPT2*). To make lipoyl-H, lipoate synthase (*LIAS*) donates the sulfur atoms from its iron-sulfur cluster, which were synthesized through multiple genes including *GLRX5*, *BOLA3*, *NFU1*, *ISCA2* and *IBA57*. They also create iron-sulfur clusters for respiratory chain complex I and II and aconitase. Lipoyltransferase 1 (*LIPT1*) transfers the lipoate group from H-protein to the E2 component of pyruvate dehydrogenase (PDH), 2-ketoglutarate dehydrogenase (2KDH), branched chain ketoacid dehydrogenase (BCKDH), and 2-ketoadipate dehydrogenase (2KADH). *LIPT1* deficiency causes dysfunction of the E2 components; *LIPT2* and *LIAS* add deficiency of the glycine cleavage enzyme (GCS). Some disorders of iron sulfur cluster biogenesis genes add deficient activity of complexes I and II of the respiratory chain, which carry iron sulfur clusters (► Chapter 14)

Nonketotic hyperglycinemia (NKH) is caused by defective glycine cleavage enzyme activity (► Glycine metabolic scheme). Classic NKH is caused by mutations in protein coding genes (*GLDC* and *AMT*). Disorders of lipoate synthesis and transport are caused by mutations in *LIAS*, *BOLA3*, *NFU1*, *GLRX5*, *ISCA2*, *IBA57*, *LIPT1*, and *LIPT2* and can affect glycine metabolism referred to as variant NKH. Patients with the severe form of classic NKH have minimal psychomotor development and develop therapy resistant epilepsy. Patients with the attenuated form of classic NKH make variable developmental progress, and present with attention deficits, hyperactivity, chorea, and episodic lethargy. Treatment consists of reduction of glycine levels with benzoate and sometimes diet, and of blocking the excitatory effect of glycine on NMDA receptors with either dextromethorphan or ketamine. Therapy is most effective in patients with attenuated NKH where it can improve development particularly when treatment is initiated early. High levels of CSF glycine and presence of brain malformations are indicative of severe classic NKH, whereas low CSF:plasma glycine ratios, onset after 4 months, or absence of epilepsy are indicators of attenuated classic NKH. A specific pattern of diffusion restriction on brain MRI is nearly always present in the first months of life and is useful in distinction with non-genetic causes of elevated CSF glycine.

23.1 Introduction: Definitions

Nonketotic hyperglycinemia (NKH) is a genetic disorder characterized by deficient activity of the glycine cleavage enzyme. Classic NKH is caused by mutations in genes that encode the protein components of the glycine cleavage enzyme system (GCS). Based on the clinical severity and developmental outcome, classic NKH is further divided into severe classic NKH and attenuated classic NKH [1][2]. Variant NKH has an overlapping phenotype to classic NKH and is caused by mutations affecting the biosynthesis of the main cofactor lipoic acid, and fit into the larger group of the lipoate synthesis disorders. Lipoate is not only cofactor for the GCS but also for pyruvate dehydrogenase, 2-ketoglutarate dehydrogenase (► Chapter 11), and branched chain ketoacid dehydrogenase (► Chapter 18). These disorders are also re-

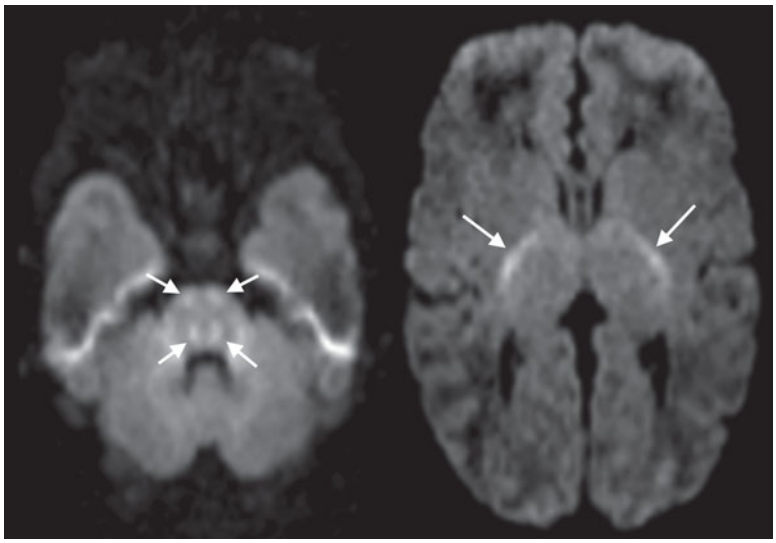

Fig. 23.3 Brain MRI with diffusion restriction of a newborn with classic nonketotic hyperglycinemia. Diffusion restriction is seen in the corticospinal and central tegmental tract in the brain stem and in the posterior limb of the internal capsule

ferred to as multiple mitochondrial dysfunction syndromes (▶ Chapter 14).

Pyridox(am)ine 5′-phosphate oxidase deficiency (resulting from *PNPO* mutations) leads to reduced activity of a large number of pyridoxal-P-dependent reactions including the GCS (▶ Chapter 28). Phenocopies of NKH, historically called transient NKH, are non-genetic secondary causes of elevated plasma and CSF glycine levels. Atypical NKH is a heterogeneous historic term, which should no longer be used. Deficiency of glycine synthesis is very rare and the genetic cause has not been identified, and will not be further discussed here.

23.2 Clinical Presentation

23.2.1 Severe Classic NKH

Onset of severe classic NKH generally is during the neonatal period with a few patients presenting in early infancy. Initial signs and symptoms include hypotonia, increasing lethargy, coma, apnea, myoclonic jerks, seizures, and frequent hiccupping, which may have already started during the prenatal period, and sometimes pin-point pupils. Patients typically require ventilatory support for the first 10 to 20 days, although not in the 15% of patients who do not develop apnea. Seizures may not be present at diagnosis but manifest in infancy. The initial EEG pattern often includes burst-suppression, and can progress in infancy to hypsarrhythmia followed by multifocal epilepsy. Coma and apnea are rarely described in patients who present after the neonatal period. At least 15–30% of patients with classic NKH die during neonatal period often due to withdrawal of intensive care and ventilatory support [1][2][3]. Patients with severe classic NKH can present with congenital malformations including club feet and cleft lip/cleft palate. Brain malformations have sometimes been noted including agenesis of the corpus callosum, retrocerebellar cysts with hydrocephalus, and cerebellar hypoplasia [1][2]. The corpus callosum often remains thin. By MRS, elevated glycine peaks may be detected in the white and grey matter. Brain MRI shows a typical pattern of diffusion restriction in the corticospinal tract, the posterior tegmental tracts, and the cerebellar white matter (Fig. 23.3).

All children with severe classic NKH have severe developmental delay with a developmental age of approximately six weeks [2]. Patients can learn to smile, coo, roll over, but they do not learn to sit, reach and grasp objects, or to communicate. During the first year, patients usually develop spastic quadriparesis and truncal hypotonia. Seizures gradually worsen in infancy and progress into intractable seizures, requiring multiple anticonvulsant treatment [1][2]. Cortical blindness is very frequent and microcephaly often develops over time. Further long-term problems include feeding difficulties requiring tube feeding, gastroesophageal reflux often requiring Nissen fundoplication, and multiple orthopedic problems in childhood such as hip dislocation and scoliosis often requiring surgical intervention [1]. Airway maintenance becomes poor over time, ultimately leading to the child's demise.

23.2.2 Attenuated Classic NKH

Patients with attenuated classic NKH attain developmental milestones with varying degrees of developmental and intellectual progress. Patients may present in the neonatal period, resembling severe classic NKH, or later in infancy with hypotonia, lethargy, coma, and seizures [1][2]. Developmental progress has been reported in 15–20% of patients with neonatal onset and in 50% of patients presenting in infancy [1]

[2][3]. Patients do not present with congenital brain malformations, but they may also have thinning of the corpus callosum [1][2]. Usually, MRS does not reveal the elevated glycine peak detectable in severe classic NKH. However, a similar pattern of diffusion restriction is often recognized in both cohorts.

Children with attenuated classic NKH develop hyperactivity, which is often severe, as well as chorea, behavioral problems, and intermittent episodes of lethargy and ataxia [1][2][4]. Patients often ambulate and achieve various motor skills. Expressive speech is much more delayed than receptive speech. Seizures may occur, but they are often less severe requiring only one or even no anticonvulsant [2]. The EEG pattern of burst-suppression occurs less frequently [1]. The degree of intellectual impairment in attenuated classic NKH is variable: depending on the intellectual outcome, patients may be differentiated into attenuated good, intermediate, or poor classic NKH [2]. Patients with attenuated good NKH reach a DQ of 50 to 80 and have mostly hyperactivity and behavioral issues with no epilepsy. They can have late onset and intermittent episodes of chorea only [2][4][5]. Patients with attenuated intermediate NKH have a DQ between 20 and 50 and have severe hyperactivity, chorea, and poor to absent speech with mild to no seizures. Patients with poor attenuated NKH make some developmental progress but have a DQ <20 and exhibit seizures manageable with medications, autistic features, absent speech, and moderate spasticity.

Rarely, patients with treated classic NKH, both severe and attenuated, have presented systemic phenotypic features the cause of which is not yet known. These include: gastric problems with delayed emptying, severe intestinal dysmotility up to pseudoileus, sudden life threatening electrolyte imbalances of hypokalemia or hypernatremia, and unexplained prolonged crying.

23.2.3 Lipoate Disorders Including Variant NKH

Variant NKH is caused by a deficiency of the cofactor lipoate and combines the clinical findings of severe NKH with mitochondriopathies, such as leukoencephalopathy, optical atrophy, cardiomyopathy, and sometimes episodes of severe lactic acidosis. Some patients formerly classified as atypical NKH must retrospectively be reclassified as variant NKH. Our knowledge of the clinical phenotype of the newly described group of lipoate disorders is still rapidly evolving.

Patients with mutations in *LIAS* presented during the neonatal period with a clinical picture resembling severe NKH including seizures, myoclonus, burst-suppression pattern on EEG, hypotonia, and apnea, but also revealing leukodystrophy and Leigh-like lesions on cerebral MRI. Two patients presenting with intractable seizures died during infancy. Another mildly affected patient had stable developmental delay and transient seizures only [6][7][8]. A patient with *LIPT2* deficiency showed encephalopathy with cortical atrophy, hypotonia and spasticity [9]. Glycine and lactate were both increased. Patients with *LIPT1* deficiency have elevated lactate but do not have increased glycine [10][11]. One patient presented neonatally with severe fatal disease of hypertonia, dystonia, pulmonary hypertension, whereas another presented at age 18 months with sudden psychomotor regression, spasticity, and an extrapyramidal syndrome with cerebral and cerebellar atrophy, thalamic lesions and white matter disease.

Several iron sulfur cluster biogenesis disorders impair lipoate synthesis (mutations in *BOLA3, NFU1, ISCA2, GLRX5, IBA57)* (Fig. 23.2; ▶ Chapter 14; ▶ Table 14.5). Mutations in *BOLA3* have been identified in infants presenting within the first 8 months of life with progressive encephalopathy with seizures and central cavitating white matter lesions, hypotonia, cardiomyopathy, sometimes severe lactic acidosis episodes, and early death between the ages 3 months to 11 years [6][12]. Mutations in *NFU1* have been described most commonly. Predominant signs in these patients include cavitating leukodystrophy, infantile encephalopathy, pulmonary hypertension, and early death between neonatal period and infancy. One patient with a milder phenotype surviving more than three decades has been described [12][13][14]. Patients with *ISCA2* mutations presented after an uneventful neonatal period at the age of 3–7 months with progressive central leukodystrophy causing spastic paresis and optic atrophy, and in the majority of cases leading to early death at the age of 11 months to 5 years [15]. In contrast, mutations in *GLRX5* were noted in children presenting at the age of 2–7 years, and were all reported to be alive even into adulthood. All patients developed progressive spasticity, ataxia, and sometimes optic atrophy, but none of them showed seizures, myoclonus, or cognitive decline. MRI predominantly revealed leukodystrophy and central lesions in the cervical spinal cord [6]. Two siblings with mutations in *IBA57* presented with a neonatal lethal condition of severe hypotonia, respiratory insufficiency, arthrogryposis, microcephaly, dysmorphic features, and congenital brain malformations [16]. Another infant presented with white matter abnormalities and psychomotor retardation [17]. In contrast, multiple members of a family were reported with slowly progressing childhood onset spastic paraplegia, optic atrophy, and peripheral neuropathy [18].

PNPO deficiency may also resembles classic NKH but with a much more complex metabolic profile that includes a mildly elevated CSF glycine. Symptoms are partly responsive to pyridoxal-P (▶ Chapter 28).

23.3 Metabolic Derangement

Glycine is an amino acid which is involved in many biochemical reactions. Multiple biochemical pathways intersect in the synthesis as well as in the use of glycine. The most important pathway for the synthesis of glycine is from serine through the serine-hydroxymethyltransferases. Serine itself is synthesized from the glycolytic pathway. The most important catabolic pathway of glycine is the GCS. The glycine content of protein in food differs significantly. The glycine content per gram of

total protein is low in milk, higher in soy, but particularly high in meat and gelatin [19]. When excluding the GCS, the excess flux of glycine synthesized in the body over its degradation is far larger than the amount of glycine taken in from food. Thus, the contribution of dietary glycine is only of limited impact in NKH management [19].

The GCS breaks down glycine with tetrahydrofolate into carbon dioxide, ammonia, and the generation of 5,10-methylenetetrahydrofolate (Fig. 23.1). It is a four protein complex located in the mitochondrial matrix with the H-protein carrying a lipoyl-group as the central core. Octanoate is synthesized by intramitochondrial fatty acid synthesis on the mitochondrial acylcarrier protein and is transferred to apo-H protein by lipoyltransferase 2 (*LIPT2*). Lipoate synthase (*LIAS*) donates the sulfur atoms from its iron-sulfur cluster to build lipoyl-H. Lipoyltransferase 1 (*LIPT1*) transfers the lipoate group from H-protein to the E2 component of four 2-ketodehydrogenases. *LIPT1* deficiency causes dysfunction of the E2 components; *LIPT2* and *LIAS* add deficiency of the GCS. Some disorders of iron-sulfur cluster biogenesis genes add deficient activity of complexes I and II of the respiratory chain and aconitase, which carry iron sulfur clusters (Fig. 23.2 and ▶ Chapter 14).

The GCS is expressed in liver, brain, and in placental villi in the syncytiotrophoblast. In the brain it is highly expressed in astrocytes of the cerebrum and the cerebellum, but only lowly expressed in the brain stem and not in the spinal cord. It is also expressed in neural progenitor cells and in neural stem cells [20]. Deceased neonates with classic NKH exhibit myelin spongiosis in the corticospinal tract, the optic radiation, and the brain stem, explaining the diffusion restriction seen on MRI. On electron microscopy, there is splitting of the myelin lamellae [21]. In contrast, patients with variant NKH have shown cavitating leukodystrophy and hypertrophic cardiomyopathy at autopsy [6][13]. Absence of the GCS results in increased levels of glycine in all body fluids. High concentrations of glycine outside the brain, such as seen with excessive provision of glycine, do not result in symptoms. In NKH, glycine accumulates particularly in the brain. Glycine is an inhibitory neurotransmitter on the glycinergic receptors in the brain stem and spinal cord, possibly contributing to apnea and hypotonia. However, in neuronal stem cells, the glycinergic receptor may be excitatory rather than inhibitory. In addition, glycine is an allosteric activator of the excitatory N-methyl-D-aspartate (NMDA) type glutamate receptor NR1/NR2 type. Increased levels of glycine result in overactivation of this NMDA receptor. D-serine, also an NMDA receptor activator, is synthesized from glycine in the brain and decreased levels are documented in the cortex of children with NKH [22]. Glycine is also an activator of the excitatory NR1/NR3 receptors, the function of which is unclear. Furthermore, glycine is a trophic factor for cerebellar Purkinje cells. In mice with deficient GCS, there is a deficiency of methylated folates in the brain [23]. Replenishment of the one methyl-group metabolism improves symptoms and survival in these mice. Although there is a slight increase in CSF homocysteine levels of children with classic NKH, CSF methylfolate levels are not reduced.

23.4 Genetics

Classic NKH is an autosomal recessive disorder with biallelic mutations identified in *GLDC* encoding the P-protein or in *AMT* encoding the T-protein. No mutations have been identified in *GCSH* encoding the H-protein.

The majority (80%) of patients with classic NKH have a mutation or genomic alteration within *GLDC*. There is extensive intragenic heterogeneity with over 200 mutations identified, many of them being private [2][24]. The most common disease-causing mutations reported are amino acid replacement (missense) mutations, followed by RNA splicing mutations and insertion deletion mutations (indels). A number of recurrent mutations have been reported with the p.R515S mutation comprising 10% of all disease-causing alleles. The p.A389V, p.A802V, IVS19-1G>A, and IVS22+1G>C are also relatively common and are present in >2% of disease-causing alleles. 20% of disease-causing alleles are intragenic deletions not detected by traditional sequencing techniques, but identified by multiplex ligation probe analysis or by targeted array-based genomic hybridization [2][25]. Approximately 20% of patients with classic NKH have a mutation within the T protein gene, *AMT*. The majority of the disease-causing mutations reported are missense mutations followed by indels and RNA splicing mutations. No intragenic deletions or duplications within *AMT* have been reported to date. A number of recurrent mutations have been reported including the p.R320H mutation, which is present in 15% of all disease-causing alleles, and p.R222C and p.R94W which are present in >5% of disease-causing alleles.

Testing of *GLDC* and *AMT* is negative in approximately 5% of patients with deficient GCS activity. These cases comprise the group of disorders referred to as variant NKH [6]. Variant NKH results from bialliec mutations in the genes involved in the synthesis of lipoate. Mutations have been identified in *LIAS, BOLA3, GLRX5, NFU1, ISCA2, LIPT1, LIPT2,* and *IBA57* in patients with lipoate disorders [6][7][8][9][10][11][12][13][14][15][16][17][18] (▶ Chapter 14). In contrast to classic NKH, a genotype-phenotype correlation has not been established in the genes causing lipoate deficiency syndrome.

Both classic and variant NKH are inherited in an autosomal recessive pattern. Parents of children with NKH are assumed to be heterozygous carriers of NKH. *De novo* mutations have occurred in approximately 1% of individuals with classic NKH [2]. Thus, the heterozygote state should be confirmed in parents as a *de novo* mutation dramatically reduces recurrence risk.

23.5 Diagnostic Tests

Increased levels of glycine are found in plasma, urine, and cerebrospinal fluid. Increased plasma glycine levels are usually the first indication of a glycine disorder, but they have a low specificity and always require confirmatory testing. Most infants identified on newborn screening with highly elevated

blood glycine levels did not have NKH and remained asymptomatic [26]. Excessive glycine intake such as seen with glycine buffered intravenous gamma globulin infusion can cause very elevated plasma glycine levels. Extremely high glycine levels are also seen in neonatal disseminated herpes virus infection. Branched chain organic acidurias often cause elevated plasma glycine levels and must be excluded. Hyperglycinuria without hyperglycinemia is noted in disorders of the renal carrier of glycine, proline, and hydroxyproline.

Increased levels of CSF glycine are highly indicative of nonketotic hyperglycinemia. The lowest CSF glycine found in a series of 124 patients with classic NKH was 40 μM [2]. An elevated CSF:plasma glycine level is a further indication of NKH, but is only valid if the CSF glycine level is elevated. Contamination of CSF with blood or with serum, as evidence by increased CSF protein levels, can cause false elevation of both CSF glycine and the CSF:plasma glycine ratio, and should never be relied upon for diagnosis [27]. Valproate inhibits the GCS and raises both the plasma and CSF glycine levels and the CSF:plasma glycine ratio. Exceptional patients with attenuated NKH have had normal CSF glycine levels, but this is very rare and the sensitivity of elevated CSF glycine is >99%, making it the preferred diagnostic test.

Mutation analysis of the genes involved in the GCS is an excellent confirmatory test. With both sequencing and deletion/duplication analysis of the *GLDC* and *AMT* genes, 98% of the alleles are identified. The high rate of exonic deletions in *GLDC* makes the use of a deletion/duplication test absolutely necessary. Despite the identification of mutations in over 500 families, the frequent identification of variants of unknown significance makes it not a good primary test, but its place in diagnosis is best after identification of elevated CSF glycine when classic NKH is suspected. For prenatal diagnosis, mutation analysis can be used once the familial mutation is known, or if only one allele is known and thus identifies the gene, intragenic linkage can be used for the unknown allele. Preimplantation diagnosis has also been performed successfully in some families with a known molecular genetic diagnosis.

Measurement of the GCS activity is possible in liver tissue and placenta. Enzymatic analysis may be used for prenatal diagnosis, when the mutation in the family is unknown, but is not recommended as it has at least a 1% false negative diagnostic rate [28]. Due to cycling over the P-protein, patients with T-protein defects show 10% residual activity in measurements of whole GCS activity. The glycine exchange assay is deficient in defects of the P-protein or the H-protein, but is normal in defects of the T-protein. A whole body glycine metabolism analysis is done by measuring exhaled carbon dioxide ^{13}C isotope enrichment after an enteral dose of 1-^{13}C-glycine [29].

Transient NKH is a phenocopy. In this clinical setting, neonates present with acute neurological symptoms and have elevated CSF glycine with often elevated CSF:plasma glycine levels. These elevated CSF glycine levels disappear spontaneously over the next days to weeks. There are no mutations present in the genes of the glycine cleavage enzyme and the GCS activity is normal. This feature can be seen in a variety of clinical settings, most commonly in hypoxic ischemic injury in a neonate [30]. The absence of the typical pattern of diffusion restriction of NKH indicates a phenocopy.

Most patients with variant NKH manifest only mild elevations of glycine in plasma and CSF and may also have an increase of plasma alanine and serum lactate and pyruvate concentrations with 2-ketoglutarate in urine organic acids. Patients with NFU1 may in addition have elevated 2-aminoadipic acid and 2-ketoadipic acid. Enzymatic analyses reveal deficient GCS activity, but also consistently a deficiency in PDH activity, and in certain disorders of iron sulfur cluster synthesis (*NFU1*, *ISCA2*, *IBA57*, *BOLA3*) a deficiency of complexes I and II of the respiratory chain. Western blot analysis can demonstrate reduced amounts of lipoylated H-protein, E2 of PDH, and 2KDH [2][3][4][5][6][7][8][9][10][11][12][13][14][15][16][17]. In PNPO deficiency other results point to low pyridoxal phosphate in cells including low CSF HVA and 5HIAA with high CSF 3-methoxytyrosine, glycine, threonine, histidine and taurine and low plasma arginine (▶ Chapter 28). Identification of the correct genetic defect by mutation analysis is important since treatment and prognosis of classic NKH are different from that of variant NKH.

23.6 Treatment

Withdrawal of intensive care in the neonatal period is an ethical consideration given the very poor outcome in severe classic NKH [31]. Correct distinction between severe and attenuated NKH can aid in this decision making [2]. Once breathing resumes, children with classic NKH will not die and adequate supportive care must be provided.

First principle of medical treatment in classic NKH is reduction of glycine plasma levels by benzoate with 250–750 mg/kg/day in 3–6 daily doses, with higher doses in severely affected patients (500–750) compared to attenuated patients (250–500) [1][2][4][19][32]. Monitoring of benzoate treatment should include regular measurement of plasma glycine obtained 1 hour after a dose (goal of treatment ≤300 μmol/L), and benzoate levels if glycine is low (non-toxic level ≤2.5 mmol/L) [1][19]. Side effects of benzoate are incompliance due to unpalatability often requiring administration via G-tube, esophagitis or gastritis, which should always be avoided by prophylactic treatment with proton pump inhibitors, and carnitine deficiency, which should be monitored for [33]. Overdosage of benzoate may result in toxicity, beginning as nausea, vomiting, lethargy, hypocalcemia, and leading to coma, seizures, acidosis, hypernatremia, hypokalemia, and death [19]. Dosing over 750 mg/kg/day can cause renal dysfunction syndrome [32]. Children with severe NKH requiring high doses of benzoate can benefit from a glycine- and serine-restricted diet, which can be assisted by a glycine-free amino acid formula [19]. Dietary gelatin and high protein intake should always be avoided. Benzoate treatment results in a reduction of seizure frequency and an improvement of alertness [1][32][33]. In severe NKH, it does not ameliorate developmental delay.

The second principle of medical treatment in classic NKH is the use of receptor antagonists to block the effects of glycine

at the neurotransmitter receptors. Dextromethorphan blocks the glutamate binding site of the excitatory NMDA receptor. In doses of 3–15 mg/kg/day it reduces seizures and improves alertness [34][35]. Its importance is most striking in attenuated NKH where early treatment has resulted in substantially increased cognitive outcome, and late treatment has at times resulted in resolution of chorea, increased alertness, and improved school function. In patients with severe NKH, dextromethorphan is less effective or ineffective and may result in recurrent pneumonia, presumably due to decreased coughing [1][34]. An alternative lipophilic non-competitive NMDA receptor blocker is ketamine, used in oral application at daily doses of 15 mg/kg in neonates, reduced to 9 mg/kg/day in infants (range: 1–32 mg/kg) with improved cognitive outcome by early treatment in attenuated mutation carriers [5][35]. Treatment with strychnine, a competitive antagonist of inhibitory GlyR receptor, resulted in some clinical improvement in a few historical cases.

Symptomatic epilepsy treatment with anticonvulsants is challenging in patients with severe classic NKH, and multiple anticonvulsants are required. A systematic study of anticonvulsant effectiveness is not available. Benzodiazepines (clonazepam, clobazam) are most effective for treating myoclonic seizures in early infancy. Levetiracetam, topiramate, and phenobarbital are most commonly used in older children. Felbamate, an NMDA-receptor-blocker, may be considered for treating recalcitrant seizures, although serious side effects limit its wider use [36]. Vigabatrin may rarely cause rapidly progressive deterioration in patients with severe NKH, most likely due to a secondary increase of GABA concentrations [37]. Valproate is contraindicated in all patients with NKH. It inhibits residual GCS enzyme activity, increases glycine plasma and brain levels in patients with NKH, leading to encephalopathy with chorea and a paradoxical increase in seizures [38]. In PNPO, seizures are only pyridoxal-P responsive. A further treatment is a ketogenic diet, which decreases plasma and CSF glycine levels, and hence concomitant benzoate dosing should be reduced. It sometimes resulted in decreased seizures and increased alertness, but the effect on EEG pattern is variable and hypsarrhythmia has not often resolved [39]. A vagal nerve stimulator has been effective in several older patients with severe NKH [40]. In attenuated NKH, hyperactivity is difficult to control medically. Hyperactivity and behavioral problems respond best to Applied Behavioral Analysis therapy, resulting in substantial developmental progress in some children. Scoliosis does not respond to bracing and surgical options for scoliosis and hip dysplasia have to be weighted with the quality of life [41]. Respiratory secretion management is challenging in older severe NKH patients and careful management can be helpful in avoiding recurrent pneumonia, often a terminal complication. The role of one methylgroup donors such as folinic acid has not been proven in humans, in contrast to mice with NKH. Patients with certain rare mutations could potentially benefit from co-factor therapy with pyridoxine (*GLDC*) or folinic acid (*AMT*).

No effective treatment currently exists for variant NKH and lipoate synthesis defects. Benzoate and dextromethorphan have no apparent effect. Avoidance of catabolism can aid in preventing regressive episodes in patients with *BOLA3* mutations. High dose lipoate can be tried but results are generally disappointing.

23.7 Prognosis

Prognostic indicators of severe outcome include high CSF glycine level (>230 μM) and presence of malformations on brain MRI, whereas prognostic indicators of attenuated NKH are low CSF:plasma glycine ratio (<0.08), late onset (≥4 months), and absence of epilepsy [2]. Frequent hiccupping and EEG patterns of burst suppression and hypsarrhythmia tend to indicate severe outcome [1]. In classic NKH, a genotype-phenotype correlation has been established following the hypothesis that residual enzyme activity was associated with an improved developmental outcome [2]. The presence of two mutations without residual activity always predicts severe NKH. At least one mutation with residual activity is required for attenuated NKH. Patients with two residual activity conferring mutations have the best neurocognitive outcome [2]. Early treatment in attenuated patients with a mutation with residual activity is paramount for achieving the optimal developmental outcome, such as seen in four patients homozygous for *GLDC* p.A802V mutation and treated aggressively with sodium benzoate and ketamine very early on [5][42]. This emphasizes the importance of early genotyping of the patients for an accurate prognosis. Survival of patients with severe NKH varies from months to over two decades when seizure management and supportive care are done effectively. Patients with attenuated NKH often survive for decades.

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Disorders of Glutamine, Serine and Asparagine Metabolism

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24

Glutamine, Serine and Asparagine Metabolism

Glutamine Metabolism

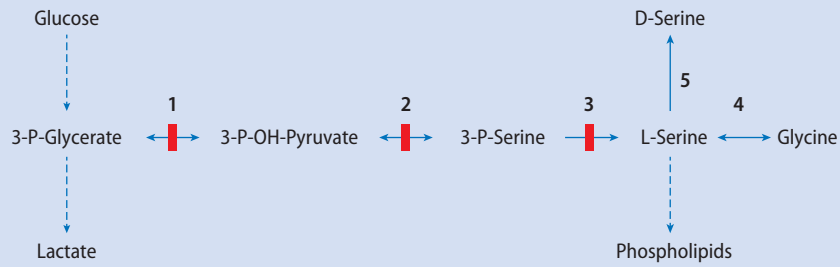
Glutamine, the most abundant amino acid in the human organism, is synthesized *de novo* from glutamate and ammonia by the enzyme glutamine synthetase. This reaction is the only way to produce glutamine in the human body and there is no net absorption from the intestine. Glutamine plays a pivotal role in metabolism as constituent, precursor, and amino moiety donor for many proteins, purines and pyrimidines, nicotinamide adenine dinucleotide, adenosine-monophosphate, amino acids and glucose.

Serine Metabolism

Serine is a non-essential amino acid and has important functions besides its role in protein synthesis. It is a precursor of a number of compounds (partly illustrated in ◘ Fig. 24.1), including D-serine, glycine (► Chapter 23), cysteine, serine phospholipids (► Chapter 34), sphingomyelins, and cerebrosides (► Chapter 38) that play an essential role in neuronal development and function. It must be synthesized within the brain because of its poor permeability across the blood-brain barrier. This synthesis is confined to astrocytes, and its shuttle to neuronal cells is performed by a dedicated neutral amino acid transporter, ASCT1. Moreover, it is a major source of N^5,N^{10}-methylene-tetrahydrofolate (THF) and of other one-carbon donors that are required for the synthesis of purines and thymidine. Serine is synthesized *de novo* from a glycolytic intermediate, 3-phosphoglycerate and can also be synthesized from glycine by reversal of the reaction catalyzed by serine hydroxymethyltransferase, which thereby converts N^5,N^{10}-methylene-THF into THF. It has recently been demonstrated that there is a rheostat-like mechanistic relationship between pyruvate kinase activity and serine biosynthesis and that the serine biosynthesis pathway is crucial for cancer cell survival [22].

Asparagine Metabolism

Asparagine is synthesized *de novo* by asparagine synthetase (ASNS) which catalyzes the transfer of ammonia from glutamine to aspartic acid via a β-aspartyl-AMP intermediate. Nutritional intake is another source of asparagine. Because of the poor transport of asparagine across the blood-brain barrier (the level of cerebrospinal asparagine is only 1 to 10 % of the plasma level), the brain depends on local *de novo* synthesis. Thus asparagine synthesis is essential for the development and function of the brain but not of other organs. ASNS is expressed at low levels in most tissues except the brain that exhibits a brain-specific splice variant. Besides its role as a constituent of proteins, asparagine is a precursor of the glucogenic amino acid L-aspartate, itself a precursor of the neurotransmitter D-aspartate. It also plays an important role in ammonia detoxification and in asparagine-linked protein glycosylation.



◘ **Fig. 24.1 Pathway of de novo serine synthesis**. *P*, phosphate; *1*, 3-phosphoglycerate dehydrogenase; *2*, phosphoserine aminotransferase; *3*, 3-phosphoserine phosphatase; *4*, serine hydroxymethyltransferase (utilizes tetrahydrofolate); *5*, serine racemase. Glycine is synthesized from serine, but also from other sources. Red bars across arrows indicate the known defects in serine synthesis

One disorder of ***glutamine*** synthesis is known, due to glutamine synthetase deficiency. It has been reported in only three patients who presented as newborns with epileptic encephalopathy. The outcome is very poor, and it is not yet known whether early start of glutamine therapy can improve the clinical prognosis.

Four disorders of ***serine*** metabolism are known. Three are in its biosynthesis: namely, 3-phosphoglycerate dehydrogenase deficiency, phosphoserine aminotransferase deficiency and phosphoserine phosphatase deficiency. Most patients with 3-phosphoglycerate dehydrogenase deficiency have a severe infantile phenotype with congenital microcephaly, intellectual disability and intractable seizures or Neu-Laxova syndrome, a severe dysmorphism syndrome with, as a rule, perinatal lethality. In patients with the severe, infantile form, the treatment is oral L-serine, supplemented with glycine in case of unsatisfactory clinical response. Phosphoserine aminotransferase deficiency has also been reported in Neu-Laxova syndrome, and in a boy with a milder phenotype. His affected sister was treated from birth on with serine and glycine, and this resulted in a normal outcome at the age of 15 years. Phosphoserine phosphatase deficiency has been described in Neu-Laxova syndrome, but also in a child with a mild phenotype. Mutations have recently been reported in the *SLC1A4* gene, encoding the brain serine transporter. They are associated with a phenotype that closely resembles that of severe 3-phosphoglycerate dehydrogenase deficiency.

One disorder of ***asparagine*** synthesis has been reported, due to asparagine synthetase deficiency. Fourteen patients are known. All showed severe psychomotor/intellectual disability, progressive microcephaly, limb hypertonia, hyperreflexia and mostly also intractable epilepsy. Six patients died within the first year of life. The poor permeability of the blood-brain barrier for asparagine leaves little hope for a therapeutic effect of substitution (► Glutamine, Serine and Asparagine Metabolism).

24.1 Glutamine Synthetase Deficiency

Glutamine Synthetase (GS) deficiency is an ultra-rare disease, first described in 2005 [1].

Clinical Presentation

GS deficiency has only been reported in three patients, two of them with Turkish and one with Sudanese origin [1][3]. They all presented as newborns with epileptic encephalopathy, as the only sign or in combination with other symptoms including diarrhoea, erythematous skin rash, and multiorgan failure. Two patients died during the newborn period while the patient with Sudanese origin survived until age six years. This patient was affected by chronic epileptic encephalopathy with severe mental retardation. In all patients, the disease started prenatally as illustrated by brain magnetic resonance imaging (MRI) which showed cerebral and cerebellar atrophy associated with an almost complete agyria [2].

Metabolic Derangement

The main **biochemical findings** in GS deficiency are the low glutamine concentrations in all body fluids. Remarkably, plasma glutamine concentrations were initially only borderline low in one patient but continuously decreased during infancy [3]. All other amino acids including glutamate are normal. The only other constant finding is chronic moderate hyperammonemia with most ammonia levels between 100–200 μM [3]. This reflects the failure to clear, within the perivenous hepatocytes, ammonia that escaped the urea cycle in periportal hepatocytes. Pathophysiology of GS deficiency is not completely understood but it is assumed that, in addition to the developmental abnormalities caused by glutamine deficiency during fetal and embryonic stages, a combination of systemic glutamine and nicotinamide adenine dinucleotide (NAD^+) deficiency, and chronic hyperammonemia lead to the severe phenotype [4].

Genetics

Defects in *GLUL* cause GS deficiency. The disease is transmitted in an autosomal-recessive mode. The mutations known in the three patients were all missense changes presumably allowing some, albeit little, residual GS activity and it can be assumed that a complete loss of GS function would be lethal during embryonic or fetal development. Prenatal diagnosis is feasible by genetic means.

Diagnostic Tests

GS deficiency is characterized by decreased levels of glutamine in all body fluids. Glutamine levels may be only slightly decreased in the newborn period and infancy but may become as low as <10 μM in plasma. Thus, any slight decrease in plasma glutamine concentration in an infant with epileptic encephalopathy should be taken seriously and other body fluids should be investigated. Since GS is ubiquitously expressed, enzyme studies can be done in many tissues but are not routine tests.

Confirmation of the diagnosis should be undertaken by mutation analysis. Newborn screening is currently not available and would require reliable assays for glutamine in dried blood spots.

Treatment and Prognosis

Patients with GS deficiency are not at risk of acute metabolic decompensations. Glutamine deficiency and hyperammonemia are chronic and difficult to treat. In a single patient, enteral glutamine substitution (given every two hours or as continuous gastric tube feeding at a dose of up to 1020 mg/kg/day) led to correction of glutamine in plasma and partly also in the CSF [5]. Whether early start of glutamine therapy can improve the clinical prognosis is unknown. In addition to glutamine substitution, correction of systemic NAD^+ deficiency by nicotinamide treatment may be beneficial but this has not yet been tested prospectively.

Based on the three patients reported, the outcome of GS deficiency is poor with death in the newborn period or in early childhood. It is however unknown whether early treated or mildly affected patients may survive longer.

24.2 Inborn Errors of Serine Metabolism

24.2.1 3-Phosphoglycerate Dehydrogenase Deficiency

Clinical Presentation

Thirty-three (reported and non-reported) patients (from 25 families) are known, with four different clinical presentations [6][7][8][9]. The most severe end of the known clinical spectrum is *Neu-Laxova syndrome* first reported in 1971. It is characterized by severe fetal growth restriction, microcephaly, a distinct facial dysmorphism, ichthyosis, skeletal anomalies and perinatal lethality. Patients with a defect in one of the other serine biosynthesis steps can also have this syndrome (see further). The majority of the patients with 3-phosphoglycerate dehydrogenase deficiency had the *severe, infantile phenotype* characterized by congenital microcephaly, intractable seizures, and pronounced intellectual disability. From birth on, these patients suffer from feeding difficulties with vomiting and from irritability with inconsolable crying. Therapy-resistant convulsions appear in the first weeks to months of life and show great variation. The psychomotor development of these patients is extremely poor; those reported (aged up to 24 years) had a developmental age of less than one year. Hypertonia is present before the age of one year and evolves into spastic tetraplegia. Other symptoms, reported in a minority of the patients, are congenital cataracts, growth retardation, abnormal hair, inguinal and umbilical hernias, hypogonadism, and megaloblastic anemia. Magnetic resonance imaging (MRI) of the brain revealed cortical and subcortical hypotrophy and evidence of disturbed myelination.

A *mild, juvenile phenotype* has been described in a brother and sister presenting with intellectual disability and therapy-responsive absence seizures at 5 and 9 years. Brain MRI was normal in both [10].

Finally, a *Charcot-Marie-Tooth phenotype* has been reported in a man who was diagnosed at 31 years with a progressive, severe, axonal sensorimotor polyneuropathy compatible with Charcot-Marie-Tooth disease type 2. Brain MRI showed non-specific T2-weighted hyperintensities [11].

Metabolic Derangement

The deficiency of 3-phosphoglycerate dehydrogenase, the first step of serine biosynthesis (enzyme 1 ◘ Fig. 24.1), causes decreased concentrations of serine and, to a lesser extent, of glycine in CSF and in fasting plasma. Serine thus becomes an essential amino acid in these patients. A significant accumulation of the substrate, 3-phosphoglycerate, is unlikely since it is an intermediate of the glycolytic pathway. Therefore, the deficiency of brain serine seems to be the main determinant of the disease. Serine plays a major role in the synthesis of important brain and myelin constituents, such as proteins, glycine, cysteine, serine phospholipids, sphingomyelins and cerebrosides.

In the two patients with megaloblastic anemia, decreased methyltetrahydrofolate was found in CSF. This can be explained by the fact that serine is converted into glycine by a reaction that forms methylenetetrahydrofolate, which is further reduced to methyltetrahydrofolate.

Genetics

This is an autosomal-recessive disease. To date fourteen mutations in *PHGDH* have been identified. Prenatal diagnosis is only possible by mutation analysis as there is a lack of data on enzyme activity in chorionic villi and amniocytes.

Diagnostic Tests

This disease should be considered in patients with Neu-Laxova syndrome and in encephalopathy comprising congenital microcephaly, epilepsy and intellectual disability. Plasma amino acids must be measured in the fasting state (range of serine in patients: 28–64 μM; normal range: 70–187 μM), since serine and glycine levels can be normal after feeding. In CSF, serine levels are always decreased (6–8 μM; control range 35–80 μM), as are glycine levels, but to a lesser extent. The diagnosis is confirmed by finding a deficient activity of 3-phosphoglycerate dehydrogenase in fibroblasts (reported residual activities from 6–22%) or by mutation analysis. In the patients with the milder, juvenile phenotype, the metabolite and enzymatic findings were indistinguishable from the findings in the patients with the severe phenotype.

Treatment and Prognosis

Treatment with L-serine has a beneficial effect on the convulsions, spasticity, feeding and behaviour. Oral L-serine treatment (500–700 mg/kg/day in six divided doses) corrected the biochemical abnormalities in all reported patients and abolished the convulsions in most, even in those in whom many anti-epileptic treatment regimens had failed previously. During treatment with L-serine, a marked increase in the white matter volume was observed, and in some patients a progression of myelination. In a few patients, convulsions stopped only after adding glycine (200–300 mg/kg/day).

In a girl diagnosed prenatally, because of decelerating head growth, L-serine was given to the mother at 190 mg/kg/day in 3 divided doses from the 27th week of gestation. This normalized fetal head growth and with subsequent postnatal therapy the girl at 16 years of age shows normal development.

The patients with the milder phenotypes responded well to lower L-serine doses (100–150 mg/kg/day), without glycine.

24.2.2 Phosphoserine Aminotransferase Deficiency

This disorder in the second step of the serine biosynthesis (enzyme 2 ◘ Fig. 24.1) has been reported in a brother and sister who showed decreased concentrations of serine and glycine in plasma and cerebrospinal fluid [12]. The index patient presented with intractable seizures, acquired microcephaly, hypertonia and psychomotor retardation, and died at the age of 7 months despite supplementation with serine (500 mg/kg/day) and glycine (200 mg/kg/day) from the age of 7 weeks. The younger sibling received treatment from birth, which led to a normal outcome at the age of 3 years. Enzyme activity measurement in cultured fibroblasts was inconclusive but mutation analysis revealed compound heterozygosity in *PSAT1* in both children. Recently, Neu-Laxova syndrome has been found to be caused by mutations in any of the three enzymes of serine biosynthesis (▶ Section 24.2.1).

24.2.3 3-Phosphoserine Phosphatase Deficiency

Decreased serine levels were found in plasma (53–80 μM; normal range 70–187 μM) and CSF (18 μM; control range 27–57 μM) of a patient with Williams syndrome [13]. Phosphoserine phosphatase activity in lymphoblasts and fibroblasts amounted to about 25% of normal (enzyme 3 ◘ Fig. 24.1). Oral serine normalized plasma and CSF levels of this amino acid and seemed to have some beneficial clinical effect. The gene was mapped to 7p11, and the patient was found to be a compound heterozygote for two missense mutations, excluding a link with Williams syndrome [14]. Recently, Neu-Laxova syndrome has been found to be caused by mutations in any of the three enzymes of the serine biosynthesis (▶ Section 24.2.1).

24.2.4 Brain Serine Transporter Deficiency

In 2015 several families have been reported with mutations in *SLC4A1*, encoding the ASCT1 brain transporter of serine and other neutral amino acids [15][16][17]. They presented with significant intellectual disability, severe progressive microcephaly, epilepsy, spasticity and thin corpus callosum. This phenotype is strongly reminiscent of the severe presentation of phosphoglycerate dehydrogenase deficiency and

suggests that serine deficiency is the main determinant of the disease.

24.2.5 Serine Palmitoyltransferase Defects

These cause the most frequent subtype of hereditary sensory and autonomic neuropathy, HSAN type 1 (HSAN1), an autosomal dominant disease. The disorder is caused by mutations in the *SPTLC* gene, encoding three subunits of serine palmitoyltransferase (SPT), the first step in the de novo synthesis of sphingolipids (▶ Chapter 38).

24.3 Asparagine Synthetase Deficiency

Fourteen patients (from 8 families; Bangladeshi, Chinese/Brunei, Emirati, French Canadian, Iranian Jews, Saudi Arabian) are known (including an unreported patient) with this disease, that was first described in 2013 [18][19][20][21].

■ Clinical Presentation

All patients showed severe psychomotor/intellectual disability, progressive (mostly congenital) microcephaly, limb hypertonia and hyperreflexia. In most patients there was in addition early-onset intractable epilepsy and axial hypotonia. Six patients died at ages from 9 days to 12 months. The oldest reported patient was 14 years. There was a broad range of epileptic manifestations (spasms, tonic seizures, generalized tonic-clonic seizures, partial complex seizures) and EEG patterns (hypsarrythmia, multiple independent spike foci, disorganized background activity, burst suppression). In our patient, there was a striking sequence from myoclonus with clonic seizures often turning to status epilepticus with focal or multifocal spikes followed by spasms with modified hypsarrhythmia and then discontinuous EEG recordings most often distinct from suppression bursts. This sequence was similar to what has been seen in pyridoxine dependency. Brain MRI showed a decreased cerebral volume, and in most cerebellar atrophy, a decreased size of the pons, a thin corpus callosum, simplified gyri and evidence of deficient myelination. Post mortem findings, available from two patients (siblings), were cortical dysgenesis, periventricular leukomalacia, mesial temporal sclerosis, gliosis, neuronal loss and hydromelia of the spinal cord [21]. In our patient, there was gliosis of the grey matter and simplified gyration with poor white matter clearly resulting from loss of cortical neurons (personal communication of Gataullina S et al.).

■ Metabolic Derangement

Plasma levels of asparagine were normal to decreased while plasma glutamine levels were normal to increased. Plasma aspartate (the other substrate of ASNS) was normal. There was no information on whether the blood was taken in the fed or in the fasting state. Our experience with the serine synthesis diseases indicates that plasma serine is only decreased in the fasting state. Thus a normal plasma asparagine level does not rule out ASNS deficiency. Cerebrospinal fluid asparagine levels were measured in only two patients (siblings) and found decreased in both (0 and 1 µmol/l; normal range: 1.1–6.9) [19]. The cerebrospinal glutamine level was increased in the first patient and normal in the other. There is no information on aspartate levels in this fluid. As to the pathophysiology, it is assumed that the brain abnormalities are caused by asparagine deficiency during intrauterine development, and maybe by excitotoxicity due to a possible accumulation of aspartate, a substrate of ASNS.

■ Genetics

This disease shows an autosomal recessive inheritance. Consanguinity was present in over half of the reported families. The eight mutations in *ASNS* were all missense changes presumably allowing some residual ASNS activity. It can be assumed that a complete loss of ASNS function would be lethal during embryonic of fetal development [18]. Prenatal diagnosis is feasible by mutation analysis.

■ Diagnostic Tests

Blood for asparagine measurement has to be taken in the fasting state. Finding a decreased plasma asparagine level should be followed by amino acid analysis of the cerebrospinal fluid but there is only a small difference between normal and decreased levels. Therefore, in the absence of a clear metabolic marker, mutation analysis is the main diagnostic test.

■ Treatment and Prognosis

In the absence of effective substitution, the outcome is very poor, and six patients died within the first year of life. The epilepsy is generally pharmacoresistant. One patient showed a relatively good response to lamotrigine, an antiepileptic compound that reduces glutamate release. Unfortunately, there are no data available on the potential effect of asparagine substitution. The poor permeability of the blood-brain barrier leaves poor hope for an effect, unless very high doses can be administered without toxicity. This remains to be investigated.

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Disorders of Amino Acid Transport at the Cell Membrane

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Transepithelial Transport of Amino Acids

Epithelial cells in (for example) renal tubules and intestinal mucosa utilise several different amino acid transport systems (Fig. 25.1), which prefer amino acids with certain physicochemical properties. Cystine and the structurally related dibasic cationic amino acids lysine, arginine and ornithine are transported from the intestinal or renal tubular lumen into epithelial cells by an ***apical transporter*** (***system b$^{0,+}$***) in exchange for neutral amino acids. The dibasic amino acids are then transported from the epithelial cell into the tissues by a ***basolateral dibasic amino acid transporter*** (***system y$^+$L***) in exchange for neutral amino acids and sodium. Both these transporters are heteromers of a heavy subunit (*N*-glycosylated type 2 membrane glycoprotein) and a light subunit (nonglycosylated polytopic membrane protein) linked by a disulfide bridge. The subunits of an active transporter colocalize in the plasma membrane, but the exact process of dimerization is unclear since direct evidence for the assembly of the transporter in intact human cells has not been available. A third ***transporter system for neutral amino acids*** is expressed only at the luminal border of epithelial cells. It transports neutral amino acids *i.e.* alanine, asparagine, citrulline, glutamine, histidine, isoleucine, leucine, phenylalanine, serine, threonine, tryptophan, tyrosine and valine into epithelial cells. A specific renal transporter for the imino acids glycine, proline and hydroxyproline probably exists. Dicarboxylic aminoacids (aspartate, glutamate) have a specific transporter EAAT3 (encoded by *SLC1A1)*, located at the luminal border of epithelial cells and *also expressed in neurons (not shown on* Fig. 25.1*)*.

Cystinuria, lysinuric protein intolerance and Hartnup disorder are caused by defects of the apical ***cystine/dibasic amino acid transporter***(upper left arrow), the ***antiluminal dibasic amino acid transporter*** (lower left arrow), and the ***luminal neutral amino acid transporter*** (upper right arrow), respectively.

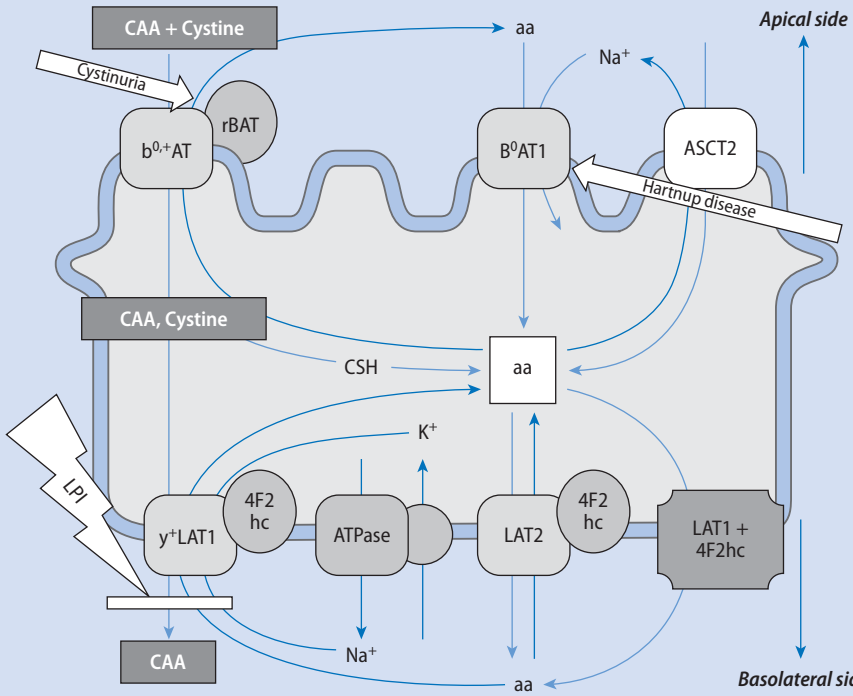

Fig. 25.1 Simplified schematic representation of cationic and neutral amino acid transport in epithelial cells. Courtesy of G. Sebastio.; *aa,* amino acids; *CAA,* cationic amino acids; *rBAT and b$^{0,+}$AT,* heavy and light subunits of the high-affinity luminal transporter system b$^{0,+}$; *y$^+$LAT,* system y+L amino acid transporter; *4F2hc,* 4F2 cell surface antigen heavy chain; *ASCT2,* alanine serine cysteine transporter 2; *CSH*, cysteine

Inherited defects in amino acid transport at the cell membrane are usually expressed as selective renal aminoaciduria, i.e., the concentration of the affected amino acids is high in the urine while it is normal or low in plasma. Intestinal absorption of the affected amino acids is also almost always impaired. The clinical symptoms thus result from excess amounts of certain amino acids in the urine or lack of them in the tissues. Consequently, in cystinuria renal stones may be formed because of high urinary concentration of poorly soluble cystine. In lysinuric protein intolerance (LPI), the transporter defect for the dibasic cationic amino acids leads to poor intestinal absorption and urinary loss of arginine, ornithine and lysine. Subsequently, the patients develop protein intolerance with hyperammonaemia, growth retardation and skeletal and immunological manifestations. The pellagra-like dermatitis and ataxia in Hartnup disease are attributed to deficiency of tryptophan, the precursor of niacin synthesis. Iminoglycinuria and dicarboxylic aciduria are mostly asymptomatic (► Transepithelial Transport of Amino Acids and ◘ Table 25.1).

25.1 Cystinuria

25.1.1 Clinical Presentation

Cystinuria is linked to a life-long risk of urolithiasis. It is responsible for 1-2% of kidney stones in adults and 6-8% in children. First stones typically manifest during the second decade. Some patients never develop any problems, but others may have recurrent symptoms from early childhood. Acute episodes of abdominal or lower back pain, haematuria, pyuria or spontaneous passing of stones may be the presenting sign. Symptomatic stones often appear in clusters between long asymptomatic periods. Microscopic nephronal obstruction by cystine crystals associated with inflammation may lead to renal injury. Recurrent urinary tract infections, urinary obstruction and, finally, renal failure are possible complications. Transient cystinuria due to an immature transport system may appear during the first years of life [1][2].

Cystinuria associated with severe neurological findings or Prader-Willi-like syndrome suggests a contiguous gene deletion on chromosome 2p16 or 2p21 (► Section 25.1.3).

25.1.2 Metabolic Derangement

In cystinuria, the high-affinity luminal transporter (system $b^{0,+}$; consisting of two protein subunits rBAT and $b^{0,+}$AT; ◘ Fig. 25.1) for cystine and the dibasic amino acids at the apical side of the epithelial cells of the proximal renal tubule and in jejunal mucosa is defective. Subsequently, absorption of cystine in the intestine and its reabsorption in the kidney is reduced. Normally, 99% of the filtered cystine is reabsorbed, while homozygotes with cystinuria excrete 600-1,400 mg of cystine into the urine per day. Cystine is poorly soluble at neutral or low urinary pH and crystals and stones may be formed [1]. Specific proteins may serve as promoters of cystine precipitation, aggregation or epithelial adherence [3]. No signs of cystine deficiency have been described.

25.1.3 Genetics

The average incidence of cystinuria is 1 in 7,000 but varies considerably between different populations. Cystinuria type A (type 1 cystinuria by former classification based on the phenotype of the parents as obligatory carriers) is the autosomal recessive form of the disease and represents over 60% of the cases. It is caused by mutations in *SLC3A1* on chromosome 2p16 that encodes rBAT, the heavy subunit of the amino acid transporter. At least 152 mutations have been reported. Cystinuria type B (non-type 1 cystinuria) is inherited in a dominant mode but with incomplete penetrance and is due to mutations in *SLC7A9* on chromosome 19q13.11. The gene product is the light subunit of the transporter, $b^{0,+}$AT. At least 104 mutations have been described. Thus, individuals harboring one mutated allele in *SLC7A9* may exhibit an abnormal urinary cystine excretion but generally remain free of clinical symptoms as opposed to clinically symptomatic individuals who exhibit two *SLC7A9* mutations. Individuals with the rare type AB cystinuria have two mutated alleles in the same gene in addition to a mutated allele in the other gene and so are actually AAB or ABB. Most of the mutations have been detected only in single patients, and the distribution of the more frequent ones is associated with ethnic background. There seems to be no genotype-phenotype correlation [4].

Homozygous contiguous gene deletions on chromosome 2p21 lead to three syndromes all presenting with cystinuria but otherwise distinct phenotypes. Homozygous deletions involving *SLC3A1* and the adjacent *PREPL* cause the hypotonia-cystinuria syndrome (HCS) that mimics the Prader-Willi syndrome with hypotonia, feeding difficulties and growth hormone deficiency in infancy and hyperphagia and obesity later in childhood [5]. Larger homozygous deletions in this region result in more severe neurological phenotypes, atypical HCS and the 2p21 deletion syndrome [6][7]. Recessive contiguous gene deletions in chromosome 2p16 lead to a combination of cystinuria and mitochondrial disease [8].

25.1.4 Diagnostic Tests

Cystine stones are usually radio-opaque and also visible on ultrasonography. Hexagonal cystine crystals in urine analysis are pathognomonic for cystinuria. Positive nitroprusside test and analysis of urinary amino acids lead to the diagnosis. Daily urinary cystine excretion is generally more than 400 mg (1.7 mmol)/1.73 m^2/d while the normal amount is less than 50–60 mg (0.26 mmol)/1.73 m^2/d. A cut-off value of 150 µmol/mmol creatinine has been suggested. However, the excretion of cystine varies markedly. Proper alkalinisation of the urine

Table 25.1 Inherited defects in amino acid transport at the cell membrane (modified from [62])

Aminoaciduria	Key clinical characteristics	Excess amino acids in urine	Protein	Solute carrier family	Gene
Cystinuria A	Urolithiasis	Cystine, lysine, arginine, ornithine	rBAT	Solute carrier family 3 (cystine, dibasic, and neutral amino-acid transporters), member 1	*SLC3A1*
Cystinuria B		Cystine, lysine, arginine, ornithine	$b^{0,+}$AT	Solute carrier family 7 (cationic amino-acid transporter, y^+ system), member 9	*SLC7A9*
Cystinuria AB		Cystine, lysine, arginine, ornithine			*SLC3A1* and *SLC7A9*: AAB or ABB
Lysinuric protein intolerance	Hyperammonemia after protein load, failure to thrive, renal problems, risk of alveolar proteinosis	Lysine, arginine, ornithine	y^+LAT1	Solute carrier family 7 (cationic amino-acid transporter, y^+ system), member 7	*SLC7A7*
Hartnup disorder	Mostly asymptomatic; pellagra-like dermatitis, ataxia, neuropsychiatric symptoms, mental retardation in some patients	Neutral amino acids	B^0AT1	Solute carrier family 6 (neutral amino-acid transporter), member 19	*SLC6A19*
Iminoglycinuria	Mostly asymptomatic	Proline, hydroxyproline, glycine	?		?
Dicarboxylic aminoaciduria	Asymptomatic? Neuropsychiatric symptoms?	Aspartate, glutamate	SLCA1 (EAAT3)	Solute carrier family 1, (dicarboxylic amino acid transporter; excitatory amino acid transporter)	*SLC1A1*

sample after voiding is a prerequisite for correct results. Plasma concentrations of cystine and the dibasic amino acids are normal or slightly decreased [9].

Chemical analysis of the stones alone may be misleading, because mixed stones are not uncommon in cystinuria, and some stones may contain no cystine at all. Hyperechogenic colon due to accumulated cysteine crystals in foetal ultrasonography has lead to prenatal diagnosis [10].

25.1.5 Treatment and Prognosis

Increased fluid intake to dilute the urine and alkalinisation to improve cystine solubility are the cornerstones of therapy. Moderate sodium restriction is recommended to reduce cystine excretion. Adults should drink 3000–4000 ml/24 h (1.75–2 l/m^2/24 h), 500 ml of this before bedtime and, if possible, 500 ml during the night to dilute urinary cystine concentration below saturation (less than 243 mg/L or 1 mmol/L). The amount of fluids should be further increased in warmer temperatures or with exercise. Permanent alkalinisation of the urine with a goal of a pH over 7.0 is best achieved by potassium citrate (2–4 mEq/kg/day, adults 30–90 mEq/day, increased if necessary on the basis of urinary pH monitoring).It may be helpful to avoid excessive dietary animal protein [11].

If the standard therapy fails to prevent new or dissolve pre-existing stones, a thiol derivative is added to decrease urinary free cystine concentration. The thiol drugs split the cystine molecule into two cysteines that form highly soluble drug-cysteine disulfide compounds that are then excreted in the urine. D-Penicillamine (30 mg/kg/day up to 3–4 g divided in 3–4 doses) has a large number of side effects including hypersensitivity reactions, bone marrow suppression, liver and kidney problems, trace metal deficiencies and disturbances in taste. In children, an initial dose of 5 mg/kg/day for 1 week and gradual increase up to 20–40 mg/kg/day has been proposed. The better tolerated mercaptopropionylglycine (tiopronin) may be the first option. The dose (10–20 mg/kg/day up to 1,000 mg/day in three doses) [12] should be increased gradually and adjusted individually. Captopril, while not as effective, is less toxic, and may have a role as an alternative therapy. Alkaline pH of the urine enhances the efficacy of the thiols. Liver enzymes, complete blood count, zinc and copper levels and urinary protein excretion should be monitored. Future therapeutic approaches may include crystal growth inhibitors [1][2][9].

Percutaneous nephrolithotomy and extracorporeal shockwave lithotripsy are seldom effective in stone removal, because cystine stones are extremely hard. New, minimally invasive urological techniques minimize the need for open surgery

[13][14]. Surgical procedures should always be combined with preventive therapy.

Regular follow-up is mandatory to support compliance, to monitor renal function and to detect developing stones early. Determination of cystine crystal volume in morning urine in addition to urinary pH and specific gravity [15] or direct assessment of urinary supersaturation [16] may prove helpful. Early detection of the disease by screening the family members of a patient is also essential.

25.2 Lysinuric Protein Intolerance

25.2.1 Clinical Presentation

The natural history of LPI still remains to be fully characterized, as only few of the oldest patients have reached the age of 50 years. Breast-fed newborns and infants are usually asymptomatic. Postprandial episodes of hyperammonaemia usually emerge when formula with higher protein content or supplementary high-protein foods are introduced [17]. Hyperammonaemia may present as refusal to eat, vomiting, stupor and drowsiness leading to coma, and can be misdiagnosed as food protein-induced enterocolitis syndrome. In that setting, forced tube feeding may be fatal. Strong aversion to high-protein foods with failure to thrive usually develops around the age of 1 year. The liver and spleen may be moderately enlarged.

In toddlers and school-age children, the presenting signs are most often growth failure and hepatosplenomegaly. The children are usually hypotonic with poor muscular strength. They show easy bruising and may have fractures after minor traumas. Neurological development is normal if severe or prolonged hyperammonaemia has been avoided. Bone maturation is retarded, and there is often pubertal delay.

The clinical heterogeneity of LPI is obvious in adult patients. Some are of moderately short stature, with abundant subcutaneous fat on a square trunk and thin extremities. They may have marked hepatomegaly with or without splenomegaly. Two thirds have osteopenia [18], but pathological fractures seldom occur in appropriately treated patients. Radiological signs of pulmonary fibrosis are common, but few patients suffer from symptomatic interstitial lung disease [19]. Mental capacity varies from normal to moderate impairment depending on previous history of hyperammonaemia.

Some patients have mild normochromic or hypochromic anaemia, leukopenia and thrombocytopenia, and their reticulocyte count is often slightly elevated. Serum ferritin, zinc and lactate dehydrogenase values are constantly elevated, while serum iron and transferrin concentrations are normal. Most adult patients have combined hyperlipidaemia [20]. High serum immunoglobulin-G concentrations and abnormalities in the distribution of lymphocyte subpopulations as well as in humoral immune responses [21] have been reported. Varicella infections are usually severe. Several cases of systemic lupus erythematosus have been reported. Bone marrow involvement with haemophagocytic lymphohistiocytosis (HLH) and interstitial pulmonary disease with alveolar proteinosis are quite frequent complications, and can occasionally be presenting signs. While most if not all LPI patients exhibit biological features of HLH (hypertriglyceridemia, hypofibrinogenemia and other coagulation alterations, hyperferritinemia, hypoalbuminemia, and elevated serum transaminases and LDH), a few develop a true clinical HLH syndrome with fever, hepatosplenomegaly and haemophagocytosis in bone marrow, spleen or lymph nodes [22][23][24].

Disturbed proximal tubular function with mild proteinuria, glucosuria, phosphaturia, tubular acidosis and microscopic haematuria may appear in childhood and often progress to glomerular dysfunction and end-stage renal failure. Systematic screening has revealed renal dysfunction of variable degree in the majority of adult Finnish patients, with rapid deterioration in glomerular filtration in some cases [25]. Urine beta2-microglobulin seems to be a sensitive early marker of renal tubular involvement in LPI [26].

A few children and adults have died after a very uniform course of progressive multiorgan failure, often starting with interstitial lung involvement and alveolar proteinosis, progressive glomerulonephritis that leads to renal insufficiency, and a severe bleeding diathesis [24]. In a French cohort, lung involvement has been observed in as many as 71% of LPI children, often with dismal prognosis [27]. One child with alveolar proteinosis went through an initially successful heart-lung transplantation, but died later after a recurrent disease [28].

Pregnancies of patients with LPI have been complicated by toxaemia, anaemia or bleeding during delivery and variable degrees of intrauterine growth retardation, but many have been completed successfully without any major problems [29].

25.2.2 Metabolic Derangement

In LPI, transport of the dibasic cationic amino acids lysine, arginine and ornithine (system y^+L; ◘ Fig. 25.1) is defective at the basolateral membrane of epithelial cells in the renal tubules and small intestine [30], where y^+LAT1 combines with 4F2hc to generate an active amino acid transporter [31].

Massive amounts of lysine and more moderate amounts of arginine and ornithine are lost in the urine, and their intestinal absorption is limited, resulting in low plasma concentrations. Glutamine, glycine and alanine concentrations are often clearly elevated owing to malfunction of the urea cycle. It is still unclear whether the transport defect is also expressed in non-epithelial cells. Contrary to an earlier report [32], more recent data indicate that fibroblasts and erythrocytes from LPI patients have normal cationic amino acid transport, probably via other transporter isoforms [33][34]. A transport defect in hepatocytes has been postulated because of normal or even paradoxically elevated cationic amino acid concentrations in liver biopsy in LPI, and abnormal cationic amino acid transport between various intracellular compartments has been suggested [35].

Hyperammonaemia after protein ingestion and diminished protein tolerance in LPI resemble the symptoms of urea cycle enzyme deficiencies (▶ Chapter 19). This is best explained by functional deficiency of the intermediates arginine

and ornithine in the hepatocytes [35]. Most patients develop a protective aversion to high-protein foods, which further impairs their amino acid intake, aggravating the amino acid deficiencies. As arginine is the rate-limiting precursor of nitric oxide synthesis, extracellular arginine deficiency may also result in persistently low nitric oxide concentrations that may influence vascular and immunological functions [36]. Reduced availability of lysine, an essential amino acid, probably has a prominent role in the poor growth and skeletal and immunological manifestations in LPI. Occasional patients have exhibited severe carnitine deficiency [37] that may be of dietary origin: the principal dietary source of carnitine is red meat, which is consumed in very small amounts by most patients with LPI. Chronic lysine deficiency may also limit endogenous carnitine biosynthesis.

The pathogenic mechanisms of several clinical manifestations of LPI are still unknown. Macrophages along with altered regulation of nitric oxide synthesis may play a central role in the development of alveolar proteinosis and nephropathy: LPI macrophages secrete less nitric oxide than control macrophages while several inflammatory chemokines are elevated [38]. Impaired phagocytic function [39] and abnormal inflammatory and immune responses may contribute to lung injury [27]. Conversely, intracellular nitric oxide accumulation secondary to intracellular arginine trapping might also explain some LPI complications [40]; e.g. intracellular nitric oxide excess in kidney cells might contribute to kidney damage. The role of HLH is unclear. High lysine concentration in proximal tubular cells in LPI may induce ROS and NADPH oxidase generation and thus make the tubular cells susceptible to apoptosis [41], potentially contributing to the proximal tubular dysfunction.

25.2.3 Genetics

LPI is a rare autosomal-recessive disease, with less than 200 patients reported, over 50 of them from Finland. The incidence is highest in Finland (1 in 60,000); clusters of families are also known at least in Italy, Norway and Japan, and sporadic cases have been reported on all continents. *SLC7A7* encodes the light subunit of the dibasic amino acid transporter y^+LAT-1. At least 50 different mutations spread along the entire gene have been reported [42][43].

All Finnish patients share the same founder mutation, 1181-2A>T, which causes a frame shift leading to a premature stop codon. The phenotypic variability is wide within the genetically homogeneous Finnish patients, as well as in homozygous patients with other mutations, and no genotype/phenotype correlation has been established.

25.2.4 Diagnostic Tests

The diagnosis LPI is based on the combination of increased urinary excretion and low plasma concentrations of the cationic amino acids, especially lysine. The concentrations of plasma lysine, arginine and ornithine are usually less than 80 µmol/l, 40 µmol/l, and 30 µmol/l, respectively. If plasma amino acid concentrations are exceptionally low owing to very limited protein intake, urinary cationic amino acid excretion may on rare occasions be within the reference range.

Blood ammonia concentration increases after protein-rich meals. Postprandial orotic aciduria is practically always seen in untreated patients (see hyperammonaemia algorithm in ▶ Chapter 19). Nonspecific but almost constant findings include elevated serum lactate dehydrogenase activity and increased ferritin and triglyceride concentrations due to secondary HLH (see above).

In the genetically homogenous Finnish population, the diagnosis is easily confirmed by mutation analysis. In all other patients molecular analysis is necessary to confirm the diagnosis whenever the biochemical data are unclear that is not rare or if the clinical presentation is that of isolated HLH or alveolar proteinosis that might be erroneously ascribed to other aetiologies.

25.2.5 Treatment and Prognosis

The principal aims of the treatment are to prevent hyperammonaemia and to provide a sufficient supply of protein and essential amino acids for normal metabolism and growth. Protein tolerance in LPI can be improved with supplementary low-dose citrulline, a neutral amino acid that is also an intermediate of the urea cycle. Citrulline is readily absorbed and partially converted to arginine and ornithine, all of which improve the function of the urea cycle. Approximately 50–100 mg/kg/day of L-citrulline is given in three to five doses in association with protein-containing meals [44], with a target of maintaining plasma citulline within the high-normal range. On such a regimen, children usually tolerate 1.0–1.5 and adults 0.5–0.8 g/kg/day of natural protein [45]. There is marked interindividual variation in protein tolerance, and infections, pregnancy and lactation may alter it extensively. Frequent monitoring of urinary orotic acid excretion is necessary. In patients with constantly highly elevated glutamine and glycine levels, sodium benzoate or sodium phenylbutyrate (both up to 250 mg/kg/day or 13 g/m^2/day) help to reduce the nitrogen load.

A carefully titrated dose of L-lysine-HCl (20–30 mg/kg/day in three doses) is able to elevate the plasma lysine concentrations to low-normal range without side effects.

Carnitine supplementation is indicated for the patients with carnitine deficiency [46]. Owing to their restricted diet, all patients need regular supplementation with calcium, vitamins and trace elements, and the involvement of an experienced nutritionist is essential. Growth hormone therapy has been used in four children with growth retardation, with a promising response and no side effects [47]. Hypercholesterolemia has successfully been treated with statins, and high triglyceride levels may also need dietary and/or pharmacological treatment.

The rare cases of acute hyperammonaemia in LPI patients should be treated as in other urea cycle defects (▶ Chapter 19).

LPI patients should be immunised against varicella zoster, and non-immunised patients should be treated immediately

with acyclovir if they get the infection [21]. The treatment of the immunological and bone marrow complications, including HLH, is still experimental. Good responses have been reported in individual cases with immunosuppressive drugs and with immunoglobulin infusion [48]. In alveolar proteinosis, bronchoalveolar lavage and steroid therapy have been effective in some cases [23]. Granulocyte-macrophage colony-stimulating factor therapy in LPI proteinosis has thus far given contradictory results [49][50]. Heart-lung transplantation is probably contraindicated due to the risk of a relapse of proteinosis in the graft.

Although hyperammonaemia and the associated mental retardation can be avoided with citrulline treatment, renal and other complications of LPI develop and progress during current therapy. Large dosages of citrulline (>100 mg/kg/day) increase the intracellular synthesis of arginine and may further stimulate the immune cascade in tubular, glomerular and mesangial cells in the kidney, in alveolar macrophages and epithelial cells in the lung, and in reticular endothelial cells, with overt clinical complications [40].

25.3 Hartnup Disease

25.3.1 Clinical Presentation

The classical symptoms of Hartnup disease, pellagra-like dermatitis, intermittent ataxia and neuropsychiatric abnormalities, closely resemble those of nutritional niacin (nicotinic acid and nicotinamide) deficiency. Since the first description of the syndrome in several members of the Hartnup family in 1956 [51], an extensive number of subjects who fulfil the biochemical diagnostic criteria have been reported, mostly detected in newborn screening programmes. However, most of them remain asymptomatic.

In the few patients who develop clinical symptoms, the skin lesions and neurological problems usually appear in early childhood [52] and tend to ameliorate with age. Exposure to sunlight, fever, diarrhoea, inadequate diet or psychological stress may precipitate the symptoms. Pellagra-like skin changes are found on light-exposed areas. Eruptions may mimic those seen in zinc deficiency, and the rare combination of coeliac disease and Hartnup disorder has led to severe skin problems [53], intermittent cerebellar ataxia, attacks of headache, muscle pain and weakness may appear. Occasionally, patients present with mental retardation, seizures or psychosis-like symptoms [54]. Maternal Hartnup disorder seems to be harmless to the foetus [55].

25.3.2 Metabolic Derangement

The molecular defect involves a sodium-dependent and chloride-independent neutral amino acid transporter, B(0)AT1 (*SLC6A19*) in the apical brush border membrane of renal proximal tubule and intestinal epithelium [56][57]. Mutations in *SLC6A19* impair intestinal uptake and tubular reabsorption of all the neutral amino acids, *i.e.* alanine, serine, threonine, valine, leucine, isoleucine, phenylalanine, tyrosine, tryptophan, histidine and citrulline and the monoamino-dicarboxylic amides asparagine and glutamine. The transporter is associated with partner proteins that are necessary for its expression, collectrin (Tmem27) in the kidney and angiotensin-converting enzyme 2 (ACE2) in the intestine, both components of the renin angiotensin system [58].

The affected amino acids are readily absorbed in the intestine as short oligopeptides but not as free amino acids. They are excreted in 5- to 20-fold excess into the urine, leading to decreased or low normal plasma concentrations. The stools of patients contain increased amounts of free amino acids, closely reflecting the urinary excretion pattern [56][57]. The unabsorbed amino acids in the colon are exposed to bacterial degradation. Degradation of tryptophan produces large amounts of indole compounds, which are then excreted in the urine.

Systemic tryptophan deficiency plays a crucial role in the development of clinical symptoms such as neuropsychiatric signs since tryptophan is the precursor of the neurotransmitter serotonin. Most importantly, tryptophan deficiency leads to reduced availability of nicotinic acid, the precursor of NAD(P)H. Subsequent deficiency of nicotinic acid (or niacin) and its amide, nicotinamide, may explain the skin abnormalities resembling pellagra that is due to nutritional niacin deficiency. The wide phenotypic variability of Hartnup disorder may be explained by nutritional factors and genetic differences owing to the high frequency of compound heterozygotes. Tissue specific partner proteins may also play a role.

25.3.3 Genetics

The reported incidence of Hartnup disorder in newborns screened for aminoaciduria varies from 1 in 14,000 to 1 in 45,000. Hartnup disease is caused by mutations in *SLC6A19* and follows an autosomal recessive pattern of inheritance. At least 21 mutations have been identified, including missense and splicing as well as small deletions and insertions. The most common allele D173N does not completely inactivate the transport mechanism. Most patients are compound heterozygotes [59][60][61].

25.3.4 Diagnostic Tests

The characteristic excess of neutral amino acids in the urine and their normal or low-normal concentrations in plasma confirm the diagnosis. Urinary excretion of indole compounds may be within the normal range if the patient consumes normal or low amounts of dietary protein, but an oral load of L-tryptophan (100 mg/kg) in most cases leads to a supranormal increase in indole excretion. Genetic testing is available.

25.3.5 Treatment and Prognosis

Clinical symptoms may be prevented by sufficient dietary intake of niacin or adequate supply of high-quality protein that allows the necessary amount of tryptophan to be absorbed in oligopeptide form. Dermatitis and neurological symptoms usually but not invariably disappear rapidly with oral nicotinamide (50–300 mg/day). Tryptophan ethyl ester has been successfully used to circumvent the transport defect. Oral neomycin reduces intestinal degradation of tryptophan and decreases indole production; however, the role of the indole compounds in the disease has been poorly characterized [56] [62]. Early recognition of the condition in newborn screening programmes permits adequate follow-up and prevention of symptomatic disease.

25.4 Asymptomatic Aminoacidurias: Iminoglycinuria and Dicarboxylic Aminoaciduria

Urine screening programmes have detected asymptomatic patients with iminoglycinuria and dicarboxylic aminoaciduria. In iminoglycinuria, the excretion of glycine, proline and hydroxyproline is increased. As iminoglycinuria is normal in newborns, probably reflecting renal immaturity, the finding needs to be confirmed later in life.

The incidence of iminoglycinuria is 1 in 10,000. It is inherited in an autosomal recessive mode. Interestingly, the parents of the homozygous individuals (obligate heterozygotes) show glycinuria only. The molecular cause is not known, but candidate genes include *SLC36A1* and *SLC6A20*, encoding a proton-dependent amino acid transporter, PAT-1, and a sodium-dependent iminoacid transporter, SIT-1, respectively, and *SLC6A19* encoding the transporter B^0AT1. Although iminoglycinuria has occasionally been linked to other diseases in case reports, the present view is that it does not lead to clinical symptoms in spite of constant urinary loss of the three amino acids [63][64].

Dicarboxylic aminoaciduria i.e. excess urinary excretion of the acidic amino acids (aspartate and glutamate), has also been considered to be a largely asymptomatic condition but has recently been linked in the pathogenesis of some neuropsychiatric disorders. The estimated incidence of dicarboxylic aminoaciduria is 1 in 36,000. It is caused by loss-of-function mutations in *SLC1A1 (EAAT3)* encoding the glutamate transporter, which is also expressed in neurons [65].

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Section V Vitamin-Responsive Disorders

Biotin-responsive Disorders

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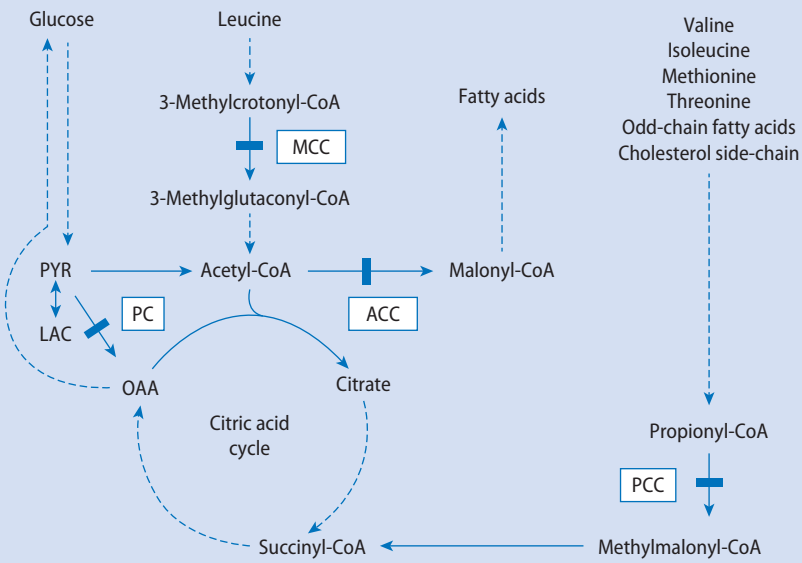
The Biotin Cycle and Biotin-dependent Enzymes

Biotin is a water-soluble vitamin widely present in small amounts in natural foodstuffs, in which it is mostly protein bound. The classic role of biotin is to function as the coenzyme of five important carboxylases involved in gluconeogenesis, fatty acid synthesis and the catabolism of several amino acids (■ Fig. 26.1). Covalent binding of biotin to the inactive apocarboxylases, catalysed by *holocarboxylase synthetase* (HCS), is required to generate the active holocarboxylases (■ Fig. 26.2). Recycling of biotin first involves proteolytic degradation of the holocarboxylases, yielding biotin bound to lysine (biocytin) or to short biotinyl peptides. *Biotinidase* then releases biotin from the latter compounds, which are derived from either endogenous or dietary sources. Both HCS and biotinidase have been shown to bind biotin covalently to histones *in vitro*. However, the physiological significance of this biotinyltransferase activity of biotinidase in not known [1], and the *in vivo* biotinylation of histones by HCS has been discussed controversially [2]. Recent studies indicate that HCS is a part of a chromatin-based regulatory complex in association with histone deacylases, and functions possibly as a general co-transcriptional repressor [2]. In addition, the transcription of a large number of genes, including those encoding HCS and the biotin-dependent carboxylases, is regulated by biotin in a process that requires biotinyl-5′-AMP, the intermediate of the HCS reaction [3]. A further biotin-regulated gene is *SLC19A3* encoding the thiamine transporter hTHTR2. *SLC19A3* mutations cause biotin-responsive basal ganglia disease [4] (▶ Chapter 28). However, since biotin is not a substrate for this transporter, the rescue of clinical symptoms through high doses of biotin is likely to be the result of increased expression of the receptor leading to restoration of some function [5]. Recent data suggest that high doses of biotin has an impact on disability and progression of the demyelinating process in progressive multiple sclerosis raising many questions on the potential role of this vitamin in neuronal energetic metabolism [6].



■ **Fig. 26.1 Location of the biotin-dependent carboxylases in intermediary metabolism**. *ACC*, acetyl-CoA carboxylases (ACC-1, cytosolic; ACC-2, outer mitochondrial membrane); *CoA*, coenzyme A; *HCS*, holocarboxylase synthetase; *LAC*, lactate; *MCC*, 3-methylcrotonyl-CoA carboxylase; *OAA*, oxaloacetate; *PC*, pyruvate carboxylase; *PCC*, propionyl-CoA carboxylase; *PYR*, pyruvate. *Full lines* indicate one enzyme, and dotted lines indicate that several enzymes are involved. Sites of the enzyme defects are indicated by **solid bars**

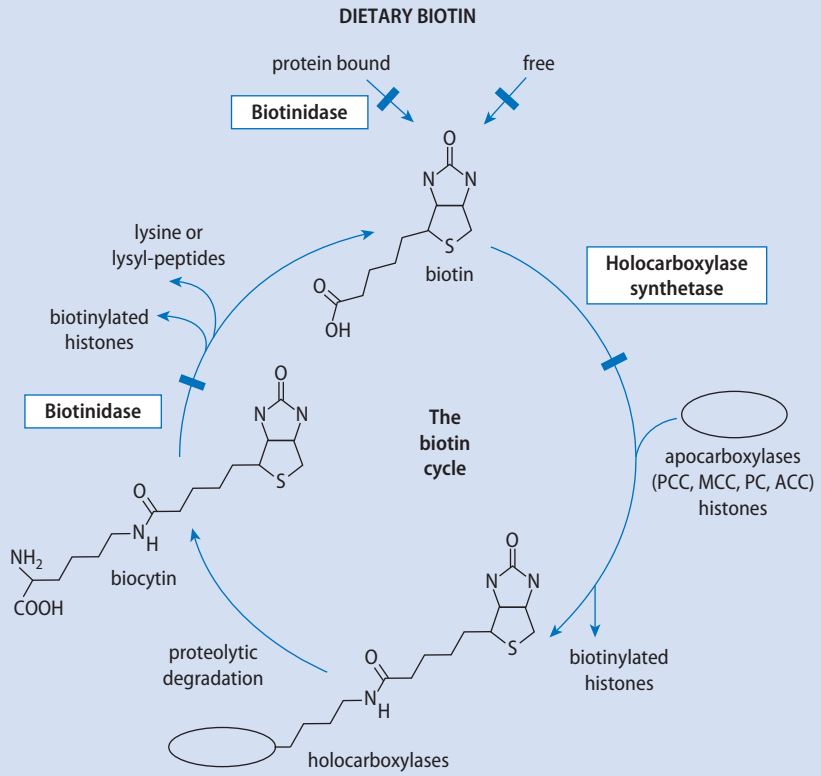


Fig. 26.2 The biotin cycle. Abbreviations as Fig. 26.1. Sites of the enzyme and transport defects are indicated by **solid bars**

Two inherited defects affecting the coenzyme function of biotin are known: *holocarboxylase synthetase* (HCS) *deficiency* and *biotinidase deficiency*. Both lead to deficiency of all biotin-dependent carboxylases, i.e. to *multiple carboxylase deficiency* (MCD). In HCS deficiency, the binding of biotin to apocarboxylases is impaired. In biotinidase deficiency, biotin depletion ensues from the inability to recycle endogenous biotin and to utilise protein-bound biotin from the diet. As the carboxylases play an essential role in the catabolism of several amino acids, in gluconeogenesis and in fatty-acid synthesis, their deficiency provokes multiple, life-threatening metabolic derangements, eliciting characteristic organic aciduria and neurological symptoms. The clinical presentation is extremely variable in both disorders. Characteristic symptoms include metabolic acidosis, hypotonia, seizures, ataxia, impaired consciousness and cutaneous symptoms, such as skin rash and alopecia. All patients with biotinidase and a majority of patients with HCS deficiency respond dramatically to oral therapy with pharmacological doses of biotin. Delayed diagnosis and treatment in biotinidase deficiency may result in irreversible neurological damage.
A few patients with HCS deficiency show only a partial or even no response to biotin and seem to have an impaired long-term outcome. *Acquired biotin deficiency,* which also causes MCD, is extremely rare. Dietary deficiency of biotin was documented in an 11-year-old retarded boy as a consequence of a dietary prescription containing raw eggs rich in avidin that chelates biotin and avoids its intestinal absorption [7]. A defect in *biotin transport* has been reported in a single child; however, the genetic defect remains unresolved to date [8].

26.1 Clinical Presentation

The characteristic manifestation of multiple carboxylase deficiency (MCD) is metabolic acidosis associated with neurological abnormalities and skin disease. The expression of the clinical and biochemical features is variable in both holocarboxylase synthetase (HCS) and biotinidase deficiency [9]. While patients with HCS deficiency commonly present with the typical symptoms of MCD, those with biotinidase deficiency show a less consistent clinical picture, particularly during the early stage of the disease. The onset in biotinidase deficiency may be insidious, and the manifestation is usually very variable, neurological symptoms often being prominent without markedly abnormal organic acid excretion or metabolic acidosis. Later-onset forms of HCS deficiency cannot be clinically distinguished from biotinidase deficiency, neces-

sitating confirmation of the diagnosis by enzyme or molecular genetic analysis.

26.1.1 Holocarboxylase Synthetase Deficiency

Although HCS deficiency was initially termed early-onset MCD, experience shows that the age of onset varies widely, from a few hours after birth to 8 years of age [10]. Nevertheless, about half of the patients have presented acutely in the first days of life with symptoms very similar to those observed in other severe organic acidurias, i.e. lethargy, hypotonia, vomiting, seizures and hypothermia. The most common initial clinical features consist of respiratory difficulties, such as tachypnoea or Kussmaul breathing associated with severe metabolic acidosis, ketosis and hyperammonaemia that – without biotin supplementation – may lead to coma and early death. Patients with a less severe defect and later onset may also present with recurrent life-threatening attacks of metabolic acidosis and typical organic aciduria [11][12].

Episodes of acute illness are often precipitated by catabolism during intercurrent infections or by a higher protein intake. Early-onset patients who recover without biotin therapy and untreated patients with a less severe defect may additionally develop psychomotor retardation, hair loss and skin lesions. These include an erythematous, scaly skin rash that spreads over the whole body but is particularly prominent in the diaper and intertriginous areas; alternatively, the rash may resemble seborrhoeic dermatitis or ichthyosis [13]. In contrast to biotinidase deficiency, deafness has not been reported in patients with HCS.

26.1.2 Biotinidase Deficiency

Important features are the gradual development of symptoms and episodes of remission, which may be related to increased free biotin in the diet. The full clinical picture has been reported as early as 7 weeks, but discrete neurological symptoms may occur much earlier, even in the neonatal period [14]. Neurological manifestations (lethargy, muscular hypotonia, grand mal and myoclonic seizures, ataxia) are the most frequent initial symptoms. In addition, many children have developmental delay, hearing loss, conjunctivitis and visual problems, including optic atrophy. Biotinidase deficiency should be considered in any child with unexplained developmental delay and particularly in those with sensorineural hearing loss. Skin rash and/or alopecia are hallmarks of the disease; however, these may develop late or not at all [1][15]. Skin lesions are usually patchy, erythematous/exudative and typically localised periorificially. Eczematoid dermatitis or an erythematous rash covering large parts of the body has also been observed, as has keratoconjunctivitis. Hair loss is usually discrete but may, in severe cases, become complete, including the eyelashes and eyebrows. Immunological dysfunction may occur in acutely ill patients.

Some children with profound biotinidase deficiency may not develop symptoms until later in childhood or during adolescence [1][16]. Their symptoms are usually less characteristic and may include motor limb weakness, spastic paraparesis, spinal cord demyelination and unusual symmetrical findings on brain MRI [1][16][17], or eye problems such as optic neuropathy, loss of visual acuity and scotomata [1][16].

Asymptomatic adults and siblings with profound biotinidase deficiency, some with very low serum or plasma biotinidase activity of 1.2–3.1% of the mean control value, were ascertained after identification of their affected children/siblings by newborn screening [1][18][19]. Therefore, investigation of all family members of patients with biotinidase deficiency is very important for the detection of asymptomatic individuals who are at risk of exhibiting symptoms at any age.

Because of the variability and nonspecificity of clinical manifestations, there is a very high risk of a delay in diagnosis [17][20][21][22]. Late-diagnosed patients often have psychomotor retardation and neurological symptoms, such as leukoencephalopathy, hearing loss and optic atrophy, which may be irreversible [15][17][21][22][23][24]. Outcome may even be fatal.

Metabolic acidosis and the characteristic organic aciduria of MCD are frequently lacking in the early stages of the disease. Plasma lactate and 3-hydroxyisovalerate may be only slightly elevated, whereas cerebrospinal fluid levels may be significantly higher [25]. This fact and the finding of severely decreased carboxylase activities in brain but moderately deficient activity in liver and kidney in a patient with lethal outcome [20] are in accordance with the predominance of neurological symptoms and show that, in biotinidase deficiency, the brain is affected earlier and more severely than other organs. The threat of irreversible brain damage demands that biotinidase deficiency should be considered in all children with neurological problems including a therapeutic trial with oral biotin (10 mg/day for 5 days), even if obvious organic aciduria and/or cutaneous findings are not present. Sadly, in regions where no neonatal screening for biotinidase deficiency is performed there seems to have been little improvement in the diagnostic delay over the last two decades [21][23]. Therefore, neonatal screening provides the best chance of improving outcome in biotinidase deficiency. Importantly, treatment should be instituted without delay, since patients may become biotin depleted within a few days after birth [14].

26.2 Metabolic Derangement

In HCS deficiency, a decreased affinity of the enzyme for biotin and/or a decreased maximal velocity lead to reduced formation of the five holocarboxylases from their corresponding inactive apocarboxylases at physiological biotin concentrations (Fig. 26.2) [26][27][28]. In biotinidase deficiency, biotin cannot be released from biocytin and short biotinyl peptides. Thus, patients with biotinidase deficiency are unable to recycle endogenous biotin and use protein-bound dietary

biotin (Fig. 26.2) [9]. Consequently, biotin is lost in the urine, mainly in the form of biocytin [14][29], and progressive biotin depletion occurs. Depending on the amount of free biotin in the diet and the severity of the enzyme defect, the disease becomes clinically manifest during the first months of life or later in infancy or childhood.

Deficient activity of biotin-dependent carboxylases in both HCS and biotinidase deficiencies (Fig. 26.1) results in accumulation of lactic acid and derivatives of 3-methylcrotonyl-coenzyme A (CoA) and propionyl-CoA (▶ Section 26.4).

Isolated inherited deficiencies of each of the three mitochondrial carboxylases, propionyl-CoA carboxylase (PCC; ▶ Chapter 18), 3-methylcrotonyl-CoA carboxylase (MCC; ▶ Chapter 18), and pyruvate carboxylase (PC; ▶ Chapter 11), are also known. A single patient with an isolated defect of acetyl-CoA carboxylase (ACC-1, cytosolic) has been reported [30]. These isolated deficiencies are due to absence or abnormal structure of the apoenzyme and usually do not respond to biotin therapy. A patient with isolated partial MCC deficiency and partial responsiveness to biotin therapy has been reported [31].

Acquired biotin deficiency is rare, but may result from excessive consumption of raw egg white [7], malabsorption, long-term parenteral nutrition, haemodialysis and long-term anticonvulsant therapy.

Biotin dependency due to a defect in biotin transport has been suggested in a 3-year-old boy with normal biotinidase activity and nutritional biotin intake [8], but the genetic defect remains unresolved to date.

26.3 Genetics

Both HCS and biotinidase deficiency are inherited as autosomal recessive traits. HCS deficiency seems to be rarer than biotinidase deficiency with the exception of the isolated small population in Faroe Islands where the incidence was calculated to be 1 in 1200 newborns [32].The incidences of profound (<10% residual activity) and partial (10-30% residual activity) biotinidase deficiencies are, on average, 1:112,000 and 1:129,000, respectively [33]. The incidence of combined profound and partial deficiency is about 1 in 60,000. The cDNAs for human HCS [34] and biotinidase [35][36] have been cloned. In both genes, multiple disease causing mutations have been identified.

26.3.1 Holocarboxylase Synthetase Deficiency

More than 40 different disease-causing mutations have been reported in the *HLCS* gene [37][38][39]. About two-thirds of them are within the putative biotin-binding region of HCS and some mutations have been shown to result in decreased affinity of the enzyme for biotin [26][28][40]; this is in accordance with elevated Km values for biotin measured in fibroblasts of several HCS deficient patients [27][41], and probably accounts for the *in vivo* responsiveness to biotin therapy of these patients. The degree of abnormality of the K_m values correlates well with the time of onset and severity of illness, i.e. highest K_m with early onset and severe disease [27].

Other mutations, located outside the biotin-binding site in the N-terminal region, are associated with virtually normal K_m for biotin but decreased V_{max} [28]. Most patients with this type of mutation also respond to biotin, although higher doses are usually required and residual biochemical and clinical abnormalities mostly persist. Biotin responsiveness in such patients most probably derives from a positive effect of biotin on *HLCS* mRNA transcription and thus on the level of HCS protein [42]. However, since this mechanism involves HCS protein itself, it requires the presence of residual HCS activity. One mutant allele, p.L216R, when present in the homozygous state, has been associated with a virtually biotin-unresponsive, severe clinical phenotype [43]. This mutation leads to unstable enzyme with reduced affinity for the protein substrate [44] and nearly absent activity [28], and seems to be prevalent in Polynesian patients of Samoan origin [43].

26.3.2 Biotinidase Deficiency

Over 165 mutations in *BTD* have been reported [45]. The two most common mutations detected in symptomatic patients with profound biotinidase deficiency in the USA, accounting for about one-third of the alleles, are c.98-104del7ins3 and p.R538C [46][47]. In contrast, in patients with profound biotinidase deficiency detected by newborn screening, three mutations – p.Q456H, the double-mutant allele p.A171T + p.D444H, and p.D252G – accounted for about half of the mutant alleles detected [46]. Strikingly, these mutations were not detected in any of the symptomatic patients [46][47].

A comparison of all mutations identified in children detected by newborn screening and in symptomatic children revealed six mutations comprising 60% of all mutant alleles [1].

Almost all individuals with partial biotinidase deficiency have the p.D444H mutation in combination with a mutation causing profound biotinidase deficiency on the second allele [1][48].

26.4 Diagnostic Tests

A characteristic organic aciduria due to systemic deficiency of the carboxylases is the key feature of MCD. In severe cases, an unpleasant urine odour (cat's urine) may even be suggestive of the defect. MCD is reflected in elevated urinary and plasma concentrations of organic acids as follows:

- Deficiency of MCC: 3-hydroxyisovaleric acid and 3-hydroxyisovalerylcarnitine (C5-OH) in high concentrations, 3-methylcrotonylglycine and tiglylcarnitine (C5:1) in smaller amounts.
- Deficiency of PCC: methylcitrate, 3-hydroxypropionate, propionylglycine, tiglylglycine, propionic acid and propionylcarnitine (C3) in small to moderate amounts.

- Deficiency of PC: lactate in high concentrations, pyruvate in smaller amounts.
- It should be noted that a similar organic acid profile can occur in patients with hyperammonemia due to carbonic anhydrase VA deficiency (▶ Chapter 19).

The majority of HCS-deficient patients excrete all of the typical organic acids in elevated concentrations, provided that the urine sample has been taken during an episode of acute illness. In contrast, in biotinidase deficiency elevated excretion of only 3-hydroxyisovalerate may be found, especially in early stages of the disease. In 20% of untreated biotinidase-deficient children urinary organic acid excretion was normal when they were symptomatic [1].

The measurement of carboxylase activities in lymphocytes provides direct evidence of MCD. These activities are low in HCS deficiency but may be normal in biotinidase deficiency, depending on the degree of biotin deficiency [11][19].

Determination of biotin concentrations in plasma and/or urine has little diagnostic value but can be used in evaluation of therapeutic compliance. Untreated, biotin concentrations are normal in HCS deficiency and usually decreased in symptomatic patients with biotinidase deficiency [14][19], provided that an assay method that does not detect biocytin is used [49].

The two inherited disorders can easily be distinguished by the assay of biotinidase activity in serum. Today, this assay is included in the neonatal screening programmes in many countries worldwide.

26.4.1 Holocarboxylase Synthetase Deficiency

- The diagnosis can be confirmed by molecular genetic analysis of the *HLCS* gene or indirect assay of HCS activity by demonstrating severely decreased activity of at least 2 mitochondrial biotin-dependent carboxylases in skin fibroblasts cultured in a medium with low biotin concentration (0.1 nM) and normalization (or at least an increase) of the activities in cells cultured in a medium supplemented with a high biotin concentration (0.2–10 μM) [11][27].
- It must be noted that fibroblasts of some late-onset patients may exhibit normal levels of carboxylase activities when cultured in standard media supplemented with 10% fetal calf serum (FCS), which results in a final biotin concentration of about 10 nM [11], corresponding to about 5 times the normal plasma biotin concentration in man. Carboxylase activities in lymphocytes are deficient and may remain reduced even during prolonged biotin supplementation [9]. Direct measurement of HCS activity requires a protein substrate, e.g. an apocarboxylase or a subunit or fragment of an apocarboxylase that contains the biotin attachment site [27][50]; therefore, it is not routinely performed.

26.4.2 Biotinidase Deficiency

- Biotinidase activity in serum is absent or decreased [1][19]. Many patients have measurable residual activity. The level of residual activity should be confirmed in a second serum sample obtained at the age of 4 month or later. Mutation analysis of the entire *BTD* gene is rarely necessary, because there are no therapeutic consequences.
- Carboxylase activities in lymphocytes are decreased in those patients that have biotin deficiency, but are normalised within hours after a single dose of oral biotin [14]. Carboxylase activities in fibroblasts cultured in low-biotin medium are similar to those in control fibroblasts, and are always normal in fibroblasts cultured in standard FCS based medium.

26.4.3 Acquired Biotin Deficiency

- Biotinidase activity is normal while biotin concentrations in plasma and urine are low. Carboxylase activities in lymphocytes are decreased and are promptly normalised after biotin supplementation [9].

26.4.4 Prenatal Diagnosis

Prenatal diagnosis of HCS deficiency is possible by mutation analysis if mutations of an index patient are known, by enzymatic studies in cultured chorionic villi or amniotic fluid cells, or by demonstration of elevated concentrations of metabolites by stable isotope dilution techniques in amniotic fluid. In milder forms of HCS deficiency organic acid analysis may fail to show an affected fetus, necessitating molecular genetic or enzymatic investigation [51]. Prenatal diagnosis allows rational prenatal therapy, preventing severe metabolic derangement in the early neonatal period [51][52]. Prenatal diagnosis of biotinidase deficiency is possible by mutation analysis but, in our opinion, not warranted, because prenatal treatment is not necessary.

26.5 Treatment and Prognosis

With the exception of some cases of HCS deficiency, both inherited disorders can be treated effectively with oral biotin in pharmacological doses as long as treatment is started before irreversible neurological damage has occurred, e.g. deafness in biotinidase deficiency. No adverse effects have been observed from such therapy over a more than 30-year experience of treating biotinidase deficiency [48][53] and, importantly, there is no accumulation of biocytin in body fluids [29], which was previously suspected to be a possible risk.

Restriction of protein intake is not necessary except in very severe cases of HCS deficiency. Raw eggs should be avoided because they contain avidin, an egg-white protein that binds biotin, thereby decreasing the vitamin's bioavailability

[1]. Acutely ill patients with metabolic decompensation require general emergency treatment in addition to biotin therapy (► Chapter 4).

26.5.1 Holocarboxylase Synthetase Deficiency

The required dose of biotin is dependent on the severity of the enzyme defect and has to be assessed individually [9]. Most patients have shown a good clinical response to 10–20 mg/day, although some may require higher doses, i.e. 40–200 mg/day or even higher doses [9][11][43][52][53][54][55]. In spite of apparently complete clinical recovery, some patients continue to excrete abnormal metabolites (particularly 3-hydroxyisovalerate), a finding that correlates inversely with the actual level of carboxylase activities in lymphocytes. Exceptionally, persistent clinical and biochemical abnormalities have been observed despite treatment with very high doses of biotin [9][43][52][54][55]. All patients with HCS deficiency have at least partially responded to pharmacological doses of biotin with the exception of the majority of those homozygous for the missense mutation p.L216R [43][54]. Patients homozygous for the IVS10+5G>A mutation, a founder mutation in Scandinavia originating from Faroe Islands, show a unique clinical character with onset between 2 months and 8 years, and a slow and in some patients only partial clinical response to biotin treatment [32][55].

To date, the prognosis for most surviving, well-treated patients with HCS deficiency seems to be good, with the exception of those who show only a partial or no response to biotin [9][43][52][54][55]. Careful follow-up studies are needed to judge the long-term outcome. In one patient, followed for 9 years and treated prenatally and from the age of 3.5 months with 6 mg biotin/day, some difficulties in fine motor tasks were obvious at the age of 9 years [56]. In five Japanese patients (four families) the intelligence quotient (IQ) at the age of 5–10 years varied between 64 and 80 [52]. Four of these patients had a severe neonatal-onset form, and one of them (IQ=64) was treated prenatally. Three showed recurrent respiratory infections, metabolic acidosis and organic aciduria despite high-dose (20–60 mg/day) biotin therapy. However, irreversible neurological auditory-visual deficits, as described for biotinidase deficiency, have not been reported. Prenatal biotin treatment (10 mg/day) has been reported in a few pregnancies [52]. It is unclear whether prenatal treatment is essential; treatment of at-risk children immediately after birth may be sufficient.

26.5.2 Biotinidase Deficiency

The introduction of neonatal screening programmes has resulted in the detection of asymptomatic patients with residual biotinidase activity [53]. Based on measurement of serum biotinidase activity, the patients are classified into those with profound biotinidase deficiency, with less than 10% of mean normal biotinidase activity, and those with partial biotinidase deficiency, with 10–30% residual activity. Because genotype–phenotype correlations in biotinidase deficiency are not well established, decisions regarding treatment should be based on enzymatic activity [1].

■ Profound Biotinidase Deficiency

In early-diagnosed children with complete biotinidase deficiency, 5–10 mg of oral biotin per day promptly reverses or prevents all clinical and biochemical abnormalities. For chronic treatment, the same dose is recommended. Under careful clinical and biochemical control, it may be possible to reduce the daily dose of biotin to 2.5 mg. However, biotin has to be given throughout life and regularly each day, since biotin depletion develops rapidly [14]. Some patients with profound deficiency have been reported to develop symptoms, e.g. hair loss, during puberty and adulthood that could be resolved when biotin dosage was increased to 15 or 20 mg [1].

Neonatal screening for biotinidase deficiency [33] allows early diagnosis and effective treatment. In such patients, the diagnosis must be confirmed by quantitative measurement of biotinidase activity. Treatment should be instituted without delay, since patients may become biotin deficient within a few days after birth [14].

In patients who are diagnosed late, irreversible brain damage may have occurred before the commencement of treatment. In particular, auditory and visual deficits often persist in spite of biotin therapy [15][23][24][25], and intellectual impairment and ataxia have been observed as long-term complications [15][21][23][24].

Patients with residual activity up to 10%, usually detected by neonatal screening or family studies, may remain asymptomatic for several years or even until adulthood [18][19]. According to our experience with 61 such patients (52 families), however, they show a very high risk of becoming biotin deficient and should be treated [1][19][33].

■ Partial Biotinidase Deficiency

Patients with partial biotinidase deficiency (10–30% residual activity) are mostly detected by neonatal screening or in family studies and usually remain asymptomatic. However, over 20 children with partial deficiency who were identified by newborn screening but were not treated with biotin eventually did develop symptoms typical of profound biotinidase deficiency, such as hypotonia, skin rashes and loss of hair, particularly when they were stressed by an infectious disease or moderate gastroenteritis. In the vast majority all symptoms were readily reversed upon biotin treatment [48][53]. Thus, because some untreated children will develop symptoms and conclusive evidence is lacking, it seems prudent to supplement patients with 10-30% of residual activity with biotin, e.g. 2.5–5 mg/day [48][53].

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Disorders of Cobalamin and Folate Transport and Metabolism

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Cobalamin Transport and Metabolism

Cobalamin (Cbl or vitamin B_{12}) is a cobalt-containing water-soluble vitamin that is synthesised by lower organisms but not by higher plants and animals (Fig. 27.1). The only source of Cbl in the human diet is animal products. Cbl is needed for only two reactions in man, but its metabolism involves complex absorption and transport systems and multiple intracellular conversions. As methylcobalamin (MeCbl), it is a cofactor of the cytoplasmic enzyme methionine synthase, which converts homocysteine to methionine. As adenosylcobalamin (AdoCbl), it is a cofactor of the mitochondrial enzyme methylmalonyl-coenzyme A mutase, which is involved in the catabolism of valine, threonine and odd-chain fatty acids into succinyl-CoA, an intermediate of the Krebs cycle.

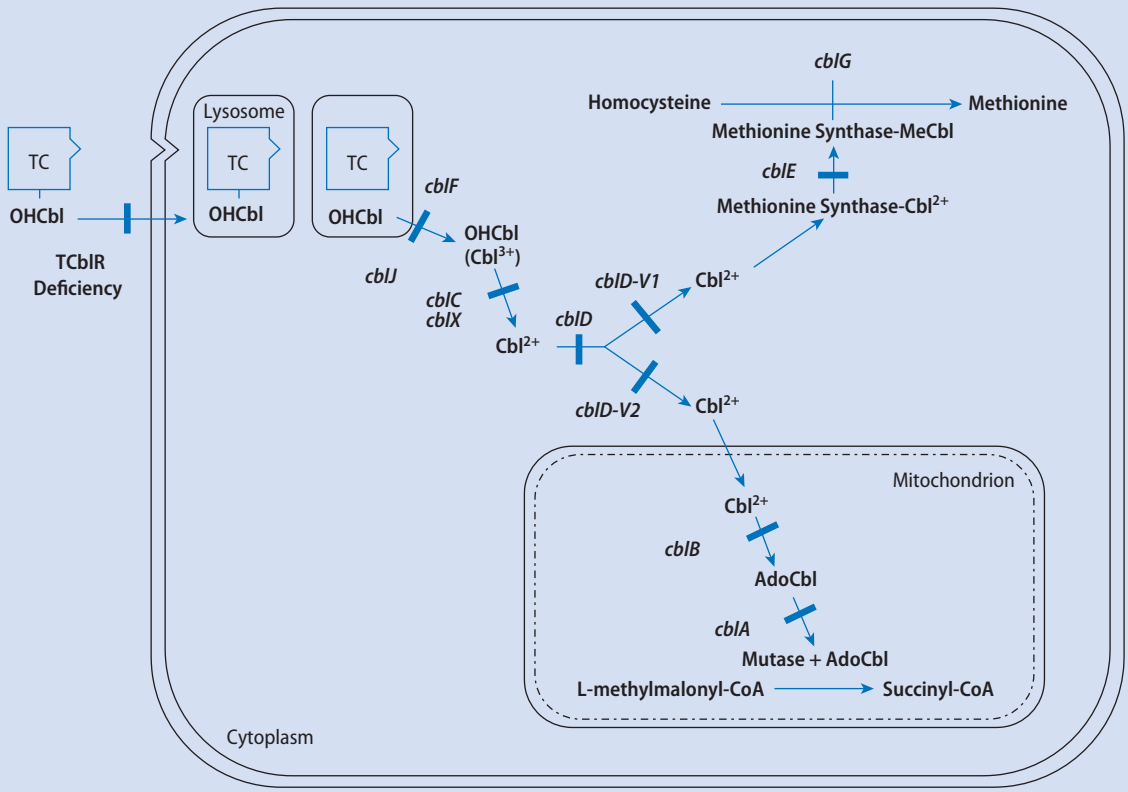


Fig. 27.1 Cobalamin (Cbl) endocytosis and intracellular metabolism. The cytoplasmic, lysosomal, and mitochondrial compartments are indicated: *AdoCbl*, adenosylcobalamin; *CoA*, coenzyme A; *MeCbl*, methylcobalamin; *OHCbl*, hydroxocobalamin; *TC*, transcobalamin (previously TCII); *V1*, variant 1; *V2*, variant 2; $^{1+, 2+, 3+}$ refer to the oxidation state of the central cobalt of Cbl; *cblA-cblG*, *cblJ* and *cblX*, refer to the sites of blocks. »Mutase + AdoCbl« refers to the active form of methylmalonyl-CoA mutase, which contains a molecule AdoCbl. Inborn errors are indicated by **solid bars**

The serum Cbl level is usually low in patients with disorders affecting absorption and transport of Cbl, with the exception of transcobalamin (TC) deficiency (▶ Cobalamin Transport and Metabolism). Patients with disorders of intracellular Cbl metabolism typically have serum Cbl levels within the reference range, although levels may be reduced in the cblF and cblJ disorders. Homocystinuria (Hcy) and hyperhomocysteinaemia, as well as megaloblastic anaemia and neurological disorders, are major clinical findings in patients with disorders of Cbl absorption and transport, as well as those with defects of cellular metabolism that affect synthesis of MeCbl. Methylmalonic aciduria and acidaemia, (MMA) resulting in metabolic acidosis, are seen in disorders that result in decreased synthesis of AdoCbl.

Increased urine MMA and plasma Hcy are also found in nutritional vitamin B_{12} deficiency. Severe vitamin B_{12} deficiency in newborn infants, which may occur in breast fed infants born to vegan mothers or those with sub-clinical pernicious anaemia, can result in a disorder that ranges from an elevation in serum concentration of propionylcarnitine detected by newborn screening, to one presenting with severe neonatal encephalopathy. The mother does not necessarily have a very low serum

concentration of vitamin B_{12}. IM vitamin B_{12} replacement therapy to normalize vitamin B_{12} serum concentration reverses the metabolic abnormality [1].
Inherited disorders of Cbl metabolism are divided into those involving absorption and transport and those involving intracellular utilisation [2][3][4][5].

27.1 Disorders of Absorption and Transport of Cobalamin

Absorption of dietary Cbl first involves binding to a glycoprotein (haptocorrin, R binder) in the saliva. In the intestine, haptocorrin is digested by proteases, allowing the Cbl to bind to intrinsic factor (IF), which is produced in the stomach by parietal cells. Using the specific receptor cubam, the IF-Cbl complex enters the enterocyte. Following release from this complex Cbl enters the portal circulation bound to transcobalamin (TC), the physiologically important circulating Cbl-binding protein. Inherited defects of several of these steps are known.

27.1.1 Hereditary Intrinsic Factor Deficiency

Clinical Presentation

Presentation is usually from 1 to 5 years of age, but in cases of partial deficiency can be delayed until adolescence or adulthood. Patients present with megaloblastic anaemia as the main finding, together with failure to thrive, often with vomiting, alternating diarrhoea and constipation, anorexia and irritability. Hepatosplenomegaly, stomatitis or atrophic glossitis, developmental delay and myelopathy or peripheral neuropathy may also be found.

Metabolic Derangement

IF is either absent or immunologically detectable but non functional. There have been reports of IF with reduced affinity for Cbl or cubam, or with increased susceptibility to proteolysis.

Genetics

Fewer than 50 patients of both sexes have been reported. Inheritance is autosomal recessive. Mutations in the gastric IF (GIF) gene have been identified in several patients with IF deficiency [6][7].

Diagnostic Tests

The haematological abnormalities in the defects of Cbl absorption and transport should be detected by measurement of red blood cell indices, complete blood count and bone marrow examination. Low serum Cbl levels are present. In contrast to acquired forms of pernicious anaemia, there is normal gastric acidity and cytology, and anti-IF antibodies are absent. Cbl absorption, as measured by the Schilling test, is abnormal and normalised by exogenous IF. Because the Schilling test is rarely available, and because differentiation between hereditary IF deficiency and Imerslund-Gräsbeck syndrome on the basis of other clinical findings has proven difficult in some cases, sequencing of the *GIF*, *AMN* and *CUBN* genes may represent an appropriate first-line means of correctly diagnosing these disorders [6]. The reliability of a proposed alternative test to the Schilling test remains to be determined for the diagnosis of this disorder [8].

Treatment and Prognosis

IF deficiency can be treated initially with hydroxocobalamin (OHCbl, 1 mg/day i.m.) to replenish body stores until biochemical and haematological values become normal. The subsequent dose of OHCbl required to maintain values within or above the reference range may be as low as 0.25 mg every 3 months.

27.1.2 Defective Transport of Cobalamin by Enterocytes (Imerslund-Gräsbeck Syndrome)

Clinical Presentation

Defective transport of Cbl by enterocytes, also known as Imerslund-Gräsbeck syndrome or megaloblastic anaemia 1 (MGA1), is characterised by prominent megaloblastic anaemia manifesting once foetal hepatic Cbl stores have been depleted. The disease usually appears between the ages of 1 year and 5 years, but onset may be even later [9]. Many patients have proteinuria that is not of the classic glomerular or tubular types, does not respond to therapy with Cbl and is not progressive [10]. This apparently reflects the role of cubam in reabsorption of specific proteins in the kidney. There may be small changes in renal ultrastructure that, unlike the proteinuria, respond to Cbl therapy. Neurological abnormalities, such as spasticity, truncal ataxia and cerebral atrophy, may be present as a consequence of the Cbl deficiency.

Metabolic Derangement

This disorder is caused by defects of the IF-Cbl receptor, cubam, which comprises two components. Cubilin was first purified as the IF-Cbl receptor from the proximal renal tubule. A second component, amnionless, colocalises with cubilin in the endocytic apparatus of polarised epithelial cells, forming a tightly bound complex that is essential for endocytosis of IF-Cbl and other molecules, including vitamin D-binding protein, albumin, transferrin and apolipoprotein A [11]. Thus defective function of either protein may cause this disorder.

Genetics

Over 400 cases have been reported. Inheritance is autosomal recessive, with environmental factors affecting expression [12]. Most patients are found in Finland, Norway, Saudi Arabia and Turkey, and among Sephardic Jews. A P1297L mutation in the cubilin gene (*CUBN*) was the most common causal variant in Finnish families, while mutations in the amnionless gene (*AMN*) were identified in Norwegian patients. Mutations of both *CUBN* and *AMN* have been identified in patients of Eastern Mediterranean origin [7].

Diagnostic Tests

The diagnosis is aided by finding low serum Cbl levels, megaloblastic anaemia and proteinuria. Most of the reports in the literature do not comment on the levels of homocysteine and methylmalonic acid. Gastric morphology and pancreatic function are normal; there are no IF autoantibodies and IF levels are normal. As previously noted, in the absence of the Schilling test, molecular analysis of the *GIF*, *CUBN* and *AMN* genes may be the best means of differentiating between hereditary IF deficiency and Imerslund-Gräsbeck syndrome [6]. Reliability of a proposed non-radioactive alternative to the Schilling test remains to be determined for the diagnosis of this disorder.

Treatment and Prognosis

Treatment with systemic OHCbl corrects the anaemia and the neurological findings, but not the proteinuria. As with hereditary IF deficiency, once Cbl stores are replete, low doses of systemic OHCbl may be sufficient to maintain normal haematological and biochemical values.

27.1.3 Haptocorrin (R Binder) Deficiency

Clinical Presentation

Very few cases have been described, and it is not clear whether this entity has a distinct phenotype. Haematological findings are absent and neurological findings such as subacute combined degeneration of the spinal cord in one man in the 5th decade of life and optic atrophy, ataxia, long-tract signs and dementia in another may be coincidental. It has been suggested that a deficiency of haptocorrin may be responsible for a number of patients with unexplained low serum Cbl levels. Haptocorrin deficiency has also been identified in individuals with serum Cbl levels within the reference range [13][14].

Metabolic Derangement

The role of haptocorrin is uncertain, but it could be involved in the scavenging of toxic Cbl analogues or in protecting circulating MeCbl from photolysis. Deficiency of haptocorrin has been described in isolation and in association with deficiency of other specific granule proteins such as lactoferrin [15].

Genetics

A patient with severe deficiency of haptocorrin was shown to be compound heterozygous for two nonsense mutations (c.270delG and c.315C→T) in the *TCN1* gene, which encodes haptocorrin. Members of this patient's family with moderate haptocorrin deficiency, as well as unrelated individuals with moderate deficiency, were found to be heterozygous for one of the mutations [14].

Diagnostic Tests

Serum Cbl levels are low because most circulating Cbl is bound to haptocorrin. TC-Cbl levels are normal, and there are no haematological findings of Cbl deficiency. A deficiency or absence of haptocorrin is found in plasma, saliva and leukocytes.

Treatment and Prognosis

It is likely that no treatment is needed because of the lack of a clearly defined phenotype.

27.1.4 Transcobalamin Deficiency

Clinical Presentation

In transcobalamin (TC) deficiency, symptoms usually develop much earlier than in other disorders of Cbl absorption, typically within the first few months of life. Even though the only TC in cord blood is of foetal origin, patients are not sick at birth. Presenting findings include pallor, failure to thrive, weakness and diarrhoea. Although the anaemia is usually megaloblastic, patients with pancytopenia or isolated erythroid hypoplasia have been described [16]. Leukaemia may be mistakenly diagnosed because of the presence of immature white cell precursors in an otherwise hypocellular marrow [17]. Neurological disease is not an initial finding but may develop with delayed treatment, with administration of folate in the absence of Cbl, or with inadequate Cbl treatment. Neurological features include developmental delay, weakness, hypotonia, neuropathy, myelopathy and encephalopathy and, rarely, retinal degeneration. Immunologic abnormalities including agammaglobulinaemia, low IgG and low T and B cell counts may be present; some patients have had recurrent infections.

Metabolic Derangement

The majority of patients have no immunologically detectable TC, although others have some detectable TC that is able to bind Cbl but cannot support cellular Cbl uptake.

Genetics

Inheritance is autosomal recessive. There have been at least 50 cases, including both twins and siblings. Disease-causing deletions, nonsense mutations and activation of an intra exonic cryptic splice site have been described in *TCN2* [16].

Diagnostic Tests

Serum Cbl levels are not usually low, because the majority of serum Cbl is bound to haptocorrin and not to TC. Cbl bound to TC, as reflected by the unsaturated vitamin B_{12}-binding capacity, is low provided that the test is performed before Cbl treatment is started. Reports of levels of Cbl-related metabolites are inconsistent. Patients with plasma total homocysteine within the reference range and moderately increased urine methylmalonic acid have been reported, as well as patients with methylmalonic aciduria and homocystinuria. Study of TC synthesis in cultured fibroblasts or amniocytes allows both pre- and postnatal diagnosis in patients who do not synthesise TC [18]. DNA testing is possible for both diagnosis and heterozygote detection in families in which the molecular defect has been identified. Assays using antibodies generated against recombinant human TC allow reliable measurement of serum TC even in patients who have been treated with Cbl [19].

Treatment and Prognosis

Adequate treatment requires administration of oral or systemic OHCbl or cyanocobalamin (CNCbl) at a dose of 0.5–1 mg, initially daily then twice weekly, to maintain serum Cbl levels in the range of 1000–10,000 pg/ml. Intravenous Cbl is not recommended because of its rapid loss in the urine. Folic acid or folinic acid can reverse the megaloblastic anaemia and has been used in doses up to 15 mg p.o. four times daily. However, folates must never be given as the only therapy in TC deficiency, because of the danger of neurological deterioration. Treatment with Cbl, particularly when instituted during the first months of life, has been associated with favourable patient outcomes. A review of TC-deficient patients found a single patient, among 19 older than 6 years old at the latest follow-up, with significant intellectual deficits, possibly due to sub-optimal therapy. A second patient had neurological findings that responded to treatment optimization [16].

27.1.5 Transcobalamin Receptor Deficiency

Clinical Presentation

Several subjects with a defect affecting the cell surface receptor that recognises the TC-Cbl complex and modulates its uptake by carrier-mediated endocytosis have been identified on newborn screening. They had moderate elevations of serum methylmalonic acid and, in most cases, also of homocysteine, but otherwise most did not show clinical signs of Cbl deficiency [20]. A single patient presented with bilateral central artery occlusions that were attributed to hyperhomocysteinemia [21].

Metabolic Derangement

Uptake of TC-bound radioactive Cbl was decreased in cultured fibroblasts from all of those investigated, but synthesis of MeCbl and AdoCbl occurred normally and no decrease in function of methionine synthase or methylmalonyl-CoA mutase could be detected.

Genetics

Several individuals with this autosomal recessive state are homozygous for a 3-bp deletion (c.262_264delGAG) in *CD320* that encodes the TC receptor. This mutation has been shown to diminish Cbl uptake in an *in vitro* system [20]. It was present at a frequency of 3% in an Irish control population [22].

Treatment and Prognosis

Since most of the affected individuals lack clinical signs of Cbl deficiency, it is likely that treatment is not necessary.

27.2 Disorders of Intracellular Utilisation of Cobalamin

A number of disorders of intracellular metabolism of Cbl have been classified as *cbl* mutants (*A-G, J, X*), based on the biochemical phenotype and on somatic cell analysis (Fig. 27.1). Precise diagnosis of the inborn errors of Cbl metabolism requires either tests in cultured fibroblasts or identification of causal mutations. Complementation analysis can be used to reliably assign a patient to one of the known classes of inborn error if function of either methylmalonyl-Coenzyme A (CoA) mutase or methionine synthase is reduced in patient fibroblasts. The one exception is the *cblX* disorder which cannot be differentiated from *cblC* by complementation analysis.

27.2.1 Combined Deficiencies of Adenosylcobalamin and Methylcobalamin

Five distinct disorders are associated with functional defects in both methylmalonyl-CoA mutase and methionine synthase. They are characterised by both methylmalonic aciduria and homocystinuria.

Cobalamin-F

Clinical Presentation

Most patients with *cblF* disease have presented in the 1st year of life. Frequent findings have included intrauterine growth retardation, feeding difficulties, failure to thrive, developmental delay and persistent stomatitis. A complete blood count and bone marrow examination may reveal megaloblastic anaemia, neutropenia and thrombocytopenia. Two patients have had minor facial anomalies including pegged teeth and bifid incisors; four have had structural heart defects. One patient died suddenly at home in the 1st year of life; two others died after cardiac surgery [23].

Metabolic Derangement

The defect in *cblF* appears to be a failure of Cbl transport across the lysosomal membrane following its release from TC in the lysosome. As a result, Cbl accumulates in lysosomes and cannot be converted to either AdoCbl or MeCbl. The inability of *cblF* patients to absorb oral Cbl suggests that IF-Cbl also has to pass through a lysosomal stage in the enterocyte before Cbl is released into the portal circulation.

Genetics

Fifteen patients with the *cblF* disorder have been reported. Mutations in *LMBRD1* have been identified in all reported patients [23][24][25]. This gene encodes a lysosomal membrane protein that is presumed to function in transport of free Cbl across the lysosomal membrane into the cytoplasm. A deletion mutation (c.1056delG), which is found on a common haplotype (26), occurs in patients from different ethnic groups and represents two-thirds of disease-causing alleles that have been identified.

Diagnostic Tests

The serum Cbl level may be low, and the Schilling test has been abnormal when tested. Usually, increased plasma total homocysteine, methylmalonic acid and C3 acylcarnitine, low to

normal plasma methionine, homocystinuria and methylmalonic aciduria are found, although urine and plasma elevations of homocysteine were not reported in the original patient. In fibroblasts from *cblF* patients, total incorporation of labelled CNCbl is elevated, but CNCbl is not converted to either AdoCbl or MeCbl. Most of the label is found as free CNCbl in lysosomes. There is decreased function of both Cbl-dependent enzymes.

Treatment and Prognosis

Treatment with parenteral OHCbl (first daily and then biweekly, or even less frequently) at a dose of 1 mg/day seems to be effective in correcting the metabolic and clinical findings. The original patient responded to oral Cbl before being switched to parenteral Cbl, despite the fact that the Schilling test performed on two occasions showed an inability to absorb Cbl with or without IF.

Cobalamin-J

Clinical Presentation

Four patients have been reported with the *cblJ* disorder. The first two patients presented in the newborn period. One had feeding difficulties, hypotonia, lethargy and bone marrow suppression; the second had feeding difficulties, macrocytic anemia and congenital heart defects [27]. Subsequently, two Taiwanese patients were reported with later onset (4 and 6 years of age), hyperpigmentation and prematurely grey hair; one additionally reported dizziness and headaches [28][29]. Macrocytic anemia, methylmalonic aciduria and hyperhomocysteinemia were present in all cases.

Metabolic Derangement

As in the *cblF* disorder, there is decreased ability to transfer Cbl across the lysosomal membrane into the cytoplasm, resulting in accumulation of free Cbl in lysosomes. The exact role of the two proteins involved in lysosomal transport of Cbl and which are defective in the *cblF* and *cblJ* disorders remains to be elucidated.

Genetics

Mutations in *ABCD4* have been identified in all cases. The gene encodes an ATP binding cassette transporter that has been localized to the lysosomal membrane and is believed to play a role, in conjunction with the product of *LMBRD1*, in Cbl transport.

Diagnostic Tests

Patients have had elevated urine methylmalonic acid and hyperhomocysteinemia. Serum Cbl was low in several patients; intestinal absorption has not been investigated. Results of studies of cultured fibroblasts in the early-onset patients were identical to those of cblF fibroblasts; studies of the first Taiwanese patient showed a milder cellular phenotype, with moderately reduced MeCbl synthesis and apparently normal AdoCbl synthesis.

Cobalamin-C

Clinical Presentation

This is the most frequent inborn error of Cbl metabolism, and over 550 patients are known [30][31][32]. Many were acutely ill in the 1st month of life, and most were diagnosed within the 1st year. This early-onset group shows feeding difficulties and lethargy, followed by progressive neurological deterioration. This may include hypotonia, hypertonia or both, abnormal movements or seizures and coma. Severe pancytopenia or a non-regenerative anaemia, which is not always associated with macrocytosis and hypersegmented neutrophils, but which is megaloblastic on bone marrow examination, may be present. Patients may develop multisystem pathology, such as renal failure, hepatic dysfunction, cardiomyopathy, interstitial pneumonia or the haemolytic uraemic syndrome characterised by widespread microangiopathy. Additional features include an unusual retinopathy consisting of perimacular hypopigmentation surrounded by a hyperpigmented ring and a more peripheral salt-and-pepper retinopathy sometimes accompanied by nystagmus, microcephaly and hydrocephalus [32][33]. Congenital structural heart defects may be present [34]. A small number of *cblC* patients were not diagnosed until after the 1st year of life and some as late as the end of the 4th decade of life. The patients in this group who were diagnosed earlier had findings overlapping those found in the younger onset group. Major clinical findings in the late-onset *cblC* group included confusion, disorientation and gait abnormalities and incontinence. Macrocytic anaemia was seen in only about a third of the oldest patients [32][33]. Therefore, it is important to search for the *cblC* disorder by determination of metabolite levels in the presence of neurological findings alone.

Metabolic Derangement

The *cblC* disorder is caused by defects in MMACHC, a protein that plays a role in the early steps of cellular Cbl metabolism [30]. MMACHC binds Cbl and catalyses removal of upper axial ligands from alkylcobalamins (including the methyl group from MeCbl and the adenosyl group from AdoCbl) and from CNCbl.

Genetics

A common *MMACHC* mutation, c.271dupA, accounts for 40% or more of all disease alleles in patient populations of European origin [31]. A different mutation, c.609G→A (p.W203X), represents over 50% of disease-causing alleles in Chinese *cblC* patients [35]. Inheritance is autosomal recessive. Prenatal diagnosis can be performed by mutation analysis, by *in vitro* studies in cultured chorionic villus cells (but not chorionic villus biopsies) and amniocytes, and by measuring methylmalonic acid and total homocysteine levels in amniotic fluid. These techniques cannot detect heterozygotes.

Diagnostic Tests

Increased plasma total homocysteine, low to normal plasma methionine, homocystinuria and methylmalonic acidaemia and aciduria are the biochemical hallmarks of this disease. In general, the methylmalonic acid levels seen are lower than

those found in patients with methylmalonyl-CoA mutase deficiency but higher than those seen in the Cbl transport defects. A complete blood count and bone marrow examination allow detection of the haematological abnormalities.

Fibroblast studies show decreased accumulation of CNCbl, decreased synthesis of both AdoCbl and MeCbl, and decreased function of both methylmalonyl-CoA mutase and methionine synthase. Cells fail to complement those of other *cblC* patients and patients with mutations in *HCFC1*. Differentiation between *cblC* and *cblX* disorders requires sequencing of *MMACHC* and *HCFC1*.

Treatment and Prognosis

Treatment is usually with 1 mg/day OHCbl (parenteral) in combination with oral betaine. Elevated metabolite levels improve, but are not usually completely normalised. Oral OHCbl has been found to be insufficient, and neither folinic acid nor carnitine was effective. One study found that as much as 20 mg OHCbl a day was necessary to entirely correct methylmalonate and homocysteine levels in one patient [36]. Even though oral administration of OHCbl generally appears not to be effective, this route was reported to be successful in one patient. Both *in vitro* studies and studies of patients indicate that CNCbl is less effective than OHCbl in treatment of this disease, possibly reflecting the role of the MMACHC protein in decyanation of CNCbl.

12-30% of early-onset *cblC* patients have died, and most survivors have had moderate or severe neurological impairment despite treatment [32][37]. Patients with later onset tend to have better outcomes. Treatment starting early in life, before neurologic impairment becomes established, is important for optimal patient response, but long-term outcome remains uncertain.

Cobalamin-X

Clinical Presentation

Exome sequencing of a male patient with a diagnosis of *cblC* disease based on complementation analysis, in whom no *MMACHC* mutations could be detected, identified a hemizygous mutation in *HCFC1* on the X chromosome. Subsequently, *HCFC1* mutations were identified in an additional 14 patients [38][39]. Patients have presented in the first months of life with a similar clinical presentation to *cblC* patients, although the metabolic abnormalities are milder and the neurologic presentation is more severe, with choreoathetosis, intractable epilepsy and severe developmental delay and sometimes with manifestations before birth.

Metabolic Derangement

The *cblX* disorder is caused by mutations at *HCFC1*, which encodes a transcription regulator that affects expression of a number of genes, including *MMACHC*. The metabolic consequences of mutations stem from decreased MMACHC expression leading to decreased synthesis of both AdoCbl and MeCbl.

Genetics

All patients have been male, and all have been hemizygous for *HCFC1* mutations affecting the kelch domain near the N-terminus of the protein.

Diagnostic Tests

Patients have moderately elevated serum and urine levels of methylmalonic acid that are usually lower than those seen in other inborn errors of Cbl metabolism. Serum total homocysteine has been elevated in some patients, but others had values within the reference range. Fibroblasts studies place these patients within the *cblC* complementation group and diagnosis therefore depends on identification of mutations affecting the 5' end of *HCFC1*.

Cobalamin-D

Clinical Presentation

This defect was first described in two brothers. The elder sibling had behavioural problems and mild mental retardation at the age of 14 years, and also ataxia and nystagmus. Heterogeneity of the *cblD* defect was established by the description of one patient with isolated methylmalonic aciduria who presented prematurely with respiratory distress, cranial haemorrhage, necrotising enterocolitis and convulsions but without anaemia, and two unrelated patients with isolated homocystinuria, megaloblastic anaemia and neurological changes but without metabolic decompensation [40]. Following the discovery of the *cblD* gene [41] further patients with diverse phenotypes were described. A total of 21 cblD patients are now known, 6 with isolated homocystinuria, 9 with isolated methylmalonic aciduria and 6 with combined methylmalonic aciduria/homocystinuria [41][42].

Metabolic Derangement

The cblD defect is caused by mutations in *MMADHC* and can cause deficient synthesis of both AdoCbl and MeCbl together, or of either in isolation. This suggests that the product of *MMADHC* plays a role in directing Cbl from the MMACHC protein to the two Cbl-dependent enzymes.

Genetics

Biallelic *MMADHC* mutations have been found in all patients belonging to the *cblD* complementation group regardless of the phenotype. The nature and location of mutations within the gene seem to determine the phenotype. Thus the combined-defect patients have crippling mutations towards the C-terminus; isolated homocystinuria patients have missense mutations towards the C-terminus; and isolated methylmalonic aciduria patients have mutations leading to a stop codon toward the N-terminus, in which case re-initiation of translation occurs at one of two downstream start codons.

Diagnostic Tests

Methylmalonic aciduria with or without increased plasma total homocysteine and homocystinuria, or isolated homocystinuria may be found. Although the original patient showed no megaloblastic anaemia, the deoxyuridine-suppression test

was abnormal. In fibroblast studies findings can be similar to those of the *cblC*, *cblA/B* or *cblE/G* defects although differences in the severity and responsiveness to addition of OHCbl to the culture medium may be seen. This heterogeneity emphasises the necessity of complementation or genetic analysis to make a specific diagnosis in the *cbl* defects.

27.2.2 Adenosylcobalamin Deficiency

Clinical Presentation

Adenosylcobalamin (AdoCbl) deficiency comprises *cblA* and *cblB*, two disorders characterised by methylmalonic aciduria (MMA) which is often Cbl-responsive. The phenotype resembles methylmalonyl-CoA mutase deficiency (▶ Chapter 19).

Metabolic Derangement

The defect in *cblB* is deficiency of Cbl adenosyltransferase, which catalyses the final step in intramitochondrial synthesis of AdoCbl, the cofactor for methylmalonyl-CoA mutase [43]. The defect in *cblA* results from mutations in *MMAA* [44]. Studies of the bacterial homologue of the *MMAA* gene product suggest that this protein is involved in transfer of AdoCbl from adenosyltransferase to methylmalonyl-CoA mutase and in maintaining mutase-bound AdoCbl in its active form.

Genetics

MMAA encodes a polypeptide belonging to the G3E family of GTP-binding proteins. Over 40 mutations in *MMAA* have now been described among *cblA* patients [44][45].The most common of these is a c.433C→T (p.R145X) nonsense mutation that represented 43% of mutant alleles identified in the largest of these studies.

MMAB encodes cobalamin adenosyltransferase. A number of mutations in *MMAB* have been identified in *cblB* patients [43][46]. Virtually all of these mutations are clustered in the regions of the protein identified as the active site of adenosyltransferase.

Diagnostic Tests

Total serum Cbl is usually normal. Urinary methylmalonic acid levels are elevated above reference values (typically <5 μmol/mmol creatinine), sometimes to greater than 20,000 μmol/mmol creatinine [47][48], but there is no increase of plasma total homocysteine or homocystinuria. Methylmalonic acid excretion may decrease in response to Cbl therapy in some patients although many *cblB* patients do not respond. There has been marked variation in the form and dosage of Cbl used and its mode of administration, as well as in the parameters used to assess response. A standardised protocol involving administration of 1 mg OHCbl i.m. on 3 consecutive days has been suggested, with a decrease in plasma or urine methylmalonic acid of 50% or more over 10 days considered a positive result [48].

The differentiation of *cblA* and *cblB* from mutase deficiency depends on fibroblast studies or sequencing of *MMAA* and *MMAB*. In both *cblA* and *cblB* methylmalonyl-CoA mutase activity is within the reference range in the presence of added AdoCbl. Methylmalonyl-CoA function in fibroblasts is decreased in both *cblA* and *cblB*; this is usually responsive to the addition of OHCbl to the culture medium in *cblA*, while many *cblB* patients do not show a response to OHCbl supplementation. Uptake of labelled CNCbl is within the reference range, but there is decreased synthesis of AdoCbl. Adenosyltransferase specific activity is clearly deficient in *cblB*, but normal in *cblA* fibroblast extracts. Complementation or sequence analysis allows confirmation of the mutant class.

Treatment and Prognosis

Most of these patients respond to protein restriction and to OHCbl treatment, with either 10 mg p.o. daily or 1 mg i.m. once or twice weekly. For details of the planning of a protein-restricted diet, see ▶ Chapter 19. Some patients appear to become resistant to Cbl treatment. Therapy with AdoCbl has been attempted in *cblB* with and without success, possibly reflecting removal of the adenosyl group by the MMACHC protein after entry into cells. There have been reports of prenatal therapy with Cbl in AdoCbl deficiency. Most (90%) *cblA* patients improve on Cbl therapy, with 70% doing well long term. However, late severe renal and neurological complications, including optic atrophy, have been observed. Only 40% of *cblB* patients respond to Cbl, and the long-term survival of *cblB* patients is poorer than that of *cblA* patients [49][50].

Renal transplantation may be required in patients with end-stage renal disease. Liver transplantation has resulted in prevention of metabolic decompensation, although serum methylmalonic acid levels remain markedly elevated and neurological and renal deterioration may continue even in the absence of decompensation [51].

27.2.3 Methylcobalamin Deficiency

Clinical Presentation

Formation of MeCbl is disturbed in the *cblE* and *cblG* disorders. The most common clinical findings are megaloblastic anaemia and neurological disease [52]. The latter includes poor feeding, vomiting, failure to thrive, developmental delay, nystagmus, hypotonia or hypertonia, ataxia, seizures and blindness. Cerebral atrophy may be seen on imaging studies of the central nervous system, and at least one *cblE* patient showed a spinal cord cystic lesion on autopsy. Most patients are symptomatic in the 1st year of life, but one *cblG* patient was not diagnosed until the age of 21 years and carried a misdiagnosis of multiple sclerosis. Another *cblG* patient, who was diagnosed during his 4th decade of life, had mainly psychiatric symptoms. Patients with minimal findings and without clear neurological features have also been reported.

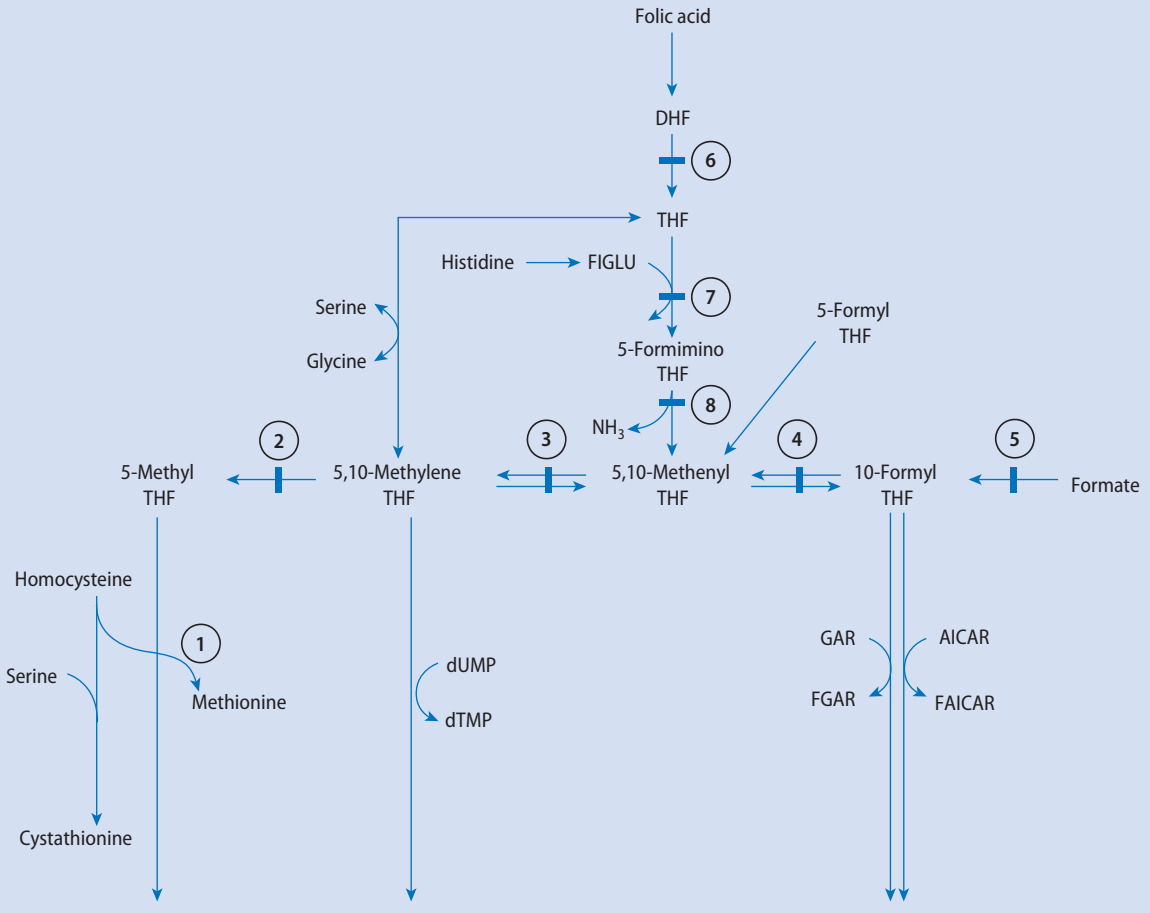
Metabolic Derangement

The defect in *cblE* is deficiency of the enzyme methionine synthase reductase, which is required for the activation by reductive methylation of the methionine synthase apoen-

Folate Metabolism

Folic acid (pteroylglutamic acid) is plentiful in foods such as liver, leafy vegetables, legumes and some fruits. Its metabolism involves reduction to dihydrofolate (DHF) and tetrahydrofolate (THF), followed by addition of a single-carbon unit, which is provided by serine or histidine; this carbon unit occurs in various redox states (methyl, methylene, methenyl or formyl). Transfer of this single-carbon unit is essential for the endogenous formation of methionine, thymidylate (dTMP) and formylglycineamide ribotide (FGAR) and formylaminoimidazolecarboxamide ribotide (FAICAR), two intermediates of purine synthesis (◘ Fig. 27.2). These reactions also allow regeneration of DHF and THF. The predominant folate derivative in blood and in cerebrospinal fluid is 5-methyltetrahydrofolate (the product of the methylenetetrahydrofolate reductase reaction).

Several proteins have been shown to play a role in transport of folates across cellular membranes [55]. The reduced folate carrier (RFC) supports a low-affinity high-capacity system for uptake of reduced folates at micromolar concentrations. It appears to play an important role in folate uptake by many types of cells, including haematopoietic cells. The folate receptors (FRα and FRβ) are a family of folate-binding proteins that are attached to the cell surface by a glycosylphosphatidylinositol anchor; they support a high-affinity low-capacity uptake system for 5-methyltetrahydrofolate and folic acid that is active at nanomolar concentrations of folate. The protein-coupled folate transporter (PCFT) supports uptake of reduced and oxidised folates at acid pH [56]. Uptake of folate in the intestine appears to depend on function of the PCFT and not RFC whereas transport of folate across the blood-brain barrier at the choroid plexus requires both PCFT and FRα [57].



◘ **Fig. 27.2 Folic acid metabolism: 1**, methionine synthase; **2**, methylenetetrahydrofolate reductase; **3**, methylenetetrahydrofolate dehydrogenase (*MTHFD1* gene); **4**, methenyltetrahydrofolate cyclohydrolase (*MTHFD1* gene) **5**, formyltetrahydrofolate synthetase (*MTHFD1* gene); **6**, dihydrofolate reductase; **7**, glutamate formiminotransferase (*FTCD* gene); **8**, formiminotetrahydrofolate cyclodeaminase (*FTCD* gene); *AICAR*, aminoimidazole carboxamide ribotide; *DHF*, dihydrofolate, *dTMP*, deoxythymidine monophosphate; *dUMP*, deoxyuridine monophosphate; *FAICAR*, formylaminoimidazole carboxamide ribotide; *FGAR*, formylglycinamide ribotide; *FIGLU*, formiminoglutamate; *GAR*, glycinamide ribotide; *THF*, tetrahydrofolate. Enzyme defects are indicated by **solid bars**

zyme. The *cblG* defect is caused by deficient activity of the methionine synthase apoenzyme itself.

Genetics

Over 30 patients are known with each of the *cblE* and *cblG* disorders. A number of mutations have been identified in the methionine synthase reductase gene, *MTRR*, in *cblE* patients [53]. The most common of these mutations is c.903+469T→C, which represents 25% of identified mutant alleles. Mutations in the methionine synthase gene, *MTR*, have been found in *cblG* patients [54]. The most common of these mutations, c.3518C→T (p.1173L), represents over 40% of identified disease-causing alleles.

Diagnostic Tests

Homocystinuria and hyperhomocysteinaemia are almost always found in the absence of methylmalonic acidaemia, although one *cblE* patient had transient unexplained methylmalonic aciduria. Hypomethioninaemia and cystathioninaemia may be present, and there may be increased serine in the urine. Methionine synthase function is decreased in cultured fibroblasts from both *cblE* and *cblG* patients. Uptake of CNCbl is normal but synthesis of MeCbl is decreased in both disorders. Complementation analysis distinguishes *cblE* from *cblG* and *cblD*-HC patients.

Treatment and Prognosis

Both disorders are treated with OHCbl or MeCbl, 1 mg i.m., first daily and then once or twice weekly. Although the metabolic abnormalities are nearly always corrected, it is difficult to reverse existing neurological findings. Treatment with betaine (250 mg/kg/day) has been used, and one *cblG* patient was treated with L-methionine (40 mg/kg/day) and showed neurological improvement. Despite therapy, many patients have a poor outcome. In one family with *cblE*, following a prenatal diagnosis, the mother was treated with OHCbl during the 2nd trimester, and the baby from birth. This boy has developed normally to the age of 14 years, in contrast to his older brother, who was not treated until after showing metabolic decompensation in infancy and had significant developmental delay at 18 years. Some patients may benefit from high-dose folic or folinic acid treatment.

27.3 Disorders of Absorption and Metabolism of Folate

27.3.1 Hereditary Folate Malabsorption

Clinical Presentation

This rare condition presents in the 1st months of life with severe megaloblastic anaemia, diarrhoea, stomatitis, failure to thrive and usually progressive neurological deterioration with seizures and sometimes with intracranial calcifications. Peripheral neuropathy has been seen, as have partial defects in humoral and cellular immunity [58][59].

Metabolic Derangement

All patients have severely decreased intestinal absorption of oral folic acid or reduced folates, such as formyltetrahydrofolic acid (formyl-THF, folinic acid) or methyltetrahydrofolic acid. There is also decreased transport of folate across the blood-brain barrier. Transport of folates across other cell membranes is not affected in this disorder. The disorder is the result of decreased function of the proton-coupled folate transporter (PCFT) [56]. Folate metabolism in cultured fibroblasts is normal.

Genetics

Approximately 30 patients with this disorder have been reported. It is caused by mutations affecting *SLC46A1*, which encodes the PCFT. A number of different mutations have been described.

Diagnostic Tests

Measurement of serum, red blood cell and CSF folate levels and a complete blood count and bone marrow analysis should be performed. The most important diagnostic features are the severe megaloblastic anaemia in the first few months of life, together with low serum folate levels. Measurements of related metabolite levels have been sporadically reported and inconsistently found abnormalities include increased excretion of formiminoglutamate, orotic aciduria, increased plasma sarcosine and cystathionine and low plasma methionine. Folate levels in CSF remain low even when blood levels are high enough to correct the megaloblastic anaemia [58]. Folate absorption can be investigated by measuring serum folate levels following an oral dose of between 5 and 100 mg of folic acid.

Treatment and Prognosis

High-dose oral folic acid (up to 60 mg daily) or lower parenteral doses in the physiological range correct the haematological and gastrointestinal abnormalities but are less effective in correcting the neurological findings and in raising the level of folate in the CSF. Folinic acid is more effective in raising CSF levels and has been given in combination with high-dose oral folic acid. The clinical response to folates has varied among patients; in some cases seizures were worse after folate therapy was started. It is important to maintain both blood and CSF folate in the normal range. If oral therapy does not raise CSF folate levels, parenteral therapy should be used. Intrathecal folate therapy may be considered if CSF levels of folate cannot be raised by other treatments, although the required dose of folate is unknown. In some cases high oral doses of folinic acid (up to 400 mg orally daily) may eliminate the need for parenteral therapy.

27.3.2 Cerebral Folate Deficiency

Clinical Presentation

This disorder presents in the 1st year of life, with psychomotor retardation, spastic paraplegia, cerebellar ataxia and dyskine-

sia, associated with normal blood folate levels and low folate levels only in the cerebrospinal fluid (CSF) [60][61]. Several affected children have developed autistic features. This disorder should be differentiated from patients with acquired (perinatal asphyxia, CNS infection) and genetic (Rett syndrome, Kearn Sayre disease, MTHFR deficiency, white matter disease) disorders which also have decreased cerebral folate levels.

Metabolic Derangement

There is a decreased level of 5-methyl THF, the major circulating form of folate in the CSF, with normal blood levels of the vitamin. This is the result of decreased FRα (folate receptor α) function at the choroid plexus.

Genetics

Mutations in *FOLR1*, which encodes FRα, have been identified in a small number of families with cerebral folate deficiency [62][63]. The disorder segregates as an autosomal recessive trait in these families. Cerebral folate deficiency without *FOLR1* mutations has been attributed to antibodies directed against FRα.

Diagnostic Tests

Patients are characterised by decreased CSF levels of folate in the presence of normal serum folate levels.

Treatment and Prognosis

The cerebral folate deficiency syndrome responds exclusively to folinic acid (10–20 mg/day) and not to folic acid [61]. Folinic acid therapy can restore CSF folate concentrations, reverse white matter choline and inositol depletion and improve clinical symptoms [62].

27.3.3 Methylenetetrahydrofolate Dehydrogenase (MTHFD1) Deficiency

Clinical Presentation

Five individuals from three families have been reported. Affected individuals have had megaloblastic anaemia, severe combined immunodeficiency in all but one and atypical haemolytic uraemic syndrome [64][65]. Two untreated patients died at 9 weeks of age.

Metabolic Derangement

Serum folate levels are within the reference range, while cerebrospinal fluid folate levels are reduced. The product of *MTHFD1* is a trifunctional cytoplasmic enzyme that catalyzes synthesis of 10-formyl-THF from THF and formate and its conversion to 5,10-methylene-THF. Biochemical studies in fibroblasts from the first identified patient showed adequate function of 10-formyl-THF-dependent purine biosynthesis with impairment of methylene-THF-dependent thymidylate synthesis and methyl-THF-dependent conversion of homocysteine to methionine [65]. Synthesis of MeCbl from exogenous CNCbl is somewhat reduced due to deficiency of methyl-THF.

Genetics

Mutations in *MTHFD1* have been identified in affected individuals in all three families, consistent with autosomal recessive inheritance [66][67]. Fibroblast studies in the first patient demonstrated decreased function of thymidylate synthase and methionine synthase in the presence of normal purine biosynthesis, indicating that in this case synthesis of 10-formyl-THF was unaffected [66].

Diagnostic Tests

Patients have normal serum folate levels and decreased cerebral folate levels. Serum total homocysteine is elevated, with normal or low-normal methionine levels.

Treatment and Prognosis

Treatment with folinic acid has been associated with reduction of total homocysteine to within the reference range and correction of megaloblastic marrow morphology, and with improved neurological function, although seizures in the initial patient were not corrected and maculopathy with retinal atrophy in a second patient was resistant to therapy. Two patients that had been treated long-term with folinic acid had normal neurological development at 8 and 22 years.

27.3.4 Dihydrofolate Reductase Deficiency

Clinical Presentation

Three families with apparent dihydrofolate reductase deficiency have been described [68][69]. Findings included megaloblastic anaemia, cerebral folate deficiency and seizures, and in severe cases, pancytopenia, cerebral atrophy and severe developmental delay,

Metabolic Derangement

Plasma and red cell folate levels are within the normal range However, there are relatively high levels of the oxidised forms of folates (dihydrofolate and folic acid), reflecting the deficiency in dihydrofolate reductase, which catalyses reduction of dihydrofolate to tetrahydrofolate, and (at a slower rate) folic acid to dihydrofolate.

Genetics

Homozygous mutations in *DHFR* have been identified in affected individuals in all three families, consistent with autosomal recessive inheritance. The mutations affect well-conserved amino acid residues, and decreased dihydrofolate reductase function has been shown in affected individuals.

Diagnostic Tests

Patients have decreased cerebral folate levels. Serum and red cell folate levels are normal, but the proportion of tetrahydrofolate derivatives is decreased. This disorder can be differentiated from cerebral folate deficiency due to mutations in *FOLR1* by the presence of megaloblastic anaemia.

Treatment and Prognosis

Treatment with folinic acid has been associated with normalisation of red cell volume and of megaloblastic marrow morphology, and with improved neurological function. There may be transient improvement of seizures, but ultimately folinic acid therapy has not proved effective in seizure control. In severely affected individuals, neurological dysfunction and developmental delay persist despite therapy.

27.3.5 Glutamate Formiminotransferase Deficiency

Clinical Presentation

Over a dozen patients have been described, but the clinical significance of this disorder is still unclear [70]. A mild and a severe form have been postulated, including patients with mental and physical retardation and folate-responsive megaloblastic anaemia with macrocytosis and hypersegmentation of neutrophils.

Metabolic Derangement

Histidine catabolism is associated with a formimino group transfer to THF, with the subsequent release of ammonia and the formation of 5,10-methenyl-THF. A single octameric enzyme catalyses two different activities: glutamate formiminotransferase and formiminotetrahydrofolate cyclodeaminase. These activities are found only in the liver and kidney, and defects in either of them will result in formiminoglutamate excretion.

Genetics

Hilton et al. found mutant alleles in *FTCD* in three patients and concluded that they represent the molecular basis for this disease, although expressed enzyme activity was 60% of controls [71].

Diagnostic Tests

Elevated formiminoglutamate and hydantoin propionate excretion and elevated levels of formiminoglutamate in the blood, only following a histidine load in the severe form, help to establish the diagnosis. Normal to high serum folate levels are found, particularly in the mild form. Hyperhistidinaemia and histidinuria have been reported.

Treatment and Prognosis

Although two patients in one family responded to folate therapy with reduced excretion of formiminoglutamate, six others did not. Pyridoxine and folic acid have been used to correct the megaloblastic anaemia in one infant.

27.3.6 Methylenetetrahydrofolate Reductase Deficiency

This section is restricted to the severe form of this deficiency. The role of polymorphisms in methylenetetrahydrofolate reductase (MTHFR) with respect to the risk for common disease, such as neural tube defects or cardiovascular disease, is beyond the scope of this chapter [72].

Clinical Presentation

Over 200 patients with severe MTHFR deficiency have been described [73][74][75]. Most were diagnosed in infancy, and more than half presented in the 1st year of life. The most common early manifestation was progressive encephalopathy with apnoea, seizures and microcephaly. However, patients became symptomatic at any time from infancy to adulthood, and in the older patients ataxic gait, psychiatric disorders (schizophrenia) and symptoms related to cerebrovascular events have been reported. At least one adult with severe enzyme deficiency was completely asymptomatic. Autopsy findings have included dilated cerebral vessels, microgyria, hydrocephalus, perivascular changes, demyelination, gliosis, astrocytosis and macrophage infiltration. In some patients, thrombosis of both cerebral arteries and veins was the major cause of death. There have been reports of patients with findings similar to those seen in subacute degeneration of the spinal cord due to Cbl deficiency. It is important to note that MTHFR deficiency is not associated with megaloblastic anaemia.

Metabolic Derangement

Methyl-THF is the methyl donor for the conversion of homocysteine to methionine, and in MTHFR deficiency its lack results in an elevation of total plasma homocysteine levels and decreased levels of methionine. Total CSF folate levels are also severely reduced. The block in the conversion of methylene-THF to methyl-THF does not result in the trapping of folates as methyl-THF and does not interfere with the availability of reduced folates for purine and pyrimidine synthesis in contrast to disorders at the level of methionine synthase. This explains why patients do not have megaloblastic anaemia. It is not clear whether the neuropathology in this disease results from the elevated homocysteine levels, from decreased methionine and resulting interference with methylation reactions or from some other metabolic effect. It has been reported that individuals with a severe deficiency in MTHFR may be at increased risk following exposure to nitrous oxide anaesthesia [76].

Genetics

MTHFR deficiency is inherited as an autosomal recessive disorder. Prenatal diagnosis has been reported using amniocytes, and the enzyme is present in chorionic villi. Over 100 mutations causing severe deficiency have been described in *MTHFR*, in addition to polymorphisms that result in intermediate enzyme activity and that may contribute to disease in the general population [74][75]. Most of these mutations are restricted to one or two families. Exceptions are a c.1542G>A muta-

tion that results in a splicing mutation, that was seen in 21 of 152 mutant alleles in a large study [74]; and a C.1117c>T mutation that is present at high frequency in the Old Order Amish [77]. A recent review has summarized all known mutations [75]; mutations in this publication are listed using an alternative nucleotide numbering system.

Diagnostic Tests

Because methyl-THF is the major circulating form of folate, serum folate levels may sometimes be low. There is a severe increase of plasma total homocysteine (60–320 µmol/l, with controls less than 14 µmol/l), together with plasma methionine levels ranging from zero to 18 µmol/l (mean: 12 µmol/l, range of control means from different laboratories: 23–35 µmol/l). Although neurotransmitter levels have been measured in only a few patients, they are usually low. Direct measurement of MTHFR specific activity can be performed in liver, leukocytes, lymphocytes and cultured fibroblasts. In cultured fibroblasts, the specific activity is heavily dependent on the stage of the culture cycle, with activity highest in confluent cells. Severe MTHFR deficiency has been associated with complete lack of enzyme activity, and with mutations causing reduced affinity for NADPH, decreased FAD responsiveness, abnormal inhibition of enzyme activity by S-adenosylmethionine and reduced affinity for methylene-THF [74].

Treatment and Prognosis

It is important to diagnose MTHFR deficiency early because, in the infantile forms, the only patients who have done well are those who were treated from birth. Early treatment with betaine following prenatal diagnosis has resulted in the best outcome [78]. Suggested doses have been in the range of 2–3 g/day (divided twice daily) in young infants and 6–9 g/day in children and adults. Betaine is a substrate for betaine methyltransferase, which converts homocysteine to methionine but is mainly active in the liver. Therefore, betaine may be expected to have the doubly beneficial effect of lowering homocysteine levels and raising methionine levels. Because betaine methyltransferase is not present in the brain, the central nervous system effects must be mediated through the effects of the circulating levels of metabolites. The dose of betaine should be modified according to plasma levels of homocysteine and methionine. Other therapeutic agents that have been used in MTHFR deficiency include folic acid or reduced folates, methionine, pyridoxine, Cbl and carnitine. In 3 patients with severe MTHFR deficiency measurable 5-methyltetrahydrofolate in cerebrospinal fluid was only achieved with mefolinate (5-methyltetrahydrofolate) supplements and not with either folic acid or folinic acid [79]. Most of the treatment protocols omitting betaine have not been effective. Dramatic improvement was reported in a patient with severe enzyme deficiency following early introduction of methionine supplements.

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Disorders of Thiamine and Pyridoxine Metabolism

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Thiamine Metabolism

Thiamine is transported across cell membranes by two closely related transporters, THTR1 and THTR2, encoded by the *SLC19A2* and *SLC19A3* genes (Fig. 28.1). Both transporters are widely expressed in the body, but they differ in kinetic properties and in the level of expression in different tissues. In the upper small intestine, where dietary thiamine is absorbed, THTR2 is the major transporter at the luminal surface whereas THTR1 predominates at the basal surface. The active cofactor of thiamine, TPP, is formed in the cytoplasm by the enzyme thiamine pyrophosphokinase. There the cofactor is attached directly to the transketolase and 2-hydroxyacyl CoA lyase apoproteins, while a TPP transporter (MTPPT) in the inner mitochondrial membrane delivers the cofactor to the α-ketoacid dehydrogenases in the mitochondrial matrix.

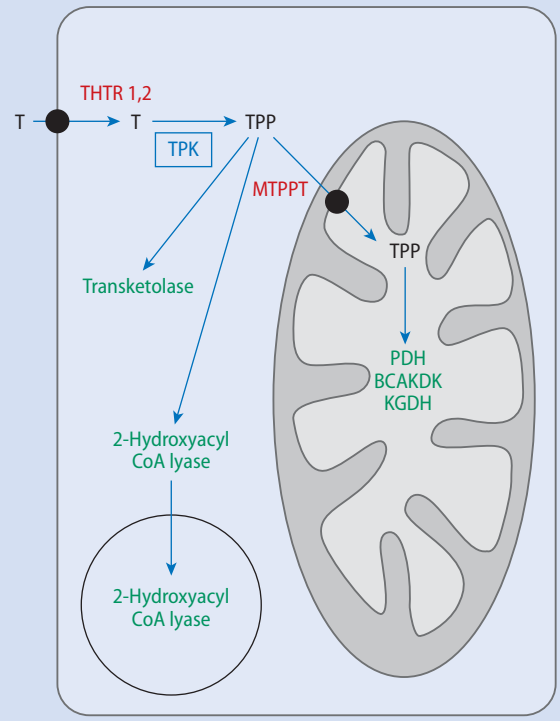

Fig. 28.1 Thiamine transport. *THTR1* and *THTR2,* thiamine transporter 1 and 2; *TPP,* thiamine pyrophosphate; *MTPPT,* mitochondrial TPP transporter; *TPK*, thiamine pyrophosphate kinase; *PDH,* pyruvate dehydrogenase; *BCAKDK,* branched chain aminoacid dehydrogenase kinase; *KGDH,* ketoglutarate dehydrogenase. The black circle represents the peroxisomal membrane

Thiamine (vitamin B_1) is a water-soluble vitamin transported across cell membranes by two closely related transporters, THTR1 and THTR2. The active cofactor of thiamine, thiamine pyrophosphate (TPP), is formed in the cytoplasm by the enzyme thiamine pyrophosphokinase. TPP enters mitochondria with a specific TPP transporter. Pyridoxine (vitamin B_6) is a water-soluble vitamin with broad availability from various food sources, including dairy products, meat, cereals and vegetables. The three vitamers, pyridoxal, pyridoxamine and pyridoxine and their phosphorylated esters are absorbed in the small intestine. Within the cells vitamers are rephosphorylated by kinases and further oxidised to the active cofactor pyridoxal 5′-phosphate (PLP) by pyridox(am)ine 5′-phosphate oxidase (PNPO).

28.1 Disorders of Thiamine (Vitamin B_1) Metabolism

Thiamine (vitamin B_1) has long been recognised as an essential dietary component. The minimal daily requirement is about 0.5 mg/1000 Kcal and this is normally provided by a well-balanced diet. Requirements do vary, however, and are increased in parallel with carbohydrate intake, during pregnancy and lactation, in hypermetabolic states and in infants. The active form of the vitamin is thiamine pyrophosphate (TPP) and this is a coenzyme for a number of important metabolic enzymes: pyruvate dehydrogenase, branched chain α-ketoacid dehydrogenase, α-ketoglutarate dehydrogenase, transketolase and the peroxisomal enzyme, 2-hydroxyacyl

Table 28.1 Disorders of thiamine metabolism

Defect (mechanism)	Disorder	Diagnostic tests	Effective dose of thiamine
Defective intake	Total parental nutrition without B_1 supplementation Breast-fed babies of B_1-deficient mothers Beri-Beri, Wernicke encephalopathy	Raised blood lactate Excretion of α-ketoacids in urine Low erythrocyte transketolase	2–4 mg/day (20 mg in emergency)
Defective transport	Thiamine transporter 1 (THTR1) deficiency Thiamine-responsive megaloblastic anaemia with diabetes and deafness	Megaloblastic anaemia Hyperlycaemia No specific biochemical markers DNA testing	25–50 mg/day
Defective transport	Thiamine transporter 2 (THTR2) deficiency Biotin/thiamine-responsive basal ganglia disease	Raised blood and CSF lactate Reduced muscle pyruvate dehydrogenase and α-ketoglutarate dehydrogenase (only in some patients) DNA testing	Biotin 2–10 mg/day Thiamine 100–400 mg/day (combination claimed to be more effective)
Defective cofactor biosynthesis	Thiamine pyrophosphokinase (TPK1) deficiency	Raised blood and CSF lactate Urinary excretion of α-ketoglutarate Low blood TPP	100–200 mg/day Higher doses (500 mg/day) may be more effective
Defective mitochondrial TPP transport	Mitochondrial thiamine pyrophosphate transporter deficiency Amish lethal microcephaly	Urinary excretion of α-ketoglutarate DNA testing	No evidence as yet of responsiveness
Defective binding of TPP to apoenzyme	Thiamine-responsive pyruvate dehydrogenase deficiency	Raised blood lactate and pyruvate, normal L/P ratio	50–1000 mg/day
Defective binding of TPP to apoenzyme	Thiamine-responsive maple syrup urine disease	Raised plasma leucine, isoleucine, valine and alloisoleucine	50–1000 mg/day

CoA lyase. As an essential component of these highly regulated enzymatic reactions, thiamine plays a crucial role in carbohydrate metabolism and the metabolic switch from the fed to the fasting state. Acute thiamine deficiency states (such as total parenteral nutrition without thiamine supplement) are life threatening emergencies and present as cardiac failure, Gayet Wernicke encephalopathy, or lactic acidosis [1][2]. Metabolic markers are hyperlactatemia with hyperpyruvic acidaemia, a normal lactate to pyruvate ratio, slight elevation of branched chain amino acids in plasma, presence of α-ketoglutarate, pyruvate and branched chain α-ketoacids in urine, with a positive DNPH reaction, and low transketolase activity in red blood cells. However, these markers are rarely available under emergency conditions and diagnosis relies on primary care physicians in the emergency room and the life-saving therapeutic test of administration of thiamine intravenously at a dose of 5 mg/kg/day. This dose may be given without risk of adverse effects.

Thiamine-dependent inborn errors of metabolism are very rare and can arise from defects in thiamine transport or the biosynthesis and intracellular transport of thiamine pyrophosphate. They can also be due to intrinsic structural defects in thiamine-dependent enzymes which alter the affinity of the enzyme for the cofactor (Table 28.1). Patients with these different conditions present with a wide range of clinical and biochemical manifestations, reflecting different patterns and degrees of involvement of the thiamine-dependent enzymes. Most of the conditions have only recently been identified and many details about their natural history and response to treatment remain to be elucidated. In many cases, patients only respond to high doses of thiamine, however, these are readily tolerated and can be used safely.

28.1.1 Thiamine Transporter 1 (THTR1) Deficiency

Clinical Presentation

THTR1 deficiency results in thiamine-responsive megaloblastic anaemia [3][4]. The hallmarks of this condition are megaloblastic anaemia, diabetes mellitus and sensorineural deafness. The anaemia is often the first manifestation and develops during infancy or early childhood. Although the anaemia is megaloblastic in character, ringed sideroblasts may be present in the marrow and some patients develop thrombocytopaenia. Diabetes usually develops later in childhood, although patients have been reported with neonatal diabetes. Other manifestations of the condition include cardiac abnormalities, short stature, retinal abnormalities, optic atrophy and stroke-like episodes. Cardiac involvement

includes arrhythmias, congenital malformations and cardiomyopathy [5].

Metabolic Derangement

There have been few biochemical studies in patients with THTR1 deficiency. It is likely that the megaloblastic erythropoiesis is related to deficiency of transketolase in the pentose phosphate shunt, with impaired synthesis of ribose-5-phosphate. The blood thiamine concentration is normal, supporting experimental evidence that intestinal absorption of thiamine does not depend on this transporter. Apart from the anaemia, many of the features of THTR1 deficiency are shared with various mitochondrial diseases, however, biochemical defects in energy metabolism have not been widely documented. There is a single report of a patient with raised blood and cerebrospinal fluid lactate concentration and reduced activities of complex I and pyruvate dehydrogenase in muscle which returned to normal with thiamine supplementation [6].

Genetics

Various missense mutations, small deletions and duplications and nonsense mutations have been identified in *SLC19A2* in these patients. Most have been found in single individuals and there is so far little information about possible genotype/phenotype correlations.

Treatment and Prognosis

Patients with THTR1 deficiency generally respond well to thiamine supplementation, but not all clinical manifestations respond to the same extent. Treatment with doses of thiamine between 25-50 mg/day usually produces a good response in the anaemia, diabetes and cardiac arrhythmias. Deafness and other neurological features do not usually respond as well, however this may be improved with early diagnosis and treatment. With long term treatment, thiamine-responsiveness may decrease and previously well-controlled patients may become transfusion and insulin dependent [4].

28.1.2 Thiamine Transporter 2 (THTR2) Deficiency

Clinical Presentation

Deficiency of this transporter most commonly results in biotin-responsive basal ganglia disease [7]. Onset is usually during childhood when patients develop a subacute encephalopathy characterised by speech and swallowing difficulty, confusion, dystonia and rigidity. This is associated with symmetric lesions in the caudate nucleus and putamen. Patients with defects in THTR2 and different clinical presentations have also been identified and the clinical spectrum is continually expanding.

Early presentations of this condition include atypical infantile spasms and delayed development with progressive cerebral atrophy and bilateral lesions in the thalami and basal ganglia, [8], and severe neonatal lactic acidosis with acute encephalopathy [9]. Later presentations, in adolescence or adulthood, include Leigh-like [10], or Wernicke-like encephalopathy [11], and generalised dystonia and seizures [12].

A very severe form of this condition has been recognised recently in patients with an early onset, fatal encephalopathy. These patients had a characteristic pattern of brain MRI abnormalities with cerebral atrophy and symmetrical lesions in the thalami, basal ganglia and brain stem [13][14].

Metabolic Derangement

A number of non-specific biochemical abnormalities have been documented, including raised blood and cerebrospinal fluid lactate concentration and reduced activity of pyruvate dehydrogenase and α-ketoglutarate dehydrogenase in muscle.

Genetics

In the original cohort of patients from Saudi Arabia, there was a high degree of parental consanguinity and a common missense mutation, p.Thr422Ala, in *SLC19A3* [15]. In the remaining patients, different missense, nonsense, splicing and frameshift mutations have been identified. Several, including p.Gly23Val and p.Glu320Gln have been found in several unrelated families. There is no clear genotype-phenotype correlation in relation to course, outcome or response to treatment.

Treatment and Prognosis

The first patients to be described all had a rapid response to 5-10 mg/day biotin and remained symptom-free provided the diagnosis was established promptly and treatment was continued. Other patients have been treated effectively with a combination of biotin and thiamine (2–10 mg/day of biotin and 100–400 mg/day of thiamine). The patients with infantile spasms and delayed development did not respond to biotin and were treated with thiamine alone, as were the patients with a Wernicke-like encephalopathy. In a recent controlled study of combined biotin and thiamine versus thiamine alone, all patients diagnosed and treated early had a favourable outcome. There was no long term difference between the two treatments in terms of sequelae, but recovery from acute episodes was slightly faster in patients treated with both biotin and thiamine [16].

The favourable clinical response of many patients to biotin supplementation is unexpected as THTR2 does not transport biotin. However, in addition to its function as an enzyme cofactor, biotin also affects the expression of a number of genes, including *SLC19A3* [17]. In patients with certain types of *SLC19A3* mutations, enhanced expression of the gene induced by increased biotin intake may generate sufficient THTR2 protein to ameliorate the thiamine transport defect.

28.1.3 Thiamine Pyrophosphokinase Deficiency

Clinical Presentation

This is a recently identified condition, with reports of nine patients from six families [18][19][20]. Patients usually

present with episodic ataxia, psychomotor retardation and dystonia during early childhood. Spasticity and seizures are less common. Brain MRI changes include global atrophy and abnormal signal in the basal ganglia, cerebellum and brain stem.

Metabolic Derangement

Elevated blood and CSF lactate concentrations during episodes of ataxia, and enhanced urinary excretion of α-ketoglutarate are consistent findings. Blood and muscle TPP concentrations are significantly reduced and measurement of the blood concentration is an effective screening test. The major biochemical consequence of the enzyme defect appears to be deficiency of pyruvate and α-ketoglutarate dehydrogenases, however the activity of these enzymes *in vitro* is normal in the presence of TPP.

Genetics

Most patients have missense mutations in *TPK1* and so far there is no common mutation in unrelated families.

Treatment and Prognosis

Three of the patients died in childhood. Several patients have received thiamine supplementation at 100–200 mg/day, although this generally proved ineffective when neurological abnormalities were already established at diagnosis. Earlier intervention, with higher doses (500 mg/day) may be associated with better prognosis, with one treated patient attending normal school.

28.1.4 Mitochondrial TPP Transporter Deficiency

Clinical Presentation

Deficiency of the mitochondrial TPP carrier was first described in patients with Amish lethal microcephaly. These patients have a distinctive facial appearance and a characteristic pattern of brain abnormalities [21]. Although death usually occurs within the first six months, one patient is reported to have survived through childhood.

A defect in the TPP transporter has also been identified in one family from outside of the Amish community in which four siblings developed acute episodes of flaccid paralysis and encephalopathy, with motor and sensory neuropathy, precipitated by intercurrent illness. There was some residual weakness between episodes and a progressive polyneuropathy, but no cognitive impairment [22]. MRI changes were present in the caudate nucleus and putamen, but not the globus pallidus.

Metabolic Derangement

In patients with Amish lethal microcephaly, increased urinary excretion of α-ketoglutarate is a consistent finding, however this was absent in the siblings in the unrelated family described subsequently. In these patients, the lactate concentration in cerebrospinal fluid was raised during acute episodes. Activity of respiratory chain complexes and pyruvate dehydrogenase in a muscle sample taken between episodes in one of these patients was normal.

Genetics

Patients from the Amish community with lethal microcephaly have a common missense mutation, p.Gly177Ala in *SLC25A19* [23]. The siblings in the family described by Spiegel et al also have a missense mutation, p.Gly125Ser.

Treatment and Prognosis

All of the patients identified by Spiegel et al are receiving high dose thiamine treatment, but at present it has not been established if this prevents acute episodes or improves the neuropathy and basal ganglia abnormalities.

28.1.5 Thiamine-Responsive α-Ketoacid Dehydrogenase Deficiencies

In some individuals, the normal dietary thiamine intake is not sufficient to sustain function of some TPP-dependent enzymes. This is the case in a small number of patients with pyruvate dehydrogenase deficiency and maple syrup urine disease, in whom high doses of thiamine have been reported to improve the clinical and/or biochemical features. The impaired enzyme activity in these patients is proposed to result from a structural defect which reduces the affinity of the enzyme for the cofactor, but which can be overcome if the cofactor concentration is increased by pharmacological doses of the vitamin precursor.

28.1.6 Thiamine-Responsive Pyruvate Dehydrogenase Deficiency

Clinical Presentation

Over 20 patients with pyruvate dehydrogenase deficiency have been claimed to be thiamine-responsive [24]. They usually present later, and are less severely affected, than is usual with this condition. Most common features are delayed development and hypotonia from late infancy, sometimes with episodes of ataxia in association with intercurrent illness. The great majority have lesions in the brain characteristic of Leigh syndrome. A small number of these patients have normal development and cognition, but they may develop problems due to peripheral neuropathy. The rare adult patient with unusual presentation of pyruvate dehydrogenase deficiency may also respond to thiamine supplementation.

Biochemical Derangement

Blood and cerebrospinal fluid lactate concentrations are often normal or elevated only during acute episodes. In a number of patients, *in vitro* studies with cultured fibroblasts have been performed to correlate the clinical response with correction of the enzyme defect in the presence of excess TPP, however, these do not always yield unequivocal results.

Vitamin B_6 Metabolism

Pyridoxine (vitamin B_6) is a water-soluble vitamin with broad availability from various food sources, including dairy products, meat, cereals and vegetables. The three vitamers, pyridoxal, pyridoxamine and pyridoxine and their phosphorylated esters are absorbed in the small intestine. For cellular uptake and transport across the blood-brain barrier, phosphorylated forms undergo dephosphorylation by intestinal phosphatases and tissue non-specific alkaline phosphatase (TNSAP) respectively. The transport mechanism of B_6 vitamers across cell membranes has not yet been fully elucidated. Within the cells vitamers are rephosphorylated by kinases and further oxidised to the active cofactor pyridoxal 5′-phosphate (PLP) by pyridox-(am)ine 5′-phosphate oxidase (PNPO) (Fig. 28.2).

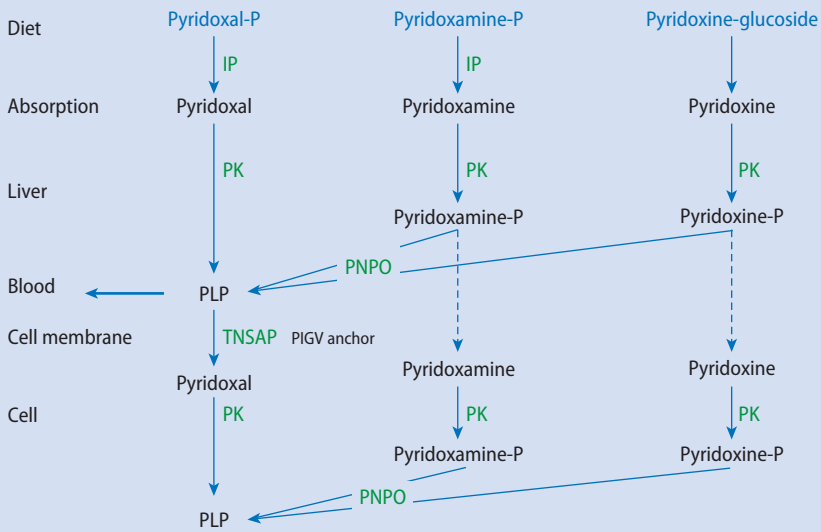

Fig. 28.2 Pyridoxine metabolism. *IP,* intestinal phosphatases; *PK,* pyridoxine kinase; *PNPO,* pyridox(am)ine 5´-phosphate oxidase; *TNSAP,* tissue non-specific alkaline phosphatase

Genetics

The TPP binding site is shared between the α and β subunits of the E1 component of the pyruvate dehydrogenase complex, however all thiamine-responsive patients identified to date have had mutations in *PDHA1* encoding the E1α subunit. The mutations are missense changes, mostly involving amino acid residues adjacent to the TPP binding site.

Treatment and Prognosis

It is difficult to establish definitively if any of the reported cases of thiamine-responsive pyruvate dehydrogenase deficiency are truly responsive. Almost all patients have received other treatments, as thiamine alone has not controlled symptoms. Doses of thiamine have varied widely from 50–1200 mg/day, and while this has led to clinical improvement in some cases, more often only the biochemical abnormalities have normalised and the clinical course has remained unaltered. Reports of initial improvement are rarely followed up with documentation of later outcome, and only a small number of patients have survived to adolescence with normal cognitive development.

28.1.7 Thiamine-Responsive Maple Syrup Urine Disease

Clinical Presentation

This has been described in over 10 patients [25]. Presentation is usually similar to the intermediate form of maple syrup urine disease, with episodes of ketoacidosis or ataxia in late infancy, and delayed development.

Metabolic Derangement

These patients have elevated branched chain amino acid and α-ketoacid concentrations in blood and urine at diagnosis which reduce, but do not necessarily normalise, with thiamine supplementation. Episodes of acute decompensation with severe ketoacidosis are less common than in classic maple syrup urine disease and are usually suppressed with thiamine treatment.

Genetics

Almost all thiamine-responsive maple syrup urine disease patients have mutations in the *DBT* gene for the E2 component of the complex [26] and this may reflect the fact that binding of the E1 enzyme to the E2 core of the complex influences its affinity for TPP. A single thiamine-responsive patient with a mutation in *BCKDHB*, encoding the E1β subunit, has been reported.

Treatment and Prognosis

It is difficult to assess the status of thiamine-responsiveness in patients with maple syrup urine disease. Patients have been given a wide range of thiamine dosage, up to 1000 mg/day, and most have also received dietary branched chain amino acid restriction. There are few long term follow up studies, although several patients remain healthy as adults, with normal cognitive function and no episodes of metabolic decompensation.

28.2 Vitamin B_6 Metabolism

While the liver seems to be the most important organ of pyridoxal 5′-phosphate (PLP) formation, pyridox(am)ine 5′-phosphate oxidase (PNPO) is expressed in various cell types including neurons. PLP is one of the most abundant cofactors and participates in over 140 reactions in aminoacid and neurotransmitter metabolism. The daily requirement is 0.1 to 0.3 mg/day in infants and 1.2-1.4 mg/day in adults.

Systemic vitamin B6 deficiency causes seizures, failure to thrive and anemia in a variety of species, including chickens and rabbits, and also human infants fed a formula with low vitamin B6 content due to overheating during sterilisation. Nutritional vitamin B6 deficiency is rarely seen nowadays and usually occurs together with other vitamin deficiencies in malnutrition or in association with severe chronic disease.

Table 28.2 Disorders of vitamin B_6 metabolism

Defects and PLP related mechanism	Biochemical abnormalities			Response to vitamin B_6
	Urine	Plasma	CSF	
Coeliac disease Chronic dialysis Malabsorption, depletion	↑ Xanthurenic acid	↑ Threonine, glycine and serine		To very low doses of pyridoxine
Drug interaction (eg. hydrazines, D-penicillamine, enzyme inducing anticonvulsants)		↑ Homocysteine		Preventive B_6 supplementation
PNPO Deficiency Reduced PLP formation	Vanillacetate*	↑ PM and PM/PA	↓ to normal PLP° sec. NT changes*	Mainly to PLP, in certain mutations also pyridoxine
Congenital Hypophosphatasia Reduced PLP uptake		↓ AP, ↓Ph, ↑ Ca		To pyridoxine (or PLP)
Congenital Hyperphosphatasia Reduced PLP uptake		↑ AP		Unknown
Antiquitin Deficiency (PDE) PLP inactivation	AASA, P6C	↑ Pipecolic acid*	↑ AASA, ↓ PLP° sec. NT changes*	To pyridoxine (or PLP)
Hyperprolinemia II PLP inactivation	↑ Prolin, P5C	↑ Prolin, P5C		To pyridoxine (or PLP)
Classical Homocystinuria Chaperone	↑ Homocyst(e)ine	↑↑ Homocysteine, methionine		To pyridoxine in about 50% of patients
Gyrate atrophy (OAT) Chaperone		↑ Ornithine		To pyridoxine in some patients
X-linked sideroblastic anaemia		Enzyme assay in RBC and DNA		To pyridoxine in about 90% of patients

AP, alkaline phosphatase; *AASA,* alpha aminoadipic acid; *Ca,* calcium; *PA,* pyridoxic acid; *PDE,* pyridoxine dependent epilepsy; *P6C,* piperideine-6-carboxylate; *P5C,* pyrrolin-5-carboxylate; *Ph,* phosphate; *PLP,* pyridoxal 5′-phosphate; *PM,* pyridoxamine; *PNPO,* pyridox(am)ine 5′-phosphate oxidase; *NT,* neurotransmitter; *OAT,* ornithine delta-aminotransferase
* Inconsistent findings, ° before specific treatment with vitamin B_6

There are several mechanisms that lead to an increased requirement for pyridoxine and/or PLP [27] (Table 28.2): (i) inborn errors affecting the pathways of B_6 vitamer metabolism: PNPO and alkaline phosphatase defects ; (ii) inborn errors that lead to accumulation of small molecules that react with PLP and inactivate it: hyperprolinemia type II and antiquitin deficiency (pyridoxine dependent epilepsy) ; (iii) inborn errors affecting specific PLP dependent enzymes : X-linked sideroblastic anemia, classical homocystinuria, gyrate atrophy of the choroid; (iv) drugs as D-penicillamine or isozianid that affect the metabolism of B_6 vitamers or react with PLP ; (v) coeliac disease, which is thought to lead to malabsorption of B_6 vitamers or renal dialysis, which leads to increased losses of B_6 vitamers from the circulation;

There are currently five known inborn errors of metabolism, all of autosomal recessive inheritance, that lead to reduced availability of the active vitamin B_6 cofactor, either by inactivation, reduced formation, or reduced cellular uptake of PLP and can be recognized by respective biomarkers (Table 28.2). In each of these entities, seizures are a hallmark of the disease, with no or incomplete response to common anticonvulsants, but a good response to pyridoxine or PLP. In these IEM, seizures typically recur upon withdrawal of vitamin B_6 and illustrate B_6 dependency in contrast to mere responsiveness, as seen in nutritional deficiencies and also as a non-specific phenomenon due to GABA-ergic effects of vitamin B_6 supplementation. As biomarkers and genetic analysis are both available to test for IEM associated with vitamin B_6 responsive seizures (Table 28.2), withdrawal in responders is no longer relevant.

28.2.1 Antiquitin Deficiency

Clinical Presentation

Antiquitin deficiency is the most common form of pyridoxine dependent epilepsy (PDE). Typically, patients present in the neonatal period with myoclonic and tonic seizures or status epilepticus, but onset up to 3 years of age has been observed [28]. About one third of affected neonates have a history of asphyxia and some may also present with encephalopathy (inconsolable crying, sleeplessness) or systemic features including hypoglycemia, lactic acidosis or acute abdomen. The EEG changes can range from non-specific slowing and discontinuity to focal discharges, or rarely, burst suppression patterns. Seizures are typically resistant to common anticonvulsants aside from a possible partial or transient response to phenobarbitone. Imaging is non-diagnostic, but may show thinning of the corpus callosum, cysts of the posterior fossa or white matter anomalies [29].

Metabolic Derangement

Antiquitin (*ALDH7A1*) encodes for α-aminoadipic semialdehyde dehydrogenase, an enzyme involved in lysine degradation (Fig. 28.3). The accumulating compound, α-aminoadipic acid semialdehyde (AASA), is in equilibrium with L-Δ^1-piperideine-6-carboxylate (PC6)Δ, which inactivates PLP by a so called Knoevenagel condensation [30]. The Knoevenagel product has not been shown in vivo to date. AASA (and P6C) in urine can be determined semiquantitatively by LC-MS-MS and serve as reliable biomarkers, even when patients are on treatment with pyridoxine. Simultaneous determination of sulfocysteine is crucial to exclude molybdenum cofactor and sulfite oxidase deficiency causing secondary inhibition of antiquitin [31]. Pipecolic acid in plasma, the first described biomarker of PDE, is less specific as it can also be found in peroxisomal disease and has been found normal in older patients while on pyridoxine [32] [33]. A number of secondary phenomena have been described in CSF of affected patients: low GABA, homovanillic acid (HVA) and hydroxyindole acetic acid (HIAA) concentrations. PLP levels in CSF, if measured pre-treatment, are markedly decreased, while PLP in plasma is low-normal. Enzymatic testing of antiquitin activity in fibroblasts, though feasible, has not been established as a routine investigation.

Genetics

Diagnosis is confirmed by Sanger sequencing of *ALDH7A1*. Over 70 different mutations have been described to date with no clear genotype/phenotype correlation. The E427Q mutation, which results in complete enzyme deficiency, accounts for about 30% of all mutant alleles within Europe. Deletions of *ALDH7A1* have been reported and can only be detected by MLPA techniques [34]. In 2009 it was shown, that Antiquitin deficiency is allelic to folinic acid responsive seizures [35].

Treatment and Prognosis

In most cases the administration of pyridoxine, 100 mg iv. or po., leads to prompt cessation of seizures. In about 14% the response to pyridoxine has been ambiguous and may be missed by a single dose administration [36]. Therefore, the administration of 30 mg/kg in 2 to 3 SD over three consecutive days has been recommended to identify patients with delayed response. As first administration of pyridoxine may lead to severe apnea, resuscitation equipment should be at hand.

About 90% of patients have complete seizure control on pyridoxine monotherapy. To prevent side effects of pyridoxine, such as peripheral neuropathy, dosages should not exceed 300 mg/day. In those with breakthrough seizures during febrile illness, doubling of the pyridoxine dose over a few days is effective. Despite complete seizure control only 25% of PDE patients show normal development, irrespective of any treatment delay. This may be due to the accumulation of potentially toxic metabolites within the L-lysine pathway that do not normalise upon pyridoxine treatment. Therefore add-on therapies, such as lysine restriction and arginine supplementation for competitive inhibition of cellular lysine uptake, are under investigation [37]. Prenatal treatment with 100 mg of pyridoxine starting from 3 months in pregnancies at risk may lead to better outcome [38], but warrants rapid confirmation testing after birth, as an unaffected offspring had pro-convulsive effects on high dose pyridoxine therapy [39]. As some patients with antiquitin deficiency have been shown to have additional benefit from folinic acid, mainly during the neonatal period or infancy, folinic acid (3–5 mg/kg/day) is recom-

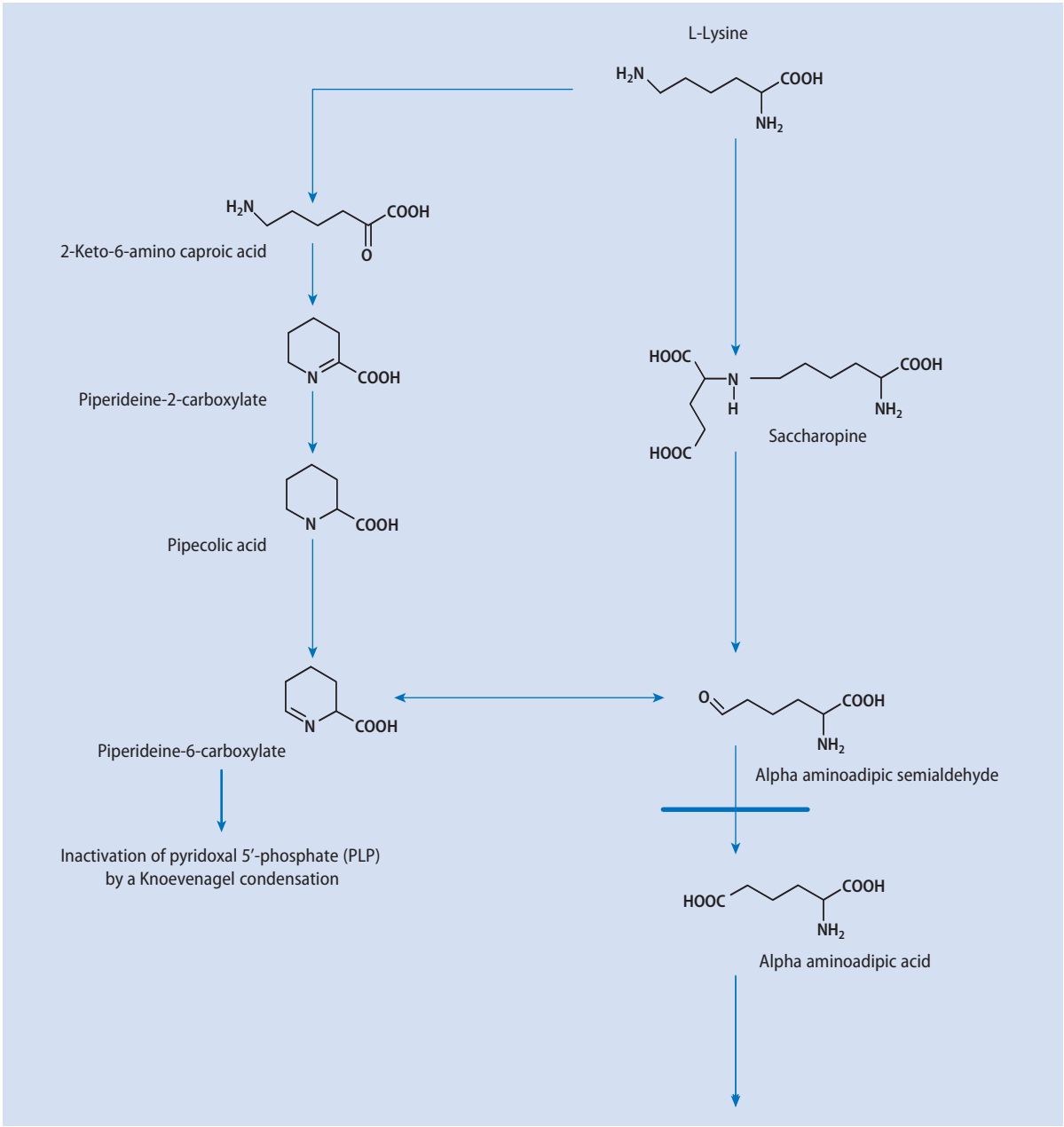

Fig. 28.3 Lysine degradation and antiquitin deficiency (blue bar)

mended for patients who fail to respond to pyridoxine alone. The role of folinic acid is not completely understood, but might be due to a »B_6 sparing effect« (P. Clayton, personal communication, 2014).

28.2.2 Hyperprolinemia Type II

Among the five IEM with vitamin B_6 dependent seizures, this has probably the most attenuated phenotype. Only about 50% of patients with hyperprolinemia type II present with seizures during infancy or childhood. These seizures may be triggered by fever and may respond to common anticonvulsants. Most individuals have accompanying learning disabilities. This disease was first identified in the Irish Traveller population [40] and may remain largely undiagnosed outside of this inbred community. It is in this IEM that the inactivating mechanism of PLP by a Knoevenagel condensation was first described [41]. The accumulated inactivating compound is Δ^1-pyrroline-5-carboxylate (P5C) due to deficiency of Δ^1-pyrroline-

5-carboxylate dehydrogenase. The diagnosis can be made by marked elevation of plasma proline concentration and the presence of P5C in urine. The disorder is described in ▶ Chapter 21 (▶ Section 21.6).

28.2.3 Pyridox(am)ine 5′-phosphate Oxidase (PNPO) Deficiency

Clinical Presentation

The clinical presentation of PNPO deficiency is indistinguishable from Antiquitin deficiency except for a higher rate of prematurity which is found in 61% of all published cases [42]. The disease was first described in Taiwan, where PLP is the first line drug to test for vitamin B_6 responsiveness [43]. Seizures recurred when patients were switched to pyridoxine and they showed a neurotransmitter profile that mimicked aromatic L-amino acid decarboxylase deficiency [44]. In contrast to Antiquitin deficiency, patients with PNPO deficiency show signs of systemic PLP deficiency beyond the neonatal period, as failure to thrive and anaemia. In the neonatal period, the EEG is usually severely abnormal and a burst suppression pattern has been described in two thirds of published cases. Brain MRI imaging can be normal, but shows white matter changes and atrophy if diagnosis and specific treatment are significantly delayed.

Metabolic Derangement

PNPO deficiency leads to severe (systemic) PLP deficiency and impaired function of PLP dependent enzymes [45]. Vanillacetate in urine reflects the buildup of dopamine metabolites, but is an inconstant finding. While the original patients with PNPO deficiency were found to have decreased urinary HVA and HIAA, there are now two reports of elevated levels of these metabolites prior to treatment, an observation which remains unexplained. PLP concentrations in CSF prior to treatment have been found to be low but this may also be an inconsistent finding [42]. In fact the function of PNPO might be much broader than previously thought as it has also been shown to be involved in the intracellular recycling and trafficking of PLP [46].

Genetics

In 2005 PLP dependent seizures were shown to be caused by PNPO deficiency [45]. In 2014, two reports documented a significant proportion of patients with novel pyridoxine-responsive *PNPO* mutations, with residual enzyme activity of 8% or above [47][48].

Treatment and Outcome

Patients with PNPO deficiency have a short time window for specific treatment in order to prevent irreversible brain damage. Outside of Asia, PLP is an unlicensed chemical and can be purchased from naturopathic stores. Effective dosages vary from 30 to 60 mg/kg/day. Patients often respond to frequent dosing of 4–6 single dosages/day. To avoid oxidation PLP should be dissolved immediately before oral administration. According to recent reports on liver toxicity, transaminases should be monitored and the lowest effective dose used [49].

28.2.4 Congenital Hypophosphatasia

Clinical Presentation

Only patients affected by the severe form of congenital hypophosphatasia (CHP) present with neonatal seizures, sometimes before the skeletal manifestation of osteomalacia due to poor bone mineralisation becomes apparent [50]. The EEG is usually severely abnormal and can show a burst suppression pattern. Prior to the availability of enzyme replacement therapy, this condition was fatal as a result of respiratory insufficiency.

Metabolic Derangement

CHP is caused by a deficiency of Tissue Non Specific Alkaline Phosphatase (TNSAP) and impaired dephosphorylation of PLP for cellular uptake, leading to intracellular PLP deficiency. The diagnosis is straight forward with markedly reduced levels of alkaline phosphatase (AP) in routine clinical chemistry, elevated serum calcium and reduced serum phosphate, and elevated phosphoethanolamine upon amino acid analysis. PLP in plasma prior to treatment is markedly elevated.

Genetics

CHP is caused by mutations in *ALPL* and there is some genotype-phenotype correlation and an increased frequency of specific mutations in some ethnic populations.

Treatment and Prognosis

There is a variable and inconsistent response to treatment with pyridoxine. The availability of enzyme replacement therapy will hopefully alter the outlook for this devastating disease as it is expected to improve cellular PLP uptake alongside the major effect of improving bone mineralization [51].

28.2.5 Hyperphosphatasia-Mental Retardation Syndrome (HPMRS)

HPMRS or Mabry syndrome, is a clinically recognizable syndrome with facial dysmorphism, brachytelephalangy and seizures of neonatal or childhood onset. The majority of cases are caused by mutations in *PIGV*, or less frequently in *PIGO* or *PGAP2*, that encode the synthesis of phosphatidylinositol (GPI) –anchors of various membrane-bound proteins such as TNSAP [52] (▶ Chapter 34). Mildly to moderately elevated AP in serum is a hallmark of the disease. To date it remains unclear, if seizures of these patients respond to pyridoxine.

28.2.6 Other B_6 Responsive Disorders

Some IEM caused by defects of PLP dependent enzymes benefit from cofactor supplementation [27]. This is true for about 50% of all cases with classical homocystinuria and warrants a pyridoxine challenge prior to the initiation of a methionine restricted diet and/or medication (▶ Chapter 20). Pyridoxine responsiveness is also seen in some cases of gyrate atrophy,

caused by deficiency of ornithine aminotransferase (▶ Chapter 21). The pyridoxine-responsive anaemia (or X-linked sideroblastic anaemia) caused by a defect in the erythroid-specific form of 5-aminolevulinate synthase presents in the 2nd decade of life with a microcytic, hypochromic anaemia with a sideroblastic marrow and other problems caused by iron overload; 90% of patients are B_6 responsive (▶ Chapter 36). In all these B_6 responsive disorders vitamin B_6 vitamers may act as a chaperone on the mutated protein [53]. A stepwise increase of pyridoxine is advised to identify the lowest effective dose. For the risk of (reversible) neuropathy pyridoxine doses should be kept below 300mg/day wherever possible.

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Section VI Neurotransmitter and Small Peptide Disorders

Disorders of Neurotransmission

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Neurotransmitters

Although chemical transmission in the nervous system is characterized by an amazing complexity, classical neurotransmitter systems involve inhibitory aminoacidergic [γ-aminobutyric acid (GABA) and glycine], excitatory aminoacidergic (aspartate and glutamate), cholinergic (acetylcholine), monoaminergic (mainly adrenaline, noradrenaline, dopamine, and serotonin), and purinergic (adenosine and adenosine mono-, di-, and triphosphate). The addition of neuropeptides (the largest family of signalling molecules in the nervous system) channels (that modulate neurotransmitter actions), and new approaches based on synaptic physiology will likely reclassify neurotransmitter systems and disorders in the future.

GABA is formed from glutamic acid by glutamic acid decarboxylase (Fig. 29.1). It is catabolized into succinic acid through the sequential action of two mitochondrial enzymes, GABA transaminase and succinic semialdehyde dehydrogenase. Glutamic acid decarboxylase and GABA transaminase require pyridoxal phosphate as a coenzyme. Pyridoxal phosphate also participates in the synthesis of dopamine and serotonin (Fig. 29.3), and in many other pathways including the glycine cleavage system. A major inhibitory neurotransmitter, GABA is present in high concentration in the central nervous system, predominantly in the gray matter. GABA modulates brain activity by binding to sodium-independent, high-affinity, mostly $GABA_A$ receptors.

Glycine, a non-essential amino acid, is an intermediate in many metabolic processes but also one of the major inhibitory neurotransmitters in the central nervous system. The inhibitory glycine receptors are mostly found in the brain stem and spinal cord.

Glutamate is the major excitatory neurotransmitter in the brain. Its function requires rapid uptake to replenish intracellular neuronal pools following extracellular release.

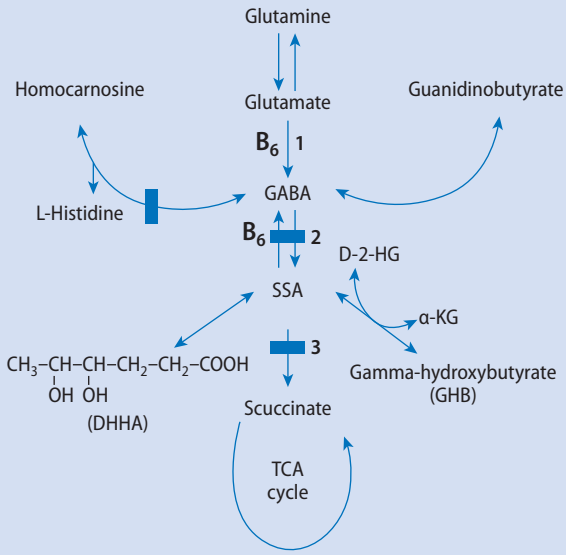

Fig. 29.1 Brain metabolism of γ-aminobutyric acid (GABA). B_6, pyridoxal phosphate. *1*, glutamic acid decarboxylase; *2*, GABA transaminase; *3*, succinic semialdehyde dehydrogenase; *D-2-HG*, D-2-hydroxybutyrate; *α-KG*, alpha ketoglutarate; *TCA*, tricarboxylic c-ycle; *DHHA*, 4,5-dihydroxyhexanoic acid; *SSA*, succinic semialdehyde. Enzyme defects are depicted by **solid bars**. The block at conversion of homocarnosine to GABA remains to be clarified

This chapter deals primarily with inborn errors of neurotransmitter metabolism (► Neurotransmitters). Defects of their receptors and transporters are also discussed.
Three defects of GABA catabolism have been reported: GABA transaminase deficiency (very rare, severe, and untreatable), succinic semialdehyde dehydrogenase (SSADH) deficiency, and homocarnosinosis (◘ Fig. 29.1). Hyperekplexia is usually due to a dominantly inherited defect of the α_1 subunit of the glycine receptor which causes excessive startle responses, and is treatable with clonazepam. Mutations in $GABA_A$ receptor are a cause of dominantly inherited epilepsy while mutations in glutamate receptors associate with neurodevelopmental and psychiatric disorders. Three transportopathies are reported: mitochondrial glutamate transporter defect, which is a cause of severe epileptic encephalopathy, and diseases that produce early parkinsonism-dystonia: dopamine transporter defect and vesicular monoamine transporter type 2 defect. Disorders of the metabolism of glycine are discussed in ► Chapter 24.

Six disorders of monoamine metabolism are discussed: Tyrosine hydroxylase (TH) deficiency impairs synthesis of dihydroxyphenylalanine (L-dopa) and causes a neurological disease with prominent extrapyramidal signs, and a variable response to L-dopa. The clinical hallmark of dopamine β-hydroxylase deficiency is severe orthostatic hypotension with sympathetic failure. The other disorders of monoamine metabolism involve both catecholamine and serotonin metabolism. Aromatic L-amino acid decarboxylase (AADC) is located upstream of the neurotransmitter amines; treatment can be challenging. Monoamine-oxidase A (MAO-A) deficiency, located downstream, mainly causes behavioral disturbances; no effective treatment is known. guanosine triphosphate cyclohydrolase-I (GTPCH-I) and sepiapterin reductase (SR) defi*ciencies* are pterin disorders upstream of L-dopa and 5-hydroxytryptophan (5-HTP) with normal baseline phenylalaninemia and effective treatment (especially GTPCH-I deficiency).

29.1 Inborn Errors of Gamma Amino Butyric Acid Metabolism

29.1.1 Gamma Amino Butyric Acid Transaminase Deficiency

GABA transaminase deficiency has been reported in four families [1][2][3].

▪ Clinical Presentation

Cardinal clinical features in the initial Flemish probands (sibship) included neonatal onset epileptic encephalopathy, macrosomia, and early mortality [1]. Postmortem examination of the brain revealed spongiform leukodystrophy. Death ensued at age 25 months and 12 months. The presenting clinical picture of a third Japanese patient was similar to that described above. Initially reported at 28 months and currently age 7 years, he manifests profound developmental impairment without mobility or language [2]. Diffusion-weighted MRI in the basal ganglia revealed hyperintense signals in both internal/external capsules with extensive subcortical white-matter involvement. Two additional patients, identified employing whole exome sequencing (WES) [3], were ages 21 months and 7 years at diagnosis. Clinical features included developmental impairment, generalized tonic-clonic seizures, choreoathetosis and myoclonus. EEG demonstrated high-voltage polymorphic delta and multifocal and generalized spike-wave.

▪ Metabolic Derangement

The cerebrospinal (CSF) and plasma concentrations of GABA, GABA conjugates, and β-alanine were increased in the Flemish sibship [1]. CSF free GABA was elevated for all patients for whom diagnostic lumbar puncture was performed (0.25–4.8 μM (reference <0.12) in three patients). Diagnosis in the Japanese patient was suggested via proton magnetic resonance spectroscopy with spectral editing for GABA in the basal ganglia at 8 months of age (patient GABA, 2.9 mmol/L; control, 0.8 mmol/L). GABA-transaminase activity was deficient in white cells derived from two patients (1.2–2 nmol/hr/mg protein (reference 20–64), with intermediate enzyme activities indicative of autosomal-recessive inheritance [4]. Growth hormone was elevated in two patients (7.9–38.4 ng/ml; reference <5), consistent with the growth hormone releasing effect of GABA. An isotope-dilution enzyme assay for GABA-transaminase suggests that GABA- and β-alanine transaminases are identical, thereby explaining the increase of β-alanine in the sibship [5].

▪ Genetics

One patient of the initial sibship was a compound heterozygote for two missense alleles (c.659G>A [p.Arg220Lys] and c.1433T>C [p.Leu478Pro]) [2][6]. For the Japanese case a single missense mutation (c.275G>A[p.Arg92Gln]) was identified, and multiplex probe ligation analysis also revealed an exon deletion [6]. In the most recently described patients [3], WES revealed compound heterozygosity for c.454C>T (p.P152S) and c.1393G>C (p.G465R) for the 21 month old and homozygous c.1129C>T (p.R377W) in the 7 year old.

▪ Diagnostic Tests

The differential diagnosis requires analysis of the relevant amino acids in CSF, primarily free and total GABA. Due to enzymatic degradation of homocarnosine, free GABA levels in the CSF show an artefactual increase unless samples are rapidly deep-frozen within a few minutes, at -20°C if analysed within a few weeks, or at -70°C if analysis is to be delayed. Control CSF free GABA levels are quite low (<175 nmol/L) and thus sensitive techniques, such as stable-isotope-dilution analysis [7], must be employed. Molecular confirmation is now the gold-standard for diagnosis (WES). Pop and colleagues [8] recently reported a high-throughput expression system for GABA-T alleles and its utility in verifying ten novel GABA-T deficiency alleles, suggesting that more patients will be identified via enhanced use of advanced generation sequencing [9].

Enzymatic confirmation remains possible in lymphocytes, lymphoblasts, and liver in specialized laboratories [8][10].

Treatment and Prognosis

No clinical or biochemical response was observed in the first three patients using pyridoxine or first-line antiepileptics (phenytoin, clonazepam, valproate, midazolam). The 7 year old patient is currently on a ketogenic diet and follow-up is in progress. In the 21 month old patient flumazenil (a$GABA_A$ receptor antagonist) infusion was undertaken and the patient tolerated 0.5 mg/kg/hr with clinical and EEG improvement, raising the potential for this agent to be further explored in GABA transaminase deficiency.

29.1.2 Succinic Semialdehyde Dehydrogenase Deficiency

Succinic semialdehyde dehydrogenase (aldhehyde dehydrogenase 5a1, ALDH5A1) deficiency (SSADHD) was first reported as γ-hydroxybutyric aciduria (4-hydroxybutyric aciduria) in 1981 [11]. It is the most prevalent of the disorders of GABA metabolism.

Clinical Presentation

SSADHD is a neurometabolic disorder with non specific clinical manifestations including developmental delay and early-onset hypotonia, later-onset expressive language impairment and obsessive–compulsive disorder, hyporeflexia, non-progressive ataxia, and epilepsy [12][13][14][15]. Imaging abnormalities may include hyperintensity of the T1-weighted signals in the globus pallidus, bilaterally, indicative of cytotoxic oedema. Abnormalities of myelination have been noted, and cerebellar atrophy in several patients has been confirmed.

Metabolic Derangement

The key feature is an accumulation of γ-hydroxybutyrate in urine, plasma, and CSF (Fig. 29.1). γ-Hydroxybutyrate and GABA (elevated three-fold in CSF) are neuropharmacologically active compounds. Additional biochemical abnormalities relate to GABA, succinic semialdehyde, and GHB. These include increased homocarnosine and guanidinobutyrate in CSF, D-2-hydroxyglutarate, succinic semialdehyde, and 4,5-dihydroxyhexanoic acid (Fig. 29.1). Many of these intermediates likely derive from succinic semialdehyde [16]. As predicted, levels of glutathione, the major intracellular antioxidant, are low in both the animal model of SSADHD and in patients [17][18].

Genetics

SSADHD is an autosomal recessive disorder caused by mutations in *ALDH5A1*. Multiple disease-associated alleles have been identified, but a mutation hotspot has not been detected [12]. Identification of consanguinity in many families indicates the presence of rare alleles in humans. The ALDH5A1 protein is a member of the aldehyde dehydrogenase protein superfamily, which has a number of highly conserve glycine residues [19]. Not surprisingly, mutations that alter these glycine residues are frequently pathogenic.

Diagnostic Tests

Diagnosis is primarily achieved by determination of γ-hydroxybutyric acid in urine, followed by molecular diagnosis via sequencing of *ALDH5A1*. Concomitant identification of 4,5-dihydroxyhexanoic acid, both the free and lactone form (*threo-*, *erythro-*) in the organic acid profile is highly suggestive. Issues with false-positives are generally not problematic, although GHB is also employed clinically (Xyrem^R (sodium salt of GHB); for cataplexy) and illicitly (for induction of euphoria). Enzyme analysis in white cells is possible, but molecular diagnosis has become the gold-standard for confirmation [12].

Treatment and Prognosis

Sudden death can occur in patients often in association with epilepsy (sudden unexplained death in epilepsy or SUDEP). One patient died with a diagnosis of SUDEP in the early third decade [20]; the authors are aware of two additional patients who died in the third and fourth decade with the probable diagnoses of SUDEP (personal communications). The non-specific clinical picture of SSADHD can lead to a diagnostic odyssey, as is evident by a recently reported patient diagnosed postmortem at age 63 [21]. Therapeutic intervention has historically employed vigabatrin (Sabril^R; gamma-vinyl GABA), an irreversible inhibitor of GABA-transaminase [12][13], which has been beneficial in some but without efficacy in others. It remains to be determined whether enhancing GABA levels in SSADHD (which are already elevated) is prudent, and the visual field disturbances associated with vigabatrin are treatment limiting. An open-label trial with taurine revealed neither clinical nor metabolic benefit [22] and a double-blind, placebo-controlled trial with the $GABA_B$ receptor antagonist SGS-742 is ongoing (www.clinicaltrials.gov; NCT02019667). The recent identification of a role for GABA in autophagy/mitophagy has raised the potential of rapalogue intervention to treat SSADHD [23][24], and studies are in progress.

29.1.3 Homocarnosinosis

Homocarnosinosis is rare and poorly defined. Two families have been identified, and it remains likely that homocarnosinosis represents the severe end of the spectrum of human carnosinase deficiency [25][26][27]. The index family was of Norwegian descent, in which the mother and three of four children manifested increased CSF homocarnosine [25]. The phenotype featured spastic paraplegia, mental deterioration and retinal pigmentation. A second case was a male of Russian descent whose phenotype included developmental delay, myasthenia, skin desquamation, hypotonia, diffuse atrophy and ataxia [26].

Metabolic Derangement

Concentration of CSF homocarnosine in the index family (mother and off spring) ranged from 50–75 μM (normal <3).

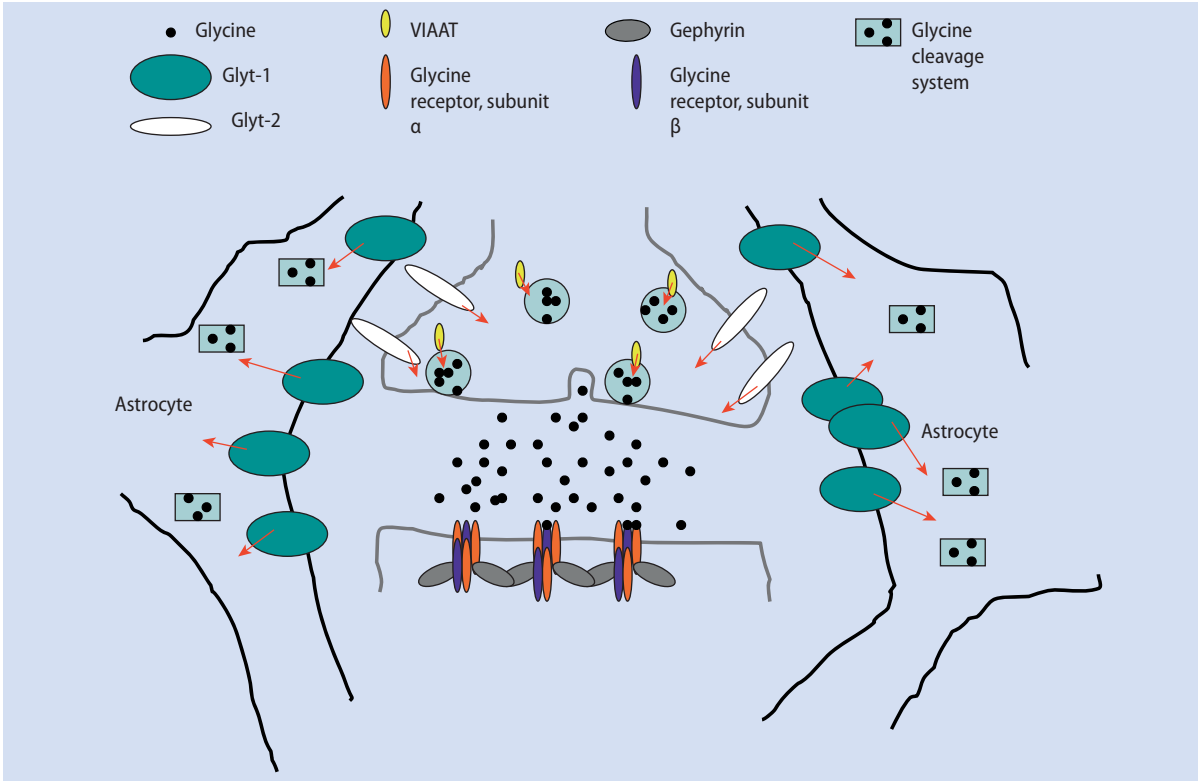

Fig. 29.2 Inhibitory glycinergic synapse. Glycine is stored in vesicles in the presynaptic neuron. Vesicle inhibitory amino acid transporter (VIAAT) transports these vesicles to the presynatic membrane, where glycine is released to the synaptic cleft. Glycine transporters (GlyT1 and 2) are members of the Na+/Cl--dependent neurotransmitter transporter superfamily. The neuronal GlyT2 is essential for glycine uptake into the presynaptic neuron and thereby provides substrate for VIAAT mediated refilling of re-endocytosed vesicles. The glial isoform GlyT1 removes released glycine from postsynaptic receptors and allows for its degradation by the glial glycine cleavage system. Glycine receptors are ligand-gated chloride channels assembled into pentameric complexes consisting of a combination of α (GLRA1) and β (GLRB) subunits. *GLRA1* mutations are the most important cause of hyperekplexia.

In the second patient, plasma homocarnosine was described as increased, but the CSF values (also reported to be »increased«) were three orders of magnitude below reported values in other publications (nM as opposed to μM). Thus, the metabolic aetiology of homocarnosinosis remains to be elucidated.

Genetics

A gene encoding a distinct homocarnosinase activity has not been reported, and current evidence suggests that homocarnosinase and carnosinase (locus 18q21.3) are identical.

Diagnostic Tests

First line investigation includes quantitation of homocarnosine in CSF, although care must be taken to avoid artifactual hydrolysis [28]. Carnosinase may be readily measured in plasma using either radiometric or amino acid quantitation methodology (hydrolysis of carnosine yields L-histidine and β-alanine, measurable by amino acid analysis).

Treatment and Prognosis

Treatment and prognosis remain to be elucidated. In the index family, dietary restriction of L-histidine led to a significant decrease in CSF homocarnosine but no improvement in clinical features [25].

29.2 Inborn Errors of Receptors and Transporters of Neurotransmitters

29.2.1 Hyperekplexia

Clinical Presentation

Three main symptoms required for the diagnosis are [29][30]: 1) Generalized stiffness immediately after birth, which normalizes during the first years of life, increases with handling and disappears during sleep; 2) excessive startle reflex to unexpected stimuli which, in older children causes frequent falls; 3) a short period of generalized stiffness (during which voluntary movements are impossible) following the startle response. Associated features such as exaggerated head retraction reflex elicited by tapping the tip of the nose, periodic limb movements during sleep, and hypnagogic myoclonus (myoclonus occurring when falling asleep) may occur. Other symptoms include inguinal, umbilical, or epigastric herniations, congen-

ital hip dislocation, and epilepsy. Sudden infant death has been reported. Psychomotor development is usually normal or mildly delayed. Brain MR spectroscopy has shown a reduction of N-acetylaspartate / creatine ratio and choline, in the frontal cortex, suggesting frontal neuronal dysfunction [31].

Metabolic Derangement

Hyperekplexia is caused by defective inhibitory glycinergic neurotransmission (Fig. 29.2). This may be due to mutations in the genes encoding the α1 subunit of the glycine receptor (GLRA1) [32], the β subunit of the glycine receptor (GLRB) [33], the gene encoding the presynaptic sodium- and chloride-dependent glycine transporter, GlyT2 (SLC6A5) [34], and the gene encoding the glycinergic clustering molecule, gephyrin (GPHN) [35]. Gephyrin is also involved in molybdenum cofactor (MoCo) synthesis and mutations in *GPHN* can also lead to one form of MoCo deficiency [36] (▶ Chapter 20). In one individual with hyperexplexia plus severe epilepsy and severe developmental delay, mutations were found in the X-linked *ARHGEF9* encoding collybistin which is also involved in glycinergic receptor clustering [37]. *GLRB* mutations are strongly associated with delays in gross motor development and speech acquisition since β subunits are expressed at a much earlier developmental stage than α1 subunits [38].

Genetics

Hyperekplexia has, in the great majority of the patients, an autosomal dominant inheritance with nearly complete penetrance and variable expression in most pedigrees. *GLRA1* gene study for point mutations and deletion of exons 1–6 will detect the mutation in approximately 80% of cases [30]. In cases without a family history suggesting dominant inheritance, screening all the genes listed above will detect mutations in approximately 20% of cases (mostly recessive) [30].

Diagnostic Tests

Clinical diagnosis is based on the unique neurological features and the response to medication: clonazepam reduces the frequency and magnitude of startle responses and diminishes the frequency of falls. Confirmation of the clinical diagnosis requires DNA sequencing, particularly of *GLRA1*. In cases of negative results for all the known genes of hyperkeplexia, the analysis of other proteins involved in the development or function of glycinergic synapses could reveal further susceptibility genes.

Treatment and Prognosis

The stiffness decreases during the first years of life, but the excessive startle responses remain. Clonazepam significantly reduces the startle responses but has less effect on the stiffness. The mechanism of the beneficial effect of clonazepam is not known but it binds to the benzodiazepine site of the GABA(A) receptor [39].

29.2.2 GABA Receptor Mutations

Multiple mutations in GABA(A) receptor subunits have been reported in different causes of rare epilepsies: childhood absence epilepsy (CAE), autosomal dominant epilepsy with febrile seizures plus (ADEFS+), autosomal dominant juvenile myoclonic epilepsy [40][41][42], and Dravet syndrome, a severe form of infantile myoclonic epilepsy (GABRG2 and GABRA1) [43]. Recently, mutations in *GABRA1*, *GABRB2*, and *GABRB3* have been associated with infantile spasms and Lennox-Gastaut syndrome [44]. A patient with *GABRD* mutations has been reported to have Rett-like features [45].

Mutations in the GABA receptor alter fast inhibitory neurotransmission facilitated by GABA and altering chloride homeostasis. Mutations associated with epilepsy have been located at genes encoding α1, α6, β2, β3, γ2, or δ subunits (*GABRA1, GABRA6, GABRB2, GABRB3, GABRG2,* and *GABRD*, respectively) [40][42][44]. Interestingly, other than impaired neurotransmission, mutant GABA(A) receptor γ2(Q390X) subunits, related to Dravet syndrome, accumulate and aggregate intracellularly, activate caspase 3 and causes age-dependent neurodegeneration [46].

The diagnosis is based on molecular genetic analysis of different GABAA receptor subunits. Treatment is generally tailored to the symptoms of the particular epilepsy, but may also address febrile episodes in patients with ADEFS+. The prognosis also is dependent upon the epileptic syndrome involved.

29.2.3 Glutamate Receptor Mutations

Alterations in glutamatergic neurotransmission have long been associated with neurodevelopmental and psychiatric disorders. Although there are no strict relationships between receptor subunit or type and disease, ionotropic glutamate receptors (iG-luRs: NMDA, AMPA and kainaite receptors) which are central to synaptic transmission and plasticity, are mostly associated with intellectual disability, epileptic encephalopathies and autism spectrum disorders. In contrast, mutations of metabotropic GluRs, having a role on modulating neural transmission, are preferentially associated with psychiatric disorders [47].

29.2.4 Mitochondrial Glutamate Transporter Defect

This disorder, first described in 2005 [48], is characterized by severe, neonatal onset epileptic spasms and focal seizures with a burst-suppression EEG pattern, microcephaly, hypotonia, an abnormal electroretinogram, and severe psychomotor delay. MRI imaging in childhood shows cerebellar hypoplasia, an abnormal corpus callosum, abnormal gyration of temporo-parietal regions and abnormal myelination of temporal poles. Other than early myoclonic epilepsy, mutations in this transporter have been related to migrating partial seizures in

infancy [49]. This is a recessive disorder caused by missense mutations in *SLC25A22* which encodes a mitochondrial glutamate transporter specifically expressed in the brain during development. The defect impairs oxidation of glutamate. Diagnosis is based on measurement of defective glutamate oxidation in cultured skin fibroblasts and *SLC25A22* mutation analysis [48][50]. Recently, biochemical abnormalities such as hyperprolinaemia and lipid vacuolated fibroblasts have been reported in three patients, indicating impairment of the proline/pyrroline-5-carboxylate (P5C) shuttle [51]. There is no specific treatment.

29.2.5 Dopamine Transporter Defect

Dopamine transporter (DAT) deficiency syndrome due to *SLC6A3* mutations is a recessive disorder presenting as early infantile progressive parkinsonism dystonia [52]. Atypical presentations appear later in childhood including juvenile onset with a milder disease course [53]. Overall, the clinical picture shows progressive parkinsonism-dystonia that is medically refractory. CSF concentrations of HVA (homovanillic acid) are high. *SLC6A3* mutations reduce levels of DAT and the binding affinity of dopamine. Genotype-phenotype analysis suggests that higher residual DAT activity is likely to contribute to postponing disease presentation in later-onset adult cases. Recently, *SLC6A3* missense mutations have been linked to adult parkinsonism and ADHD (attention deficit hyperactivity disorder) [54].

29.2.6 Brain Dopamine-Serotonin Vesicular Transport Defect

Mutations in *SLC18A2*, which encodes vesicular monoamine transporter 2 (VMAT2), have been described to produce a severe infantile parkinsonism with autonomic instability and developmental delay. VMAT2 transports dopamine and serotonin into synaptic vesicles. CSF neurotransmitter metabolites were normal. Treatment with levodopa was associated with worsening, whereas dopamine agonists notably improved the symptoms in patients [55].

29.3 Inborn Errors of Monoamine Metabolism

Monoamines

The monoamines, adrenaline, noradrenaline, dopamine, and serotonin, are metabolites of the amino acids tyrosine and tryptophan. The first step in their formation is catalysed by amino-acid-specific hydroxylases, which require tetrahydrobiopterin (BH_4) as a cofactor. BH_4 is also a cofactor of phenylalanine hydroxylase (▶ Chapter 16). Its synthesis from GTP is initiated by the rate-limiting GTP cyclohydrolase-1 (GTPCH-I), which forms dihydroneopterin triphosphate (NH2TP). L-dopa and 5-hydroxytryptophan (5-HTP) are metabolized by a common B6-dependent aromatic L-amino acid decarboxylase (AADC) into dopamine (the precursor of the catecholamines, adrenaline and noradrenaline) and serotonin (5-hydroxytryptamine), respectively. Adrenaline and noradrenaline are catabolized into vanillylmandelic acid (VMA) and 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG) via monoamino oxidase A (MAO-A). This enzyme is also involved in the catabolism of both dopamine into homovanillic acid (HVA) via 3-methoxytyramine, and of serotonin into 5-hydroxyindoleacetic acid (5-HIAA). Dopaminergic modulation of ion fluxes regulates emotion, activity, behaviour, nerve conduction, and the release of a number of hormones via G-protein-coupled cell-surface dopamine receptors. Serotoninergic neurotransmission modulates body temperature, blood pressure, endocrine secretion, appetite, sexual behaviour, movement, emesis, and pain.

29.3.1 Tyrosine Hydroxylase Deficiency

Clinical Presentation

Around sixty patients with tyrosine hydroxylase (TH) deficiency (THD) have been reported worldwide [56][57]. Clinically, it causes a neurological disease with predominant extrapyramidal signs and a variable response to L-dopa. Although different phenotypes have been described in the largest series of cases reported, patients were classified into two main forms [57]: Type A – hypokinetic-rigid syndrome plus dystonia, with onset in infancy or childhood and Type B – complex encephalopathy with neonatal or early infancy onset (hypokinetic-rigid syndrome plus developmental delay, a variety of movement disorders and sometimes epilepsy). Non-progressive mental retardation, tremor, chorea, oculogyric crises, ptosis, fluctuation of signs, autonomic dysfunction and poor response to L-dopa, can be present in both groups but are more likely in type B [57]. Motor and cognitive prognosis is worse in type B, however, it is likely that there is a phenotypic continuum from the milder type A THD to more severe type B THD. Atypical clinical cases of THD have also been reported, including those presenting with early-onset spastic paraplegia and dopa responsive myoclonus dystonia [58].

Metabolic Derangement

TH converts tyrosine into L-dopa, the direct precursor of catecholamine biosynthesis (◘ Fig. 29.3). This enzymatic step is rate-limiting in the biosynthesis of the catecholamines. The enzyme is expressed in the brain and in the adrenals but also in non-neuronal tissues, such as kidney, intestine and lymphoid nodes [57]. BH_4 is the co-factor. The biochemical hallmarks of the disease are low CSF levels of HVA and MHPG, the catabolites of dopamine and norepinephrine, respectively, with normal 5-HIAA levels [62][64]. Pterin values are also normal.

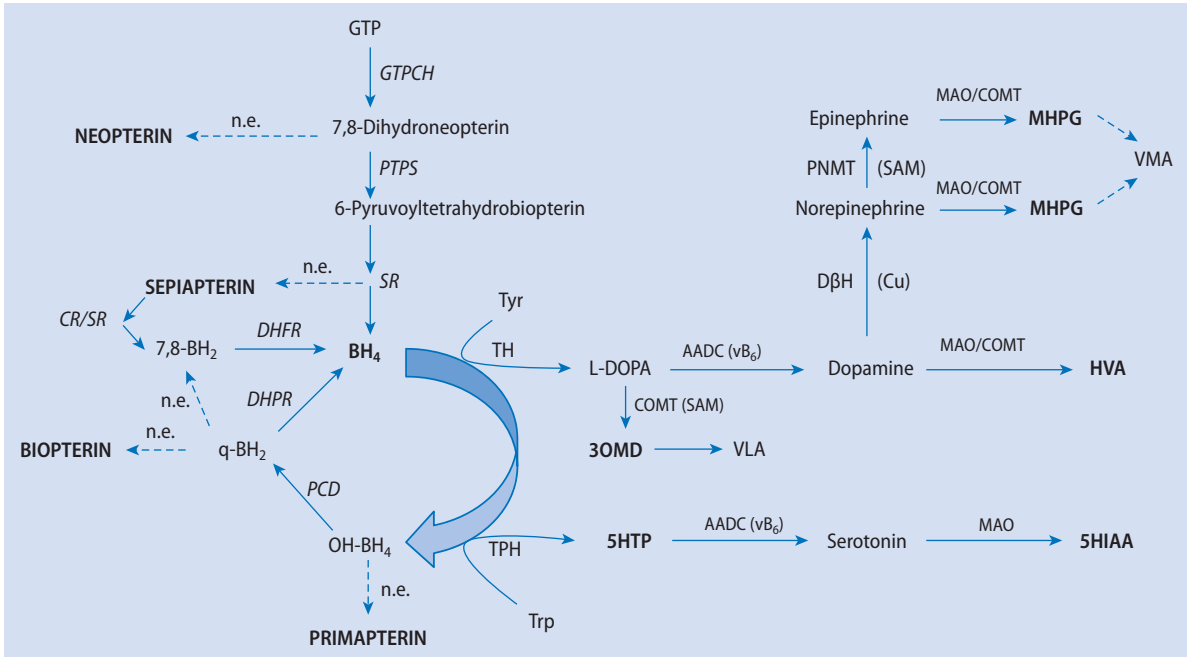

Fig. 29.3 Metabolism of adrenaline, noradrenaline, dopamine, and serotonin. The key metabolites for neurotransmitters and proteins are marked in bold letters. Their simultaneous quantification in CSF is very useful in order to make the correct diagnosis. Abbreviations: *AADC*, aromatic L-amino acid decarboxylase; *7,8-BH2*, 7,8-dihydrobiopterin; *BH4*, tetrahydrobiopterin; *COMT*, catechol O-methyltransferase; *CR*, carbonyl reductase; *DHFR*, dihydrofolate reductase; *DHPR*, dihydropteridine reductase; *DβH*, dopamine betahydroxylase; *GTP*, guanosine triphosphate; *GTPCH*, GTP cyclohydrolase I; *5HTP*, 5-hydroxytryptophan; *5HIAA*, 5-hydroxyindoleacetic acid; *HVA*, homovanillic acid; *L-DOPA*, 3,4-dihydroxyphenylalanine; *MAO*, monoamine oxidase; *MHPG*, 3-methoxy-4-hydroxyphenylglycol; *n.e.:* non-enzymatic; *3OMD*, 3-O-methyl-dopa; *OH-BH4*, hydroxy-tetrahydrobiopterin; *PCD*, pterin-4a-carbinolamine dehydratase; *PNMT*, phenylethanolamine N-methyltransferase; *PTPS*, 6-pyruvoly-tetrahydropterin synthase; *q-BH2*, quinoide-dihydrobiopterin; *SAM*, S-adenosylmethionine; *SR*, sepiapterin reductase; *TPH,* tryprophan-5-hydroxylase; *TH*, tyrosine 3-hydroxylase; *VLA*, vanillactic acid; *VMA,* vanillmandelic acid; *vB6*, vitamin B*6*

Genetics

TH deficiency is inherited as an autosomal recessive trait. Several mutations including promotor region and deletions have been described in *TH* [57]. Genotype–phenotype analysis reveals that patients with promoter mutations had type A TH deficiency [57].

Diagnostic Tests

The most important diagnostic test is the measurement of HVA, MHPG, and 5-HIAA in the CSF. The HVA/5HIAA ratio in CSF is the most sensitive marker not only for diagnosis but also to identify the severity of the phenotype [57]. Urinary measurements of HVA and 5-HIAA are not reliable in the diagnosis. Direct enzyme measurement is not a diagnostic option, as there is no enzyme activity detectable in body fluids, blood cells and fibroblasts. Hyperprolactinemia is observed in around half of cases.

Treatment and Prognosis

In most cases, TH deficiency can be treated with L-dopa in combination with a L-dopa decarboxylase inhibitor. However, the response is variable, ranging from complete remission (more likely in type A) to mild improvement. Therapy should be started with low doses to prevent dyskinesias. The initiating dose should be <0.5 mg/kg per day, with slow titration (over weeks to months) to 3–10 mg/kg per day, according to response. Dyskinesias may be triggered by L-dopa increment, intercurrent febrile illness, tiredness, and overexcitement. It may be successfully treated with amantadine [59].

29.3.2 Aromatic L-Aminoacid Decarboxylase Deficiency

Clinical Presentation

Aromatic L-aminoacid decarboxylase (AADC) deficiency has been reported in almost 100 patients worldwide [60][61][62][63][64]. Although neonatal symptoms such as poor sucking, feeding difficulties, lethargy, increased startle response, hypothermia and ptosis may be present, clinical presentation of AADC deficiency usually ranges from 4 months to adulthood (although the majority of patients present in childhood) [60]. Patients may present with a severe and progressive epileptic encephalopathy, although epilepsy is rarely a single symptom [63][64]. The most frequent signs are truncal hypotonia associated with limb rigidity, oculoryric crises and developmental delay. Dystonia, ptosis, and autonomic dysfunction (temperature instability with hypothermia, gastro intestinal

symptoms, paroxysmal sweating, impaired heart rate and blood pressure regulation) will also develop. A milder AADC phenotype consisting of fatigability, hypersomnolence, dystonia has been reported [60].

Metabolic Derangement

AADC is implicated in the biosynthesis of catecholamines and of serotonin (Fig. 29.3). The activity of the enzyme requires pyridoxal phosphate as a cofactor. A deficiency of the enzyme results in a deficiency of the catecholamines and of serotonin. The concentrations of the catabolites (HVA from dopamine, 5-HIAA from serotonin, and MHPG in the central nervous system from norepinephrine) are severely reduced in the CSF. Another biochemical hallmark of the disease is the increased concentration of metabolites upstream of the metabolic block: L-dopa, 3-O-methyl-l-dopa, vanillyllactic acid (VLA), and 5-HTP. In several patients a paradoxical hyperdopaminuria has been noted probably due to production of dopamine and metabolites in non neural cells.

Genetics

AADC deficiency is inherited as an autosomal recessive trait and it is caused by mutations in *DDC*. Several *DDC* mutations have been reported with a common founder mutation identified in Taiwanese Chinese patients (IVS6+4A>T) [60].

Diagnostic Tests

Typical CSF profile consists of markedly decreased HVA and, 5-HIAA values, with raised 3-O-methyl-l-dopa and 5-HTP, in the presence of a normal pterin concentrations [60]. Increased VLA excretion may be observed in the urinary organic acid profile. AADC deficiency can be confirmed at the enzyme level, as all patients have also shown a consistent deficiency of the enzyme activity in plasma.

Treatment and Prognosis

Treatment in AADC deficiency may be beneficial but the effects are limited and long-term prognosis is poor. Various strategies have been used including cofactor supplementation in the form of vitamin B_6, pyridoxal phosphate, MAO inhibitors (such as tranylcypromine, selegeline, phenelzine), dopamine agonists (pergolide, bromocriptine), high dose L-dopa as »substrate therapy«, serotoninergic agents (fluoxetine) or combinations of these and anticholinergic drugs (trihexylphenidyl). Only some patients with relatively mild forms clearly improved on a combined therapy with pyridoxine (B_6)/pyridoxal phosphate, dopamine agonists, and monoamine oxidase B inhibitors [62]. Transdermal rotigotine (a dopamine agonist), may benefit some patients [60]. Gene therapy is being tested with promising results [60].

29.3.3 Dopamine β-Hydroxylase Deficiency

Clinical Presentation

Congenital dopamine β-hydroxylase (DBH) deficiency is characterized by normal parasympathetic and sympathetic cholinergic function but with a lack of sympathetic noradrenergic function [65]. Its clinical hallmark is severe orthostatic hypotension. Most patients complain of fatigue and impaired exercise tolerance. Although DBH deficiency appears to be present from birth, symptoms become manifest in early childhood but may worsen in late adolescence. Perinatal hypoglycaemia, hypothermia and hypotension may occur. There is no obvious intellectual impairment. Additional symptoms in some patients are ptosis, nasal stuffiness, weak facial musculature, hyperflexible joints, brachydactyly, high palate and sluggish deep tendon reflexes. A mild normocytic anaemia has been found [66]. Differential diagnosis includes pure autonomic failure/autonomic neuropathy, familial dysautonomia, and Shy-Drager syndrome or central autonomic failure.

Metabolic Derangement

DBH converts dopamine into noradrenaline. It is present in the synapses of postganglionic sympathetic neurons. A defect in the enzyme should have consequences for (nor-) adrenergic neurons and as well for the adrenals.

Genetics

Pathogenic mutations have been found in DBH in all known patients with symptomatic DBH deficiency, and it is inherited in an autosomal recessive trait.

Diagnostic Tests

Tests of autonomic function may provide diagnostic information of great specificity [66]. The patients typically have extremely low plasma noradrenaline and adrenaline levels and increased or high-normal levels of dopamine. At the enzyme level the diagnosis can easily be confirmed by the deficiency of DBH activity in plasma. Interestingly, 4% of the population have nearly undetectable DBH activity in plasma with normal concentrations of noradrenaline and adrenaline and without clinical features of DBH deficiency. This is caused by a common allelic variant (1021 C>T) [67].

Treatment and Prognosis

Therapy with L-dihydroxyphenylserine (L-Dops) is available. This compound can be directly converted by AADC into noradrenaline, thereby by-passing the defective enzyme. Administration of 100–500 mg L-Dops orally twice or three times daily increases blood pressure and restores plasma norepinephrine levels, however plasma epinephrine concentration still remains below a detectable level [65]. The prognosis on therapy is satisfactory to good.

29.3.4 Monoamine Oxidase-A Deficiency

Clinical Presentation

Monoamine oxidase-A (MAO-A) deficiency has been identified in five generations of one Dutch family [68]. Only males were affected. They showed borderline mental retardation with behavioural disturbances, aggressive and violent behav-

iour, arson, attempted rape, and exhibitionism. Additionally, a functional polymorphism of the MAO-A gene promoter region may act as a genetic modifier of the severity of autism in males [69]. Furthermore, other MAO-A polymorphisms have been related to abnormal limbic circuitry for emotion regulation and cognitive control, explaining impulsive aggression and serious delinquency [70]. MAO exists as two X-linked isoenzymes (A and B). Patients with a contiguous gene syndrome affecting both the MAO-A and -B genes, and also the gene responsible for Norrie disease, have been described [71]. They are severely mentally retarded and blind. Patients with only the MAO-B and the Norrie genes affected were also found. These patients are not mentally retarded and do not have abnormalities of catecholamine metabolites in urine. Here, we address only the isolated deficiency of MAO-A.

Metabolic Derangement

MAO-A deficiency is a defect in the catabolism of both serotonin and the catecholamines. In patients with MAO-A deficiency, marked elevations were noted of the MAO substrates serotonin, normetanephrine, 3-methoxytyramine, and tyramine in urine. The concentrations of the metabolites downstream of the metabolic block, VMA, HVA, 5-HIAA, and MHPG, were markedly reduced.

Genetics

The locus for this X-linked inherited disease has been assigned to Xp11.21. A point mutation in the eighth exon of *MAO-A*, causing a premature truncation of the protein, has been found in this family [68].

Diagnostic Tests

Elevated urinary serotonin, normetanephrine, metanephrine, and 3-methoxytyramine is the characteristically pattern in random urine samples of the patients. The ratios in urine of normetanephrine to VMA, normetanephrine to MHPG or HVA/VMA are altered in patients with the defect [72]. The discovery of this disorder suggests that it might be worthwhile performing systematic urinary monoamine analysis when investigating unexplained, significant, behaviour disturbances, particularly when these occur in several male family members. In CSF, nearly absent HVA and 5-HIAA are observed, with no accumulation of 3-OMD and 5-HTP (differential diagnosis with AADC deficiency) and normal pterin profile.

Treatment and Prognosis

No effective treatment is known at present. Both the borderline mental retardation and the behavioural abnormalities seem to be stable with time.

29.3.5 Guanosine Triphosphate Cyclohydrolase-I Deficiency

Clinical Presentation

This is the most common dopamine-responsive dystonia form [73]. A large literature review reported 352 patients with genetically proven autosomal dominant GTPCH-I deficiency [74]. Autosomal dominant GTPCH-I deficiency was identified as the cause for dopa-responsive dystonia (DRD) [75]. Patients with this deficiency develop symptoms during the first decade of life. The onset age in childhood is around 6 years. However, there are patients who have an onset as early as the first week of life or in adulthood, or at ages older than fifty years [76]. Dystonia in the lower limbs is generally considered to be the initial and most prominent symptom. Unless treated with L-dopa, the dystonia becomes generalized. Diurnal fluctuation of the symptoms with improvement after sleep is a feature in most patients. Other clinical features of GTPCH-I deficiency include parkinsonism that manifests as rigidity, bradykinesia postural tremor, oculogyric crisis, waddling gait, generalized hypotonia, proximal weakness, paroxysmal exercise-induced dystonia, sleep disturbances and impaired intelligence [76]. The disease is classified into two types, the postural dystonia type and the action dystonia type, with association of vigorous dystonic movements. Dystonia might also have a relapsing and remitting course, and may be associated with oculogyric crises, depression and migraine. Adult onset patients can start with hand tremor and gait disturbance due to generalized rigidity.

Metabolic Derangement

GTPCH-I is the initial and rate-limiting step in the biosynthesis of BH_4, the essential cofactor of various aromatic amino acid hydroxylases (Fig. 29.3) with the highest affinity for TH. The deficiency is characterized by defective biosynthesis of serotonin and catecholamines.

Genetics

GTPCH-I deficiency can be inherited as an autosomal-dominant trait with 30% penetrance. The incidence of autosomal dominant GTPCH-I is generally reported to be 2.5–4.0 fold greater among females than among males [76]. A dominant negative mechanism has been proposed and it would mean that a single mutation in GCH1 decreases GTPCH-I activity by over 50% [76]. This effect might also account for the phenotypic heterogeneity of DRD, as the degree of enzyme inactivation depends on the specific genetic abnormality. Point mutations and large rearrangements have been reported [76].

Diagnostic Tests

Patients with dominant GTPCH-1 deficiency have normal Phe levels in body fluids. The following tests may be helpful in reaching the correct diagnosis: 1) Measurement of pterines especially in CSF (biopterin and neopterin; both are decreased, from 20 to 50% of normal levels, and are the biochemical hallmarks of the disease). 2) Measurement of CSF HVA and 5-HIAA. A normal or slightly low CSF HVA in combination with a low 5-HIAA is observed, with no accumulation of biogenic amine precursors (3-OMD and 5-HTP). 3) An oral Phe-loading test. In general, it reveals a 2 to 6 hour increase in Phe levels and Phe/Tyr ratio. 4) Mutation analysis. 5) Measurement of the enzyme activity in fibroblasts.

Treatment and Prognosis

Patients have been treated with a combination of low dose L-dopa (4 to 5 mg/kg/day) and a dopa-decarboxylase inhibitor. There is normally a complete or near-complete response of motor problems soon after the start of the therapy. Even when the therapy is started after a diagnostic delay of several years, the results are satisfactory. However, in cases of action dystonia and adult onset cases, levodopa does not always show complete effects [76].

29.3.6 Sepiapterine Reductase Deficiency

Clinical Presentation

Sepiapterin reductase deficiency (SRD) is a disease implicated in the final step of BH_4 synthesis. Friedman et al. [77] reported the largest SRD series to date highlighting the significant delay in diagnosis for many patients, with frequent misdiagnosis of cerebral palsy. Frequent clinical features of SRD are axial hypotonia, motor and language delay, oculogyric crisis, weakness, dystonia with diurnal fluctuation of symptoms, parkinsonian features, sleep disturbance, behavioral, and psychiatric abnormalities [77]. Tremor of the limbs and head at rest, inhibited by skin contact and spontaneous movement as presenting symptoms of the disease during the first months of life have been reported [78].

Metabolic Derangement

Central nervous system BH_4 depletion contributes to the deficient dopamine and serotonine biosynthesis [77]. Conversely, BH_4 availability is normal in peripheral tissues due to alternative metabolic pathways that overpass the SR deficiency. Thus, phenylalanine values are normal in the newborn screening programs.

Genetics

SRD is inherited as an autosomal recessive trait. Different mutations in *SPR* have been reported [77].

Diagnostic tests

CSF study shows high levels of biopterin and sepiapterin (the hallmark of the disease) with normal levels of neopterin, and very low concentrations of HVA and 5-HIAA. Urine pterins and plasma phenylalanine values are normal. The phenylalanine loading test is frequently positive. Enzyme activity in fibroblasts is reduced. Recently, accumulation of sepiapterin in urine of patients has been found as a potential biomarker [79].

Treatment and Prognosis

For SRD, therapeutic approaches involve dopamine and serotonin precursor supplementation, and most patients respond well to L-dopa and 5-hydroxytryptophan combination [77]. As regards dopaminergic disturbances, improvement in motor and sleep symptoms have been reported with the combination of L-dopa and carbidopa. Since dyskinesias may appear after treatment, a very low starting dosage of L-dopa (around 0.5 mg/kg per day) with slow increment is advised. Regarding 5-HTP treatment (a precursor of serotonin), improvement in sleep, motor and cognitive aspects have been reported (doses ranging from 1 to 6 mg/kg per day).

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Trimethylaminuria, Dimethylglycine Dehydrogenase Deficiency and Disorders in the Metabolism of Glutathione

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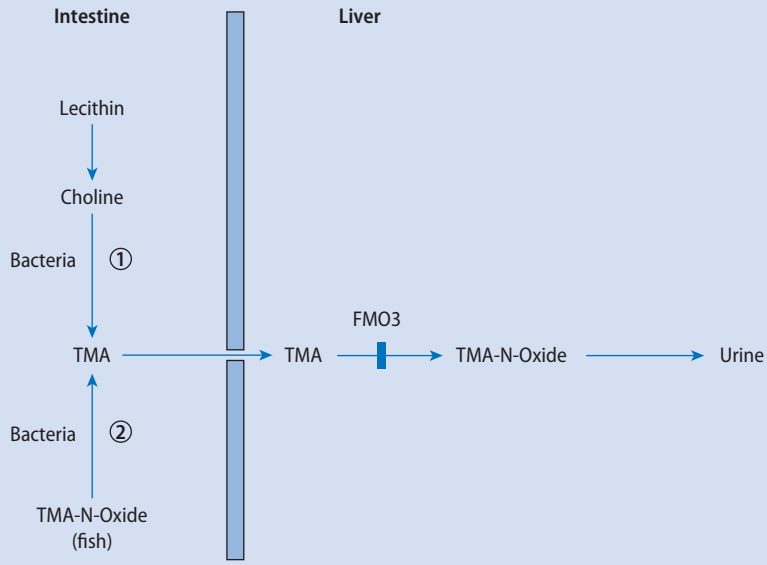
Trimethylamine Metabolism and Choline Catabolism

Trimethylamine Metabolism

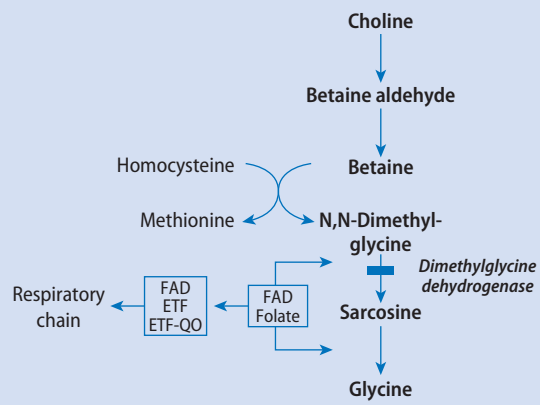
Trimethylamine (TMA) is a volatile tertiary amine which smells of rotting fish. It is a bacterial metabolite which is produced by anaerobes resident in the human colon from choline (present in lecithin), carnitine and betaine, and from trimethylamine-N-oxide (TMAO) in salt water fish and shellfish. TMA is absorbed from the intestine and oxidised in the liver by flavin-containing monooxygenase 3 (FMO3), a microsomal NADPH-dependent enzyme. The product is TMA-*N*-oxide (TMAO) which is non-odorous and is excreted in urine (◘ Fig. 30.1). Normally, more than 90% of the absorbed TMA is oxidised and the urinary output of unmetabolised TMA in adults on a Western diet is only around 17–35 μmol/24 h [1]. FMO3 is not produced by the fetus after 15 weeks of gestation or, usually, in the first month of life. Levels increase to around 8% of adult values by nine months of age and 20% by 11 years [2]. FMO3 activity is inhibited by indoles from brassicas [3] and is reduced perimenstrually [1].

Catabolism of Choline

This process (◘ Fig. 30.2) occurs within the mitochondria and involves the sequential removal of two methyl groups by dimethylglycine dehydrogenase (DMGDH) and sarcosine dehydrogenase (SDH). These are related flavin enzymes with covalently linked FAD which use folate as co-factor. The methyl groups from dimethylglycine and sarcosine are transferred to tetrahydrofolate (THF), forming 5,10-methylene THF. The electrons are transferred from FAD to electron transfer flavoprotein (ETF) and thence to the mitochondrial respiratory chain.



◘ **Fig. 30.1 Metabolism of trimethylamine**. *FMO3*, flavin-containing monooxygenase 3; *TMA*, trimethylamine; ①, bacterial choline TMA-lyase; ②, bacterial TMA-N-oxide reductase. The enzyme defect in trimethylaminuria is indicated by a **solid bar**



◘ **Fig. 30.2 Catabolism of choline**. *ETF*, electron transfer flavoprotein; *ETF-QO*, ETF-ubiquinone oxidoreductase; *FAD*, flavin adenine dinucleotide. The enzyme defect in dimethylglycine dehydrogenase deficiency is indicated by a **solid bar**

30.1 Trimethylaminuria (Fish Malodour Syndrome)

30.1.1 Clinical Presentation

TMA is excreted in the breath, sweat, urine and vaginal secretions. Individuals with trimethylaminuria (TMAU) excrete excessive amounts of TMA and when this happens have an unpleasant, pervasive body odour of decaying fish. Although readily detected by others, subjects may be unaware of their own odour. There are no physical symptoms. However, the disorder may seriously impact on the life of the individual, causing depression, obsessional behavior, social isolation and problems in forming relationships. The presentation varies according to the severity of FMO3 deficiency, and the amount of TMA presented to the enzyme for oxidation (reviewed [1][3][4][5]).

Severe TMAU This is usually due to a null mutation of the *FMO3* gene. The onset is generally in infancy or early childhood, but sometimes later. Presentation is with a severe persisting malodour, which is exacerbated by sweating and in some women increases just before, and during, menstruation.

Mild/intermittent TMAU Some individuals have first presented with TMAU during adult life. Increasingly, genetic testing has demonstrated that this is associated with polymorphisms of the *FMO3* gene which cause mild reduction in enzyme activity. The malodour may be precipitated by an excessive influx of TMA from the intestine due to abnormal bacterial colonisation of the bowel, or to high intakes of choline, carnitine or betaine taken as ›health supplements‹ or prescribed for Huntington's chorea, Alzheimers disease or inherited metabolic disorders.

▪ Transient Childhood Presentations

Neonatal Preterm neonates have developed TMAU when fed on choline-supplemented milk formulae. The problem resolved with withdrawal of the supplement and did not recur. The abnormality can be explained by the normal neonatal FMO3 deficiency coupled with a heavy TMA substrate load.

Childhood Rarely, TMAU occurs transiently in infants and young children, sometimes as intermittent episodes. Such children have mild variants of the *FMO3* gene which reduced FMO3 activity [6][7]. The minor genetic abnormalities coupled with low enzyme expression in childhood would explain the findings. In view of the genetic defect, however, there may be a life-long risk for recurrence if TMA production becomes excessive.

Secondary to medical disorders TMAU may occur in viral hepatitis, severe chronic liver disease, advanced renal failure, and with anatomical abnormalities of the intestine such as blind loops with abnormal bacterial colonisation. Bacterial vaginosis, cervical carcinoma and urinary tract infections may increase urine TMA.

30.1.2 Metabolic Derangement

TMAU results when the oxidative capacity of FMO3 is overwhelmed by TMA substrate. This is most often caused by a deficiency of FMO3 attributable to mutations of the *FMO3* gene or to reduced enzyme expression. With the mildest deficiencies, TMAU may only be manifest when there is an excessive load of TMA from the intestine, as a result of abnormal gut bacterial metabolism or ingestion of large amounts of choline or TMAO in fish.

A high influx of TMA from the bowel, when hepatic activity of FMO3 is normal, increases TMAO production. Urinary excretion of both TMA and TMAO is increased, but the ratio of free TMA to total TMA remains at <10%. Although TMAO production is a beneficial detoxification process, TMAO may be proatherogenic and increase cardiovascular risk [8]. Further, recent studies indicate that FMO3 may modulate glucose and lipid homeostasis in addition to its activity in TMA metabolism [9].

As well as TMA, substrates of FMO3 include tyramine and phenylethylamine, (*S*)-nicotine, and many medications including cysteamine, methimazole, ranitidine, cimetidine, benzydamine and tricyclic antidepressants [3][5][10][11]. The relative importance of FMO3 to the cytochrome P_{450} enzymes and monoamine oxidase inhibitors in drug and tyramine metabolism, respectively, is unknown. Drug toxicity has not been demonstrated unequivocally yet in FMO3 deficiency. However, severe FMO3 deficiency is associated with reduced metabolism of benzydamine in vivo [11], and genetic polymorphisms of *FMO3* may contribute to interethnic variation of metabolism of some drugs. There is no strong evidence to date that inherited FMO3 deficiency increases the risk for hypertension [3].

30.1.3 Genetics

TMAU is an autosomal recessive disorder with an estimated carrier frequency in the United Kingdom of around 1% [1]. There are five *FMO* genes (*FMO1-5*) and six pseudogenes located in two clusters on chromosome 1 (q23-q25) [5]. FMO3 is the main isoform of the enzyme in adult liver and the only isoform which oxidises TMA. The *FMO3* gene is highly polymorphic. More than 300 variants have been reported and more than 40 mutations are associated with TMAU [5]. Most of these are missense mutations or minor deletions causing a frame-shift. With very rare exceptions, heterozygotes for a mutant and a normal allele do not develop TMAU, although TMA loading reveals a decreased enzyme capacity [1]. The commonest severe mutations are P153L and E305X. Some polymorphisms which have no, or little, effect on FMO3 activity when present alone in an otherwise normal allele, significantly reduce enzyme activity *in vitro* when two of them are present in *cis* on a single allele. Enzyme activity is reduced severely by V187A in *cis* with E158K and moderately by E158K in *cis* with E308G [4]. Homozygosity for the E158K/E308G allele, or compound heterozygosity for this variant and

an allele with a severe mutation, were reported in individuals with mild TMAU [6]. As many as 2–5% of the general population in Germany may be homozygous for the E158K/E308G allele [6][7].

30.1.4 Diagnostic Tests

Common causes of abnormal body odour and urine infection are excluded. A random urine sample is collected if the odour is obvious, if not menstruating. Otherwise, urine is collected after a high choline meal (for example two eggs and baked beans) or a 300 g marine fish meal, either as a timed 6–8 hour collection, or as a random sample after 2–12 h [5]. Alternatively, urine is collected for 24 h after a standard oral choline or TMA load [1]. Samples should be collected on two separate occasions for confirmation and to detect episodic TMAU. The urine is acidified quickly to pH 2.0 and frozen until analysis by NMR spectroscopy or mass spectrometric procedures to obtain values for TMA, TMAO and total TMA [5]. A TMA to total TMAO ratio of 10–39% is consistent with mild, and one of 40% and over with severe, TMAU. A ratio of <10% with normal TMA excretion is found in normal and heterozygous individuals and some patients with intermittent TMAU [3][4]. A ratio of <10% with increased TMA excretion indicates excessive intestinal TMA production. If this is not due to ingestion of choline-containing supplements, the test should be repeated after a one-week course of metronidazole or neomycin to alter the gut microflora. An oral TMA-loading test may be useful when investigating the functional significance of new mutations [6]. Gene sequencing is recommended when the TMA/total TMA ratio is > 10% on a normal diet [4], but may not be necessary for diagnosis of severe, persistent TMAU, and might be most useful in those with mild or intermittent symptoms [5].

30.1.5 Treatment

Explanation of the problem provides insight. Non-prescribed lecithin-containing and carnitine health supplements must be discontinued. Dietary restrictions may reduce the odour. Foods to avoid include eggs, mayonnaise, liver, kidney, peas, beans, peanuts, soya products, seafish, shellfish, squid, octopus and cuttlefish, and also brassicas (brussel sprouts, broccoli, cabbage, cauliflower). Choline intake should be adequate for health and must not be restricted during pregnancy and lactation because of the high requirements of the fetus and infant [3][4]. Folate requirement is increased, necessitating a good dietary intake. Riboflavin supplements may enhance residual FMO3 activity in those with mild *FMO3* defects. Appropriate clothing and room ventilation minimise sweating, and the use of acid soap or body lotions (pH 5.5–6.5) traps TMA in its protonated, less volatile form. Short courses of copper chlorophyllin or activated charcoal to sequester TMA in the gut may help [4]. Long-term use of antibiotics to reduce enteric TMA production should be avoided, but a 2-week course of metronidazole or short courses of lactulose may provide some protection for special occasions and holidays [3][4][5].

30.2 Dimethylglycine Dehydrogenase Deficiency

30.2.1 Clinical Presentation

An adult patient with this defect was investigated for an abnormal body odour resembling fish, which was present from 5 years of age, was increased by stress and effort and caused him major social, psychological and professional problems [12]. The patient had chronic muscle fatigue with persistent elevation of creatine kinase to around 4 times normal. Intelligence was normal. Since this first description no further patients have been reported making some authors doubt about the clinical significance of defects in this gene [13].

30.2.2 Metabolic Derangement

Dimethylglycine dehydrogenase (DMGDH) is involved in choline and in 1-carbon metabolism (Fig. 30.2). It catalyzes the oxidation of N,N-dimethylglycine and the formation of 5,10-methylene tetrahydrofolate. The crystal structure of the enzyme has been published [14]. Dimethylglycine accumulated in body fluids of the patient (around 100-fold in plasma and 20-fold in urine) explaining the malodour [12].

30.2.3 Genetics

The pedigree of the patient with DMGDH deficiency suggests autosomal recessive inheritance. The *DMGDH* gene is on chromosome 5q12.2-12.3 [15]. Sequence analysis suggests that the genes for DMGDH and SDH have diverged from a common ancestor. The affected patient is homozygous for an inactivating point mutation (A326G leading to H109R at the protein level) of the *DMDGH* gene. From expression studies, this mutated gene codes for a stable protein lacking enzyme activity [16]. The mutated enzyme has a reduced affinity for FAD, a 27-fold decrease in specific activity and a 65-fold increase in Km, explaining the pathogenicity of the mutation [17].

30.2.4 Diagnostic Tests

The diagnosis is made by finding raised levels of dimethylglycine in plasma and urine, preferably collected when the odour is present. Proton NMR spectroscopy is a good method for this, and it will also detect TMA and TMA-N-oxide which are increased in trimethylaminuria, the other inherited cause of a fishy odour [12]. A UPLC quadrupole linear ion trap MS method for comprehensive analysis of acylglycines can also be

used [18]. Dimethylglycine is not detected with gas chromatography-mass spectrometry procedures using solvent extraction, used routinely in metabolic laboratories. Normal urine excretion is age-dependent.

Reference values for dimethylglycine are as follows:

- Plasma: healthy adults: 1–5 µmol/L
- Urine:
  - infants (birth to 2 months): <550 mmol/mol creatinine
  - from 2 months of age (children and adults): <26 mmol/mol creatinine

Increased serum dimethylglycine levels have been observed in folate deficiency (up to 10-fold), cobalamin deficiency (up to 2-fold) and renal failure (up to 2-fold) [12].

DMGDH activity is present in liver but not in blood cells and fibroblasts.

30.2.5 Treatment

Management is by counselling and minimising the odour by restriction of dietary choline, and avoiding excessive sweating, as outlined for trimethylaminuria. Antibiotics to modify the intestinal microflora are not indicated. The reported patient did not benefit from riboflavin supplements. A trial of folate with riboflavin was suggested [12].

30.3 Disorders in the Metabolism of Glutathione

▶ Glutathione Metabolism.

Glutathione Metabolism

Glutathione (GSH) is a tripeptide consisting of glutamate, cysteine and glycine. It is ubiquitous in the eukaryotic organism and plays a role in many fundamental cellular processes. Apart from being one of the most important antioxidants, it participates in drug metabolism, free-radical scavenging, biosynthesis of DNA and proteins as well as amino acid transport. GSH is synthesized and metabolized in the γ-glutamyl cycle in which six enzymes take part in its synthesis and turnover (Fig. 30.3). It is synthesised from glutamate by sequential actions of γ-glutamylcysteine synthetase and glutathione synthetase. Degradation of GSH involves four enzymes. γ-Glutamyl transpeptidase initiates the breakdown by catalysing the transfer of its γ-glutamyl-group to acceptors. The γ-glutamyl residues are substrates of the γ-glutamyl-cyclotransferase which converts them to 5-oxoproline and the corresponding amino acids. Conversion of 5-oxoproline to glutamate is catalysed by 5-oxoprolinase. A dipeptidase splits cysteinylglycine, which is formed in the transpeptidation reaction, into glycine and cysteine. The biosynthesis of GSH is feedback regulated, i.e. GSH acts as an inhibitor of γ-glutamylcysteine synthetase. Genetic defects have been described in five of the six enzymes of the γ-glutamyl cycle.

30.3.1 γ-Glutamylcysteine Synthetase Deficiency

Clinical Presentation

Up to now, 9 patients have been identified. The disease is characterized by hemolytic anemia, usually rather mild. In two patients spinocerebellar degeneration, peripheral neuropathy and myopathy have been reported as additional symptoms [19]. Further symptoms included transient jaundice, reticulocytosis, hepatosplenomegaly, and delayed psychomotor development.

Metabolic Derangement

γ-Glutamylcysteine synthetase catalyzes the first and rate-limiting step in the synthesis of GSH (Fig. 30.3). Its deficiency results in low levels of cellular GSH and γ-glutamylcysteine. Knock-down of γ-glutamylcysteine synthetase in rat causes acetaminophen-induced hepatotoxicity [20].

Genetics

This disease is transmitted as an autosomal recessive trait. γ-Glutamylcysteine synthetase is a dimer consisting of two non-identical subunits encoded by two separate genes which are located on chromosomes 1p21 (light or regulatory subunit) and 6p12 (heavy or catalytic subunit), respectively [21]. Up to now, 4 different mutations all in the heavy subunit have been identified in 4 families.

Homozygous γ-glutamylcysteine synthetase knockout mice of the heavy subunit fail to gastrulate already at the embryo state and die before day 8.5 of gestation [22]. Knockout mice of the light subunit are viable and fertile without any significant abnormal phenotype.

Diagnostic Tests

Diagnosis is established by low activity of γ-glutamylcysteine synthetase in erythrocytes, leukocytes and/or cultured skin fibroblasts. Low levels of GSH and γ-glutamylcysteine are found in erythrocytes and/or cultured skin fibroblasts. Mutation analysis confirms the diagnosis.

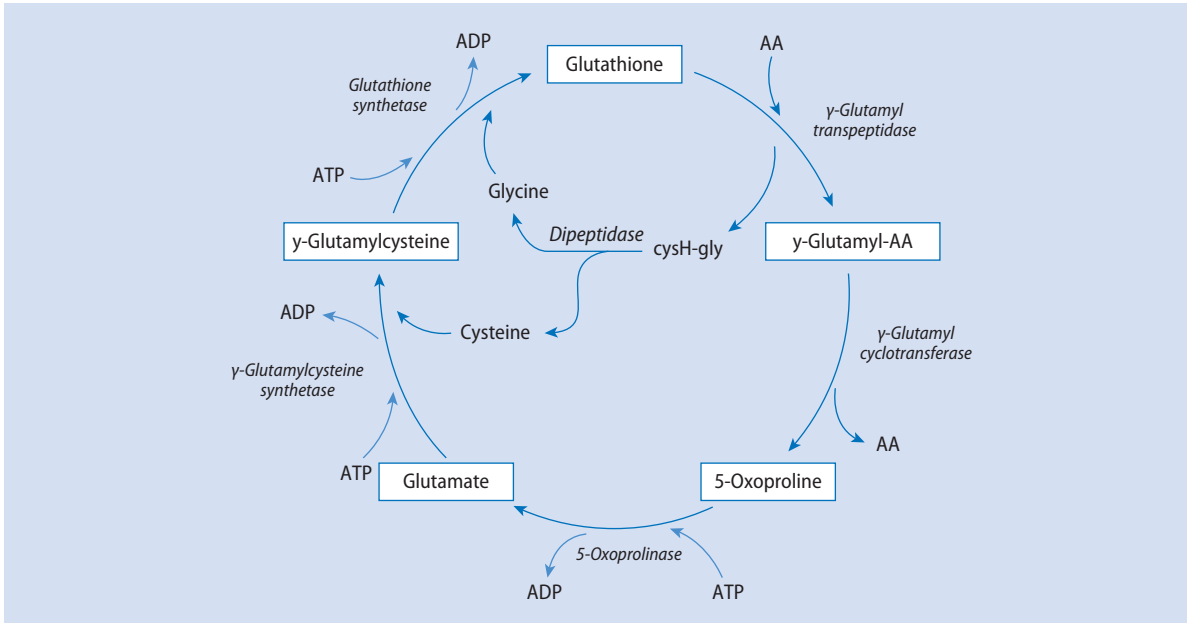


Fig. 30.3 The γ-glutamyl cycle. *AA,* amino acid; *cysH-gly*, cysteine-glycine

Treatment and Prognosis

Patients should avoid food and drugs known to precipitate hemolytic crises in glucose-6-phosphate dehydrogenase deficiency, e.g. fava beans, sulfonamides, acetylsalicylic acid, phenobarbital. Prognosis remains to be established.

30.3.2 Glutathione Synthetase Deficiency

Clinical Presentation

Deficiency of glutathione synthetase (GS) is the most common inborn error of GSH metabolism. More than 80 patients have been reported. According to the severity of clinical symptoms, patients with GS deficiency are classified as mild, moderate or severe [23][24]. Patients with mild GS deficiency show mild hemolytic anemia as their only clinical symptom. Cellular levels of GSH are usually sufficient to prevent accumulation of 5-oxoproline in body fluids. Patients with the moderate variant usually present during the neonatal period with severe and chronic metabolic acidosis, mild to moderate hemolytic anemia, jaundice and 5-oxoprolinuria. After the neonatal period, the condition usually stabilises, but patients may become critically ill during infections due to pronounced acidosis and electrolyte imbalances. Several patients died during such episodes [25]. Patients with severe GS deficiency develop also progressive CNS symptoms, e.g. mental retardation, seizures, spasticity, ataxia, and intention tremor. Some patients suffer from recurrent severe bacterial infections, probably due to defective granulocyte function.

Ophthalmological abnormalities, e.g. fundus lesions, retinal dystrophy or crystalline opacities in the lenses have been described in some patients. Antenatal cerebral haemorrhage has been reported in a patient with moderate GS deficiency, and two further cases of cerebral haemorrhages in two neonates were observed in post-mortem investigations [23][24]. Although the association between peripartal cerebral haemorrhage and GS deficiency might be coincidental in these cases, *in vitro* studies suggest that platelet function might be altered in GS deficiency.

The first pregnancy in a woman with moderate GS deficiency has been reported to be uneventful resulting in an unaffected infant [26].

Metabolic Derangement

As the enzyme catalyses the last step of GSH synthesis, its deficiency leads to low cellular GSH and excessive production of γ-glutamylcysteine, the metabolite before the enzyme defect. Reduced feedback inhibition of γ-glutamylcysteine synthetase leads to overproduction of γ-glutamylcysteine which is converted into 5-oxoproline by action of γ-glutamyl cyclotransferase. The excessive formation of 5-oxoproline exceeds the capacity of 5-oxoprolinase leading to accumulation of 5-oxoproline causing metabolic acidosis and 5-oxoprolinuria (formerly known as pyroglutamic aciduria). γ-Glutamylcysteine contains both reactive groups of GSH (i.e. the γ-glutamyl and the sulfhydryl residues). It accumulates in fibroblasts of patients and may to some extent compensate for lack of GSH. GSH also takes part in the synthesis of leukotriene C4, the primary cysteinyl LT. It has been shown that the synthesis of lipoxygenase products is impaired in affected patients [27].

Genetics

GS deficiency is inherited in an autosomal recessive manner. The GS gene is localised on chromosome 20q11.2 and consists

of 13 exons distributed over 32 kb. Since the human genome contains only one GS gene, the various clinical forms of GS deficiency reflect different mutations as epigenetic modifications in the GS gene. More than 30 different mutations in the GS gene have been identified. Because of the high frequency of splice mutations (approximately 40%), it is recommended that mutation analysis at the genomic level is completed by analyses of RNA transcripts. Heterozygous carriers of GS deficiency are healthy and show an enzyme activity of about 55% of the normal mean and normal levels of GSH. Although no definite correlation between genotype and phenotype could be established, mutations causing aberrant splicing, frameshift or premature stop codons seem to be associated with the moderate or severe clinical phenotypes, but additional genetic or epigenetic factors seem to alter the phenotypes. The milder forms of the disease are usually caused by mutations mainly affecting the enzyme stability.

▪ Diagnostic Tests

Laboratory findings include increased urinary excretion of 5-oxoproline (up to 1 g/kg/day), low levels of GSH in erythrocytes and/or cultured fibroblasts and decreased activity of GS in erythrocytes and/or cultured fibroblasts. Enzyme activities of 1–30% of healthy controls are found in affected patients. Mutation analysis confirms the diagnosis. A symptomatic patient with GS deficiency has been identified through tandem mass spectrometry-based newborn screening [28]. Antenatal diagnosis can be performed by mutation analysis of chorionic villi, analysis of GS activity in cultured amniocytes or chorionic villi, or by measuring 5-oxoproline in amniotic fluid.

▪ Treatment and Prognosis

The clinical management of GS deficient patients is aimed at correction of acidosis, prevention of hemolytic crises and support of endogenous defence against reactive oxygen species (ROS). In the neonatal period, correction of metabolic acidosis, electrolyte imbalances, treatment of anemia and excessive hyperbilirubinemia are of crucial importance.

Correction of acidosis can be reached through bicarbonate, citrate or tris(hydroxymethyl)aminoethane (THAM). Doses of up to 10 mmol/kg/day or even higher in episodes of acute infections may be required.

Repeated blood transfusions may be necessary in patients with massive hemolysis. Drugs and foods known to precipitate hemolytic crises in glucose-6-phosphatase dehydrogenase deficiency should be avoided. Successful treatment with erythropoietin has been reported in one patient.

Early supplementation with vitamin E and vitamin C are thought to replenish the lack of GSH as a scavenger of free radicals. Recommended doses are 10 mg/kg/day for vitamin E and 100 mg/kg/day for vitamin C. A long-term follow-up study of 28 patients suggested that early supplementation with both vitamins may prevent CNS damage and improve the long-term clinical outcome [23][24].

Supplementation with N-acetylcysteine should not be recommended because it was shown at least in cultured fibroblasts that patients with GS deficiency accumulate cysteine which is known to be neurotoxic in excessive amounts. A therapeutic trial with orally administered GSH showed no lasting benefit in two patients with GS deficiency. GSH esters, lipid soluble preparations which are easily transported into cells where they are converted into GSH, have been tried in animal models of GSH deficiency and in some patients with GS deficiency. However, associated toxic effects due to production of alcohols as a by-product during hydrolysis to release GSH make them of limited use. In vitro studies have shown that addition of S-acetylglutathione to the medium of cultured fibroblasts from patients with GS deficiency normalized intracellular GSH content [29].

Early diagnosis, correction of acidosis and early supplementation with vitamin E and vitamin C appear to be the most important factors regarding survival and long-term outcome.

30.3.3 γ-Glutamyl Transpeptidase Deficiency

▪ Clinical Presentation

Up to now, 7 patients have been reported worldwide. Five of them were characterized by CNS involvement. However, two affected siblings presented without any signs of CNS involvement at age of 11 and 13 years, respectively [30]. Therefore, it is yet not clear whether CNS symptoms are part of the clinical picture.

▪ Metabolic Derangement

γ-Glutamyl transpeptidase is a membrane-bound enzyme with subunits of 21 kDa and 38 kDa with its active site. It catalyses the first step in the degradation of GSH. In addition to high levels of GSH in plasma and urine, the enzyme deficiency leads to increased urinary levels of γ-glutamylcysteine and cysteine as well as a deficiency of leukotriene D4.

Knock-out mice with γ-glutamyl transpeptidase deficiency present with glutathionuria, glutathionemia, growth failure, cataracts, lethargy, shortened life span, and infertility [31].

▪ Genetics

The disease is transmitted as an autosomal recessive trait. The human gene for the γ-glutamyl transpeptidase family is composed of at least seven different gene loci, several of them are located on the long arm of chromosome 22.

▪ Diagnostic Tests

High levels of GSH are present in plasma and urine (up to 1 g/day in urine; controls <10 mg) whereas cellular levels of GSH are normal. Decreased activity of γ-glutamyl transpeptidase is found in nucleated cells such as leukocytes or fibroblasts. Erythrocytes are not useful for diagnostic purposes since they also lack γ-glutamyl transpeptidase under normal conditions.

▪ Treatment and Prognosis

There exists no specific treatment or management for a better long-term prognosis. However, administration of N-acetyl-

cysteine to γ-glutamyl transpeptidase deficient mice led to restoration of their fertility [31].

30.3.4 5-Oxoprolinase Deficiency

Clinical Presentation

Up to now, about 15 patients have been described. The clinical symptoms are inconstant and very heterogeneous including renal stone formation, enterocolitis, neonatal hypoglycemia, microcytic anemia, microcephaly and mental retardation. So far, it seems to be a benign biochemical condition and clinical symptoms are merely a coincidence or an epiphenomenon in several pathological conditions [32].

Metabolic Derangement

5-Oxoprolinase catalyses the ring-opening of 5-oxoproline yielding glutamate as a step in the γ-glutamyl cycle. It is the enyzme with the lowest capacity in the γ-glutamyl cycle. Apparently it is composed of two identical 142 kDa subunits. Decreased activity leads to decreased conversion of 5-oxoproline to glutamate resulting in elevated levels of 5-oxoproline in body fluids.

Genetics

The mode of inheritance is autosomal recessive. The condition is caused by mutations of the 5-oxoprolinase (*OPLAH*) gene.

Diagnostic Tests

Elevated levels of 5-oxoproline are found in urine (4–10 g/day; controls <0.1 mol/mol creatinine) and other body fluids. Cellular levels of GSH and acid-base balance are normal. 5-oxoprolinase activity in nucleated cells is decreased. Erythrocytes are not a suitable diagnostic tool because the enzyme is even not present under normal conditions.

Treatment and Prognosis

No specific treatment has been proposed. Since it seems to be a benign condition prognosis depends on an underlying pathological condition.

30.3.5 Dipeptidase Deficiency

Clinical Presentation

So far, dipeptidase deficiency has been suggested in only one patient [33]. The 15-year-old boy presented with mental retardation, mild motor impairment, and partial deafness.

Metabolic Derangement

Dipeptidase is a membrane-bound enzyme that hydrolyses dipeptides, including cysteinylglycine compounds, such as the oxidized γ-glutamyl transpeptidase product cystinyl-bisglycine and the conversion of leukotriene D4 to E4. This leads to cystinylglycinuria and increased excretion of leukotriene D4.

Genetics

The crystal structure of human membrane-bound dipeptidase has been reported [34]. This enzyme is 42 kDa underglycosylated and 63 kDa when glycosylated. Renal dipeptidase has been mapped to human chromosome 16 at q24. No genetic studies have been performed.

Diagnostic Tests

Diagnosis in the described patient was based on increased urinary excretion of cystinyl glycine and leukotriene D4, which is usually not detectable. Leukotriene E4, the major urinary metabolite in humans, was completely absent.

Treatment and Prognosis

No specific treatment has been proposed. Prognosis remains to be established.

30.3.6 Secondary 5-Oxoprolinuria

5-Oxoprolinuria has been described in conditions other than GS deficiency and 5-oxoprolinase deficiency. It is also present in some inborn errors of metabolism not involving the γ-glutamyl cycle and other conditions which should be considered during diagnostic work [35]. Excessive formation of 5-oxoproline has been described e.g. in some patients with urea cycle defects, homocystinuria or nephropathic cystinosis. Transient 5-oxoprolinuria of unknown cause has been reported in very preterm infants. Particular infant formulas and tomato juice may contain modified proteins with increased content of 5-oxoproline. Limited availability of glycine in malnutrition and pregnancy as well as increased turnover of collagen, fibrinogen and other proteins containing considerable amounts of 5-oxoproline in patients with severe burns or Stevens-Johnson syndrome may lead to 5-oxoprolinuria. In addition, certain drugs like paracetamol, vigabatrin or some antibiotics (flucloxacillin, netilmicin) are known to induce 5-oxoprolinuria.

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Section VII Disorders of Lipid and Bile Acid Metabolism

Inborn Errors of Lipoprotein Metabolism Presenting in Childhood

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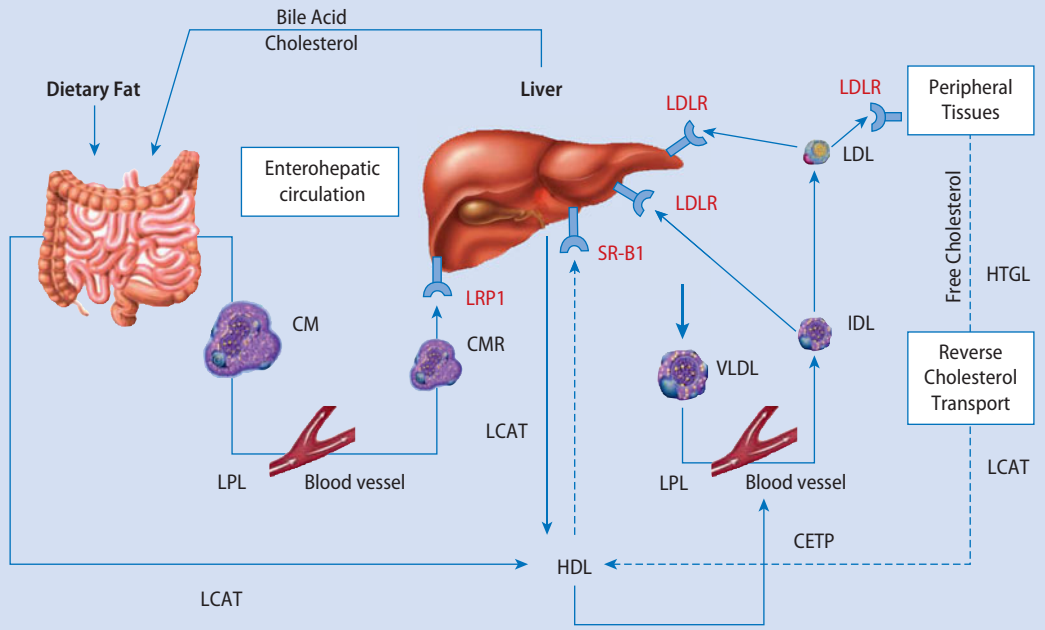
Lipid and Lipoprotein Metabolism (Fig. 31.1)

Very low-density lipoprotein (VLDL) particles are relatively large particles, which are produced in the liver. The function of VLDL is to transport endogenously synthesized triglycerides (TG) and cholesterol to the peripheral tissue. Intermediate density lipoproteins (IDL) are created with the metabolism of VLDL by lipoprotein lipase. IDL particles may be removed by the liver or are converted to low-density lipoprotein (LDL) particles by hepatic triglyceride lipase. LDL particles contain ~ 45% cholesterol and they are the major carrier of cholesterol to peripheral tissues. LDL particles are heterogeneous. Small dense LDL particles have been associated with increased risk for cardiovascular disease. Increased concentrations of small dense LDL particles are associated with male gender and diabetes, particularly in adults. LDL particles are recognized by specific LDL receptors that are highly expressed in the liver. Once LDL particles bind to the receptor they are internalized into the cell. This pathway removes approximately 75% of LDL particles. The remaining LDL particles are removed by macrophages.

High density lipoproteins are produced by the liver and the gastrointestinal tract, as well as by peripheral catabolism of chylomicrons and VLDL particles. HDL particles are also heterogeneous. HDL2 is the sub-fraction that is associated with protection against atherosclerosis. HDL3 is a smaller particle and increased in alcohol consumption, obesity, diabetes, cigarette smoking, uraemia and hypertriglyceridemia. HDL particles are involved in reverse transport of free cholesterol from peripheral tissues to the liver providing an explanation for the protective effect of HDL particles against atherosclerosis. Lipoprotein (a) or Lp(a) has also been found to be associated with the atherosclerotic process. The structure of Lp(a) is similar to LDL, but with the addition of a large »little a« protein bound to apoB via a single cysteine-mediated disulphide bond. Its plasma levels are regulated independently from LDL, and risk of coronary heart disease is greatly increased if both LDL and Lp(a) are elevated. One way Lp(a) may be related to atherosclerosis is because the (a) protein has structural similarities to plasminogen, and it may inhibit the thrombolytic activity of plasminogen.



CETP	Cholesteryl Ester Transfer Protein	HDL	High density lipoprotein	CM	Chylomicrons	LDLR	LDl receptor
HTGL	Hepatic triglyceride lipase	LDL	low density lipoprotein	CMR	Chylomicron remnants	SRBP	Scavenger receptor B1
LCAT	lecithin cholesterol acyltransferase	IDL	Intermediate density lipoprotein			LRP1	LDLR-related protein 1
LPL	lipoprotein lipase	VLDL	very low density lipoprotein				

Fig. 31.1 Schematic diagram of lipid and lipoprotein metabolism. *Adapted from Daniels SR. Pediatric Cardiology 2003*. This figure demonstrates five major lipoprotein classes. These are chylomicrons, very low-density lipoprotein (VLDL), intermediate density lipoprotein (IDL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL). Chylomicrons are triglyceride-rich particles produced by the intestine. They are the largest of the lipoproteins. Their primary function is to transport dietary triglyceride and cholesterol from the intestinal lumen to sites of storage or metabolism. Chylomicrons are rapidly cleared and are usually absent after fasting. The clearance of chylomicrons occurs through the action of lipoprotein lipase (LPL), which creates chylomicron remnants. These chylomicron remnants are cleared from the circulation by the liver. These remnants are thought to be atherogenic by damaging the endothelium

Lipids are highly diverse molecules that are traditionally best known for their role in the formation of biological membranes and cellular systems and as a way to store energy. In the last decade, lipids have taken a more centre stage in apoptosis, cell signaling, inflammation, immunity and inborn errors of metabolism (IEMs). Inborn errors of lipoprotein metabolism are a group of genetic disorders exemplified by changes in plasma lipids due to defects in the protein lipid-carriers (lipoproteins), lipoprotein receptors, or enzymes responsible for the hydrolysis and clearance of lipoprotein-lipid complexes [1]. The proteins responsible for the maintenance of normal plasma and tissue lipids, which are primarily triglycerides and free and esterified cholesterol, include the apolipoproteins A-I, A-II, A-IV, A-V, B, C-I, C-II, C-III, and E with key enzymes including lipoprotein lipase (LPL), hepatic triglyceride lipase (LIPC), lecithin cholesterol acyltransferase (LCAT), and cholesterol ester transfer protein (CETP); and key receptors being the low-density lipoprotein receptor (LDL-R) for LDL-cholesterol (LDL-c), and the ATP-binding cassette transporter 1 (*ABC1A)* for HDL-cholesterol (HDL-c) levels [2][3]. A number of genetic abnormalities of lipoprotein metabolism have been described in childhood (► Lipid and lipoprotein metabolism; ◘ Fig. 31.1, ◘ Table 31.1).

▪ Plasma Lipid and Lipoprotein Metabolism

The major classes of lipids circulating in plasma are cholesterol, cholesteryl ester, triglycerides and phospholipids. Lipids are important components of many of the body's tissues. They serve as building blocks for hormones and are a vital component of cell membranes. However, lipids are insoluble in water. Thus, to be transported in the blood stream, they must be packaged into large, complex water-soluble molecules called lipoproteins. The structure of a lipoprotein is made up of a core consisting of cholesteryl ester and triglyceride covered by a polar surface layer consisting of phospholipids, free cholesterol and protein moieties called apolipoproteins. These lipoprotein particles have differing densities, which are determined by the relative content of protein and lipid. The apolipoproteins perform functions related to transport and uptake into cells.

▪ Lipoprotein Disorders Presenting in Childhood

A number of genetic abnormalities that results in dyslipidemia in childhood have been described, of which heterozygous familial hypercholesterolaemia is the most common inherited lipid disorder with a prevalence of 1 in 500. The responsible genes, inheritance and the observed plasma lipoprotein patterns for lipoprotein disorders manifesting in childhood are listed in ◘ Table 31.1.

31.1 Disorders of Low Density Lipoprotein Metabolism

In the majority of these disorders, the atherosclerotic process begins in childhood, and the extent and rate of progression has a direct relationship with increases in lipid levels. Secondary causes of hyperlipidemia, including obesity, hypothyroidism,metabolic syndromes are not discussed in this chapter but form an important differential diagnosis in disorders of lipoprotein metabolism presenting in children.

Whilst healthy lifestyles and a lower saturated fat intake is the cornerstone of treatment of lipid disorders in children, lipid-lowering therapies are becoming increasingly more important, with minimal adverse effects and no short-term effect on growth and development. There are many established therapies and emerging therapies for lipoprotein disorders and these are detailed in ◘ Table 31.2 and ◘ Table 31.3.

There are five known genetic disorders causing elevated LDL-C that are expressed in children and that cause early atherosclerosis and premature coronary artery disease (CAD). These include familial hypercholesterolaemia (FH), familial ligand defective apo-B (FLDB), autosomal recessive hypercholesterolaemia, sitosterolemia, and mutations in proprotein convertase subtilisin-like kexin type 9 (PCSK9). These disorders arise from either gene mutations that affect LDL receptor activity or abnormalities in the LDL receptor itself. The presence of these disorders indicates a significantly elevated risk for premature atherosclerosis and CAD in adulthood.Of these genetic disorders affecting LDL receptor activity, FH is the most common disorder diagnosed in childhood, and usually identified by cascade screening.

Identifying children and adolescents at enhanced risk for atherosclerosis is important for long-term cardiovascular health and prevention of early coronary heart disease from both inherited and secondary causes of lipoprotein disorders.

Homozygous FH is a severe and rare genetic disorder of lipid metabolism, resulting in extremely elevated LDL-C levels and increased cardiovascular risk. Although the clinical phenotype of this disease is highly variable, recommended target LDL-C levels are hardly ever reached even with widely used lipid-lowering medication. In these children, early lipoprotein apheresis can provide further time-averaged LDL-C level reductions by up to 48% but this therapy may not be appropriate for all children and there are emerging therapies discussed in ◘ Table 31.2, which may be beneficial. Liver transplant is a therapeutic option that should be considered in all children with homozygous FH and appropriate and early assessment is recommended.

Heterozygous FH is more common and early intervention with lipid lowering therapies should be considered by the age of 10 years as is recommended by the UK National Institute for Health and Care Excellence (NICE) guidelines to prevent early onset coronary artery disease.

Dietary and healthy lifestyle measure, with particular emphasis on exercise and avoidance of smoking are the first form of treatment. Monitoring carotid intima media thickness (CIMT) may be a way forward for all children with heterozygous FH to determine the rate of disease progression and to objectively influence treatment options, in addition to careful attention given to family history of early CAD. Dyslipidemic lipoprotein levels in adolescence have been shown to be as-

Table 31.1 Monogenic lipoprotein associated disorders presenting in childhood

Disorder	Inheritance	Protein & Gene Responsible	Observed Plasma Lipoprotein Pattern	Frequency; Ethnicity	Key References
Disorders affecting low density lipoprotein metabolism					
AD Familial hypercholesterolemia	AD*	LDL receptor – *LDLR* (heterozygous mutations)	↑ LDL	1 in 250 to 1 in 500	[4][5]
AR Familial hypercholesterolemia	AR	LDL receptor – *LDLR* (homozygous/compound heterozygous mutations)	↑ LDL	1 in10^6	[4][5]
AD Familial hypercholesterolemia	AD*	Proprotein convertase subtilisin/kexin 9 – *PCSK9*	↑ LDL		[4][5]
Familial ligand-defective apo-B, FLBD	AD*	Apo-B – *APOB*	↑ LDL	1 in 500. Northwest Switzerland, 1 in 114. Four common mutations: R3480P; R3500W; R3531C; R3500Q (R3527Q). Identical phenotype to LDLR mutation FH	[4][5][6]
AD Familial hypercholesterolemia	AD*	Signal transducing adaptor protein family member 1 – *STAP1*	↑ LDL	1 in 1 million	[7]
AR Familial hypercholesterolemia	AR*	LDL-receptor adaptor protein 1 – *LDLRAP1* Commonly truncation mutations	↑ LDL	Rare except in Sardinia	[8][9]
AR Familial hypercholesterolemia	AR*	Lysosomal acid lipase – *LIPA*[b]	↑ LDL		[10]
Familial Hypobetalipoproteinemia (FHBL)	AD*	Apo-B – *APOB* subjects generally have truncation mutations	↓ Apo-B lipoproteins (chylomicrons, VLDL, LDL)	Rare	[11][12]
Abetalipoproteinemia	AR*	Large sub-unit of microsomal triglyceride transfer protein – *MTTP*	↓ Apo-B lipoproteins, no chylomicrons, ↓ HDL	< 1 in 100,000 pan ethnic	[11][12]
Chylomicron Retention Disorder	AR	Deficiency of a GTPase (Sar1b) – *SAR1B*	↓ apo-B48 lipoproteins (chylomicrons), ↓ HDL	Rare Consanguinity frequent	[13]
Disorders affecting triglyceride metabolism					
Type-I hyperlipidaemia – Familial lipoprotein lipase deficiency	AR*	Lipoprotein lipase – *LPL*	↑ Chylomicron, VLDL	Consanguinity 1 in 1 million (homozygous) Founder effect in French Canadian population in Quebec; carrier frequency 1 in 40	[2][14]
Type-1 hyperlipidaemia – Apolipoprotein C-II deficiency	AR*	Apo-C-II – *APOC2*	↑ Chylomicrons, VLDL	Very rare	[2][14]
Type-I hyperlipidaemia – Apo AV deficiency	AR*	Apo-A5 – *APOAV*	↑ Chylomicrons, VLDL	Very rare Consanguinity	[15][16]

Table 31.1 (continued)

Disorder	Inheritance	Protein & Gene Responsible	Observed Plasma Lipoprotein Pattern	Frequency; Ethnicity	Key References
Hypertriglyceridaemia	AD*	Angiopoietin-like proteins -3 -4 and -5 *ANGPTL3/ANGPTL4/ANGPTL5*	↑ Chylomicrons, VLDL	Rare	[17]
Type-I hyperlipidaemia	AR*	Glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 – *GP1HBP1*	↑ Chylomicrons, VLDL	Very rare	[18]
Combined lipase deficiency	AR*	Lipase maturation factor –*LMF1*	↑ Chylomicrons, VLDL		[13]
Disorders affecting high density lipoprotein					
Familial LCAT deficiency	AR*	Lecithin cholesterol acyl transferase – *LCAT*	↓HDL	Rare <100 patients reported	[19][20]
Sterol storage disorders					
Wolman disease	AR*	Lipase A, lysosomal acid, cholesterol esterase – *LIPA*	Dyslipidaemia infrequent: ↑ LDL ↓↓ HDL ↑ to normal TG	1 in 500,000 Null allelic variants with no residual enzyme function	[21]
Cholesterol ester storage disease	AR*	Lipase A, lysosomal acid, cholesterol esterase – *LIPA*	↑ LDL ↓↓ HDL ↑ to normal TG	Precise prevalence unknown; 90 to 170,000; German Cohort 1 in 50,000 Nearly all individuals with CESD reported in the published literature are compound heterozygous or homozygous for c.894G>A.	[22]

*Clinical phenotypes expressed in childhood
[a] *APOA1, APOC3* and *APOA4* are adjacent genes on chromosome 11
[b] Mutations in *LIPA* are known to cause cholesterol ester storage disease
[c] Subjects are homozygous for the APOE e2 variant and require another genetic or environmental factor to express dyslipidaemia

sociated with an increased risk of high CIMT in young adulthood [23].

Some children and adolescents with more marked dyslipidemia will require drug treatment. Treatment with both diet and drugs appear safe and efficacious in childhood, but longer-term safety data are needed. Disease registries are important to collate data on the natural history, family history, life style choices, therapeutic benefits of lipid lowering benefits and side effects of lipid lowering drugs.

31.2 Disorders of Triglyceride (TG) Metabolism

Hypertriglyceridemia may present with life-threatening pancreatitis, a well-recognised and medical emergency in children. Inborn errors of triglyceride metabolism may present early in childhood with faltering growth, hepatosplenomegaly and life threatening pancreatitis. Severe elevation in TG (>500 mg/dL) is rare in childhood and is usually associated with genetically based recessive metabolic defects, including defects in lipoprotein lipase (LPL) and apoCII (the activator of LPL). With LPL and apoCII deficiency, massive increases in chylomicrons and VLDL-C can occur, producing

Table 31.2 Lipoprotein disorders presenting in childhood: Clinical manifestations and therapie

Disorder	Key Manifestations	Therapies		Emerging Therapies	Key References
		Diet	Drugs/Other		
Autosomal dominant familial hypercholesterolaemia (FH) due to heterozygous *LDLR* mutation	Diagnostic criteria * Screening criteria** Increased carotid intima-media thickness (CIMT) and distal coronary artery calcification detected by computed tomography (CT) scanning are confirmed markers of early atherogenesis. Coronary calcification is present in 25% of 11–23 year olds with phenotypic hetrozygous FH **Laboratory:** lipid profile – elevated total and LDL-C; decreased HDL-C; increased non-HDL-C; lipoprotein 9a) [lp(a)] for risk stratification. For children aged 8–10 years, LDL-C is ideally reduced by 50% from pre-treatment levels. For children aged ≥10 years, especially if there are additional cardiovascular risk factors, including elevated Lp(a), the target LDL-C should be <3.5 mmol/L (130 mg/dL). **Monitoring:** Hepatic aminotransferases, creatine kinase (CK) and creatinine levels should be measured before starting treatment and regularly during statin therapy.	Culturally acceptable heart healthy diets from early childhood. Healthy lifestyle advice from early childhood. Encourage physical activity. Smoking to be strictly discouraged.	Lipid lowering therapies in both boys and girls. Early treatment of FH can reduce LDL-C burden, improve endothelial function, substantially attenuate the progression of atherosclerosis, and improve coronary outcomes. The benefits of LDL-C reduction should be balanced against the long-term risk of treatment side effects	Cholesterol transfer protein inhibitors, Anacetrapib	[23][24]
Autosomal dominant familial hypercholesterolaemia heterozygous *PCSK9* mutations	<10% of heterozygous FH are due to *PCSK9* mutations. Management as above.	As above	As above		

Autosomal recessive familial hypercholesterolaemia due to homozygous/ compound heterozygous, *LDLR* mutations	Early cardiac features: aortic stenosis and regurgitation; coronary ostial stenosis Angina pectoris, myocardial infarction and death in early childhood have been reported. But first major cardiovascular events usually occur during adolescence, depending on the severity of the mutation.		An aggressive cholesterol-lowering approach should be initiated as soon as possible to prevent or delay the development of CHD: lipoprotein apheresis Extensive cardiovascular evaluation is imperative: paediatric cardiologist assessment and follow up; coronary CT angiography and MRI to evaluate coronary arteries and aorta is recommended. Invasive coronary angiography case by case as indicated. Lipid lowering drugs Liver transplant	**a.** Oral MTTP Inhibitor (Lomitapide) especially for patients intolerant to LDL apheresis (>12 years of age) **b**Antisense RNA therapy (Injectable Mipomersin) Both drugs decrease hepatic production of apo B lipoproteins, which are atherogenic (>18 years of age) PCSK9 targeted therapy inhibiting PCSK9 activity and reducing LDL-C especially in LDLR mutations (Evolocumab) Cholesterol transfer protein (CETP) inhibitors Pre-HDL infusion	[23][24]
Familial ligand-defective apo-B (FLDB)	**Phenotype:** similar to LDLR heterozygous FH with risk of coronary heart disease but less severe. FLDB homozygotes have later onset and less severe CHD than FH homozygotes **Laboratory:** similar to LDLR heterozygous FH with increased LDL	From the age of 8 onwards: 30% of total calories from fat; <10% of total intake of unsaturated fat	Lipid lowering therapies Liver transplant (homozygous FLDB)		[25]
Autosomal recessive familial hypercholesterolaemia	Phenocopy of LDLR homozygous FH but less severe. **Childhood**: 97% developing planar, tuberous, tendon xanthomas; nearly 50% have coronary artery disease; arcus cornea. Aortic valve disease; aortic root disease; ascending atherosclerosis rare and presents later in life Parents usually have normal lipid profile	Low fat diet	Lipid lowering drugs LDL apheresis Liver transplant		[26]
Familial hypobetalipoproteinaemia (FHBL)	Heterozygous FHBL: **Infancy:** asymptomatic; **Childhood and adults:** partial fat malabsorption; gallstones in adults; hepatic steatosis and fatty liver. **Homozygous FHBL:** similar to abetalipoproteinaemia **Laboratory:** <5th percentile plasma total cholesterol, LDL cholesterol, triglycerides and total apolipoprotein B; truncated apoB on lipid intake. Homozygous/compound heterozygous FHBL: similar to abetalipoproteinaemia	Low fat diet in homozygous/ compound heterozygous FHBL	Heterozygotes: Vit. E to prevent adult onset neuropathy. Homozygous/compound heterozygous FHBL: adequate fat soluble vitamins		[27]

Table 31.2 (continued)

Disorder	Key Manifestations	Therapies		Emerging Therapies	Key References
		Diet	Drugs/Other		
Abetalipoproteinaemia	**Infancy:** fat malabsorption; faltering growth; hypocholesterolaemia; ApoB deficiency **Childhood and adults:** atypical retinitis pigmentosa; ataxia; posterior column neuropathy; myopathy; loss of night vision; hepatomegaly; cirrhosis **Laboratory:** acanthocytosis; hepatic steatosis; elevated transaminases; hypocholesterolaemia; apo B deficiency coagulopathy; significant fat soluble vitamin deficiency	Low fat diet	Fat soluble vitamins with high dose Vitamin A and E Liver transplant	Microsomal transfer protein (MTTP) inhibitors	[28]
Chylomicron retention disorder	**Infancy:** fat malabsorption with chronic diarrhoea, vomiting and abdominal distension; cardiomyopathy; hepatomegaly; retinopathy; delayed light adaptation; micro-nystagmus; Endoscopy – fat laden enterocytes; **Childhood and adults**: hepatic cirrhosis rare; poor bone mineralisation; areflexia; proprioception abnormalities; ataxia; myopathy; sensory neuropathy; **Laboratory:** hypocholesterolaemia (>50% reduction); normal triglycerides; Vit E deficiency invariable; elevated creatine kinase; macrovesicular hepatic steatosis common; elevated transaminases; essential fatty acid deficiency; normal lipids in parents	Low long chain fat diet	Adequate fat soluble vitamins supplement		[13]
Familial lipoprotein lipase disease (LPL)	**Infancy:** abdominal pain; vomiting; faltering growth; hepatosplenomegaly; eruptive xanthomas; lipaemia retinalis; life threatening acute pancreatitis frequent; fever; hyperviscosity syndromes; intestinal bleeds and nephromegaly are rare. Homozygous LPL: 25% present before 1 year of age and majority are symptomatic by 10 years of age. **Adult:** heterozygous LPL **Laboratory:** lactescent plasma/milky serum appearance; hypertriglyceridaemia (HTG); hypercholesterolaemia (HC); very low HDL-C; hyperchlomicronaemia; normocytic anaemia	Very low fat diet; Medium chain fats used as a source of fat as not reliant on chylomicron formation	Lipid lowering Drugs – fibrates as primary therapy Thiazolidinedione drugs (e.g. pioglitazone) Fat soluble vitamins and essential fatty acids Plasmapheresis	Gene therapy (homozygous LPL) Decrease in chylomicron production: MTPI directed to the gut; Inhibition of DGAT-1	[11][29] [30]

Apolipoprotein C-II deficiency	Clinical heterogeneity common. **Infancy and Childhood:** severe cases have similar phenotype to familial LPL deficiency. **Adults:** persistent hepatosplenomegaly; pancreatitis, eruptive xanthomas. Risk of atherosclerosis rare. **Laboratory**: severe hypertriglyceridemia; hyperchylomicronaemia; raised total cholesterol and VLDL; decreased LDL-C and HDL-C	Severe cases and homozygous mutations: As for familial LPL deficiency	Lipid lowering drugs – fibrates as primary therapy Essential fatty acids Plasmapheresis		[31]
Apo AV deficiency	**Childhood:** recurrent abdominal pain; eruptive xanthomas; hepatosplenomegaly; faltering growth; pancreatitis **Laboratory:** severe hypertriglyceridaemia (>10mmol/l)	Low fat diet, MCT containing diet ω-3 fatty acid supplements (accelerates triglyceride clearance by increased LPL activity)	Lipid lowering drugs – fibrates as primary therapy Essential fatty acids Plasmapheresis		[32]
Hypertriglyceridaemia (angiopoietin-like proteins (*ANGTL3, -4, -5*)	Clinical presentation: As above **Laboratory:** Suppression of LPL activity; Hypertriglyceridaemia; increased non esterfied fatty acids	Low fat diet, MCT containing diet ω-3 fatty acid supplements	Lipid lowering drugs – fibrates as primary therapy		[17]
Glycosylphoshatidylinositol-anchored high-density lipoprotein-binding protein 1 deficiency (*GPI-HBPI*)	**Later childhood and adults: homozygous GPI-HBPI** relapsing, severe pancreatitis resistant to conventional treatment; early coronary artery disease **Laboratory:** severe triglyceridaemia; hyperchylomicronaemia	Low fat diet, MCT containing diet ω-3 fatty acid supplements	Lipid lowering drugs – fibrates Essential fatty acids Plasmapheresis		[33]
Familial LCAT deficiency (α & β LCAT deficiency) Norum-Gjone (severe form of LCAT deficiency)	**Childhood and adults:** proteinuria; retinal haemorrhage; aneurysmal dilatation of retinal vessels; angioid streaks; corneal opacities; early atherosclerosis; hepatosplenomegaly; end stage renal failure is a common symptom of disease progression **Laboratory:** normocytic anaemia; increased red cell free cholesterol and lecithin; reduced red cell half-life; moderate haemolysis; sea blue histiocytes on bone marrow examination Serum cholesterol ester (<50%); increased serum nonesterified cholesterol; increased serum phospholipids; decreased lysophosphatidylcholine; decreased high-density lipoproteins (HDL); increased triglycerides		Supportive treatment for anaemia and renal disease Corneal transplant		[19][20]

Table 31.2 (continued)

Disorder	Key Manifestations	Therapies		Emerging Therapies	Key References
		Diet	Drugs/Other		
Wolman disease	Rapidly progressive infantile disorder presenting as early as day 1 of life to a few months old, with death in early infancy in untreated infants. Infantile-onset malabsorption. Persistent vomiting, steatorrhoea, abdominal distension. Severe malnutrition/cachexia. Profound growth failure. Hepatosplenomegaly, liver failure. Adrenal calcification: not always present with absence of adrenal calcification associated with delayed diagnosis. Adreno cortical insufficiency (rare). **Laboratory:** anaemia, liver dysfunction, hyperlipidaemia (unusual and more typical of CESD); abdominal X Ray/CT abdomen – adrenal calcification (absence does not exclude Wolman).	Early metabolic dietetic involvement recommended: Electrolyte replacement Total parental nutrition Liver specific formula feeds for liver failure and malabsorption	**Supportive:** corticosteroid & mineralocorticoid replacement for adrenal insufficiency. Vitamin and mineral supplementation Specific: haematopoetic stem cell transplantation (variable success) Liver transplantation	Enzyme replacement therapy (Sebelipase Alfa)	[34][35] [36][37]
Cholesteryl ester storage disorder	Liver disease is common. It may present as altered liver function with or without jaundice, hepatomegaly, splenomegaly, heaptic steatosis, fibrosis, or cirrhosis. Liver disease can lead to esophageal varices, which are associated with risk of hemorrhage and can be life-threatening, with liver failure and hepatocellular carcinoma experienced in some patients. Lipid deposition in the wall of the intestinal tract results in diarrhoea and weight loss. With significant hyperlipidaemia, xanthelasma in the palpebral fissures, atherosclerosis and coronary heart disease are well recognised manifestations. Enlarged adrenal glands with punctate calcifications has been described in severe disease. Gall bladder disease with cholesterol gall stones has been noted with sever hyperlipidaemia. **Laboratory:** ↑ LDL, ↓↓ HDL, ↑ to normal TG, liver dysfunction; Liver Ultrasound – fatty liver (differential diagnosis of NAFLD); liver histology: microvescicular steatosis with foam cells. Immunohistochemistry: lysosomal markers cathepsin D, lysosomal-associated membrane protein 1 (LAMP1), LAMP2, and lysosomal integral membrane protein 2	Low fat diet (variable response)	**Supportive:** treatment for liver dysfunction and liver failure as in Wolman disease. Lipid lowering therapies (limited response) Specific: liver transplantation	Enzyme replacement therapy (Sebelipase Alfa)	[34][35] [36][37]

Sitosterolaemia	Tendon and tuberous xanthomas, premature atherosclerosis **Laboratory:** haematological abnormalities invariable and maybe only clinical feature in the early stages: abnormally shaped erythrocytes (stomatocytes), thrombocytopenia, giant platelets (macrothrombocytopenia); elevated plant sterols and elevated LDL	Low-plant-sterol diet: restriction of shellfish intake, which contains high amounts algae-derived plant sterol, brassicasterol	Bile acid binding resins; statins; sitostanol; Ezetimibe: NPC1L1 transporter has a role in absorption of plant sterols. Ezetimibe specifically inhibits intestinal cholesterol absorption, targeting NPC1L1 Ileal bypass surgery		[38]

***Diagnosis of familial hypercholesterolaemia in children and adolescents** [23]
- Family history of premature CHD plus high LDL-C levels are the two key selective screening criteria.
- Cholesterol testing should be used to make a phenotypic diagnosis. An LDL-C level ≥5 mmol/L (190 mg/dL) on two successive occasions after 3 months diet indicates a high probability of FH.
- A family history of premature CHD in close relative(s) and/or baseline high cholesterol in one parent, together with an LDL-C ≥4 mmol/L (160 mg/dL) indicates a high probability of FH.
- If the parent has a genetic diagnosis, an LDL-C ≥3.5 mmol/L (130 mg/dL) suggests FH in the child.
- Secondary causes of hypercholesterolaemia should be ruled out.
- DNA testing establishes the diagnosis.
- If a pathogenic *LDLR/APOB/PCSK9* mutation is identified in a first-degree relative, children may also be genetically tested.
- If a parent died from CHD, a child even with moderate hypercholesterolaemia should be tested genetically for FH and inherited elevation in Lp(a).

****Screening criteria for paediatric FH (children and adolescents)** [23]
- If DNA testing is available, cascade screening of families is recommended using both a phenotypic and genotypic strategy.
- If DNA testing is not available, a phenotypic strategy based on country, age- and gender-specific LDL-C levels should be used.
- Children with suspected HeFH should be screened from the age of 5 years; screening for HeFH should be undertaken when clinically suspected (both parents affected or xanthoma present) and as early as possible.
- Age at screening should be similar for boys and girls. † Universal screening in childhood may also be considered.

Unit conversion from mmol/L to mg/dL:
For total, HDL, and LDL cholesterol multiply mmol/L by 38.67, e.g. 3.5 mmol/L = 3.5 mmol/L * 38.67 = 135 mg/dL
For triglycerides multiply mmol/L by 88.57, e.g. 1.9 mmol/L = 1.9 mmol/L * 88.57 = 168 mg/dL

Unit conversion from mg/dL to mmol/L:
For total, HDL, and LDL cholesterol divide mg/dL by 38.67, e.g. 135 mg/dL = 135 mg/dL/38.67 = 3.5 mmol/L
For triglycerides divide mg/dL by 88.57, e.g. 168 mg/dL = 168 mg/dL/88.57 = 1.9 mmol/L

Table 31.3 Lipid lowering drugs

Drug class	Drug function	Drug names licensed in childhood*	Benefits	Possible side effects*
Statins	Inhibits the enzyme the body needs to make cholesterol	Lovastatin* Rosuvastatin* Fluvastatin* Atorvastatin* Pravastatin* (preferred choice under 10 years of age) Simvastatin*	Decrease LDL and triglycerides; slightly increase HDL	Constipation, nausea, diarrhea, stomach pain, cramps, muscle soreness and possible damage, memory loss, forgetfulness, confusion, pain and weakness, increased risk of diabetes; possible interaction with grapefruit juice
Bile acid binding resins	Prevents bile from being reabsorbed into the circulatory system	Colestipol* Cholestyramine* Colesevelam*	Decrease LDL	Constipation, bloating, nausea, gas; may increase triglycerides
Cholesterol absorption inhibitors	Blocks the amount of cholesterol that is absorbed by the small intestine	Ezetimibe*	Decrease LDL; slightly decrease triglycerides; slightly increase HDL Often used as an adjunctive therapy with statins	Stomach pain, fatigue, muscle soreness
Combination cholesterol absorption inhibitor and statin	Inhibits production of cholesterol and blocks absorption of cholesterol by the small intestine	Ezetimibe and simvastatin*	Decreases LDL and triglycerides, increases HDL	Stomach pain, fatigue, gas, constipation, abdominal pain, cramps, muscle soreness, pain and weakness; possible interaction with grapefruit juice
Fibrates	Reduces production of triglycerides	Bezabifrate Fenofibrate* Gemfibrozil	Decrease triglycerides; increase HDL	Nausea, stomach pain, gallstones
Niacin	Lowers the liver's ability to produce LDL	Niaspan	Decreases LDL and triglycerides; increases HDL	Seldom used due to significant side effects. Facial and neck flushing, nausea, vomiting, diarrhea, gout, high blood sugar, peptic ulcers
Combination statin and niacin	Inhibits production of cholesterol	Niacin and lovastatin	Decreases LDL and triglycerides; increases HDL	Seldom used due to significant side effects. Facial and neck flushing, dizziness, heart palpitations, shortness of breath, sweating, chills; possible interaction with grapefruit juice
Omega-3 fatty acids	Inhibits production of triglycerides in the liver	Lovaza (prescription omega-3 fatty acid supplement)	Decreases triglycerides	Belching, fishy taste, increased infection risk
Lomitapide	Microsomal Transfer Protein Inhibition	Lomitapide*	Decreases cholesterol Used as an adjunctive therapy and for patients who are unable to tolerate lipoprotein apheresis.	Abnormal liver function tests, gastrointestinal.

Reference to Summary of Product Characteristics (SPC) recommended prior to licensed indication of lipid lowering therapies in children and for complete list of side effects.

TG >1,000 mg/dL and as high as 5,000 to10,000 mg/dL. Such profound increases in TG can produce pancreatitis and eruptive xanthomas, but are not associated with premature atherosclerosis because the TG-enriched particles are too large to enter the vascular wall.

These children require a very low-fat diet (<10% fat) to ensure adequate intake of essential fatty acids. Medium-chain TG, which are absorbed directly into the portal system and do not require chylomicrons for transport to the liver, can have a significant effect on TG, especially in those with an LPL defect. Neither LPL nor ApoCII deficiency responds to lipid-altering medications [39].

Management of these disorders is often a combination of dietary restrictions and drugs with variable success.

The first commercially approved human gene therapy is Glybera (alipogene tiparvovec), an adeno associated viral vector encoding the lipoprotein lipase gene. Glybera is designed to restore the LPL enzyme activity required to enable the processing, or clearance, of fat-carrying chylomicron particles formed in the intestine after a fat-containing meal. In October 2012, the European Commission granted marketing authorisation for Glybera under exceptional circumstances as a treatment for adult patients diagnosed with familial lipoprotein lipase deficiency (LPLD) confirmed by genetic testing, and suffering from severe or multiple pancreatitis attacks despite dietary fat restrictions.

Prompt referral to a metabolic/lipid specialist is necessary to prevent pancreatitis (Table 31.2).

31.3 Disorders of High Density Lipoprotein Metabolism

HDLs are highly heterogeneous and a dynamic group of the smallest and densest lipoproteins present in the circulation. Disorders of HDL are very rare with only three Mendelian autosomal recessive inherited disorders described: Apolipoprotein A-1 deficiency; familial hypoalphalipoproteinaemia (Tangier's disease) and lecithin:cholesterol acyltranferase (LCAT) deficiency. Patients typically have decreased HDL and Apo A-I and raised cholesterol and triglycerides. The disorders cause premature atherosclerosis. LCAT presents in childhood with a wide range of manifestations (Table 31.2). Elevation of HDL is noted in cholesterol ester transfer protein (CETP) deficiency but these patients are mostly asymptomatic.

31.4 Disorders of Sterol Storage

Intracellular accumulation of cholesterol in certain tissues may occur either due to excessive amounts of cholesterol rich lipids or lipoproteins in plasma; for example cholesterol deposits in xanthomata in familial hypercholesterolaemia.

Intracellular accumulation of cholesterol can also occur when there is an intrinsic abnormality in metabolism of lipids in the cells in which the abnormal storage occurs and are typically seen in inherited deficiencies of lysosomal enzymes catalyzing the breakdown of a complex lipids, for example Nieman Pick Disease (▶ Chapter 30).

Lysosomal Acid Lipase (LAL) Deficiency, a lysosomal storage disorder encompasses the acute infantile onset form, Wolman disease, and the cholesteryl ester storage disease (CESD) presenting in childhood/adulthood [21]. In both Wolman disease and CESD, the accumulation of cholesteryl ester in the lysosomes is secondary to a deficiency of an esterase that is responsible for hydrolysis of esterified cholesterol in the normal lysosome. Wolman disease presents with extreme faltering growth, malabsorption, hepatosplenomegaly, adrenal calcification and death in early infancy. CESD has a relatively slow course of disease progression with hepatosplenomegaly and microvesicular cirrhosis, premature atherosclerosis and hypercholesterolaemia (elevated LDL-C and decreased HDL-C, with absence of hypertriglyceridaemia) are characteristic feature [40]. Sebelipase alfa (recombinant human lysosomal acid lipase (LAL) enzyme) is licensed for both Wolman disease and CESD, with the potential to improve significantly the life expectancy in Wolman disease and alter the natural history of CESD disease [41].

31.5 Conclusion

Inborn errors of lipoprotein metabolism in childhood are mostly rare and cause significant morbidity and mortality. Heterozygous FH is the most common of these disorders with potential benefit on cardiovascular outcomes when a healthy lifestyle is adopted and lipid lowering therapies are started in a timely manner. Most inborn errors of lipoprotein metabolism require specialist input from metabolic dietitians and physicians. There are several emerging therapies including gene therapy that is likely to alter the natural history of these disorders.

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Disorders of Isoprenoid/ Cholesterol Synthesis

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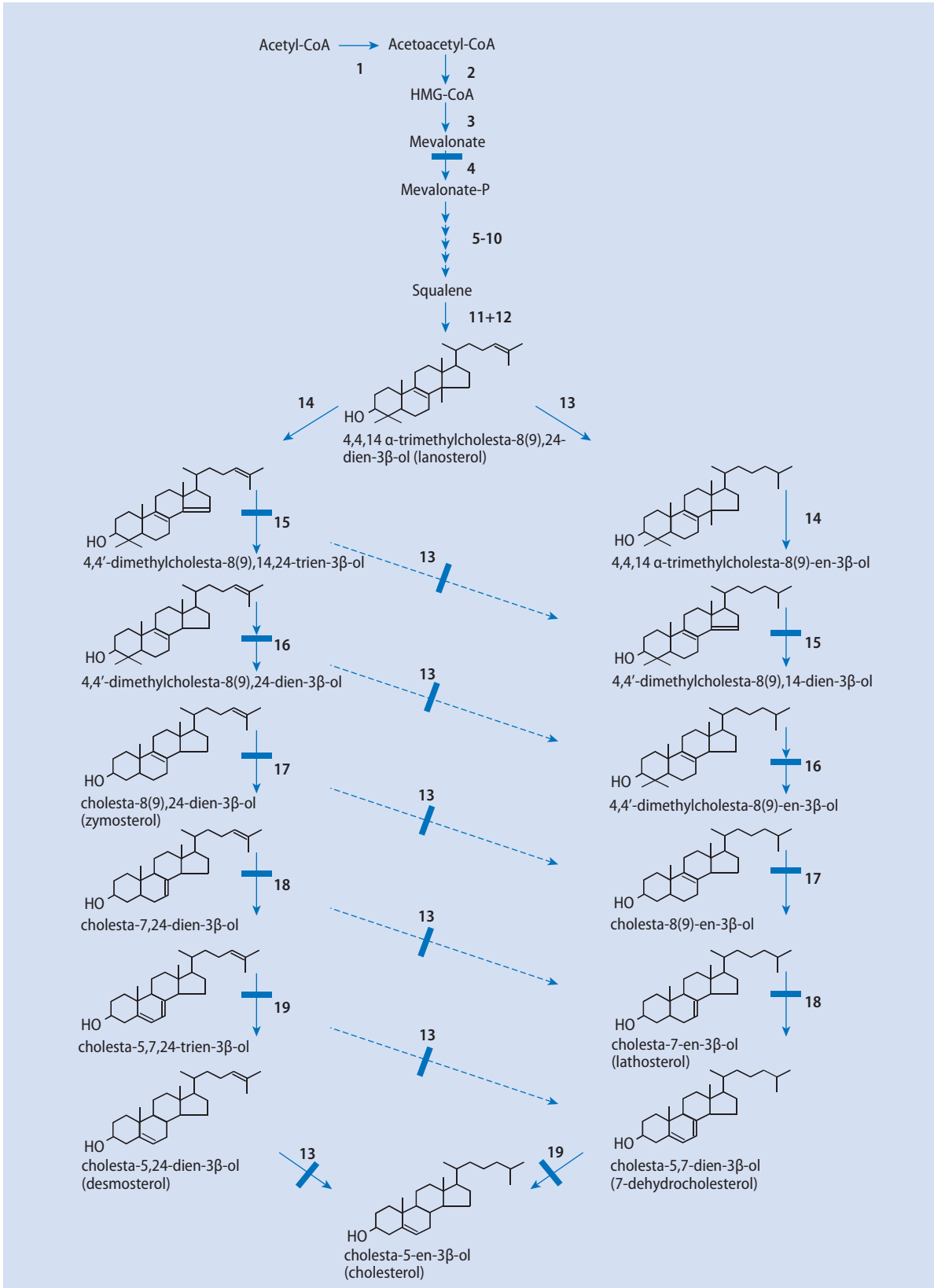
Acetyl-CoA
Acetoacetyl-CoA
1
2
HMG-CoA
3
Mevalonate
4
Mevalonate-P
5-10
Squalene
11+12
HO
4,4,14 α-trimethylcholesta-8(9),24-dien-3β-ol (lanosterol)
14
13
15
4,4′-dimethylcholesta-8(9),14,24-trien-3β-ol
16
4,4′-dimethylcholesta-8(9),24-dien-3β-ol
17
cholesta-8(9),24-dien-3β-ol (zymosterol)
18
cholesta-7,24-dien-3β-ol
19
cholesta-5,7,24-trien-3β-ol
cholesta-5,24-dien-3β-ol (desmosterol)
cholesta-5-en-3β-ol (cholesterol)
4,4,14 α-trimethylcholesta-8(9)-en-3β-ol
4,4′-dimethylcholesta-8(9),14-dien-3β-ol
4,4′-dimethylcholesta-8(9)-en-3β-ol
cholesta-8(9)-en-3β-ol
cholesta-7-en-3β-ol (lathosterol)
cholesta-5,7-dien-3β-ol (7-dehydrocholesterol)

Fig. 32.1 Isoprenoid/cholesterol synthesis pathway. *CoA*, coenzyme A; *HMG*, 3-hydroxy-3-methylglutaryl; *P*, phosphate; *PP*, pyrophosphate. *1*, acetyl-CoA acetyltransferase; *2*, HMG-CoA synthase; *3*, HMG-CoA reductase; *4*, mevalonate kinase; *5*, mevalonate-P kinase; *6*, mevalonate-PP decarboxylase; *7*, isopentenyl-PP isomerase; *8*, geranyl-PP synthase; *9*, farnesyl-PP synthase; *10*, squalene synthase; *11*, squalene epoxidase; *12*, 2,3-oxidosqualene sterol cyclase; *13*, sterol Δ24-reductase; *14*, sterol C-14 demethylase; *15*, sterol Δ14-reductase; *16*, sterol C-4 demethylase complex; *17*, sterol Δ8-Δ7 isomerase; *18*, sterol Δ5-desaturase; *19*, sterol Δ7-reductase. Enzyme deficiencies are indicated by **solid bars** across the arrows

Isoprenoid/Cholesterol Synthesis

The isoprenoid synthesis pathway produces numerous biomolecules, named isoprenoids, with pivotal functions in a variety of cellular processes including cell growth and differentiation, protein glycosylation, signal transduction pathways, etc. [1]. Isoprenoid synthesis starts from acetyl-CoA, which in a series of six enzyme reactions is converted into isopentenyl-PP, the basic isoprene unit used for the synthesis of subsequent isoprenoids (Fig. 32.1). The first committed intermediate in sterol isoprenoid synthesis is squalene, which after cyclisation is converted into lanosterol. Conversion of lanosterol into cholesterol may occur via two major routes involving the same enzymes which, depending on the timing of reduction of the Δ^{24} double bond, postulate either 7-dehydrocholesterol or desmosterol as the ultimate precursor of cholesterol.

Currently, eleven different disorders have been linked to eight specific enzyme defects in the isoprenoid/cholesterol biosynthetic pathway [2][3][4]. Two disorders are caused by mevalonate kinase deficiency and affect the synthesis of all isoprenoids. Patients characteristically present with recurrent episodes of high fever and inflammation and may have additional congenital anomalies.

The remaining enzyme defects affect the synthesis of cholesterol only. Patients with one of these defects may present with various multiple congenital and morphogenic anomalies, including internal organ, skeletal and/or skin abnormalities, and/or a marked delay in psychomotor development (► Isoprenoid/cholesterol Synthesis).

32.1 Mevalonate Kinase Deficiency

Clinical Presentation

The autoinflammatory metabolic disorder Mevalonate Kinase Deficiency (MKD) [5][6][7][8][9] is characterized by lifelong episodes of fever and inflammation accompanied by a chronic proinflammatory state of monocytes [9][10]. These episodes last 3–7 days, recur on average every 4–6 weeks and are associated with abdominal pain, vomiting and diarrhoea, (cervical) lymphadenopathy, hepatosplenomegaly, arthralgia and skin rash [9]. Onset of disease occurs mostly in the 1st year of life, often triggered by childhood vaccinations. The episodes often occur without clear cause but may be provoked by vaccinations, physical and emotional stress and minor trauma [9]. MKD includes the two previously defined clinical entities, classic mevalonic aciduria (MKD-MA) and hyper-IgD and periodic fever syndrome (MKD-HIDS), which represent the severe and mild ends, respectively, of a clinical and biochemical continuum [6][11][12]. MKD-MA patients can present with additional congenital anomalies such as mental retardation, ataxia, cerebellar atrophy, hypotonia, severe failure to thrive and dysmorphic features, and have a high risk of death in early infancy [12][13].

Heterozygous mutations in *MVK* have been reported to cause disseminated superficial actinic porokeratosis in Chinese individuals [14], but this is not observed in heterozygous parents or relatives of MKD patients.

Metabolic Derangement

The clinical presentation of patients correlates well with the residual MK enzyme activity. In cells from MKD-MA patients, this activity is often below detection limit, while in cells from MKD-HIDS patients, residual MK activity of 1–10% of that found in control cells is found [5][7][15]. MK catalyses the phosphorylation of mevalonate to produce 5-phosphomevalonate. As a consequence of the MK deficiency, high and moderately elevated levels of mevalonic acid can be detected in plasma and urine of MKD-MA and MKD-HIDS patients, respectively [7][16]. In principle, the synthesis of all isoprenoids will be affected to a certain extent. The inflammatory manifestations are thought to arise predominantly from a transient shortage of geranylgeranyl-PP [6][17][18]. A relative shortage of sterol isoprenoids during embryonic development may well contribute to the congenital anomalies observed in MKD-MA.

Genetics

MKD is autosomal recessively inherited and is due to mutations in *MVK* [5][7][15]. Most MKD-HIDS patients are compound heterozygotes for the V377I *MVK* allele, found exclusively in MKD-HIDS patients, and a second allele found also in MKD-MA patients [15]. Only few patients homozygous for this V377I *MVK* allele are known. The V377I *MVK* allele codes for an active MK enzyme, the correct assembly/maturation of which is disturbed and appears to be sensitive to temperature, which explains the observed residual MK enzyme activity associated with the MKD-HIDS phenotype [6][7][15]. In total, more than 170 different pathogenic mutations

have been identified that are widely distributed over the gene, most of which are listed in the *INFEVERS* database at http://fmf.igh.cnrs.fr/ISSAID/infevers.

Diagnostic Tests

A first test often involves the analysis of mevalonic acid levels in body fluids by organic acid analysis or, preferably, by stable isotope dilution gas chromatography-mass spectrometry (GC-MS) [16]. This test works best for MKD-MA patients, who have high levels of mevalonic acid (1-56 mol/mol creatinine in urine), but may not always be diagnostic for MKD-HIDS patients, who have rather low levels even during fever (urinary concentration 0.005–0.040 mol/mol creatinine while normally not detectable). The best diagnostic tests remain direct measurement of MK activities in white blood cells or primary skin fibroblasts from patients [19] and molecular analysis of *MVK* [15]. The latter two tests are also used for prenatal diagnosis. Carrier detection is best performed by molecular testing.

Treatment and Prognosis

There is currently no efficacious treatment available. Many MKD-MA patients die in infancy with respiratory failure [12] [13]. In some MKD-HIDS patients, clinical improvement with corticosteroid, colchicine, or cyclosporin has been reported, but in the majority of patients these do not have beneficial effects [9]. In a small group of MKD-HIDS patients, simvastatin treatment led to a small decrease in the number of days of illness [20], but treatment with similar statins in MKD-MA patients led to worsening of clinical symptoms. Treatment with etanercept, a soluble p75 TNF alpha receptor-Fc fusion protein, may lead to a reduction of the frequency and severity of symptoms in some MKD-HIDS patients [21]. Most promising results have been obtained by treatment with interleukin-1 (IL-1) receptor antagonists such as Anakinra, which blocks the biological activity of IL-1beta, an early proinflammatory cytokine that becomes elevated in MKD patients [9][21][22]. HLA-identical allogeneic bone marrow transplantation resulted in remission of the febrile attacks and inflammation during a 15-month follow-up period in a boy with MKD-MA [23].

The long-term outcome for MKD-HIDS patients is relatively benign as the clinical symptoms tend to become less frequent and less severe with age [9].

32.2 Smith-Lemli-Opitz Syndrome (7-Dehydrocholesterol Reductase Deficiency)

Clinical Presentation

Patients with Smith-Lemli-Opitz Syndrome (SLOS) clinically may present with a large and variable spectrum of morphogenic and congenital anomalies constituting a clinical and biochemical continuum ranging from hardly recognisable through mild to very severe (lethal in utero). Most affected patients have a characteristic craniofacial appearance with microcephaly, a short nose with broad nasal bridge and anteverted nares, a long philtrum, micro-/retrognathia and often blepharoptosis, low-set, posteriorly rotated ears, cleft or high-arched palate, pale hair and broad or irregular alveolar ridges. Common limb abnormalities include cutaneous syndactyly of the second and third toes (>97% of cases), short proximally placed thumbs and, in more severe cases, postaxial polydactyly. Genital abnormalities may include hypospadias, cryptorchidism and ambiguous or even female external genitalia in affected boys. Also common are congenital heart defects, and renal, adrenal, lung and gastrointestinal anomalies. Additional major features are profound prenatal and postnatal growth retardation, neonatal ascites, cholestatic jaundice, mental retardation, feeding difficulties and behavioural problems, sleeping disorders and sunlight sensitivity. Although none of these clinical symptoms are pathognomonic for SLOS, the presence of a combination of the more common clinical features associated with SLOS should prompt physicians to consider SLOS in the differential diagnosis [3][4][24].

SLOS may be suspected in a fetus by low maternal oestriol and ultrasound scan findings such as intrauterine growth retardation, microcephaly, increased nuchal translucency, dysmorphic facial features, ambiguous genitalia and digital abnormalities.

Metabolic Derangement

SLOS is caused by a deficiency of 7-dehydrocholesterol reductase (▶ enzyme 19 in ◘ Fig. 32.1), which catalyses the predominant final step in cholesterol biosynthesis, i.e the reduction of the C7-C8 double bond of 7-dehydrocholesterol to produce cholesterol. As a consequence, low cholesterol and elevated levels of 7-dehydrocholesterol can be detected in plasma, cells and tissues of the vast majority of SLOS patients. In addition, elevated 8-dehydrocholesterol levels can be detected in plasma, probably synthesized from the accumulating 7-dehydrocholesterol by the enzyme sterol Δ^8-Δ^7 isomerase functioning in the reverse direction. Clinical severity in SLOS appears to correlate best either with the absolute cholesterol levels or with the sum of 7-dehydrocholesterol plus 8-dehydrocholesterol expressed as the fraction of total sterol (e.g. [25]). There is also evidence that the efficiency of transfer of cholesterol from mother to fetus may play a role in determining severity [26]. 7-Dehydrocholesterol and 8-dehydrocholesterol can be further metabolized by both the bile acid and steroid pathways and can undergo free radical oxidation reactions [27]. The resulting downstream products may contribute to the pathogenesis of the disease.

Unesterified cholesterol accumulations similar to those seen in Niemann-Pick C disease have been observed in SLOS cells, based on which it has been suggested that 7-dehydrocholesterol may interact with NPC1 and/or NPC2, proteins that when defective cause Niemann-Pick C disease [28] (▶ Chapter 38).

Genetics

SLOS is the most frequently occurring defect of cholesterol biosynthesis known to date, and it is inherited as an autosomal

recessive trait. Dependent on the geographic region, incidences have been reported that range from 1:15,000 to 1:60,000 in Europe [24]. The higher incidences observed in particular in some East-European countries reflect founder effects.

Currently, over 190 different pathogenic mutations have been reported in *DHCR7* encoding 7-dehydrocholesterol reductase ([29] for current listing see http://www.hgmd.cf.ac.uk/).

Diagnostic Tests

Laboratory diagnosis includes sterol analysis of plasma or tissues of patients by GC-MS, in which the detection of elevated levels of 7-dehydrocholesterol (and 8-dehydrocholesterol) are diagnostic. Plasma cholesterol is usually low or low normal. Primary skin fibroblasts or lymphoblasts of patients can be cultured in lipoprotein-depleted medium to induce cholesterol biosynthesis, whereupon the accumulation of 7-dehydrocholesterol can be detected by sterol analysis using GC-MS. Finally, molecular analysis of *DHCR7* can be performed. Molecular analysis is the first choice for prenatal diagnosis and carrier detection. When low maternal oestriol or ultrasound features suggest SLOS in a fetus, prenatal diagnosis can be undertaken by measuring, in maternal urine, the ratios of dehydrooestriol to oestriol and of dehydropregnanetriol to preganetriol [30]. Furthermore, sterol analysis of amniotic fluid can be used for prenatal testing [31].

Treatment and Prognosis

Since most anomalies occurring in SLOS are assumed to be due to the unavailability of sufficient cholesterol during (early) embryonic development [24][26], it will most probably not be feasible to develop a postnatal therapy to entirely cure the patients. The therapy currently mostly employed aims to replenish the lowered cholesterol levels in the patients through dietary supplementation of cholesterol (at doses ranging from 25 to 300 mg/kg/d in various formulations) with or without bile acids [32]. While this treatment leads to a substantial elevation of plasma cholesterol concentrations, the plasma concentrations of 7-dehydrocholesterol and 8-dehydrocholesterol are often only marginally reduced. Moreover, this treatment probably does not significantly change the sterol levels in brain, which rely on de novo cholesterol synthesis due to the limited ability of cholesterol to cross the blood-brain barrier. The clinical effects of cholesterol supplementation have been rather disappointing. Standardized studies with SLOS patients revealed hardly any effect on developmental progress [33], and no short-term improvements in behaviour [34]. Simvastatin, an oral HMG-CoA reductase inhibitor, has been used with the aim to lower 7-dehydrocholesterol and 8-dehydrocholesterol levels, but no beneficial effects on either anthropometric measures or behaviour were seen [35].

In severe SLOS cases, reduced synthesis of cholesterol probably leads to reduced synthesis of adrenal steroids. An ACTH stimulation should be undertaken and, if abnormal, severe stresses (such as major surgery) should be covered by corticosteroid therapy in doses similar to those used for congenital adrenal hyperplasia.

Malformations in SLOS often require surgery and the disorder poses anaesthetic challenges. The airway may be compromised by micrognathia, prominent incisors, and a cleft palate, which could be managed by mask ventilation or fiberoptic tracheal intubation.

Feeding problems, structural and functional gastrointestinal problems are common. To maximize weight gain, nasogastric or gastrostomy tube feeding may be necessary. Behavioural problems present a major challenge in the care of patients and antipsychotropic medications, have been used with some effectiveness [32].

32.3 Sterol Δ8-Δ7 Isomerase Deficiency

32.3.1 X-Linked Dominant Chondrodysplasia Punctata 2 or Conradi-Hünermann Syndrome in Females

Clinical Presentation

X-linked dominant chondrodysplasia punctata 2 (CDPX2), also known as Conradi-Hünermann or Happle syndrome mostly affects females. Women affected by CDPX2 have a reduced number of male offspring indicating that most mutations causing CDPX2 in females are lethal in hemizygous males. Patients display skin defects ranging from ichthyosiform erythroderma in the neonate, through linear or whorled atrophic and pigmentary lesions in childhood to striated hyperkeratosis, coarse lustreless hair and alopecia in adults. Additional features are cataracts and skeletal abnormalities including short stature, asymmetric rhizomelic shortening of the limbs, calcific stippling of the epiphyseal regions and craniofacial defects. The pattern of the skin defects and probably also the variability in severity and asymmetry of the bone and eye abnormalities are consistent with functional X-chromosomal mosaicism. The expression of these skin and skeletal abnormalities can be bilateral and is often asymmetric [3][36].

Metabolic Derangement

CDPX2 is caused by a deficiency of sterol Δ^8-Δ^7 isomerase (▶ enzyme 17 in ◘ Fig. 32.1) [37][38]. As a consequence of the deficiency, elevated levels of cholesta-8(9)-en-3β-ol and 8-dehydrocholesterol can be detected in plasma and cells of patients. Plasma cholesterol levels are often (low) normal [39].

Genetics

CDPX2 is inherited as an X-linked dominant trait and due to mutations in *EBP* encoding sterol Δ^8-Δ^7 isomerase [37][38]. Currently, over 80 different disease-causing mutations have been identified in predominantly females (for current listing see http://www.hgmd.cf.ac.uk/). Many are heterozygous for a de novo mutation in line with the sporadic nature of the disorder, but gonadal and somatic mosaicism have been observed (e.g. [40]). Inheritance of a mutation from an affected mother usually results in a more severe disease in offspring [41].

Diagnostic Tests

Laboratory diagnosis involves GC-MS analysis of plasma sterols [39]. Also, primary skin fibroblasts or lymphoblasts of patients can be cultured in lipoprotein-depleted medium to induce cholesterol biosynthesis, whereupon the enzyme defect can be detected by sterol analysis using GC-MS. Finally, mutation analysis can be performed and this is first choice for prenatal diagnosis.

Treatment and Prognosis

Long-term outcome of CDPX2 patients depends on the severity of clinical symptoms. The majority of affected females show completely normal psychomotor development. Many need surgery for cataracts or scoliosis. Correction of scoliosis associated with hemidysplasia of vertebrae requires a special anterior strut graft and a posterior fusion.

32.3.2 Hemizygous EBP Deficiency in Males

Clinical Presentation, Diagnosis and Prognosis

Most mutations causing CDPX2 in females are lethal in hemizygous males. However, several affected males with aberrant karyotypes, somatic mosaicism and hemizygous, hypomorphic missense mutations have been described (e.g. [42][43]). Heterozygous mothers of male patients with hemizygous missense mutations are asymptomatic confirming the hypomorphic nature of the missense mutation.

Males with a 47XXY karyotype or with somatic mosaicism for an EBP mutation show typical features of CDPX2, including bone shortening, which may be asymmetrical, stippled epiphyses, ichthytosis, alopecia and mild facial dysmorphism. However, males with true hemizygous mutations may have different phenotypes ranging from i) dysmorphic features like those seen in SLOS (including the facial appearance, cryptorchidism and hypospadias, and 2/3 toe syndactyly); ii) brain abnormalities including Dandy-Walker malformation and agenesis of the corpus callosum; iii) skin abnormalities including collodion baby and diffuse congenital ichthyosis to iv) developmental delay and behavioural difficulties [42][43][44]. Skeletal abnormalities may be totally absent. There is a high mortality rate among affected males, many dying between 1 day and 4.5 years. The survivors have significant learning difficulties and may show severe developmental delay [42][43][44].

Increased levels of 8-dehydrocholesterol and 8(9)-cholestenol can be detected in plasma and cultured cells. *EBP* mutation analysis can be performed to confirm the diagnosis.

32.4 Deficiency of the C4-Demethylase Complex

The C4-demethylase complex (▶ enzyme complex 16 in ◘ Fig. 32.1) catalyses the sequential removal of the two methyl groups at the C4 position of early sterol precursors (e.g. lanosterol) and is comprised of 3 components: i) a 3β-hydroxysteroid C4-methyl oxidase (encoded by *MSMO1*) [45]; ii) a 3β-hydroxysteroid dehydrogenase (also referred to as a sterol 4α-carboxylate 3-dehydrogenase) that removes a C4 carboxyl group while at the same time oxidizing the 3β-hydroxyl group to a 3-keto group (encoded by *NSDHL*) [46], and iii) a 3-ketosteroid reductase that converts the 3-keto group back to a 3β-hydroxyl group (encoded by *HSD17B7*) [47].

32.4.1 C4-Methyl Sterol Oxidase Deficiency (SMO Deficiency)

Clinical Presentation, Diagnosis and Treatment

Mutations in *MSMO1* have been documented in only one female patient, who presented with severe psoriasiform dermatitis, arthralgias, immune dysfunction, congenital cataracts, failure to thrive, short stature, microcephaly and developmental delay [45]. Three additional patients with similar clinical features have been mentioned in a meeting abstract but these were never published and the underlying genetic cause is unknown [3].

Deficient activity of the C4-demethylase complex leads to accumulation of C4-methyl sterols that can be detected in plasma and cultured skin fibroblasts by GC-MS. Molecular analysis of *MSMO1* is required for genetic confirmation.

The published patient was treated with oral statin plus cholesterol and bile acid supplementation, which caused improvement in growth, weight gain and joint pains. Topical application of a statin and cholesterol led to an improvement of the skin disease [45].

32.4.2 Sterol 4α-Carboxylate 3-Dehydrogenase Deficiency

CHILD Syndrome in females

Clinical Presentation

Patients with CHILD syndrome (congenital hemidysplasia with ichthyosiform erythroderma and limb defects) display skin and skeletal abnormalities similar to those observed in patients with CDPX2, but with a striking unilateral distribution affecting the right side of the body more often than the left, in contrast to the bilateral distribution in CDPX2 patients [3][36]. Ichthyosiform skin lesions are usually present at birth and often involve large regions of one side of the body with a sharp line of demarcation in the midline. Alopecia, nail involvement and limb reduction defects with calcific stippling of the epiphysis are common on the affected side. In contrast to CDPX2, patients with CHILD syndrome show no cataracts, but have more obvious skin lesions and more severe limb defects. Like CDPX2, CHILD is considered lethal in males hemizygous for severe pathogenic mutations as so far only few males with hypomorphic mutations have been diagnosed.

Metabolic Derangement

CHILD syndrome is caused by a deficient activity of sterol 4α-carboxylate 3-dehydrogenase [41], which is part of the C4-demethylase complex. Theoretically, the enzyme deficiency should lead to the accumulation of 4-methyl sterol precursors; however, these precursors are hardly or not detectable in plasma of patients. Cholesterol levels are normal.

Genetics

CHILD syndrome is inherited as an X-linked dominant trait and due to heterozygous mutations in *NSDHL* encoding 3β-hydroxysteroid dehydrogenase [46]. Currently, over 25 different pathogenic mutations have been identified in *NSDHL* (for current listing see http://www.hgmd.cf.ac.uk/). In one patient clinically diagnosed with CHILD syndrome, a heterozygous mutation was identified in *EBP* [48].

Diagnostic Tests

The only diagnostic test for CHILD syndrome is mutation analysis of *NSDHL*. If no mutation is found in *NSDHL*, one should consider sequencing *EBP* [48].

Treatment and Prognosis

Since the clinical presentation in CHILD syndrome in general is usually far more severe than in CDPX2, the long-term outcome of patients is usually poor. Surgical corrections of skeletal abnormalities may be required.

CK Syndrome in males

Clinical Presentation, Diagnosis and Treatment

Most mutations causing CHILD syndrome in females are lethal in males. However, hemizygous mutations in *NSDHL* have been reported to cause CK syndrome (CKS) [3][49]. So far, thirteen hemizygous males from 2 families have been reported. These all had mild to severe intellectual disability and microcephaly. Most were hypotonic and had minimal speech development. All developed seizures during infancy ranging from brief absences to generalized tonic-clonic convulsions. MRI scans of three patients showed evidence of cerebral cortical malformation, including polymicrogyria or pachygyria. All patients had a distinctive appearance with a long thin face, almond-shaped eyes with epicanthic folds, up slanting palpebral fissures, posteriorly rotated ears, a high forehead, a high palate with dental crowding and micrognathia in childhood. All were of asthenic habitus with hyperextensible joints, and spinal abnormalities such as kyphosis or scoliosis. Behavioural problems included attention deficit hyperactivity disorder, irritability and aggression. Heterozygous females of the families did not show features of CHILD syndrome, but exhibited abnormal and antisocial behaviour, and psychological testing reveals problems with working memory.

As with CHILD syndrome, plasma sterol levels are not informative rendering sequence analysis of *NSDHL* as the only diagnostic test.

The affected males of both families survived into adult life [49]. Seizures can be treated with anticonvulsants.

32.5 Desmosterol Reductase Deficiency (Desmosterolosis)

Clinical Presentation

Currently 9 patients with desmosterolosis have been reported [3]. The first reported female infant died shortly after birth and suffered from multiple congenital malformations, including macrocephaly, hypoplastic nasal bridge, thick alveolar ridges, gingival nodules, cleft palate, total anomalous pulmonary venous drainage, ambiguous genitalia, short limbs, arthrogryposis and generalised osteosclerosis [50]. The second reported infant was a boy, who exhibited a far less severe phenotype. At 3 years of age, his clinical presentation included dysmorphic facial features, microcephaly, limb anomalies, and profound developmental delay [51]. Seven additional patients have been reported; one died at 5 days, but all others were alive at 4 months to 15 years. All 9 patients had major CNS involvement and agenesis of the corpus callosum and 8 patients had dilated ventricles (but head size varied from microcephalic to macrocephalic). Surviving patients showed learning difficulties and some patients suffered from epilepsy. Other common features include facial dysmorphic features, arthrogryposis and severe failure to thrive.

Metabolic Derangement

Desmosterolosis is due to a deficiency of sterol Δ^{24}-reductase (▶ enzyme 13 in ◘ Fig. 32.1), which catalyses the reduction of the Δ^{24} double bond of sterol intermediates (including desmosterol) [52]. The deficiency results in elevated levels of desmosterol in plasma, tissue and cultured patient cells [50][51][52].

Genetics

Desmosterolosis is an autosomal recessive disorder due to mutations in *DHCR24* encoding 3β-hydroxysterol Δ^{24}-reductase. Currently, 7 different pathogenic mutations have been identified (for current listing see http://www.hgmd.cf.ac.uk/).

Diagnostic Tests

Laboratory diagnosis includes sterol analysis of plasma, tissues or cultured cells by GC-MS (detection of desmosterol) and mutation analysis of *DHCR24* [52]. Molecular analysis is the first choice for prenatal diagnosis and carrier detection.

Treatment and Prognosis

At the severe end of the spectrum, patients die in the neonatal period. Surviving patients show moderate to severe developmental delay.

32.6 Sterol Δ5-Desaturase Deficiency (Lathosterolosis)

Clinical Presentation

Currently, four patients with lathosterolosis, including two sibs (one a fetus) have been reported [53][54][55][56]. One female patient presented at birth with severe microcephaly,

receding forehead, anteverted nares, micrognathia, prominent upper lip, high-arched palate, postaxial hexadactyly of the left foot and syndactyly between the second to fourth toes and between the fifth toe and the extra digit. From early infancy she suffered from cholestatic liver disease and, during infancy, severe psychomotor delay became apparent [53][55]. By 6 years, she had developed bilateral cataracts and osteoporosis leading to fractures. The cholestatic liver disease had progressed to cirrhosis with portal hypertension. At age 8 she received a liver transplant; at 5 year follow-up she had normal liver functions. The second patient was a boy, born with SLOS-like features, including growth failure, microcephaly, ptosis, cataracts, short nose, micrognathia, prominent alveolar ridges, ambiguous genitalia, bilateral syndactyly of the second and third toes, and bilateral postaxial hexadactyly of the feet. His clinical course was marked by failure to thrive, severe delay, increasing hepatosplenomegaly and increased gingival hypertrophy, with death at the age of 18 weeks. Autopsy disclosed widespread storage of mucopolysaccharides and lipids within the macrophages and, to a lesser extent, parenchymal cells, of all organ systems, extensive demyelination of the cerebral white matter and dystrophic calcification in the cerebrum, cerebellum, and brain stem [56]. The third patient was also a boy diagnosed at 22 months and with micrognathia, postaxial hexadactyly of the feet, syndactyly between the second and third toes, and developmental delay [54].

Metabolic Derangement

Lathosterolosis is due to a deficiency of sterol Δ^5-desaturase (▶ enzyme 18 in ◘ Fig. 32.1), which introduces the C5-C6 double bond in lathosterol to produce 7-dehydrocholesterol, the ultimate precursor of cholesterol [55][56]. As a consequence, elevated levels of lathosterol can be detected in plasma, (tissue) and cultured cells of patients. Cholesterol levels can be normal.

Genetics

Lathosterolosis is an autosomal recessive disorder due to mutations in *SC5D* encoding 3β-hydroxysterol Δ^5-desaturase. So far five different pathogenic mutations have been reported [54][55][56].

Diagnostic Tests

Laboratory diagnosis includes sterol analysis of plasma, tissues or cultured cells by GC-MS (detection of lathosterol) and mutation analysis of *SC5D* [54][55][56]. Molecular analysis is the first choice for prenatal diagnosis and carrier detection.

Treatment and Prognosis

The first surviving patient had cholestatic liver disease for which she received a liver transplant at the age of 8, which resulted in normal liver functions in 5-year follow up [53]. At the age of 6 she had cataract surgery [57]. One patient received simvastatin, which resulted in normalisation of lathosterol levels [54].

32.7 Sterol Δ14-Reductase Deficiency (Hydrops – Ectopic Calcification – Moth-eaten (HEM) Skeletal Dysplasia or Greenberg Skeletal Dysplasia)

Clinical Presentation

HEM skeletal dysplasia, or Greenberg skeletal dysplasia, is characterised by early in utero lethality. Affected fetuses typically present with severe fetal hydrops, short-limb dwarfism, an unusual ›moth-eaten‹ appearance of the markedly shortened long bones, bizarre ectopic ossification centres and a marked disorganisation of chondro osseous histology, and may present with polydactyly and additional non-skeletal malformations [58][59].

HEM skeletal dysplasia is allelic to Pelger-Huet anomaly (PHA) [58][59][60][61][62], a rare benign autosomal dominant disorder of leukocyte development characterised by hypolobulated nuclei and abnormal chromatin structure in granulocytes of heterozygous individuals. Heterozygous individuals with PHA do not show clinical symptoms, but homozygotes may have variable minor skeletal abnormalities and developmental delay [62]. Recently, two cases of anadysplasia-like spondylometaphyseal dysplasia due to mutations in *LBR* have been reported in a 12-year-old girl and a 15-year-old boy [60][63]. Thus, mutations in LBR can create a spectrum of disease ranging from benign isolated PHA through mild skeletal dysplasia to severe HEM skeletal dysplasia.

Metabolic Derangement

HEM skeletal dysplasia is due to a deficiency of sterol Δ^{14}-reductase (▶ enzyme 15 in ◘ Fig. 32.1), which catalyses the reduction of the Δ^{14} double bond in early sterol intermediates [59][61]. As a consequence, elevated levels of Δ^{14}-sterol intermediates can be detected in tissues and cells of fetuses with HEM skeletal dysplasia. Heterozygous individuals with PHA do not show aberrant sterol precursors.

Genetics

HEM skeletal dysplasia is an autosomal recessive disorder due to mutations in *LBR* encoding lamin B receptor [59]. Currently, more than twenty pathogenic mutations have been reported in *LBR* (for current listing see also http://www.hgmd.cf.ac.uk/), including heterozygous mutations causing PHA [62]. PHA may represent the carriership of sterol Δ^{14}-reductase deficiency [59], but was not observed in a parent carrying a missense mutation affecting only the sterol Δ^{14}-reductase region of *LBR* [61].

Diagnostic Tests

Affected fetuses are often detected by fetal ultrasound examination. Laboratory diagnosis includes sterol analysis of tissues or cells by GC-MS and molecular testing of *LBR*. Prenatal and carrier detection is best performed by molecular testing.

Treatment and Prognosis

Most cases terminate in early embryonic stage (10-20 weeks of gestation). One adult individual diagnosed with PHA and homozygous for a splice-site mutation in *LBR* showed developmental delay, macrocephaly and a ventricular septal defect. No information is available on the effect of the mutation on cholesterol biosynthesis in this individual. Two patients showed milder skeletal dysplasia [60][63].

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Disorders of Bile Acid Synthesis

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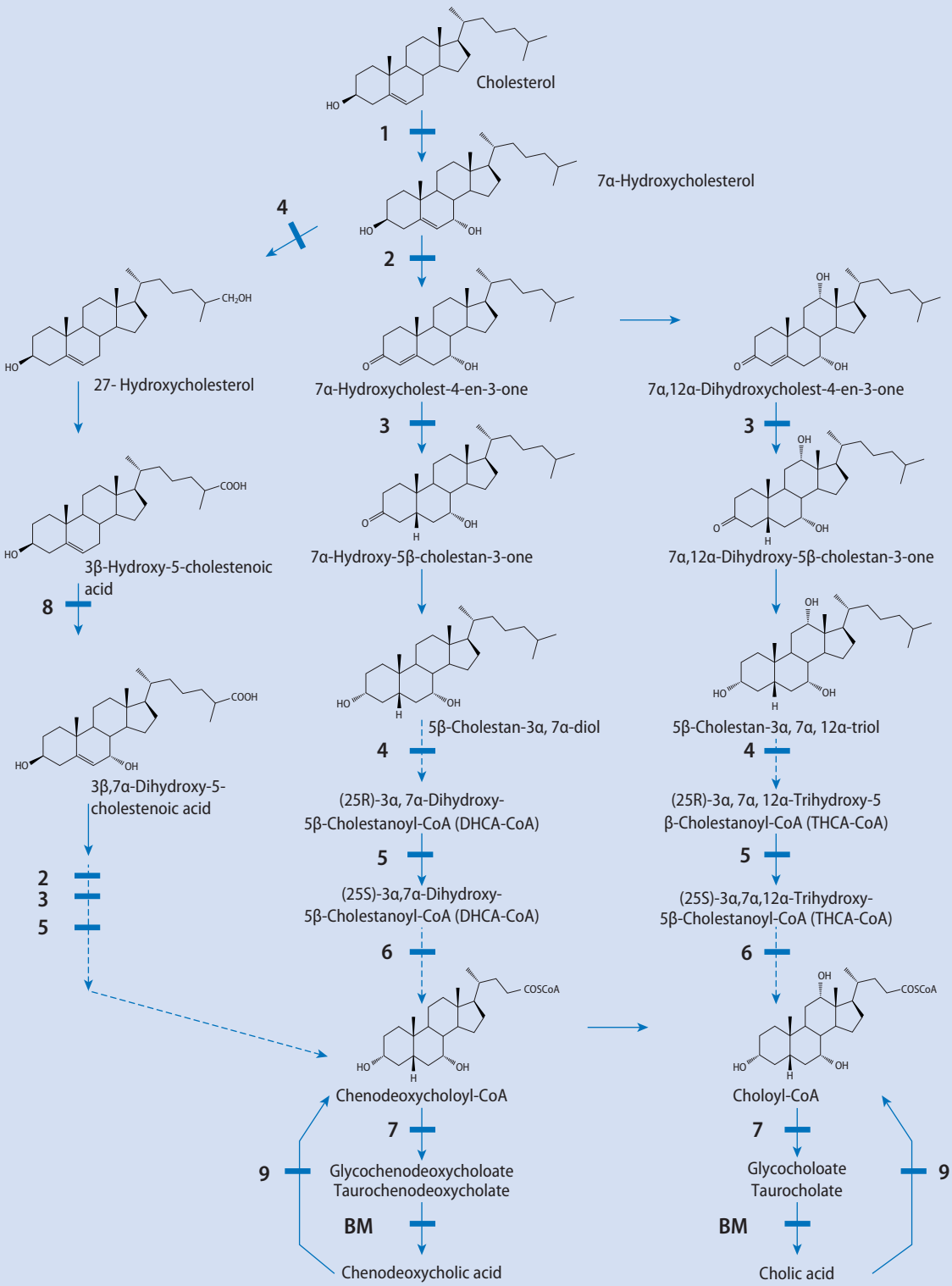

Fig. 33.1 Major reactions involved in the synthesis of bile acids from cholesterol. *1*, Cholesterol 7α-hydroxylase; *2*, 3β-hydroxy-Δ5-C27-steroid dehydrogenase/isomerase; *3*, Δ4-3-oxosteroid-5β-reductase; *4*, sterol 27-hydroxylase; *5*, α-methylacyl-CoA racemase; *6*, proteins needed for peroxisome biogenesis, peroxisomal import of THCA-CoA and DHCA-CoA and their β-oxidation; *7*, bile acid-CoA: amino acid N-acyl transferase; *8*, oxysterol 7α-hydroxylase; *9*, bile acid CoA ligase. Enzyme defects are depicted by **solid bars** across the arrows. BM indicates bacterial metabolism (in the gut)

Bile Acid Synthesis

Bile acids are biological detergents that are synthesised from cholesterol in the liver by modifications of the sterol nucleus and oxidation of the side chain. Synthesis of bile acids can occur by a number of pathways (◘ Fig. 33.1); the most important in adults starts with conversion of cholesterol to 7α-hydroxycholesterol. In infancy, other pathways are more important; one of these starts with the conversion of cholesterol to 27-hydroxycholesterol.

Two inborn errors of metabolism affect the modifications of the cholesterol nucleus in both major pathways for bile acid synthesis: 3β-hydroxy-Δ5-C27-steroid dehydrogenase (3 β-dehydrogenase) deficiency and Δ4-3-oxosteroid 5β-reductase (5β-reductase) deficiency. These disorders produce cholestatic liver disease and malabsorption of fat and fat-soluble vitamins. Onset of symptoms is usually in the 1st year of life and, if left untreated, the liver disease can progress to cirrhosis and liver failure. Treatment with chenodeoxycholic acid and cholic acid can lead to dramatic improvement in the liver disease and the malabsorption. Neonatal cholestatic liver disease can also be the presenting feature of two disorders affecting oxidation of the cholesterol side chain – sterol 27-hydroxylase deficiency (cerebrotendinous xanthomatosis [CTX]) and α-methylacyl-CoA racemase deficiency. However, these disorders more commonly present later with neurological disease. Chenodeoxycholic acid has been shown to halt or even reverse neurological dysfunction in CTX. Oxysterol 7α-hydroxylase deficiency can present with rapidly progressive liver disease in infancy or later onset hereditary spastic paraparesis. These 3 disorders can be placed in a growing list of defects that may present with transient neonatal cholestatic jaundice followed by a late onset neurodegenerative disorder. It seems likely that the 27-hydroxycholesterol pathway is important in fuelling bile flow in infancy and in the production and metabolism of important oxysterols in the brain later in life.

Other inborn errors of bile acid synthesis include two bile acid amidation defects (cholestatic liver disease and fat-soluble vitamin malabsorption) and cholesterol 7α-hydroxylase deficiency (adults with hyperlipidaemia and gallstones). In disorders of peroxisome biogenesis and peroxisomal β-oxidation, neurological disease usually predominates; these are considered in ▶ Chapter 40. A disorder affecting peroxisomal import of CoA esters of DHCA and THCA produces predominantly liver disease.

Most of the known enzyme deficiencies of bile acid synthesis affect both the 27-hydroxycholesterol and the 7α-hydroxycholesterol pathways; the exceptions are cholesterol 7α-hydroxylase deficiency and oxysterol 7α-hydroxylase deficiency [1] (▶ Bile Acid Synthesis). Because of the broad specificity of many of the enzymes, the major metabolites are often not those immediately proximal to the block. For instance, in 3β-hydroxy-Δ^5-C_{27}-steroid dehydrogenase deficiency (▶ enzyme 2 in ◘ Fig. 33.1) the major metabolite is not 7α-hydroxycholesterol but a series of unsaturated bile acids that have the normal bile acid side chain but persistence of the 3β, 7α-dihydroxy-Δ^5 structure of the nucleus.

33.1 3β-Hydroxy-Δ5-C27-Steroid Dehydrogenase Deficiency

Clinical Presentation

3β-Dehydrogenase deficiency was first described in 1987 [2]. Reviews of 38 children diagnosed in Cincinnati and 18 cases diagnosed in London were published in 2007 and 2010, respectively [3][4]. Patients presented with neonatal conjugated hyperbilirubinaemia (11/18), rickets (8/18, including 1 with hypocalcaemic tetany and seizures but normal liver function tests), hepatomegaly (7/18), pruritus (3/18), steatorrhoea and failure to thrive (3/18) [4]. Many had documented biochemical evidence of fat-soluble vitamin malabsorption (low 25-OH vitamin D in 10/18, of whom 8 also had low vitamin E levels and 6, low vitamin A levels and 1 had a prolonged prothrombin time responsive to vitamin K). The liver biopsy showed giant cell change and hepatocyte disarray in all cases, with added features of cholestasis in the majority; many had bridging fibrosis. Jacquemin et al. have described a group of patients with 3β-dehydrogenase deficiency who presented with jaundice, hepatosplenomegaly and steatorrhoea (a clinical picture resembling progressive familial intrahepatic cholestasis) between the ages of 4 months and 46 months [5]. Pruritus was absent in these children, in contrast to other children with severe cholestasis. The authors noted normal γ-glutamyl-transpeptidase activities in plasma, low serum cholesterol concentrations and low vitamin E concentrations. Presentation of 3β-dehydrogenase deficiency with chronic hepatitis / cirrhosis in adolescence/adulthood has also been described as have asymptomatic adults [6].

Metabolic Derangement

3β-Dehydrogenase catalyses the second reaction in the major pathway of synthesis of bile acids: the conversion of 7α-hydroxycholesterol to 7α-hydroxycholest-4-en-3-one (▶ enzyme 2 in ◘ Fig. 33.1). When the enzyme is deficient, the accumulating 7α-hydroxycholesterol can undergo side-chain oxidation with or without 12α-hydroxylation to produce 3β,7α-dihydroxy-5-cholenoic acid and 3β,7α,12α-trihydroxy-5-cholenoic acid, respectively. These unsaturated C_{24} bile acids are sulphated in the C3 position; a proportion is conjugated to glycine, and they can be found in high concentrations in the urine. Concentrations of bile acids in the bile are low [7]. It is probable that the sulphated Δ^5 bile acids cannot be secreted into the bile canaliculi and fuel bile flow in the same way as occurs with the normal bile acids; indeed they probably inhibit bile acid-dependent bile flow. There are at least two possible ways in which this sequence of events might lead to damage to hepatocytes and, ultimately, to cirrhosis:

1. The abnormal metabolites produced from 7α-hydroxycholesterol may be hepatotoxic as well as cholestatic.
2. Failure of bile acid-dependent bile flow may lead to hepatocyte damage, perhaps as a result of the accumulation of toxic compounds normally eliminated in the bile.

Genetics

3β-Dehydrogenase deficiency is an autosomal-recessive trait caused by mutations in *HSD3B7* located on 16p11.2–12. In 2000, Schwarz et al. showed that the original patient described by Clayton et al. in 1987 was homozygous for a 2-bp deletion in exon 6 (Δ1057–1058) [8]. In 2003, Cheng et al. reported mutations in 15 additional patients from 13 kindreds with 3β-dehydrogenase deficiency [9]. In patients with neonatal cholestasis, they identified deletions (310delC, 63delAG), a splice site mutation (340+1 G>T) and a missense mutation (E147K).

Diagnostic Tests

The diagnosis is established by demonstrating the presence of the characteristic bile acids (with a Δ^5 double bond, 3β-hydroxyl/sulphate group and 7α-hydroxyl group) in plasma or urine. It is important to remember that bile acids with a Δ^5 double bond and a 7-hydroxy group are acid labile. Analysis by fast-atom-bombardment mass spectrometry (FAB-MS) or electrospray ionisation tandem mass spectrometry (ESI-MS/MS) overcomes this problem [2][10][11].

Plasma

If plasma bile acids are analysed using a none of the other abbreviations are defind (FAB-MS, ESI-MS etc.) GC-MS method that does not include a solvolysis step, the profile of non-sulphated bile acids that is obtained shows concentrations of cholic and chenodeoxycholic acid, which are extremely low for an infant with cholestasis. The concentration of 3β,7α-dihydroxy-5-cholestenoic acid is increased. Inclusion of a solvolysis step reveals the presence of high concentrations of 3β,7α-dihydroxy-5-cholenoic acid (3-sulphate) and 3β,7α,12α-trihydroxy-5-cholenoic acid (3-sulphate). These can also be detected when plasma is analysed by FAB-MS or when a neonatal blood spot is analysed by ESI-MS [11].

Urine

Urine analysed by negative ion FAB-MS or ESI-MS shows the characteristic ions of the diagnostic unsaturated bile acids: mass/charge ratios (m/z) = 469, 485, 526 and 542. Using ESI-MS/MS, the sulphated Δ^5 bile acids (m/z 469 and 485) are detected as parents of m/z 97; glycine conjugates of sulphated Δ^5 bile acids (m/z 526 and 542) are additionally detected as parents of m/z 74. Some patients excrete the di- and trihydroxy 5-cholenoic acids largely in non-sulphated forms (unconjugated [m/z 405] and conjugated with glycine [m/z 446,462]).

Fibroblasts

3β-Dehydrogenase can be assayed in cultured skin fibroblasts using tritiated 7α-hydroxycholesterol [12]. Patients show very low activity.

Treatment and Prognosis

Emergency treatment of coagulopathy with parenteral vitamin K may be required [4]. Vitamin D deficiency may be severe enough to require intravenous calcium as well as vitamin D therapy. However, long-term treatment with fat-soluble vitamins is not required because bile acid replacement therapy corrects all the fat-soluble vitamin deficiencies. Untreated 3β-dehydrogenase deficiency has led to death from complications of cirrhosis before the age of 5 years; patients with milder forms of the disorder may survive, with a chronic hepatitis or even remain asymptomatic, into their second decade or beyond. The response to treatment depends upon the severity of the liver disease at the time of starting treatment. In patients with a bilirubin level less than 120 μM and an AST level less than 260 U/l, chenodeoxycholic acid therapy has led to a dramatic improvement in symptoms and in liver function tests within 4 weeks, and to an improvement in the liver biopsy appearances within 4 months. The dose of chenodeoxycholic acid that has been used is 12–18 mg/kg/day initially (for 2 months), followed by 9–12 mg/kg/day maintenance. In one infant with severe disease, chenodeoxycholic acid (15 mg/kg/day) led to a rise in bilirubin and AST. Her treatment regimen was changed to 7 mg chenodeoxycholic acid/kg/day plus 7 mg cholic acid/kg/day. Over the course of 15 months, her bilirubin and transaminases returned to normal, and a repeat liver biopsy showed a more normal parenchyma and less inflammation. Follow-up of patients treated with chenodeoxycholic acid has shown that, after a median follow-up of 5.5 years (range 1–17 years) 12 out of 13 treated children had no signs of liver disease or of fat-soluble vitamin deficiency [4]. Gonzalez et al. have reported treatment with cholic acid alone in 15 patients with 3β-dehydrogenase deficiency. They described normalisation of physical examination findings, laboratory test results and liver ultrasound, and improvement in liver biopsy appearances [13]. Two women had normal pregnancies during treatment. Treatment can be monitored by suppression of urinary excretion of unsaturated bile acids [7][14].

Bile-acid-replacement therapy may work in one of two ways:

1. By fuelling bile acid-dependent flow (hence directly relieving cholestasis).
2. By suppressing the activity of cholesterol 7α-hydroxylase (thereby reducing the accumulation of potentially toxic metabolites of 7α-hydroxycholesterol).

33.2 Δ4-3-Oxosteroid 5β-Reductase Deficiency

Clinical Presentation

Patients who excrete 3-oxo-Δ^4 bile acids as the major urinary bile acids can be divided into four groups – those who have proven mutations in both alleles of *SRD5B1* (*AKR1D1*, the gene encoding the 5β-reductase enzyme) [15][16][17][18][19]; those in whom this has been excluded [20]; those in whom only one mutation has been found [18][19], and those in whom the results of gene analysis have not been published

[21][22]. In the last three groups, the cause of excretion of 3-oxo-Δ^4 bile acids remains uncertain and, since this pattern of urinary metabolite excretion can be a nonspecific consequence of severe liver disease [23][24], the description in this chapter will focus on the eight patients with proven 5β-reductase mutations on both alleles.

In three of the seven families described, the parents were consanguineous [16][17][18][19]. Seven of the 8 patients presented with cholestatic jaundice in the neonatal period, the eighth at 3 months. All 8 had raised transaminases which were associated with normal γ-GT in 6 and mildly elevated GGT in 2. Low vitamin E was documented in 3 and markedly prolonged clotting times, which improved with parenteral vitamin K, were recorded in one. Liver biopsies showed giant cell transformation, canalicular and hepatocellular cholestasis, portal inflammation, septal fibrosis, occasional necrotic foci and, in some cases, increased extramedullary haemopoiesis. Two patients had significant steatosis. Without treatment, cholestasis persisted in all cases.

Metabolic Derangement

Mutations in *SRD5B1* lead to reduced activity of the hepatic enzyme, 5β-reductase (▶ enzyme 3 in ◘ Fig. 33.1), that brings about the 5β(H) saturation of the C4 double bonds of bile acid precursors such as 7α-hydroxy-cholest-4-en-3-one and 7α,12α-dihydroxy-cholest-4-en-3-one. These intermediates can then undergo side-chain oxidation to produce the corresponding C24 bile acids. The mechanism of hepatocyte damage and cholestasis in 5β-reductase deficiency is unknown; as with 3β-dehydrogenase deficiency, toxicity of unsaturated intermediates and unsaturated bile acids and loss of / inhibition of bile acid-dependent bile flow have been postulated. Deficiency of the 5β-reductase enzyme also prevents 5β(H) saturation of the Δ^4 double bond of 3-oxo-Δ^4 steroid hormones; this affects urinary steroid profiles but does not appear to have any obvious physiological effects [25].

Genetics

Primary 5β-reductase deficiency is an autosomal recessive disorder caused by mutations in *SRD5B1* [15][16][17][18][19]. The homozygous mutations that have been described are c.385C>T (p.L106F), c.850C>T (p.R261C), c.511delT (frameshift, premature stop codon), and c.662C>T (p.P198L). Documented compound heterozygous mutations include c.467C>G (p-P133R) with c.850C>T (p.R261C), c.396C>A with c.722A>T and p.G223E with p.R261C. Mutant proteins appear to have markedly reduced stability [26].

Diagnostic Tests

Plasma

GC-MS analysis of plasma bile acids reveals low or low normal concentrations of chenodeoxycholic acid (normal concentration 0.2–12.7 μM) and cholic acid (normal concentration 0.4–6.7 μM) [12]. In contrast, the plasma concentrations of 3-oxo-Δ^4 bile acids are markedly elevated, i.e. to 7α-hydroxy-3-oxo-4-cholenoic acid >1.5 μM and 7α,12α-dihydroxy-3-oxo-4-cholenoic acid >2.0 μM. Analysis of plasma bile acids by ESI-MS/MS shows taurine-conjugated (parents of m/z 80) and glycine-conjugated (parents of m/z 74) 3-oxo-Δ^4 bile acids present at concentrations similar to those of their saturated analogues [15].

Urine

Analysis of urine by FAB-MS or ESI-MS/MS shows the presence of major ions attributable to the glycine conjugates of 7α-hydroxy-3-oxo-4-cholenoic acid and 7α,12α-dihydroxy-4-cholenoic acid (m/z = 444 and 460; parents of m/z 74) and their taurine conjugates (m/z = 494 and 510; parents of m/z 80) and sometimes the taurine conjugate of 7α,12α-dihydroxy-3-oxo-4-cholestenoic acid (m/z 552; parents of 80). The normal saturated bile acids (m/z 448, 464, 498, 514) are at background level.

The identities and relative amounts of urinary bile acids can be confirmed by GC-MS analysis following enzymatic deconjugation. In patients shown to have primary 5β-reductase deficiency, the 3-oxo-Δ^4 bile acids have comprised more than 90% of the total urinary bile acids; a lower percentage is found in most children whose excretion of 3-oxo-Δ^4 bile acids is secondary to liver damage of other aetiology.

Treatment and Prognosis

Emergency treatment for vitamin K deficiency may be required. Vitamin D may be needed for rickets. 5β-Reductase deficiency can progress rapidly to liver failure. However, treatment with bile acid replacement therapy can lead to normalisation of liver function and long-term (at least 14 years) good health. Successful regimens include; i) chenodeoxycholic acid plus cholic acid (8 mg/kg/day of each) [15][16]; ii) cholic acid alone (initially 13 mg/kg/day, subsequently 6 mg/kg/day) [17]; iii) ursodeoxycholic acid (40 mg/kg/d) for 4 months followed by chenodeoxycholic acid (25 mg/kg/d) [18]; and iv) ursodeoxycholic acid (7 mg/kg/d) with chenodeoxycholic acid (10 mg/kg/d reducing to 5 mg/kg/d) [19]. The patient who responded to chenodeoxycholic acid plus cholic acid had previously failed to respond to ursodeoxycholic acid alone and logic dictates that treatment probably requires a primary bile acid that can feed back to reduce synthesis of bile acid precursors. Infants with liver failure (prothrombin ratio >1.3) at the time of starting treatment did not respond adequately and were transplanted or died.

33.3 Cerebrotendinous Xanthomatosis (Sterol 27-Hydroxylase Deficiency)

Clinical Presentation

The average age of diagnosis of cerebrotendinous xanthomatosis is (CTX) 35 years with a diagnostic delay of 16 years; the disorder has been described as a paediatric disease diagnosed in adulthood [66]. Clinical signs and symptoms include adult-onset progressive neurological dysfunction and non-neurologic manifestations i.e. tendon xanthomas, premature atherosclerosis, osteoporosis, and respiratory insufficiency [27]. Sometimes, cholestatic jaundice in infancy is the first manifestation of CTX [28][29], however, it usually improves spontane-

ously. Chronologically, the next (or the first) symptom of CTX is often mental retardation detected during the first decade of life. Cataracts may also be present as early as 5 years of age. Wevers et al. [30] have documented four Dutch patients in whom persistent diarrhoea was present from early childhood. Motor dysfunction (spastic paresis, ataxia, expressive dysphasia) develops in approximately 60% of patients in the second or third decade of life. Tendon xanthomata may be detectable during the second decade of life but usually appear in the third or fourth decade. The Achilles tendon is the most common site; other sites include the tibial tuberosities and the extensor tendons of the fingers and the triceps. Premature atherosclerosis leading to death from myocardial infarction occurs in some patients. In others, death is caused by progression of the neurological disease with increasing spasticity, tremor and ataxia and pseudobulbar palsy. It is important to recognise that the neurological deterioration is very variable [31] (▶ Chapter 2). For example, some patients are normal intellectually but suffer from a neuropathy or mild spastic paresis; others have no neurological signs but present with psychiatric symptoms resembling schizophrenia. The most serious consequences of the disease are the development of xanthomas in the brain and the neurological symptoms caused by these. The preferential site of the brain xanthomas is in the white matter of the cerebellum. Magnetic resonance imaging (MRI) of the brain in CTX may show diffuse cerebral atrophy and increased signal intensity in the cerebellar white matter on T_2-weighted scans [32]. Osteoporosis is common in CTX and may produce pathologic fractures; it is associated with low plasma concentrations of 25-hydroxy-vitamin D and 24,25-dihydroxy-vitamin D [33]. Patients with untreated CTX usually die from progressive neurological dysfunction or myocardial infarction between the ages of 30 years and 60 years.

Metabolic Derangement

CTX is caused by a defect in the gene for sterol 27-hydroxylase (▶ enzyme 4 in ◘ Fig. 33.1), the mitochondrial enzyme that catalyses the first step in the process of side-chain oxidation, which is required to convert a C27 sterol into a C24 bile acid [34]. 5β-Cholestane-3α,7α,12α-triol cannot be hydroxylated in the C27 position and accumulates in the liver. As a result, it is metabolised by an alternative pathway, starting with hydroxylation in the C25 position (in the endoplasmic reticulum). Further hydroxylations, e.g. in the C22 or C23 position, result in the synthesis of the characteristic bile alcohols that are found (as glucuronides) in the urine. Bile acid precursors other than 5β-cholestane-3α,7α,12α-triol also accumulate. Some of these (e.g. 7α-hydroxy-cholest-4-en-3-one) are probably converted to cholestanol by a pathway involving 7α-dehydroxylation. Because patients with CTX have a reduced rate of bile-acid synthesis, the normal feedback inhibition of cholesterol 7α-hydroxylase by bile acids is disrupted. This further enhances the production of bile alcohols and cholestanol from bile acid precursors. The symptoms of CTX are produced in part by accumulation of cholestanol (and cholesterol) in almost every tissue of the body, particularly in the nervous system, atherosclerotic plaques and tendon xanthomata. Lack of 3β,7α-dihydroxy-5-cholestenoic acid may contribute to motor neuron damage [35].

Sterol 27-hydroxylase is active in extrahepatic tissues, where it converts cholesterol into 27-hydroxycholesterol, which can be further metabolised and eliminated from cells. This pathway provides a route for the elimination of cholesterol; this route acts as an alternative to the high-density lipoprotein-mediated reverse cholesterol transport [36]. Disruption of this pathway in CTX provides a further explanation for the accumulation of cholesterol in the tissues.

Genetics

CTX is inherited as an autosomal recessive trait. The cDNA encoding the 27-hydroxylase enzyme has been characterised, and the gene has been localised to chromosome 2q33-qter [37]. CTX can be caused by point mutations that lead to production of an inactive enzyme (p.R362C and p.R446C). In Moroccan Jews, there appear to be two common mutations, both of which lead to failure of the production of sterol 27-hydroxylase mRNA. One is a frame-shift mutation, the other is a splice-junction mutation [38]. Many other mutations have now been described [39].

Diagnostic Tests

Plasma

The concentration of cholestanol in plasma can be determined by GC or high-performance liquid chromatography (HPLC). Patients with CTX have plasma concentrations in the range of 30-400 μM (normal range = 2.6-16 μM). The plasma cholestanol / cholesterol ratio may be a better discriminant than the absolute cholestanol concentration. Plasma 27-hydroxycholesterol is below the normal range. The following bile acid precursors have been detected at increased concentrations in plasma: 7α-hydroxycholesterol, 7α-hydroxy-cholest-4-en-3-one, 7α,12α-dihydroxy-cholest-4-en-3-one. Plasma concentrations of bile acids are low; plasma concentrations of bile alcohol glucuronides are elevated.

Urine

Negative ion FAB-MS or ESI-MS/MS indicate that major cholanoids in the urine are cholestanepentol glucuronides, giving rise to an ion with m/z ratio 627 [40]. GC-MS analysis shows that the major alcohols are 3,7,12,23,25-pentols and 3,7,12,22,25-pentols in adults. Increased urinary bile-alcohol concentrations can be detected using an enzyme assay (7α-hydroxysteroid dehydrogenase) [40]. The urinary bile-alcohol excretion following cholestyramine administration has been used as a test for carriers of CTX [42].

Fibroblasts

27-Hydroxylation of C27 sterols can be measured in cultured skin fibroblasts, and the enzyme activity is virtually absent in fibroblasts from patients with CTX [43].

DNA

In certain populations in which one or two common mutations predominate, DNA analysis may prove to be a rapid

method for diagnosis of both homozygotes and carriers of CTX (see above).

Treatment and Prognosis

The results of treatment with chenodeoxycholic acid were first reported in 1984 [44]. The rates of synthesis of cholestanol and cholesterol were reduced, and plasma cholestanol concentrations fell. A significant number of patients showed reversal of their neurological disability, with clearing of the dementia, improved orientation, a rise in intelligence quotient and enhanced strength and independence. The MRI appearances do not, however, show obvious improvement [45]. Urinary excretion of bile-alcohol glucuronides is markedly suppressed. Chenodeoxycholic acid almost certainly works by suppressing cholesterol 7α-hydroxylase activity; ursodeoxycholic acid, which does not inhibit the enzyme, is ineffective. Adults have usually been treated with a dose of 750 mg/day chenodeoxycholic acid. Other treatments that have been used in CTX include 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins such as lovastatin) [46] and low-density lipoprotein apheresis [47]. There is insufficient information available to assess these forms of treatment at the present time. Cholestatic liver disease in infancy can be self-limiting, but in those children in whom it is not, bile acid treatment has been successful; cholic acid is probably preferable to chenodoexycholic acid [28][29].

33.4 α-Methylacyl-CoA Racemase Deficiency (see also ▶ Chapter 40)

Clinical Presentation

α-Methylacyl-CoA racemase (AMACR) deficiency was first described in 2000 [48]. Neurological problems can start at any age from childhood to late adult life. They include developmental delay, epilepsy, acute encephalopathy, tremor, pigmentary retinopathy, hemiparesis, spastic paraparesis, peripheral neuropathy, depression, headache, cognitive decline and ataxia [49][50][51]. In 2001, presentation with neonatal cholestatic liver disease was documented: Van Veldhoven et al. described an infant with AMACR deficiency who presented with a coagulopathy due to vitamin K deficiency; a sibling had died of a major bleed with the same cause [52][53]. The infant had mild cholestatic jaundice with raised aspartate aminotransferase and, in contrast to 3β-dehydrogenase deficiency, 5β-reductase deficiency and CTX, a raised γ-GT. Liver biopsy showed a mild nonspecific lymphocytic portal infiltrate and abundant giant cell transformation.

Metabolic Derangement

Side-chain oxidation of cholesterol produces the 25*R* isomer of 3α,7α,12α-trihydroxycholestanoyl-CoA [(25*R*)-THC-CoA], and α-oxidation of dietary phytanic acid produces (some) (2*R*)-pristanoyl-CoA. Before these substrates can undergo peroxisomal β-oxidation they need to be converted to the *S*-isomers by AMACR (▶ enzyme 5 in ◘ Fig. 33.1). It is likely that decreased production of cholic acid and chenodeoxycholic acid contributes to cholestatic liver disease and fat-soluble vitamin malabsorption. The pathogenesis of the neurological disease is not understood.

Genetics

AMACR deficiency is caused by mutations in *AMACR* on chromosome 5p13.2–5q11.1. Pathogenic mutations in the adults with neurological disease included a common mutation, p.S52P, and p.L107P [48][49][50][51]. The p.S52P mutation was also found in the siblings who presented with neonatal coagulopathy [53].

Diagnostic Tests

Analysis of plasma bile acids by GC-MS reveals increased concentrations of DHCA and THCA; HPLC-ESI-MS/MS can be used to show that it is the (25R) isomer of THCA that is accumulating. GC-MS analysis of fatty acids in plasma shows an elevated concentration of pristanic acid with mildly elevated/normal plasma phytanic acid concentration and normal very long chain fatty acids. Studies on cultured skin fibroblasts show very low activity of AMACR.

Treatment and Prognosis

Parenteral vitamin K may be life-saving. Cholic acid therapy was important in preventing continuing fat-soluble vitamin malabsorption in the cholestatic neonate described by van Veldhoven et al. and Setchell et al. [52][53]. Its role in improving the liver disease is less certain as, given that adults with the disorder do not show signs of liver disease, there may be spontaneous resolution (as in CTX). The role of a low phytanic acid diet is uncertain; it appeared to prevent further deterioration in at least one of the adults with neurological disease. The influence of bile acid therapy on the development and progression of neurological disease is also unknown at present.

33.5 Oxysterol 7α-Hydroxylase Deficiency

Clinical Presentation

Oxysterol 7α-hydroxylase was first described in a 10-week-old male infant with severe cholestasis, cirrhosis and liver synthetic failure [54]. A second patient was also jaundiced from early infancy and died of liver failure at 11 months [55]. We have diagnosed an infant who presented with liver failure and hypoglycaemia at 3 months but recovered completely with chenodeoxycholic acid treatment [56]. Oxysterol 7α-hydroxylase deficiency has also been identified as a cause of a recessive form of hereditary spastic paraplegia (HSP) [57][58]. Patients can present with neurological dysfunction at any age from 1 to 41 years. Weakness of the lower limbs with hypertonia and hyperreflexia is associated with posterior column sensory impairment as evidenced by diminished vibration sensation and proprioception, and some degree of bladder dysfunction.

Metabolic Derangement

This recessive disorder is due to mutations in the gene encoding microsomal oxysterol 7-hydroxylase (▶ enzyme 8 in

Fig. 33.1), leading to inactivity of this enzyme and accumulation of 27-hydroxycholesterol, 3β-hydroxy-5-cholestenoic acid and 3β-hydroxy-5-cholenoic acid. The pathway of bile acid synthesis via 27-hydroxycholesterol (which is thought to be very important in infancy) is completely disrupted, and the monohydroxy bile acids that accumulate are particularly hepatotoxic. Accumulation of 3β-hydroxy-5-cholestenoic acid and deficiency of 3β,7α-dihydroxy-5-cholestenoic acid probably contribute to motor neuron damage [35].

Genetics

The two reported children with liver disease were homozygous for nonsense mutations, R388X and R112X, respectively, in *CYP7B1* on chromosome 8q21.3. The patients with HSP were mostly homozygous for missense mutations p.S363F, p.F216S, p.G57R and p.R417H. However, one individual with HSP had the p.R388X nonsense mutation.

Diagnostic Tests

Analysis of urine by FAB-MS has revealed major peaks of m/z ratio 453 and 510 attributable to 3β-hydroxy-5-cholenoic acid 3-sulphate and its glycine conjugate. However, in our patient, ESI-MS revealed taurine-conjugated 3β-hydroxy-5-cholenoic (m/z 480) as the major abnormal bile acid.GC-MS analysis of plasma indicated that the main bile acids were 3β-hydroxy-5-cholenoic acid and 3β-hydroxy-5-cholestenoic acid. 27-hydroxycholesterol was also markedly elevated in plasma [54][55][56][57][58].

Treatment and Prognosis

The first patient reported showed a deterioration with ursodeoxycholic acid and no improvement with cholic acid, and required a liver transplant for hepatic failure at the age of 4 months. The second also failed to respond to ursodeoxycholic acid, and died of liver failure at 11 months. Our patient responded rapidly to treatment with chenodeoxycholic acid (initially 25mg/kg/d) and is well at 6 years of age. Obviously, some patients must never develop significant liver disease and present later with HSP.

33.6 Bile Acid Amidation Defect 1: Bile Acid CoA: Amino Acid N-Acyl Transferase Deficiency

Clinical Presentation

Bile acid CoA: amino acid *N*-acyl transferase (BAAT) deficiency is found amongst the Amish, in whom presentation takes the form of failure to thrive, with pruritus in some cases, and occasionally coagulopathy, but without jaundice [59]. Two out of four affected patients suffered chronic upper respiratory infection. We have diagnosed BAAT deficiency in a 3-month-old infant with cholestatic jaundice, vitamin D deficiency and mild portal and focal lobular hepatitis seen on liver biopsy [60]. Setchell et al. described 8 patients with homozygous mutations in *BAAT* presenting with fat-soluble vitamin deficiency, some with growth failure or transient neonatal cholestatic hepatitis [61].

Metabolic Derangement

Without the enzyme bile acid coenzyme A: amino acid *N*-acyl transferase (▶ enzyme 7 in Fig. 33.1), encoded by *BAAT*, the CoA esters of chenodeoxycholic acid and cholic acid cannot be converted to their glycine and taurine conjugates. The unconjugated bile acids are secreted into the bile but are inefficient at solubilising lipid in the gut. Hence the failure to thrive and fat-soluble vitamin malabsorption.

Genetics

Defective amidation of bile acids in the Amish is caused by homozygosity for a missense mutation (c.226A>G; p.M76V) in *BAAT*. Our patient was homozygous for a nonsense mutation (p.R139X). The cohort described by Setchell also had homozygous mutations – c.1156C>A (p.G386R), c.206A>T (p.D69V), c.58C>T (p.R20X) and c.250C>A (p.P84T).

Diagnostic Tests

Analysis of urine by negative ion FAB-MS or ESI-MS shows that the major urinary bile acid is an unconjugated trihydroxy-cholanoic acid (m/z 407); GC-MS shows that it is unconjugated cholic acid. Other bile acids that may be detected include sulphated dihydroxycholanoic acid(s) (m/z 471) and trihydroxycholanoic acids (m/z 487) and glucuronidated dihydroxycholanoic acid(s) and trihydroxycholanoic acid(s) (m/z 567 and 583).

Treatment and Prognosis

Treatment of vitamin K deficiency may be life saving, treatment of rickets may require 1α-hydroxycholecalciferol or 1,25-dihydroxycholecalciferol. The Amish patients probably had improvement in symptoms with ursodeoxycholic acid as did our patient. Treatment with glycocholic acid (15 mg/kg/d) led to an improvement in growth in prepubertal patients with growth delay and improved vitamin D and vitamin E absorption as judged by loading tests. [62].

33.7 Bile Acid Amidation Defect 2: Bile Acid CoA Ligase Deficiency

Clinical Presentation

Mutations in the bile acid-CoA ligase encoded by *SLC27A5* lead to a urine bile acid profile dominated by unconjugated cholic acid (very similar to the profile seen in patients with *BAAT* mutations). However, whether there is a similar phenotype is currently uncertain. Two sisters born to consanguineous Pakistani parents who share the same genotype have been described. One was asymptomatic; the other had cholestatic liver disease but had two other possible causes for cholestasis – a prolonged period of parenteral nutrition as a premature neonate, and a homozygous missense mutation (c.1772A > G) in *ABCB11*, predicted to alter a highly conserved amino-acid residue (p.N591S) in bile salt export pump (BSEP) [63].

Metabolic Derangement

In the gut, taurine- and glycine-conjugated bile acids are hydolysed by bacteria, producing free cholic acid and chenodeoxycholic acid. These bile acids return to the liver in the enterohepatic circulation and must be converted to their CoA esters prior to reconjugation with taurine and glycine; this is thought to be the main role of the bile acid CoA ligase (▶ enzyme 9 in ◘ Fig. 33.1). Deficiency leads to a build-up of unconjugated bile acids in the enterohepatic circulation and, as they are less efficient detergents than the conjugated bile acids, there is malabsorption of fat and fat-soluble vitamins.

Genetics

Analysis of *SLC27A5* showed that the sisters were homozygous for a mutation in this gene – p.His338Tyr; c.1012c>t, which is in a highly conserved area of the gene, and which is probably important for protein activity.

Diagnostic Tests

The urine bile acid profile, shows the following compounds: nonamidated chenodeoxycholic acid (391) and cholic acid (407; major peak), their glucuronides (567 and 583) and chenodexocholic acid sulphate (471). Plasma bile acids are 89% unamidated (normal <20%). Screening of *SLC27A5* shows mutations.

Treatment and Prognosis

Treatment with oral glycocholic acid may be considered in view of the success reported for the BAAT defect [62].

33.8 Cholesterol 7α-Hydroxylase Deficiency

Clinical Presentation

Homozygous cholesterol 7α-hydroxylase deficiency has been detected in three adults with hypercholesterolaemia, hypertriglyceridaemia and premature gallstone disease [64]. One had premature coronary and peripheral vascular disease. Their LDL cholesterol levels were noticeably resistant to treatment with HMG-CoA reductase inhibitors (statins). A study of the kindred revealed that individuals heterozygous for the mutation were also hyperlipidaemic, indicating that this is a co-dominant disorder.

Metabolic Derangement

Cholesterol 7α-hydroxylase (▶ enzyme 1 in ◘ Fig. 33.1) is the first step in the major pathway for bile acid synthesis (and therefore for cholesterol catabolism) in adults. Reduced activity of the enzyme leads to accumulation of cholesterol in the liver, leading to down-regulation of LDL receptors and hypercholesterolaemia.

Genetics

Cholesterol 7α-hydroxylase deficiency is caused by mutations in *CYP7A1*. The only mutation described to date is a frameshift mutation (p.L413fsX414) that results in loss of the active site and enzyme function.

Diagnostic Tests

In one homozygote the cholesterol content of a liver biopsy was shown to be increased. Faecal bile acid output was reduced, and the ratio chenodeoxycholic acid-derived faecal bile acids/cholic acid-derived faecal bile acids was increased, suggesting increased activity of the alternative 27-hydroxylase pathway for bile acid (predominantly chenodeoxycholic acid) synthesis.

Treatment and Prognosis

Treatment with a powerful HMG-CoA reductase inhibitor (atorvastatin) and niacin is required to bring plasma levels of cholesterol and triglycerides under control. The variability of the disorder and the long-term prognosis are not known.

33.9 Disorders of Peroxisome Biogenesis, Peroxisomal Import and Peroxisomal β-Oxidation

These conditions are described in ▶ Chapter 40. Neurological disease usually dominates the clinical picture, but some children with Zellweger syndrome or infantile Refsum's disease have quite marked cholestatic liver disease. Mutations in *ABCD3* cause a defect involving peroxisomal import of THCA-CoA, DHCA-CoA and branched-chain fatty acyl-CoAs which was associated with liver disease with onset in infancy and needing liver transplantation by 4 years [65].

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Disorders of Intracellular Triglyceride and Phospholipid Metabolism

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Acylglycerols and phospholipids (PL) play a myriad of organ-specific roles in cell structure, biochemistry and signalling. Inborn errors of glycerolipid metabolism cause a correspondingly vast array of clinical phenotypes. Molecular analysis is currently the principal diagnostic technique. Next generation sequencing has recently led to the discovery of several genetic defects of the biosynthesis and remodelling of triglycerides (TGs), other acylglycerols [1] and of PLs [2]. Few disorders of glycerolipid metabolism have distinct metabolite patterns by conventional techniques, but lipidomic analysis is a promising new approach that may expand the role of biochemical diagnosis [3, 4].

The discovery of many new inborn errors of glycerolipid metabolism (► Phospholipid remodelling and signalling at the cell membrane) offers unprecedented opportunities for clinical description and for rethinking clinical intervention. Some of these conditions produce striking clinical syndromes identifiable in infancy; others, mildly atypical forms of common adult conditions like metabolic syndrome and type II diabetes. Inborn errors of intracellular TG metabolism, reviewed in [1], often affect adipose tissue, but non-adipose signs can dominate their clinical presentation. Disorders of extracellular TG-containing lipoproteins are discussed in Chapter 31. Inborn errors of PL metabolism often affect the central and peripheral nervous systems, but also muscle, eye, skin, bone, cartilage, liver, kidney and immune system [5]. Here we summarize the current clinical understanding of inborn errors of glycerolipid metabolism, recognizing that to date many of the conditions are only partially described in small numbers of patients and that some steps of glycerolipid metabolism are not yet associated with an inborn error.

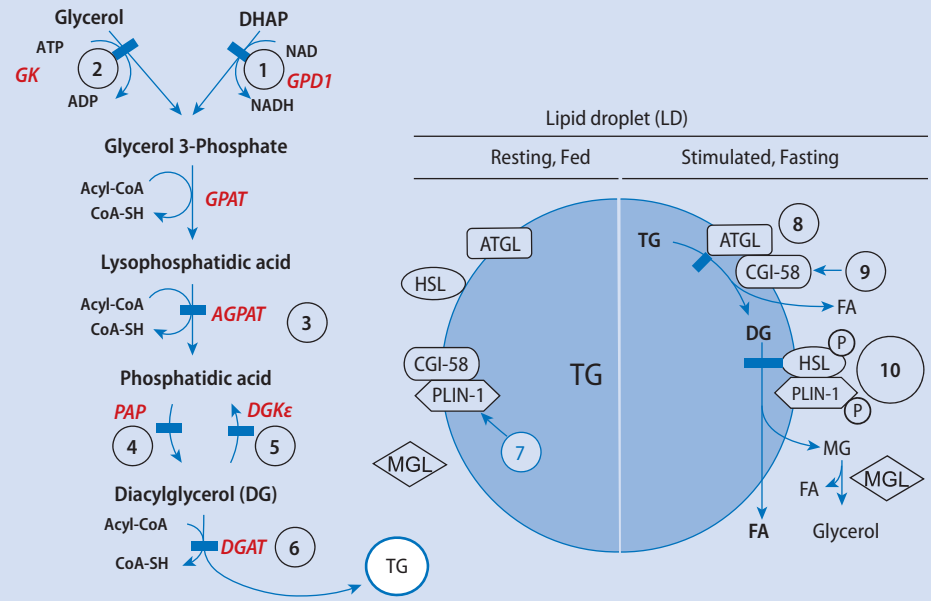

Fig. 34.1 The common pathway, triglyceride synthesis and lipolysis. Overview and common pathway. Phospholipids (PLs) form the bilayer of cell membranes. PLs all share a phosphate group that constitutes a hydrophilic head, located on the surface of the bilayer and a fatty acid chain that forms a hydrophobic tail, located inside. Triglycerides (TGs) are extremely hydrophobic, and form droplets that are surrounded by a PL monolayer. The first steps of TG and of PL synthesis are the same. De novo synthesis begins from glycerol-3-phosphate (G3P), which is formed from glycerol or from dihydroxyacetone phosphate (DHAP), an intermediate of both glycolysis and of gluconeogenesis. G3P is esterified twice, first by glycerolphosphate acyltransferases (GPATs) to form lysophosphatidic acid (LPA), then by acylglycerol acyltransferases (AGPATs) to form phosphatidic acid (PA). Phosphatidic acid can either be converted into an sn-1,2-diacylglycerol (DG) by phosphatidic acid phosphatases (PAP, lipins-1, 2 and 3) or into CDP-diacylglycerol by phosphatidic acid cytidyltransferase (CDP-DAG synthase, ◘ Fig. 34.2). DG is an essential intermediate for the synthesis of triglycerides (TG) and of three major PLs: phosphatidyl choline (P-Chol), phosphatidyl ethanolamine (P-Eth) and phosphatidyl serine (P-Ser).

TG biosynthesis and lipolysis: Phosphatidic acid phosphatases (PAPs, LPIN1-3) catalyse phosphate removal, the first dedicated step of TG synthesis, creating a sn-1,2-diacylglycerol. Then one of two diacylglycerol acyltransferases (DGATs) can catalyze TG synthesis. TGs accumulate between the leaflets of the ER lipid bilayer. The budding of lipid droplets (LDs) requires seipin. Perilipins are found on the surface of all LDs; perilipin 1 is the most abundant LD surface protein of adipocytes. Adipocyte lipolysis is activated during fasting to export fatty acids (FAs) and glycerol for fuel. The main TG lipase in adipocytes, adipocyte triglyceride lipase (ATGL) is on the LD surface. Interaction with CGI-58 enhances the lipolytic capacity of ATGL, which produces sn-1,3-DGs and to a lesser extent sn-2,3-DGs. DGs are hydrolyzed by hormone-sensitive lipase (HSL), producing mainly sn-1-MGs. Monoacylglyceride hydrolase(s) liberate glycerol and a FA. Perilipin 1 suppresses lipolysis in the fed state; beta adrenergic stimulation and low circulating insulin levels favor PLIN1 and HSL phosphorylation by protein kinase A (PKA), activating lipolysis. Solid bars indicate metabolic blocks. Circled numbers refer to the text

34.1 Inborn Errors of the Common Pathway of Acylglycerol and Phospholipid Synthesis

Fig. 34.1.

34.1.1 Glycerol-3-phosphate Dehydrogenase 1 (GPD1) Deficiency: Autosomal Recessive Hepatic Steatosis and Hypertriglyceridemia

Fig. 34.1, step 1.

Eleven patients have first described [6], ten from an Israeli Arab isolate. All had hypertriglyceridemia (2.5–70 mmol/L at presentation), fatty liver, hepatomegaly from birth, and 4- to 8-fold elevations of serum aminotransferase and gamma glutamyltransferase levels. Patients tended to improve with age although liver fibrosis was suspected in some and the long term course and full clinical spectrum are unknown. Heterozygotes were asymptomatic [6]. A recent publication enlarges somewhat the phenotypic spectrum [84].

GPD1 deficiency should be considered in the differential diagnosis of infantile hepatomegaly and fatty liver, especially if liver transaminases are increased and hypertriglyceridemia is present.

The diagnosis has been reached by gene sequencing in all patients described. Growth improved with a hypercaloric low fat, high carbohydrate diet and medium chain triglyceride supplementation. The mechanisms underlying the development of fatty liver and hypertriglyceridemia are not obvious from the pathway (Fig. 34.1) and treatments must be evaluated empirically.

34.1.2 Glycerol Kinase Deficiency is described in ▸ Chapter 7

Fig. 34.1, step 2.

34.1.3 1-Acylglycerol-3-Phosphate O-Acyltransferase 2 (AGPAT2) Deficiency: Autosomal Recessive Generalized Congenital Lipodystrophy

Fig. 34.1, step 3.

Autosomal recessive deficiency of AGPAT2 underlies nearly half of cases of severe congenital generalized lipodystrophy [7]. Patients have very low white adipose tissue (WAT) mass, visceral steatosis, insulin resistance, hypertriglyceridemia and low circulating levels of leptin and adiponectin.

Clinically, AGPAT2 deficiency cannot be reliably distinguished from other severe congenital generalized lipodystrophies [7], the commonest of which is seipin deficiency [8]. AGPAT2 deficiency may be severe, with fat loss in mechanical areas (palms, soles, orbits, scalp, periarticular regions); both AGPAT and seipin deficiencies show fat depletion in intermuscular, bone marrow, intraabdominal and intrathoracic WAT [9]. Molecular testing of the AGPAT2 and seipin genes is the preferred diagnostic method.

Adequate nutrition and treatment of insulin resistance are important. Leptin replacement therapy may improve some metabolic complications [9][10].

34.1.4 Phosphatidic Acid Phosphatase (PAP; LIPIN) Deficiencies

Fig. 34.1, step 4.

The pathophysiology of Lipin deficiencies is incompletely understood, perhaps being related in part to the non-catalytic functions of lipins. In the nucleus, lipins directly influence the transcription of target genes of PPAR alpha, PPAR gamma and nuclear factor of activated T-cells c4 (NFATc4) [11].

▪ Lipin-1 Deficiency: Recurrent Childhood Rhabdomyolysis with Myoglobinuria

Autosomal recessive deficiency of lipin-1 (*LPIN1* gene), accounts for ~10% of patients with severe recurrent childhood rhabdomyolysis, occurring particularly between 2–6 years of age [12][13]. Creatine kinase levels >10,000 IU/L can occur after exercise, fever, anaesthesia or fasting. Cardiac arrest, sometimes with hyperkalaemia, is reported. Autopsies have shown cardiomyopathy and hepatic steatosis [13]. Between episodes, clinical evaluation is usually normal but chronic myolysis with proximal weakness can occur. WAT mass and distribution, peripheral nerve function and plasma levels of cholesterol, TGs and adiponectin appear to be normal. Rarely, heterozygotes develop rhabdomyolysis under stress (e.g., statin therapy) [12].

Lipin-1 deficiency should be considered in children with recurrent rhabdomyolysis and normal plasma acylcarnitines. Molecular testing, including deletion analysis to detect a common deletion mutation, is the preferred diagnostic method. Muscle histology is not specific for LPIN1 deficiency, but may help to rule out glycogenoses and respiratory chain disorders, although ragged red fibers may occur. If performed, a muscle biopsy should be obtained after recuperation from the rhabdomyolytic episode.

Symptomatic treatment of rhabdomyolysis includes aggressive intravenous fluid administration, monitoring of electrolytes, kidney and heart function and haemodialysis, if renal function is felt to be at risk [15]. In addition to its catalytic function, lipin-1 also influences gene transcription [14] and may be related to inflammation [16]. Suppression of inflammation may have therapeutic potential, but this hypothesis is untested [16].

Lipin-2 Deficiency: Autosomal Recessive Recurrent Multifocal Non-Infectious Autoimmune Osteomyelitis, Skin Pustules and Anaemia: Majeed Syndrome

Reported only in four families to date, Majeed syndrome, an autosomal recessive disease, has a characteristic clinical triad [17]: (i) Chronic recurrent multifocal osteomyelitis of early onset (3 weeks to 2 years) and lifelong course, often recurring for a few days every 3–4 weeks, with high fever, severe pain, and periarticular tender soft tissue swelling. Standard radiography reveals irregular osteolytic (radiolucent) lesions with surrounding sclerosis. MRI of active bone lesions reveals increased signal intensity on T_2-weighted images, and decreased intensity with T_1-weighting. (ii) Congenital dyserythropoietic anemia presenting during the first year. (iii) In some individuals, transient inflammatory pustular dermatosis (Sweet syndrome). Transient hepatomegaly, neutropenia, and cholestatic jaundice have been reported in neonates. Erythrocyte sedimentation rate is elevated. Cultures of blood, bone or skin lesions are sterile.

The differential diagnosis is broad and evaluation requires expertise in immunology, haematology, and infectious disease. Similar conditions include the following [17]:

- Periodic fever syndromes, infectious osteomyelitis, and the synovitis, acne, pustulosis, hyperostosis, and osteitis (SAPHO) syndrome, found principally in adults,
- Pyogenic arthritis, pyoderma gangrenosum, and acne, (PAPA) syndrome (autosomal dominant, *PSTPIP1* mutations),
- CINCA syndrome, a congenital inflammatory disorder with cutaneous rash, neurologic impairment, and arthropathy caused by heterozygous *CIAS1* mutations,
- Deficiency of the interleukin-1 receptor antagonist (DIRA), and
- Gaucher type 1 disease

Clinical diagnosis is possible if all three elements are present. The diagnosis should be suspected in the presence of even one element and is confirmed by molecular testing of *LPIN2*.

For inflammation, nonsteroidal anti-inflammatory agents are prescribed, with physical therapy to avoid disuse atrophy for which patients are at high risk. Chronic corticosteroid treatment is avoided because of its long-term complications. Other anti-inflammatory treatments should be considered. For instance, two children with Majeed syndrome had resolution of bone inflammation when treated with an IL-1 inhibitor [18].

34.1.5 Diacylglycerol Kinase Epsilon (DGKE) Deficiency: Atypical Haemolytic Uremic Syndrome

Fig. 34.1, step 5.

In individuals with early onset, autosomal recessive atypical haemolytic-uremic syndrome (aHUS), Lemaire et al [19] found mutations in *DGKE*. Affected individuals presented in the first year of life with repeated episodes of microangiopathic haemolytic anaemia, thrombocytopenia and acute renal failure. The early onset defines a distinct subgroup of aHUS. Among subjects with *DGKE* mutations, renal function recovered after the onset of aHUS, but hypertension, microhaematuria and proteinuria persisted. Progression to chronic kidney disease was common by the second decade of life, long after the last acute episode of aHUS. Of note, nephrotic syndrome developed 3–5 years after disease onset, which is very rare in other forms of HUS.

DGKE preferentially phosphorylates arachidonic acid–containing diacylglycerols (AADAG) to their corresponding phosphatidic acids. AADAGs activate PKC9. In endothelial cells, PKC increases the production of various prothrombotic and antithrombotic factors. Phosphorylation of AADAG to phosphatidic acid by DGKE terminates AADAG signalling, it is therefore plausible that loss of DGKE results in sustained AADAG signalling, causing a prothrombotic state.

Other causes of HUS are the typical periinfectious form and genetic causes of aHUS including disorders of the complement cascade and untreated cobalamin C disease (cblC).

Anticomplement therapy may be efficacious in primary complement disorders but is ineffective in *DGKE*-related aHUS. In contrast, unlike individuals with soluble complement defects, it seems that renal transplantation can be efficacious and safe in aHUS caused by *DGKE* mutations [19].

34.2 Inborn Errors of Cytoplasmic Triglyceride Metabolism

Fig. 34.1.

34.2.1 Diacylglycerol O-Acyl Transferase 1 (DGAT1) Deficiency: Congenital Diarrhea

Fig. 34.1, step 6.

Two siblings with severe watery diarrhoea from birth and protein-losing enteropathy requiring long-term parenteral nutrition were homozygous for a deleterious splicing mutation in *DGAT1* [20]. One, a girl, died of infection aged 17 months. The other, a boy, improved after ten months of age, tolerating a normal diet by 46 months. In each, plasma TGs were two fold higher than normal and plasma cholesterol was normal.

Documentation of DGAT1 deficiency in further patients with congenital diarrhoea will be necessary before formally accepting that DGAT1 deficiency causes this phenotype. Conversely, consideration of DGAT1 deficiency is reasonable in patients with unexplained congenital diarrhoea and protein loosing enteropathy. Molecular analysis of *DGAT1* is the most convenient diagnostic method.

34.2.2 Perilipin 1 Deficiency: Autosomal Dominant Partial Lipodystrophy

Fig. 34.1, step 7.

Heterozygous frameshift mutations in *PLIN1* were described in five women aged 25-54 years, from three families, with partial lipodystrophy and android distribution of subcutaneous fat [21]. Their total fat mass was reduced by ~30% but lean masses were high, producing a normal body mass index. Other findings included fatty liver, elevated plasma glucose and insulin, 2-fold elevation of plasma TGs and low levels of HDL cholesterol and adiponectin. One patient required insulin treatment. Adipose biopsies showed reduced mean adipocyte diameter, macrophage infiltrate and fibrosis.

All of the few reported PLIN1-deficient patients show partial lipodystrophy. These patients lack the striking facial and nuchal fat accumulation seen in Dunnigan partial lipodystrophy [22] but otherwise it is difficult to distinguish perilipin deficiency from other forms of partial lipodystrophy. Diagnosis is by molecular testing of *PLIN1*.

34.2.3 Neutral Lipid Storage Diseases (NLSDs): ATGL and CGI-58 Deficiencies

Fig. 34.1, steps 8 and 9.

Two clinical forms of NLSDs are known: NLSD with ichthyosis (NLSDI) and NLSD with lipid myopathy and/or cardiomyopathy (NLSDM) [23]. NLSDI results from CGI-58 deficiency and NLSDM, from ATGL deficiency [24]. In both, the peripheral blood smear reveals Jordan's anomaly, i.e., vacuolated polymorphonuclear leucocytes. Vacuoles appear »empty« on Giemsa staining and red with Oil Red O staining for neutral lipid.

α,β-Hydrolase Domain-Containing 5 (ABHD5 or CGI-58) Deficiency (NLSDI): Neutral Lipid Storage Disease with Ichthyosis (Chanarin Dorfman Syndrome)

Nonbullous congenital ichthyosis occurs, often affecting skin flexures, scalp and face, with hyperkeratosis of palms and soles and pruritus. Neonates can present as collodion babies [25]. Liver involvement is frequent, with 2- to 4-fold elevations of plasma aminotransferases, steatosis, steatohepatitis, fibrosis and cirrhosis [26]. Myopathy may occur, with elevated serum creatine kinase, abnormal electromyographic studies and excess neutral lipid in types 1 and 2 fibres [27, 28]. Neurosensory deafness, cataract, nonprogressive psychomotor retardation, ataxia, spasticity and cardiomyopathy are reported.

The differential diagnosis of ichthyosis is vast. Lipids are essential for normal skin barrier function and several lipid metabolic disorders cause ichthyosis [29]. To diagnose NLSDI, Jordan's anomaly should be carefully searched for. Routine skin biopsy is often negative for neutral fat droplets because standard fixation procedures remove fat. Molecular diagnosis is useful (gene *ABDH5*).

Treatment is symptomatic. Case reports describe skin improvement with retinoid treatment [25][30].

Adipocyte Triglyceride Lipase (ATGL or PNPLA2) Deficiency: Neutral Lipid Storage with Myopathy (NLSDM)

ATGL deficiency [31][32] typically presents in young adults with weakness and fatty infiltration of muscle or with cardiomyopathy. Weakness can be proximal, distal or generalized. It is progressive: some patients are athletic in childhood. Among 18 patients, cardiomyopathy, 9/18 (50%); hypertriglyceridemia, 8/18 (44%); hepatomegaly, 5/14 (36%) and diabetes, 4/16 (25%) were observed [32]. Serum creatine kinase levels were elevated in all 17 patients (mostly ~200–2000 units/L; maximum 4700). TGs accumulate mainly in type I (oxidative slow twitch) fibres. Dilated cardiomyopathy may be severe and require cardiac transplantation. One patient showed coronary artery lipid accumulation [31]. Another study [33] revealed high visceral and pancreatic fat. Severe rhabdomyolytic episodes have not been reported. Some heterozygotes accumulate lipid in muscle, leukocytes (Jordan's anomaly) and basal keratinocytes [34] and some are symptomatic: lipid myopathy, 16/21 (76%); cardiomyopathy 9/21 (42%, including ventricular tachycardia requiring a pacemaker); hepatomegaly, 4/21 (19%); insulin resistance or diabetes, 3/21(14%).

In patients with myopathy or cardiomyopathy, the diagnosis is suggested by high neutral lipid content in muscle, the presence of Jordan's anomaly and with normal plasma acylcarnitine levels to exclude disorders of long chain fatty acid oxidation (▶ Chapter 12). It is confirmed by molecular testing of *PNPLA2*.

Because of the encouraging results in animals, controlled trials with PPAR alpha agonists seem indicated but presently there is no evidence of effectiveness in human ATGL deficiency [35]. High protein diet and avoidance of fasting are predicted to be beneficial in ATGL deficiency [36] but are untested in humans.

Of note, some heterozygotes are symptomatic. Siblings and parents should be tested for Jordan's anomaly, heart dysfunction and muscle weakness. If causal *PLPNA2* mutations are known, heterozygote testing to distinguish at-risk individuals may be considered.

34.2.4 Hormone-Sensitive Lipase (HSL) Deficiency; Insulin Resistance, Diabetes

HSL deficiency (Fig. 34.1, step 10) has been reported in only four individuals, diabetic Amish siblings with a frameshift deletion in *LIPE* [37]. Adipose biopsy revealed absence of immunoreactive HSL, small adipocytes, impaired lipolysis, increased DG content and inflammation. In an Amish population study, heterozygotes had insulin resistance and increased plasma TG levels (109.9±71.0, versus 84.6±59.6 mg/dL in controls and 145 in one homozygote), but had normal fat mass and blood pressure.

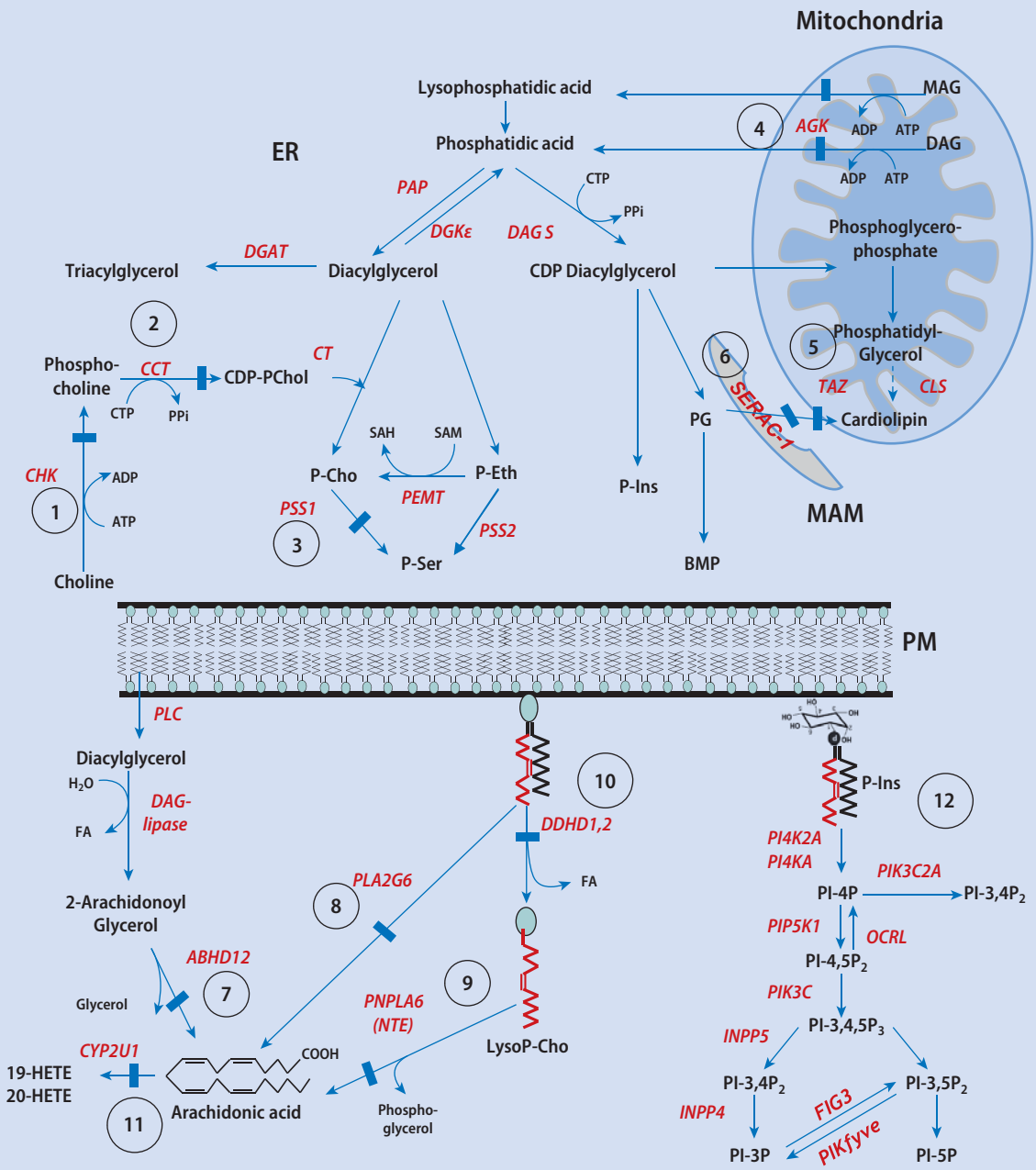

Fig. 34.2 Phospholipid biosynthesis (top of the figure) and remodelling (bottom of the figure). De novo biosynthesis of phosphatidylcholine (P-Cho), the most abundant PL in cells, requires the phosphorylation of choline into phosphocholine (PC) through a reaction catalyzed by choline kinase (CHK). Using cytidine-triphosphate (CTP), P-Chol is converted into CDP-phosphocholine (CDP-P-Choline) by phosphocholine cytidylyltransferase (CCT), a rate-limiting step in the synthesis of P-Cho. The final step in the biosynthesis of P-Chol is catalysed by choline transferase (CT) by which choline is transferred from CDP-P-Choline to DAG. This step is known as the Kennedy pathway. P-Chol can also be synthesized from phosphatidylethanolamine (P-Eth) methylation by a reaction catalysed by phosphatidylethanolamine N-methyltransferase (PEMT). The de novo biosynthesis of P-Eth also occurs via the Kennedy pathway and follows the same steps with specific enzymes. In contrast to P-Cho and P-Eth, phosphatidylserine (P-Ser) cannot be synthetized de novo but is obtained from either P-Cho and P-Eth by reactions catalyzed by phosphatidylserine synthases PSS1 and PSS2, respectively. The major site of cellular lipid synthesis is the endoplasmic reticulum (ER), however, mitochondria also synthesize certain of their structural PLs, particularly cardiolipin (CL), an important phospholipid of the inner mitochondrial membrane. Phosphatidic acid (PA), in addition to its de novo synthesis, can be recycled from other lipids in mitochondria. Mitochondrial acylglycerol kinase (AGK) can synthesize lysophosphatidic acid (LPA) and PA,

from monoacylglycerol and diacylglycerol, respectively. CDP-diacylglycerol is converted into phosphoglycerolphosphate (PGP) and then into phosphatidylglycerol (PG). Bis(monoacylglycero)-phosphate (BMP) is a unique phospholipid predominantly found in late endosomes. A protein with a lipase domain encoded by SERAC1 and localized to the mitochondria-associated membrane (MAM) is essential for PG and BMP remodelling and transport. The final step is the synthesis of CL formed by the condensation of PG with CDP-DAG, a reaction catalysed by cardiolipin synthase (CLS). CL formation as a mature and symmetric conformation requires a remodelling enzyme, monolyso-cardiolipin acyl transferase encoded by the TAZ gene. Solid bars indicate metabolic blocks. Circled numbers refer to the text

34.3 Inborn Errors of Phospholipid Biosynthesis

Seven inherited disorders of de novo PL synthesis have been described. Three involve proteins located in the endoplasmic reticulum (◘ Fig. 34.2, steps 1, 2 and 3). The other four are proteins of the mitochondrial membrane (◘ Fig. 34.2 steps 4, 5 and 6). The distinction between *de novo* synthesis and remodelling defects is unclear in some cases, such as with mutations in *PNPLA8*, a gene which encodes the main phospholipase of mammalian mitochondria.

34.4 Choline Kinase β (CHKβ) Deficiency: Congenital Muscular Dystrophy, Megaconial Type

◘ Fig. 34.2, step 1.

CHKβ deficiency is an autosomal recessive congenital muscular dystrophy, with early onset muscle wasting, mental retardation and abnormal mitochondrial morphology [38]. Some patients also had autistic features, ichthyosis-like skin abnormalities and dilated cardiomyopathy. Recently, two patients with congenital neurogenic muscular atrophy progressing to a combined neuropathic and myopathic phenotype were described [39].

CHKB encodes choline kinase β, which catalyses the first step of *de novo* biosynthesis of phosphatidylcholine, the major PL component of the external leaflet of eukaryotic plasma membranes [40]. A complete loss of CHK enzyme activity was associated with decreased levels of phosphatidylcholine and of the phosphatidylcholine/phosphatidylethanolamine ratio in muscle biopsies [38]. No phenotype-genotype correlation is known.

Brain MRI is often normal but one patient presented with thinning of corpus callosum and another with cerebral atrophy. Mildly elevated serum creatine kinase can serve as a simple screening test in patients suspected with CHK β deficiency. Histological study of biopsied muscle showed a typical pattern of enlarged mitochondria, more prevalent toward the periphery of fibres, but sparse in the centre. Dilated cardiomyopathy is a major cause of mortality in patients with *CHKB* mutations.

No specific treatment is presently known. Diagnosis is by molecular analysis.

34.4.1 Choline-PhosphateCytidylyltransferase α (CCTα) Deficiency: Spondylometaphyseal Dysplasia with Cone-Rod Dystrophy or Congenital Lipodystrophy

◘ Fig. 34.2, step 2.

Loss-of-function mutations in *PCYT1A*, which encodes CCTα have been reported in 3 independent manuscripts, in association with two distinct autosomal recessive phenotypes: spondylometaphyseal dysplasia (SMD) with cone-rod dystrophy [41][42] and congenital lipodystrophy [43]. SMD with cone-rod dystrophy, reported in 12 patients, causes postnatal growth deficiency, short stature, rhizomelic limb shortening, early bowing of the long bones of the legs, platyspondyly and shortening of all tubular bones. Patients had progressive early-onset visual impairment, related to pigmentary maculopathy and cone-rod dystrophy. Two unrelated females with *PCYT1A* mutations presented congenital lipodystrophy, insulin resistance and non-alcoholic liver steatosis, but no skeletal or visual abnormalities [43].

CCTα is the rate limiting enzyme in *de novo* biosynthesis of phosphatidylcholine (P-Cho) by the Kennedy pathway that leads from choline to P-Cho and is the main synthetic pathway of P-Cho. P-Cho is the most abundant PL in mammalian cells. Perturbation of P-Cho synthesis has dramatic effects on membrane and cell functions. PLs are important for bone formation, and mineralization by the ability of anionic phosphate group to bind calcium [44].

Low HDL cholesterol levels were seen in both clinical forms. Diagnosis is by molecular analysis.

34.4.2 PhosphatidylserineSynthase 1 (PSS1) Gain of Function (Lenz-Majewski Hyperostotic Dwarfism)

◘ Fig. 34.2, step 3.

Heterozygous *PTDSS1* mutations have recently been reported in Lenz-Majewski syndrome, also known as Lenz-Majewski hyperostotic dwarfism [45], an extremely rare condition with sclerosing bone dysplasia, intellectual disability and distinct craniofacial, dental, cutaneous (cutis laxa), distal limb anomalies (brachydactyly and symphalangism) and severe growth retardation. Skeletal radiography showed progressive generalized hyperostosis affecting the cranium, vertebrae, and diaphyses of tubular bones. The patients had a progeroid appearance. To date, all known mutations were

shown to be *de novo*. As expected, patients as shown came from diverse ethnic groups.

PTDSS1 encodes phosphatidylserine synthase 1 (PSS1). In contrast to other phospholipids, phosphatidylserine (P-Ser) cannot be synthesized *de novo* from the Kennedy pathway. It is obtained from either phosphatidylcholine (P-Chol) or phosphatidylethanolamine (P-Eth) by reactions catalysed by PSS1 or PSS2, respectively [46]. Reported mutations in *PTDSS1* activate PSS1, impairing the negative feedback exerted by its product, P-Ser, particularly in brain and in bones. P-Ser is important for bone mineralization. Its anionic character promotes osteogenesis, binding calcium within matrix vesicles and enhancing hydroxyapatite crystal formation [44]. Hydrocephalus with increased intracranial pressure is frequently reported and should be treated early to prevent neurologic morbidity.

Diagnosis is molecular (sequencing of *PTDSS1* exons and flanking regions). Interestingly, urine amino acids quantification in one Lenz-Majewski patient showed a six-fold increase of phosphoserine [47]. If confirmed in other patients, urinary phosphoserine could be a useful screening marker. Of note for genetic counselling, although the risk of recurrence appears to be low and mutations to date have been *de novo*, the small possibility of recurrence due to germinal mosaicism cannot be eliminated and future pregnancies of each parent should be followed.

34.4.3 Acylglycerol Kinase (AGK) Deficiency: Myopathy, Hypertrophic Cardiomyopathy and Congenital Cataract (Sengers Syndrome)

Fig. 34.2, step 4.

Sengers syndrome [48] is an autosomal recessive mitochondrial disorder characterized by congenital cataract, hypertrophic cardiomyopathy, skeletal myopathy, exercise intolerance, and lactic acidosis. Mental development is normal in all reported patients. Hypertrophic cardiomyopathy is the main clinical feature and the principal cause of death. Two forms are known, a neonatal-onset form with infantile death, and a late onset form with survival until the fifth or fourth decade [49]. One family with isolated cataract and a homozygous splice site mutation has been reported [50].

Sengers syndrome is caused by mutations in *AGK* [49], coding for mitochondrial acylglycerol kinase. AGK is localized in the mitochondrial intermembrane space, and phosphorylates monoacylglycerol and diacylglycerol to produce lysophosphatidic and phosphatidic acids, respectively. These compounds are signalling molecules, substrates for mitochondrial phospholipid biosynthesis, and are necessary for the correct posttranslational assembly of the adenine nucleotide translocator1 (ANT1) [49].

Elevated plasma and urinary lactate are observed. Increased urinary 3-methylglutaconic acid is reported in 70% of patients. Accumulation of lipids and glycogen is frequent in heart and skeletal muscle biopsies. Different combinations of respiratory chain complexes can be observed in biopsied muscle. However, complex I deficiency is a constant finding in myocardium [49]. A genotype-phenotype correlation has been suggested [51], with homozygous nonsense mutations in infantile Sengers syndrome but at least one *AGK* splice site variant or a start codon mutation in some patients with a milder form. No effective curative treatment is available although symptomatic measures e.g. for cataracts are useful in some patients.

34.4.4 Cardiolipin Remodeling Enzyme Deficiency: X-linked Cardiomyopathy and Neutropenia (Barth Syndrome)

Fig. 34.2, step 5.

Barth syndrome, an X-linked recessive disorder, classically presents with cardiomyopathy, skeletal muscle weakness, neutropenia, growth retardation [52], elevated urinary 3-methylglutaconic acid and hypocholesterolemia [53]. There is, however, considerable variability in the age of onset, clinical signs, and rate of progression. At its most severe, Barth syndrome can cause male fetal loss, stillbirth or neonatal death, but by contrast, patients with only mild cognitive phenotype are also described. Cardiomyopathy is the most serious manifestation and presents either as biventricular dilatation or as left-ventricular non-compaction. Episodes of sudden cardiac deterioration are common, often followed by unexplained remissions.

Barth syndrome is a mitochondrial disorder caused by mutations in *TAZ* (*Tafazzin*) on chromosome Xq28. Patients have abnormalities in cardiolipin (CL), an important phospholipid of the mitochondrial membrane. CL is primarily found in the inner mitochondrial membrane and to a lesser extent also in the outer mitochondrial membrane [54]. After primary synthesis CL acyl chains are remodelled to achieve their final mature composition. To function properly, CL must be present in a symmetric form. *TAZ* encodes monolysocardiolipin acyltransferase-1 (MLCLAT-1), a CL remodelling enzyme that maintains the symmetry of acyl chains in cardiolipin. MLCLAT-1 deficiency produces mitochondria with reduced levels and a modified acyl pattern of CL. The exact consequences for mitochondrial function remain to be established. No genotype-phenotype correlation is described [55].

Barth syndrome was previously classified as 3-methylglutaconic aciduria type II with normal 3-methylglutaconyl-CoA hydratase activity (see chapter 18). Urinary 3-methylglutaconic and 3-methylglutaric acids are normal in some proven patients [56], but elevations of these compounds should raise suspicion of Barth syndrome.

Cells from affected patients, including lymphocytes, fibroblasts and muscle, show an increased monolysocardiolipin: cardiolipin ratio (MLCL/CL). A rapid bloodspot screening method based on the measurement of this ratio by tandem mass spectrometry is available and appears to be both specific and sensitive [57]. Diagnosis is confirmed by molecular analysis of *TAZ*.

No specific treatment is available. Prognosis is highly variable and depends primarily on the severity of cardiac in-

volvement. Cardiomyopathy may respond to standard therapy but some patients have required heart transplantation. Granulocyte colony-stimulating factor can be effective for treatment of neutropenia.

34.4.5 *SERAC1* Mutation: Methylglutaconic Aciduria, Deafness, Hepatic Involvement, Encephalopathy, and Leigh Syndrome (MEGDHEL Syndrome)

◘ Fig. 34.2, step 6.

»MEGDEL« describes an autosomal recessive syndrome with 3-methylglutaconic aciduria, sensorineural deafness, encephalopathy and Leigh syndrome. Initially classified as type-IV 3-methylglutaconic aciduria (▶ Chapter 18), MEGDEL syndrome was recently linked to mutations in *SERAC1* [58]. During the first year of life hypoglycaemia, failure to thrive, and/or truncal hypotonia were observed. Patients can have hepatic involvement, ranging from neonatal hypoglycaemia, transient cholestasis to fulminant liver failure, expanding the acronym to MEGD(H)EL [59]. By two years of age, progressive deafness, dystonia, spasticity, psychomotor delay, and/or a loss of acquired skills often occur. Epilepsy can begin in the neonatal period or later [58].

MEGDEL syndrome is caused by mutations in *SERAC1*. The SERAC 1 protein is localized to the interface between the endoplasmic reticulum and mitochondria (mitochondria-associated membrane), and is hypothesized to be involved in the remodelling of phosphatidylglycerol 36:1 (PG36:1) which is a precursor for bis(monoacylglycerol) phosphate (BMP). Consequently, mutations in *SERAC1* lead to an increase in PG34:1 and a decrease in PG36:1 and BMP in fibroblasts. These biochemical abnormalities are accompanied by the accumulation of free intracellular cholesterol (producing a positive filipin test) and of abnormal cardiolipin species in mitochondria [56].

The diagnosis can be suspected clinically and by elevated urinary 3-methylglutaconic acid or abnormal cardiolipin species in cultured fibroblasts; these findings are common in MEGDEL syndrome but are not always present. Neonatal hepatic findings and positive filipin test may mimic Niemann Pick disease type C. Diagnosis is by the molecular testing of *SERAC1*.

34.4.6 Mitochondrial Calcium Independent Phospholipase A2γ (iPLA2γ): Autosomal Recessive Myopathy, Dystonia and Convulsions (not shown)

PNPLA8 mutations were recently described in a 2-year-old girl with a suspected mitochondrial myopathy that manifested as progressive muscle weakness, dystonia, seizures, poor weight gain, and hyperlacticacidemia [60]. *PNPLA8* encodes mitochondrial calcium independent phospholipase A2γ (iPLA2γ), the predominant phospholipase in mammalian mitochondria.

34.5 Inborn Errors related to Phospholipid Remodeling

Phospholipid remodelling and signalling at the cell membrane. (◘ Fig. 34.2, bottom part)

Phospholipids serve as a source of bioactive lipids released from membranes by a large family of enzymes, called phospholipases. Diacylglycerol can be formed from membrane phospholipids by phospholipase C and converted by diacylglycerol lipase into 2-arachidonoylglycerol, which can be hydrolyzed into arachidonic acid by alpha beta hydrolase 12 (ABHD12). Phospholipids can also be hydrolyzed at the sn-2 position by phospholipases A2, such as PLA2G6, or at the sn-1 position by phospholipases A1, such as DDHD1 and DDHD2, releasing free fatty acid and lysophopholipid (LysoPC). Lysophospholipids are hydrolyzed by lysophospholipases, such as PNPLA6 (NTE) into phosphoglycerol and fatty acid. Archidonic acid released by phospholipases is a precursor of eicosanoids such as hydroxyeicosatetraenoic acid (19-HETE and 20-HETE, ▶ Chapter 40).

Phosphatidylinositol (P-Ins) is a membrane phospholipid composed of diacylglycerol and a D-myo-inositol head group. In mammals, the inositol ring can be phosphorylated and dephosphorylated by a number of kinases and phosphatases to yield seven phosphoinositide derivatives (PI-3P, PI-4P, PI-5P etc…). Arachidonate-rich phosphoinositides are also believed to be another source of PLA2-mediated arachidonic acid release for the synthesis of prostaglandins and leukotrienes (◘ Fig. 34.2).

At least six inborn errors of metabolism linked to hydrolases and lipases involved in the remodelling of membrane phospholipids have been described. Most present with neurodegenerative features including spastic paraplegia, peripheral neuropathy, neuroendocrine and ophthalmologic findings. Several phospholipid disorders share many clinical symptoms (spastic paraplegia and autosomal recessive congenital ataxia) with some sphingolipid synthesis disorders (▶ Chapter 38).

34.5.1 α/β Hydrolase Domain-Containing Protein 12 (ABHD12) Deficiency: Polyneuropathy, Hearing loss, Ataxia, Retinitis Pigmentosa and Cataract (PHARC syndrome)

◘ Fig. 34.2, step7.

PHARC syndrome is an autosomal recessive neurodegenerative disease associating polyneuropathy, hearing loss, ataxia, retinitis pigmentosa, and cataracts [61]. *ABDH12* mutations have been identified in eighteen patients from 11 different families with PHARC syndrome [62]. Patients may present during adolescence with slowly progressive cataracts, hearing loss and demyelinating peripheral neuropathy. The presence and severity of ataxia are variable. Retinitis pigmentosa with cone-rod dysfunction typically develops in the second or third decade. By contrast, other families with PHARC showed an earlier onset of ataxia with both central and peripheral components. No evidence of behavioural disturbances

was detected in adult patients. Cerebral cortical function is generally spared, although single patients with mental retardation and myoclonic seizures are reported. Most patients display cerebellar atrophy on brain imaging. The phenotypic spectrum resulting from *ABHD12* mutations has recently expanded to include non-syndromic retinal degeneration [63].

ABDH12 hydrolyses the endocannabinoid 2-arachidonoyl glycerol (2-AG), releasing arachidonic acid. 2-AG and N-arachidonoylethanolamine (anandamide) are endogenous ligands of cannabinoid receptors CB1 and CB2, which mediate many physiological functions [64]. In addition, it has been demonstrated in a mouse model that ABHD12 possess lysophosphatidylserine lipase activity in brain, and that disruption of this activity causes the accumulation of proinflammatory lipids, microglial abnormalities and neurobehavioural changes [65].

Clinically, PHARC syndrome is reminiscent of Refsum disease, but affected individuals have normal phytanic and pristanic acid levels in plasma, as well as normal alpha-oxidation and other peroxisomal functions. The combination of retinitis pigmentosa and ataxia can lead to testing for the SCA7 trinucleotide expansion of spinocerebellar ataxia type 7 (SCA7) and for the mitochondrial DNA mutations of NARP syndrome (▶ Chapter 14). There is no known biochemical marker in PHARC syndrome. Diagnosis relies upon molecular analysis [62].

34.5.2 Phospholipase A2 Deficiency (PLA2G6): Autosomal Recessive Infantile Neuroaxonal Dystrophy, Neurodegeneration with Brain Iron Accumulation

Fig. 34.2, step 8.

Deficiency of phospholipase A2 (PLA2), caused by mutations in *PLA2G6*, can cause at least three autosomal recessive neurodegenerative disorders: infantile neuroaxonal dystrophy (INAD), neurodegeneration associated with brain iron accumulation (NBIA) and early-onset dystonia-parkinsonism (PARK14). These conditions have been grouped as *PLA2G6*-associated neurodegeneration (PLAN). They share many symptoms with fatty acid 2 hydroxylase deficiency (▶ Chapter 38). INAD and NBIA share distinctive pathologic features of neuroaxonal degeneration with distended axons (spheroid bodies) throughout the brain [66][67]. Patients with INAD and NBIA usually present before two years of age and die by age 10 years. Patients with INAD show progressive motor and mental deterioration, cerebellar ataxia and marked hypotonia of the trunk and early visual disturbances, later developing bilateral pyramidal tract signs with spastic tetraplegia. Seizures are not reported but electroencephalography shows characteristic high-voltage fast rhythms. Electromyography results are consistent with chronic denervation. T2-weighted cerebral magnetic resonance imaging (MRI) typically shows cerebellar atrophy, signal hyperintensity in the cerebellar cortex and occasionally, globus pallidus and substantia nigra hypointensities, corresponding to iron deposits. Neuropathology shows cerebellar atrophy, and lateral corticospinal tract degeneration. Axonal endings with spheroid bodies are often detectable in skin and conjunctival biopsies [66][67]. NBIA patients may be milder than patients with INAD, with static encephalopathy in childhood and late onset neurodegeneration in adolescence or adulthood. PARK14 is a form of autosomal recessive L-dopa–responsive early-onset dystonia-parkinsonism starting in the second or third decades. Pyramidal signs, cognitive decline and psychiatric disorders occur. Brain MRI typically shows mild generalized cerebral atrophy but no iron accumulation [66][67].

PLA2 is also known as iPLA2β and as patatin domain containing protein-9 (gene, *PNPLA9*) [70][71]. Alternative splicing produces several different PLA2 peptides that can hydrolyse phospholipids at the *sn-2* position, releasing arachidonic acid. They serve numerous functions, including phospholipid remodelling, arachidonic acid release and synthesis of leukotrienes and prostaglandins. Loss of PLA2 activity may lead to alteration in cell signalling and membrane remodelling. It is suggested that a resulting accumulation of membranes, organelles and proteins may be identified microscopically as spheroids [71]. It has been suggested that INAD/NBIA is caused by loss of PLA2G6-mediated fatty acid release. In contrast, the specific mutations responsible for dystonia-parkinsonism do not appear to decrease catalytic function, but may modify substrate preferences or regulatory mechanisms for PLA2G6 [71].

Genetic testing for *PLA2G6* mutations is replacing the biopsies traditionally used for diagnosis. This also allows for carrier detection, prenatal diagnosis and presymptomatic diagnosis in at-risk relatives. No effective specific treatment is available.

34.5.3 Deficiencies of Neuropathy Target Esterase (NTE or PNPLA-6) or Mitochondrial Calcium-independent Phospholipase A2γ (PNPLA6): Peripheral Neuropathy, Spastic Paraplegia, Chorioretinal Degeneration, Hypogonadotrophic Hypogonadism, Trichomegaly (SPG39, Boucher-Neuhauser, Gordon-Holms, Oliver-McFarlane, Laurence-Moon syndromes) or Mitochondrial Myopathy with Dystonia

Fig. 34.2, step 9.

Neuropathy Target esterase (NTE), encoded by *PNPLA6*, was initially identified as the target that is irreversibly inhibited by organophosphorus compound intoxication, causing organophosphate-induced delayed neuropathy. *PNPLA6* mutations have been shown to cause several autosomal recessive conditions initially felt to be unrelated, such as childhood onset progressive spastic paraplegia, peripheral neuropathy and distal muscle wasting, also known as SPG type 39 [73]. More recently, the clinical spectrum has expanded to include Boucher-Neuhauser (BNHS), Gordon Holmes (GHS) [74], Oliver-McFarlane and Laurence-Moon syndromes [75].

BNHS is characterized by the triad of spinocerebellar ataxia beginning between the second and fourth decades, hypogonadotropic hypogonadism, and visual impairment due to chorioretinal dystrophy detectable between the first and the sixth decades. Cerebellar atrophy and a small pituitary are seen on MRI. GHS is characterized by progressive cognitive decline, dementia, and variable adult-onset of movement disorders, associated with hypogonadotropic hypogonadism. Oliver-McFarlane syndrome is characterized by trichomegaly (i.e. long eyelashes, possibly with bushy eyebrows), severe chorioretinal atrophy typically noted in the first five years of life and multiple deficiencies of pituitary hormones including growth hormone and thyroid-stimulating hormone; hypogonadotropic hypogonadism is present in nearly all patients. Half of reported cases have spinocerebellar involvement. Laurence-Moon syndrome resembles Oliver-McFarlane syndrome, with childhood onset ataxia, peripheral neuropathy, pituitary dysfunction and spastic paraplegia, but without trichomegaly [75]. Presumably these clinical syndromes represent a continuum of the same disorder.

NTE, also called patatin domain containing protein-6 (PNPLA6), is a phospholipase B able to hydrolyse phospholipids and particularly lysophosphatidylcholine to generate glycerophosphocholine and fatty acid. NTE was reported to catalyse the hydrolysis of membrane lysophosphatidylcholine into phosphocholine and the corresponding fatty acid [76]. This reaction is hypothesized to be important for normal intracellular trafficking and to provide a source of the biosynthesis of the neurotransmitter acetylcholine (ACH). Lack of ACH and impairment of cell trafficking may explain the wide range of clinical symptoms associated with *PNPLA6* mutations.

There is no specific treatment although symptomatic measures may be of benefit. Diagnosis is by molecular testing.

34.5.4 *DDHD1* and *DDHD2* Mutations: Hereditary Spastic Paraplegias 28 and 45

Fig. 34.2, step 10.

Mutations in *DDHD1* and *DDHD2*, which each encode an A1 type phospholipase, produce autosomal recessive hereditary spastic paraplegia (HSP) with slowly progressive lower-limb spasticity due to corticospinal tract degeneration. *DDHD1* mutations have been identified in three families with adolescent-onset HSP [77]. Additionally, cerebellar oculomotor disturbance with saccadic eye pursuit were found in one patient. Brain and spin MRI were normal. *DDHD1*-related HSP is designated as spastic paraplegia 28 (SPG28) [77]. The clinical spectrum has been expanded recently to include a midlife-onset, slowly progressive HSP with cerebellar ataxia without intellectual disability [78].

DDHD2 mutations can be associated with very early onset progressive and complex HSP, classified as SPG54 [79] and two additional families with the same phenotype were identified by *DDHD2* sequencing. In addition to HSP, patients have intellectual disability, developmental delay and some have dysphagia and optic nerve hypoplasia, with marked thinning of the corpus callosum on T1-weighted MRI, subtle periventricular white-matter hyperintensities on T2-weighted MRI and an abnormal lipid peak on cerebral proton magnetic resonance spectroscopy.

DDHD1 and *DDHD2* encode DDHD-domain-containing proteins 1 and 2, respectively. DDHDs are A1 type phospholipases, serine hydrolases able to hydrolyse the acyl group of phospholipids at the sn-1 position. DDHD1 and DDHD2 enzymes have been designated as phosphatidic acid preferring phospholipases A1, but can also cleave phosphatidylethanolamine and phosphatidylinositol to form 2-arachidonoyl lysophosphatidylinositol [80]. *DDHD1* mutations have been shown to impair mitochondrial fragmentation, suggesting a role in mitochondrial fusion and fission. Recent experimental data suggest involvement of DDHD2 in a central nervous system-specific pathway of TG metabolism [81]. There is no specific treatment although symptomatic measures may be of benefit. The diagnosis is made by molecular genetic testing of *DDHD1 and DDHD2.*

34.5.5 *CYP2U1* Mutation: Spastic Paraplegia with Basal Ganglia Calcification (Hereditary Spastic Paraplegia 56, SPG56)

Fig. 34.2, step11.

Patients with *CYP2U1* mutations have presented with autosomal recessive early onset (birth to 8 years) HSP, frequently involving the upper limbs and sometimes associated with dystonic postures or cognitive alterations. On brain MRI, thinning of the corpus callosum and white matter lesions can be observed in some patients, but not all. Globus pallidus hypointensities on T1-weighted cerebral MRI of two siblings corresponded to areas of calcification on CT-scan. The severity of symptoms varied widely even among affected subjects in the same family, with no obvious genotype-phenotype relationship. In subjects from the same family, two patients never walked, while a third was only limited in his running capacities, with otherwise fully-conserved autonomy in his fourth decade [77]. Mutations in *CYP2U1* were initially designated as SPG49 [77] but finally listed in OMIM as SPG56.

CYP2U1 encodes the cytochrome P_{450} enzyme, CYP2. Cytochromes P_{450} have roles in the tissue-specific conversion of substrates into locally active compounds, including arachidonic acid derivatives. Cyp2U1 is a brain specific enzyme involved in α1- and β2-fatty acid hydroxylation, able to catalyse the hydroxylation of arachidonic acid and related long-chain fatty acids, producing 19- and 20-hydroxyeicosatetraenoic (HETE) acids. Structural abnormalities of the mitochondrial membrane were observed in patient cells. *CYP2U1* mutation should be suspected in HSP patients with basal ganglia calcifications (see also ▶ Chapter 40). Definitive diagnosis is made by molecular genetic testing of *CYP2U1*.

Table 34.1 Partial list of inherited errors linked to phosphoinositide metabolism and remodeling (Fig. 34.2). The mutant gene, the corresponding enzyme and the main clinical signs in patients are shown

Gene	Enzyme	Associated syndrome
PIK3CD	Phosphatidylinositol 3-kinase, catalytic, delta	Immunodeficiency
PIK3R1	Phosphatidylinositol 3-kinase, regulatory subunit 1	Autosomal recessive agammaglobulinemia; SHORT syndrome
PIK3R2	Phosphatidylinositol 3-kinase, regulatory subunit 2	Megalencephaly-polymicrogeria-polydactyly-hydrocephalus syndrome
PIK3R5	Phosphatidylinositol 3-kinase, regulatory subunit 5	Ataxia-oculomotor apraxia
PIKFYVE	Phosphoinositide kinase, FYVE finger-containing	Corneal dystrophy (fleck type)
PIP5K1C	Phosphatidylinositol 4-phosphate 5-kinase type I gamma	Lethal congenital contractural syndrome
PLCB1	Phospholipase C, beta-1	Epileptic encephalopathy, early infantile
PLCB4	Phospholipase C, beta-4	Auriculo-condylar syndrome
PLCD1	Phospholipase C, delta-1	Nonsyndromic congenital nail disorder
PLCE1	Phospholipase C, epsilon-1	Nephrotic syndrome type 3
PLCG2	Phospholipase C, gamma-2	Autoinflammation, antibody deficiency and immune dysregulation syndrome; Familial cold auto inflammatory syndrome
FIG4	SAC domain-containing inositol phosphatase 3	Yunis-Varon syndrome (cleidocranial dysplasia, micrognathia, absent thumbs)
INPP5E	Inositol polyphosphate-5-phosphatase	Mental retardation, truncal obesity, retinal dystrophy and micropenis
INPPL1	Inositol polyphosphate phosphatase-like 1	Opsismodysplasia, (Schneckenbecken dysplasia)
OCRL	Phosphatidylinositol 4,5-bisphosphate-5-phosphatase	Dent disease 2; Lowe oculocerebrorenal syndrome
PIK3CA	Phosphatidylinositol 3-kinase, catalytic, alpha (somatic)	Overgrowth syndrome

34.5.6 Inborn Errors of Polyphosphoinositide Metabolism

Fig. 34.2, steps 12.

Phosphatidylinositol (P-Ins) biosynthesis occurs in the ER, from cytidine diphosphate-diacylglycerol (CDP-DAG) by a reaction catalysed by P-Ins synthase. Glycosylphosphatidylinositol (GPI) is a glycosylated P-Ins, which anchors a plethora of proteins to the cell surface. Moreover, P-Ins is a source of highly bioactive polyphosphoinositide (PPInstd) molecules that account for about 10–15% of membrane phospholipids. PPInstd are phosphorylated derivatives of P-Ins generated by a number of kinases and phosphatases. They act upon membrane-bound lipid substrates [82][83]. Phosphorylation occurs at one or more of the hydroxyl groups of the inositol ring. There are seven known PPInstds, defined by the positions of inositol phosphorylation: PI4P, PI(4,5)P_2, PI(3,4)P_2, PI(3,5)P_2, PI3P, PI5P and PI(3,4,5)P_3 (Fig. 34.2). Of note, PPInstd are enriched in arachidonic acid at the *sn*-2 position, and are suspected to be a source of PLA2-mediated arachidonate release. The two main routes of PPInstd degradation are dephosphorylation by phosphoinositide phosphatases and hydrolysis at the sn-3 by phosphoinositide-specific phospholipase C (PLC). PPInstd regulate many aspects of cell function, including receptor signalling, secretion, endocytosis, ion channel regulation, intracellular vesicular trafficking and cytoskeletal organization. Inborn errors of these biochemical pathways create lipid imbalances, usually on intracellular endosomal membranes. These changes have been linked to several rare inherited disorders, some of which cause mental retardation, skeletal abnormalities, brain overgrowth, Charcot–Marie–Tooth neuropathies, and renal or immune dysfunction (briefly summarized in Table 34.1).

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Section VIII Disorders of Nucleic Acid and Heme Metabolism

Disorders of Purine and Pyrimidine Metabolism

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Purine Metabolism

Purine nucleotides are essential cellular constituents which are involved in energy transfer, metabolic regulation, and synthesis of DNA and RNA. Purine metabolism can be divided into three pathways:

- The biosynthetic pathway, often termed *de novo*, starts with the formation of phosphoribosyl pyrophosphate (PRPP) and leads to the synthesis of inosine monophosphate (IMP). From IMP, interconversions lead to adenosine monophosphate (AMP) and guanosine monophosphate (GMP). Further metabolism (not illustrated) leads to their di- and triphosphates, to their corresponding deoxyribonucleotides, and to RNA and DNA.
- The catabolic pathway starts from GMP, IMP and AMP, and produces uric acid, a poorly soluble compound, which tends to crystallize once its plasma concentration surpasses 6.5–7 mg/dl (0.38–0.47 mmol/L).
- The salvage pathway utilizes the purine bases, guanine, hypoxanthine and adenine, which are provided by food intake or the catabolic pathway, and reconverts them into GMP, IMP and AMP, respectively. Salvage of the purine nucleosides, adenosine and guanosine, and their deoxy counterparts, catalyzed by kinases, also occurs. The salvage pathway also converts several pharmacological anticancer and antiviral nucleoside analogs into their active forms.

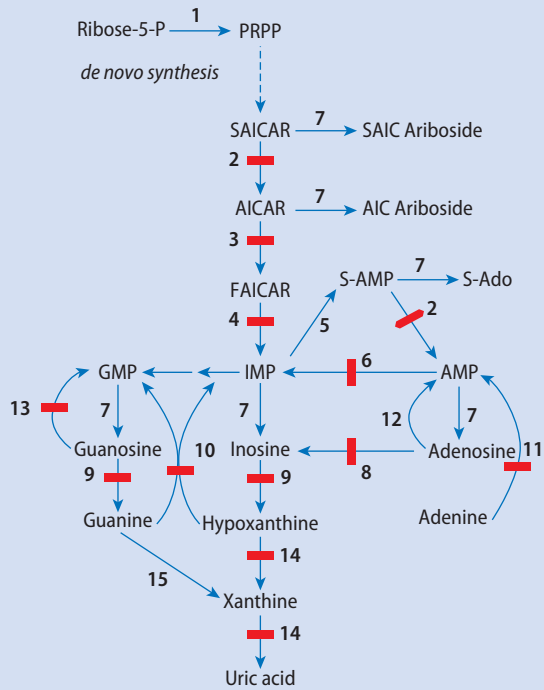


Fig. 35.1 Pathways of purine metabolism. *AICAR*, aminoimidazolecarboxamide ribotide; *AMP*, adenosine monophosphate; *FAICAR*, formylaminoimidazolecarboxamide ribotide; *GMP*, guanosine monophosphate; *IMP*, inosine monophosphate; *P*, phosphate; *PRPP*, phosphoribosyl pyrophosphate, *S-Ado*, succinyladenosine; *SAICAR*, succinylaminoimidazolecarboxamide ribotide; *S-AMP*, adenylosuccinate; *XMP*, xanthosine monophosphate. *1*, PRPP synthetase; *2*, adenylosuccinase (adenylosuccinate lyase); *3*, AICAR transformylase; *4*, IMP cyclohydrolase (3 and 4 form ATIC which stands for abbreviation of the two enzymes); *5*, adenylosuccinate synthetase; *6*, AMP deaminase; *7*, 5′-nucleotidase(s); *8*, adenosine deaminase; *9*, purine nucleoside phosphorylase; *10*, hypoxanthine-guanine phosphoribosyltransferase; *11*, adenine phosphoribosyltransferase; *12*, adenosine kinase; *13*, guanosine kinase; *14*, xanthine oxidase (dehydrogenase). Enzyme defects are indicated by **solid bars** across the arrows

Inborn errors exist of the biosynthetic, interconversion, catabolic, and salvage pathways of purine and pyrimidine metabolism (► Purine Metabolism), are depicted in ◘ Fig. 35.1, ◘ Fig. 35.2, and ◘ Fig. 35.3, respectively. For additional information, reference is made to two recent reviews [1][2].

35.1 Inborn Errors of Purine Metabolism

Inborn errors of purine metabolism (▶ Purine Metabolism) comprise defects or superactivities of:

- *purine nucleotide synthesis and interconversions*: phosphoribosyl pyrophosphate synthetase (PRS) superactivity and deficiency, adenylosuccinase (ADSL) deficiency, AICA-ribosiduria caused by ATIC deficiency;
- *purine catabolism*: the deficiencies of muscle AMP deaminase (AMPD, also termed myoadenylate deaminase), adenylate kinase (AK), adenosine deaminase (ADA), purine nucleoside phosphorylase (PNP), and xanthine oxidase (XO);
- *purine salvage*: the deficiencies of hypoxanthine-guanine phosphoribosyltransferase (HPRT), adenine phosphoribosyltransferase (APRT) and adenosine kinase (ADK). The deficiency of deoxyguanosine kinase (DGUOK) causes mitochondrial DNA depletion (see also ▶ Chapter 14).

Deficiency of thiopurine S-methyltransferase (TPMT, not shown in ◘ Fig. 35.1) results in less efficient methylation and hence in enhanced toxicity of pharmacologic thiopurine analogs. Deficiency of inosine triphosphate pyrophosphatase (ITPase, not shown in ◘ Fig. 35.1) also increases the toxicity of thiopurines. With the exception of the deficiencies of muscle AMPD and TPMT, all these inborn errors are very rare.

35.1.1 Phosphoribosyl Pyrophosphate Synthetase Superactivity

▪ Clinical Presentation

The disorder is mostly manifested by the appearance, in young adult males, of gouty arthritis and/or uric acid lithiasis, potentially leading to renal insufficiency [3][4]. Uricemia can be very high, reaching 10–15 mg/dl (0.60–0.90 mmol/L). The urinary excretion of uric acid is also increased, reaching up to 2400 mg (14 mmol)/24 h, or 2.5 mmol/mmol creatinine. A few patients present in infancy with sensorineural deafness, particularly for high tones, hypotonia, locomotor delay, ataxia and autistic features [4].

▪ Metabolic Derangement

The enzyme forms phosphoribosyl pyrophosphate (PRPP) from ribose-5-phosphate and ATP (◘ Fig. 35.1). PRPP is the first intermediate of the *de novo* synthesis of purine nucleotides. PRPP synthetase is highly regulated. Two different mechanisms have been proposed to explain PRS-I superactivity: gain-of-function point mutations in the *PRPS1* gene resulting in an altered regulatory site, and increased expression of *PRPS1* with normal kinetic properties. Because PRPP amidotransferase, the first and rate-limiting enzyme of the *de novo* pathway, is physiologically not saturated by PRPP, the synthesis of purine nucleotides increases, and hence the production of uric acid.

▪ Genetics

The various forms of PRPP synthetase superactivity are inherited as X-linked traits. In the families in which the anomaly is associated with sensorineural deafness, heterozygous females have also been found with gout and/or hearing impairment [4]. PRPP synthetase superactivity is caused either by distinctive gain-of-function point mutations in *PRPS1* (in the severe phenotype), or by acceleration of the transcription of a normal *PRPS1* isoform (in the milder phenotype) [5][6].

▪ Diagnostic Tests

Diagnosis based on extensive kinetic studies of the enzyme in erythrocytes and cultured fibroblasts is performed in only a few laboratories in the world and is now obtained by analysis of *PRPS1*. The disorder should be differentiated from HPRT deficiency, which may give similar clinical signs.

▪ Treatment and Prognosis

Patients should be treated with allopurinol, which inhibits xanthine oxidase, the last enzyme of purine catabolism (◘ Fig. 35.1). This results in a decrease of the production of uric acid and in its replacement by hypoxanthine, which is about 10-fold more soluble, and xanthine, which is slightly more soluble than uric acid. Initial dosage of allopurinol is 10–20 mg/kg per day in children and 2–10 mg/kg per day in adults. It should be adjusted to the minimum required to maintain normal uric acid levels in plasma, and reduced in subjects with renal insufficiency. In rare patients with a considerable increase in *de novo* synthesis, xanthine calculi can be formed during allopurinol therapy [7]. Consequently, additional measures to prevent crystallisation are recommended. These include a low purine diet (free of organ meats, fishes such as anchovy, herring, mackerel, salmon, sardines and tuna, dried beans and peas), high fluid intake and, since uric acid and xanthine are more soluble at alkaline than at acid pH, administration of sodium bicarbonate, potassium citrate or citrate mixtures to bring urinary pH to 6.0–6.5. Adequate control of the uricemia prevents gouty arthritis and urate nephropathy, but does not correct the neurological symptoms.

35.1.2 Phosphoribosyl Pyrophosphate Synthetase Deficiency

Several loss-of-function mutations of *PRPS1* have been identified in a number of patients with X-linked hearing impairment including Arts syndrome, Charcot-Marie Tooth disease 5 (CMTX5), and X-linked nonsyndromic hearing loss (DFNX-2) [6][8].

These form an intraindividual and intrafamilial continuum with PRPP synthetase activity varying from decreased to undetectable [9]. There is no clear reduction of serum and urinary uric acid, suggesting that the defect could be compensated. Supplementation of the diet with S-adenosylmethionine at a dose of 20 mg/kg per day which may replenish purine nucleotides independently of PRPP production, may alleviate some of the symptoms in patients with loss-of-function mutations.

35.1.3 Adenylosuccinase Deficiency

Clinical Picture

The clinical spectrum of the deficiency of adenylosuccinase (ADSL, also called adenylosuccinate lyase) is very broad, ranging from fatal neonatal convulsions to mild psychomotor retardation, muscle hypotonia and autistic behaviour. Approximately 80 affected individuals have been reported. Most patients, often referred to as type I, display variable associations of moderate to severe psychomotor retardation from birth, epilepsy after the first years, autistic features, and growth retardation associated with muscular wasting [10][11]. Other patients present with intractable convulsions starting within the first days or weeks of life [12], often with severe muscular hypotonia necessitating mechanical ventilation [13], and lead to death or severe mental retardation. Rare patients, referred to as type II, are only mildly retarded [11]. Microcephaly is often present. Computer tomography and magnetic resonance imaging of the brain show brain atrophy and hypomyelination with hypotrophy or hypoplasia of the cerebellum, particularly of the vermis [14]. The marked clinical heterogeneity justifies systematic screening for the deficiency in unexplained, profound as well as mild psychomotor retardation, in neurological disease with convulsions and/or hypotonia, and in individuals with autistic behaviour. Selective screening for purine and pyrimidine defects suggests that the disorder is not as rare as often thought [15].

Metabolic Derangement

ADSL catalyzes two steps in purine synthesis (Fig. 35.1): the conversion of SAICAR) into AICAR, along the *de novo* pathway, and that of S-AMP into AMP. Its deficiency results in accumulation in cerebrospinal fluid and urine of the succinylpurines, SAICA riboside and (S-Ado), the products of the dephosphorylation, by 5'-nucleotidase(s), of the two substrates of the enzyme. The more severe presentations of ADSL deficiency tend to be associated with S-Ado/ SAICA riboside ratios around 1, whereas in milder clinical pictures these ratios are comprised between 2 and 4. It suggests that SAICA riboside is the offending compound, and that S-Ado could protect against its toxic effects. The symptoms of the deficiency remain unexplained.

Genetics

The deficiency is transmitted as an autosomal recessive trait [10][11]. About 50 mutations have been identified, most are missense mutations but splicing errors and a mutation in the 5'UTR have also been identified [16][17]. Most frequently encountered, particularly in The Netherlands, and accounting for about one-third of the alleles investigated, is a R462H mutation.

Diagnostic Tests

Diagnosis is based on the presence in urine and cerebrospinal fluid of SAICA riboside and S-Ado, which are normally barely detectable. For systematic screening, a modified Bratton-Marshall test [18], performed on urine, appears most practical. False positive results are, however, recorded in patients who receive sulphonamides. Final diagnosis requires HPLC with UV detection [10], or tandem mass spectrometry [19][20][21]. The activity of ADSL in RBC is deficient in some patients but not in others [10]. Prenatal diagnosis of ADSL deficiency can be performed by mutation analysis on chorionic villi [22].

Treatment and Prognosis

Some patients have been treated for several months with oral supplements of adenine (10 mg/kg per day) and with allopurinol (5–10 mg/kg per day), the latter to avoid conversion of adenine by xanthine oxidase into minimally soluble 2,8-dihydroxyadenine, which forms kidney stones. No clinical or biochemical improvement was recorded, with the exception of weight gain and some acceleration of growth [12]. Oral administration of ribose (10 mmol/kg per day) [23], and S-adenosylmethionine (20–40 mg/kg per day) [21] were also without effect.

A ketogenic diet was found to greatly improve seizure control in a patient, but to require close follow-up because of significant side effects [24].

The prognosis for survival of ADSL-deficient patients is very variable. Mildly retarded patients have reached adult age, whereas several of those presenting with early epilepsy have died within the first months of life.

35.1.4 AICA-Ribosiduria

In three patients (in two families) [25][26] with profound mental retardation, epilepsy, marked dysmorphic features and vision loss, a positive urinary Bratton-Marshall test led to the identification of a massive excretion of 5-amino-4-imidazolecarboxamide (AICA) riboside, the dephosphorylated counterpart of AICAR (Fig. 35.1). Assay of ATIC, the bifunctional enzyme catalyzing the two last steps of *de novo* purine biosynthesis, revealed a profound deficiency of AICAR transformylase, and a partial deficiency of IMP cyclohydrolase [25]. The discovery of this inborn error of purine synthesis reinforces the necessity to perform a Bratton-Marshall test [18] or a screening for urinary purine metabolites in all cases of unexplained mental retardation and/or neurological symptoms. Next generation sequencing (NGS) epilepsy panels-including *ATIC* are now proposed.

35.1.5 Muscle Adenosine Monophosphate Deaminase 1 Deficiency

Clinical Picture

The deficiency of muscle adenosine monophosphate (AMP) deaminase 1 (AMPD1), frequently referred to as *myoadenylate deaminase* in the clinical literature) is present in 1–2% of the Caucasian population. The vast majority of deficient individuals are asymptomatic. Some subjects, in whom the AMPD1 defect is termed primary, present with isolated

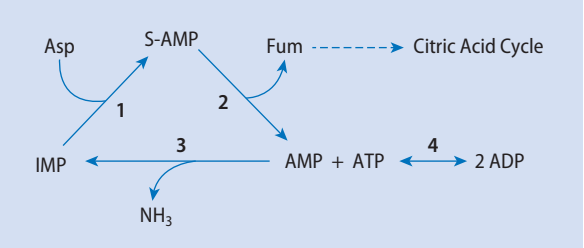


Fig. 35.2 The purine nucleotide cycle and adenylate kinase. *IMP*, inosine monophosphate; *S-AMP*, adenylosuccinate; *AMP*, adenosine monophosphate; *ADP*, adenosine diphosphate; *ATP*, adenosine triphosphate; *Asp*, aspartate; *Fum*, fumarate. 1, Adenylosuccinate synthetase; 2, Adenylosuccinase; 3, AMP deaminase; 4, adenylate kinase (also termed myokinase).

muscular weakness, fatigue, cramps or myalgias following moderate to vigorous exercise, sometimes accompanied by an increase in serum creatine kinase, myoglobinuria and minor electromyographic abnormalities [27]. Secondary AMPD1 deficiency is found in association with several neuromuscular disorders amongst which amyotrophic lateral sclerosis, fascioscapulohumeral myopathy, Kugelberg-Welander syndrome, polyneuropathies, and Werdnig-Hoffmann disease [28][29].

Metabolic Derangement

AMPD, adenylosuccinate synthetase and adenylosuccinase form the purine nucleotide cycle (Fig. 35.2). Numerous functions have been proposed for this cycle in muscle (reviewed in [30]), but the relationships between enzyme defects, clinical symptoms, and genetic findings remain uncertain [31].

Genetics

Primary AMPD deficiency is apparently transmitted as an autosomal recessive trait. In most individuals with the primary muscular deficiency the defect is caused by a c.34C->T mutation [32] which results in a nonsense codon Q12X and a severely truncated, inactive enzyme. Population studies show that this mutant allele is found with a high, 8–11% frequency in Caucasians. This accords with the finding that about 2% of diagnostic muscle biopsies are AMPD deficient. Noteworthy, AMPD activity may also be greatly diminished, to about 40% of controls, in heterozygous individuals [32][33].

Diagnostic Tests

Screening for the defect can be performed by an exercise test (▶ Chapter 3). A several-fold elevation of venous plasma ammonia, seen in normal subjects, is absent in AMPD deficiency. Final diagnosis is established by mutation analysis, histochemical or biochemical assay in a muscle biopsy. In the primary defect, the activity of AMPD is below 2% of normal, in the secondary defect the activity is 2-15% of normal [34], but in several large series of muscle biopsies for diagnostic purposes, low enzyme activities were found in about 2% of all specimens [28][29].

Treatment and Prognosis

Patients may display a gradual progression of their symptoms, which may lead to the point that even dressing and walking a few steps lead to fatigue and myalgias. Administration of ribose (2-60 g per day orally in divided doses such as to be taken constantly) has been reported to improve muscular strength and endurance [31][35].

35.1.6 Adenosine Monophosphate Deaminase 2 and 3 Deficiencies

Besides the muscular isoform AMPD1, two other isoforms exist. AMPD2 (also known as PCH9 or SPG63) is expressed in liver and brain, and mutations in the gene have been found to be linked to neurodegenerative brainstem disorders [36] and hereditary spastic paraplegia (HSP) [37].

A third isoform, AMPD3, is expressed in erythrocytes. Individuals with a complete, although totally asymptomatic deficiency of erythrocyte AMPD3 have been detected in Japan, Korea and Taiwan [38].

35.1.7 Adenosine Deaminase 1 Deficiency

There are two isoforms of adenosine deaminase (ADA). ADA1 is found in most cells, particularly lymphocytes and macrophages. ADA2 is predominant in plasma.

Clinical Picture

The clinical spectrum of adenosine deaminase 1 (ADA1) deficiency is very broad, ranging from a profound impairment of both humoral and cellular immunity in infants, known as *severe combined immunodeficienc* (SCID), to delayed and less severe later onset in older children or adults, and even benign partial ADA1 deficiency in adults [39][40]. Approximately 80–85% of patients display, within the first weeks or months after birth, multiple, recurrent opportunistic infections caused by a variety of organisms, which rapidly become life-threatening. Infections are mainly localized in the skin, the respiratory, and the gastrointestinal tract. In the latter they often lead to intractable diarrhoea, malnutrition and growth retardation. In affected children over 6 months of age, hypoplasia or apparent absence of lymphoid tissue (tonsils, lymph nodes, thymus shadow on x-ray) is a suggestive sign. Nonimmunological symptoms are also found. About half of the patients display bone abnormalities, clinically evident as prominence of the costochondral rib junctions, and radiologically as cupping and flaring thereof. In some affected children, cognitive, behavioural, and neurological abnormalities are present, including lower IQ, hyperactivity, attention deficits, spasticity, head lag, nystagmus, inability to focus, and high frequency sensorineural deafness. Hepatic dysfunction has also been reported [41].

Approximately 15–20% of ADA1-deficient children display clinical symptoms after six months of age or during the first years of life. Infections in these delayed onset patients may

initially be less severe than in those with SCID. Recurrent otitis, sinusitis, and upper-respiratory tract infections are frequent. By the time of diagnosis, these patients often have chronic respiratory insufficiency and autoimmune phenomena, including cytopenias and anti-thyroid antibodies. Allergies and elevated serum IgE are often present.

The very rare individuals who survive undiagnosed into the first decade of life or beyond often display deteriorated immune function and chronic sequelae of recurrent, particularly respiratory infections.

Metabolic Derangement

The deficiency results in the accumulation in body fluids of adenosine, normally nearly undetectable (Fig. 35.1), deoxyadenosine (not shown in Fig. 35.1) another substrate of ADA1, and their derivatives, notably deoxyadenosine triphosphate (dATP). These compounds induce the premature death of lymphoid progenitor cells, and thereby profoundly impair the generation of T, B, and NK lymphocytes. ADA deficiency has broad consequences because it moreover affects to a varying extent bone, brain, lung, liver and perhaps also epithelial functions.

Genetics

ADA1 deficiency is an autosomal recessive disorder. It accounts for about 40% of the North American, and for about 10–20% of the European patients with SCID. The frequency of the deficiency is estimated at 1 per 200,000–1,000,000 births. Over 70 mutations have been described, the majority of which are single nucleotide changes, resulting in an either inactive or unstable enzyme [40]. Most patients are compound heterozygotes. Spontaneous *in vivo* reversion to normal of a mutation on one allele, as observed in tyrosinemia type I (▶ Chapter 17), has been reported [42].

Diagnostic Tests

SCID can be confirmed by relatively simple laboratory tests: lymphopenia (usually less than 500 total lymphocytes per mm^3) involving B, T and natural killer (NK) cells, as well as hypogammaglobulinemia are almost invariably present. Whereas the IgM deficiency may be detected early, the IgG deficiency becomes manifest only after the age of 3 months, when the maternal supply has been exhausted. More elaborate tests show a deficiency of antibody formation following specific immunization and an absence or severe diminution of the lymphocyte proliferation induced by mitogens. The disease is progressive, since residual B- and T-cell function which may be found at birth, disappears later on.

The enzymatic diagnosis is mostly confirmed on red blood cells. In general, severity of disease correlates with the loss of ADA1 activity: children with neonatal onset of SCID display 0–1% residual activity; in individuals with later onset, 1–5% of normal ADA1 activity are found [39][40]. It should be noted that only about 10–20% of the patients with the clinical and hematologic picture of inherited SCID are ADA1-deficient. In the remaining patients, SCID is caused by a variety of defects of lymphocyte-specific signalling mechanisms. A few subjects have been described with ADA deficiency in red blood cells, but normal immunocompetence. This benign condition, called partial ADA1 deficiency, is explained by the presence of residual ADA1 activity in their lymphocytes.

Treatment and Prognosis

Before the advent of modern therapies, ADA1-deficient SCID as a rule invariably led to death, usually within the first year of life, unless drastic steps were taken, such as rearing in strictly sterile conditions from birth on. Treatment became possible with the advent of bone marrow transplantation. This was followed by enzyme replacement therapy (ERT) and gene therapy. The choice between the three options is difficult, depending on their accessibility, response to ERT, and potential short- and long-term risks, and has been extensively discussed in recent years [40][43].

Bone marrow transplantation This remains the first choice provided a fully matched sibling or family donor is available, and gives an approximately 70% chance for complete immunological cure. The graft provides hematopoietic stem cells, and hence T and B cells, which have sufficient ADA activity to prevent accumulation of adenosine, deoxyadenosine, and dATP. This transplantation can be performed without chemotherapeutic conditioning, probably contributing to its high rate of success. Nevertheless, some patients have delayed or incomplete recovery of immune function and there is a risk of graft-versus-host disease. Mismatched bone transplants from a »non-ideal« donor have a much lower survival, irrespective of conditioning, and are recommended to be avoided unless no other treatment is available.

Although immune recovery after fully matched transplantation is generally excellent, the prognosis for nonimmunologic clinical signs is not as good.

Enzyme replacement herapy Treatment with polyethylene glycol–modified bovine ADA1 (PEG-ADA1) has now been used in approximately 200 patients [44][45]. It corrects the metabolic alterations and improves the clinical condition but often fails to provide sustained cure of the immunodeficiency; its use is limited by neutralizing antibodies against the bovine enzyme, autoimmunity, and the high cost of lifelong therapy. Available evidence indicates that treatment with PEG-ADA1 should be started in any patient who is clinically unwell, particularly when a long wait to find a suitable donor is expected.

Gene therapy The first approved clinical trial of gene therapy was performed in 1990 in two girls with ADA1 deficiency in whom the ADA gene was inserted into peripheral blood T cells, which live a few months [46]. Later on, the gene was inserted into hematopoietic stem cells which in theory have an unlimited life span. Clinical and animal studies indicate that cytoreductive conditioning with a low, non myeloablative dose of bisulphan is absolutely needed for successful gene therapy of ADA1-deficient SCID, and that continued use of enzyme replacement therapy after gene therapy may further improve im-

mune reconstitution [47][48][49]. Since all these gene therapy studies have been conducted with gamma retroviral vectors with intact viral promotor sequences that are known to have caused insertional mutagenesis in other gene therapy trials, this issue should also be further addressed. It remains to be determined if gene therapy will be superior to fully matched sibling or family bone marrow transplantation in preventing cognitive, behavioural and neurologic abnormalities.

35.1.8 Adenosine Deaminase 2 Deficiency

Whole-exome sequencing identified recessive loss-of-function mutations in *CECR1* (cat eye syndrome chromosome region, candidate 1) encoding ADA2. Patients display a syndrome ranging from early-onset recurrent lacunar strokes and other neurovascular manifestations, to systemic vacuolopathy and polyarteritis nodosa [50][51]. Mild immunodeficiency with hypogammaglobulinemia and disturbed B-cell function are also present. Potential therapy could include fresh-frozen plasma or recombinant ADA2.

35.1.9 Adenosine Deaminase Superactivity

An autosomal dominant hereditary, approximately 50-fold elevation of red cell ADA activity, has been shown to cause non-spherocytic hemolytic anaemia [52]. The latter can be explained by an enhanced catabolism of the adenine nucleotides, including ATP, owing to the increased activity of ADA. Less pronounced, 2 to 6-fold elevations of ADA activity are found in 84% of patients with Diamond-Blackfan anaemia and some of their probands [53].

35.1.10 Purine Nucleoside Phosphorylase Deficiency

Clinical Picture

Recurrent infections are usually of later onset, starting from the end of the first year to up to 5–6 years of age, and are initially less severe than in ADA1 deficiency [54]. A strikingly enhanced susceptibility to viral diseases, such as varicella, measles, cytomegalovirus and vaccinia has been reported, but severe candida and pyogenic infections also occur. Two thirds of the patients display neurologic symptoms, including spastic tetra- or diplegia, ataxia and tremor, and mild to severe mental retardation. One third of the patients have autoimmune disorders, most commonly haemolytic anemia, but also idiopathic thrombocytopenic purpura and autoimmune neutropenia. The disorder is much less frequent than ADA1 deficiency, with about 50 patients reported.

Metabolic Derangement

The deficiency provokes an accumulation in body fluids of the 4 substrates of the enzyme which are normally nearly undetectable, namely guanosine, inosine (Fig. 35.1), and their deoxycounterparts (not shown in Fig. 35.1), the latter derived from DNA breakdown. Formation of uric acid is thus severely hampered. The profound impairment of cellular immunity, characterizing PNP deficiency, has been expounded by the greater ability of T-cells as compared to B-cells to accumulate dGTP. Formed from deoxyguanosine, dGTP inhibits ribonucleotide reductase, and hence cell division. The normally ubiquitous expression of PNP explains the presence of nonimmunologic symptoms in its deficiency.

Genetics

The deficiency is inherited in an autosomal recessive fashion. Among a number of molecular defects, a p.R234P mutation is most common [55].

Diagnostic Tests

Immunological studies reveal an increasing deficiency of cellular immunity, reflected by a marked reduction in the number of T-cells. B-lymphocyte function is deficient in about one third of the patients. Patients often display a striking decrease of the production of uric acid: plasma uric acid is usually below 1 mg/dl and may even be undetectable. However, in patients with residual PNP activity, uricemia may be at the borderline of normal. The urinary excretion of uric acid is usually also markedly diminished. Other causes of hypouricemia such as xanthine oxidase deficiency (see below), and drug administration (acetylsalicylic acid, thiazide diuretics), should be ruled out (see also hypouricemias ▶ Chapter 1). Enzymatic diagnosis of PNP deficiency is usually performed on red blood cells.

Treatment and Prognosis

Most initially diagnosed patients have died, although at a later age than untreated ADA1-deficient children, from overwhelming viral or bacterial infections. Treatments consisted of bone marrow transplantation and repeated transfusions of normal, irradiated erythrocytes [54]. More recently, successful matched bone marrow transplantation has been reported [56] without effect on neurologic symptoms in a case [57] and with improvement in another [58]. Enzyme and gene therapy might become available in the future.

35.1.11 Xanthine Oxidase Deficiency

Clinical Picture

Three types of deficiencies of xanthine oxidase (XO, also termed xanthine dehydrogenase and xanthine oxidoreductase), which all cause xanthinuria, are known: *(i)* type I classical xanthinuria, caused by isolated XO deficiency; *(ii)* type II classical xanthinuria, due to deficiency of both XO and aldehyde oxidase (AO); *(iii)* combined deficiency of XO, AO and sulfite oxidase (SO) (▶ Chapter 20). Type I and type II xanthinuria can be completely asymptomatic, although in about one third of the cases kidney stones are formed. Most often not visible on X-ray, they may appear at any age, and provoke hematuria, renal colic, and even acute renal failure. Myopathy

with pain and stiffness may also be present, caused by crystalline, birefringent xanthine deposits, and triggered by strenuous exercise. In combined XO deficiency, the devastating clinical picture of SO deficiency [which is also found as an isolated defect (▶ Chapter 20)] overrides that of XO deficiency.

Metabolic Derangement

The deficiency of XO results in the near total replacement of uric acid, in plasma and urine, by hypoxanthine and xanthine as the end products of purine catabolism (◘ Fig. 35.1). Plasma hypoxanthine is as a rule not or minimally elevated, owing to its efficient reutilization by hypoxanthine-guanine phosphoribosyltransferase. In contrast, plasma xanthine, normally below 1 μM, may rise to 10–40 μM. The very limited solubility of xanthine explains the formation of stones in kidney and deposits in muscle. The deficiency of AO results moreover in the inability to metabolize synthetic purine analogues such as allopurinol.

The combined deficiency of XO, AO, and SO is caused by failure to synthesize a molybdenum cofactor (MoCo), common to the three oxidases (▶ Chapter 20).

Genetics

The inheritance of the three types of XO deficiency is autosomal recessive. Type I xanthinuria is caused by mutations in *XO* [59]. Type II xanthinuria results from mutations of the human MoCo sulfurase (*HMCS*) gene ([60], ▶ Chapter 20).

Diagnostic Tests

In the three types of XO deficiency, plasma concentrations of uric acid below 1 mg/dl (0.06 mmol/L) are measured; they may decrease to virtually undetectable values on a low-purine diet. Urinary uric acid is reduced to a few percent of normal and replaced by hypoxanthine and xanthine. Still, patients with normal plasma uric acid have also been reported. In combined XO, AO and SO, the urinary changes are accompanied by an excessive excretion of sulfite and other sulfur-containing metabolites, such as S-sulfocysteine, thiosulfate and taurine. The enzymatic diagnosis requires liver or intestinal mucosa, the only human tissues which normally contain appreciable amounts of XO. SO and the molybdenum cofactor can be assayed in liver and fibroblasts.

Treatment and Prognosis

Type I and II XO deficiency are mostly benign but in order to prevent renal stones a low purine diet should be prescribed and fluid intake increased. The prognosis of combined XO, AO and SO deficiency has been recently improved by daily infusion of cPMP (▶ Chapter 20).

35.1.12 Hypoxanthine-Guanine Phosphoribosyltransferase Deficiency

Clinical Picture

The clinical spectrum of this disorder is very wide and determined by the residual activity of the enzyme. Patients with complete or near-complete deficiency of HPRT, sometimes abbreviated HGPRT, display the Lesch-Nyhan syndrome [61]. Affected children generally appear normal during the first months of life. At 3 to 6 months of age, a neurological syndrome evolves, which includes delayed motor development, choreo-athetoid movements, and spasticity with hyperreflexia and scissoring. This motor syndrome has been reclassified as a severe action dystonia, superimposed on a baseline hypotonia [62]. Over the years, the patients develop a striking neuropsychological profile, comprising compulsive self-destructive behaviour involving biting of their fingers and lips, which leads to mutilating loss of tissue. Aggressive, both physical and verbal acts against others such as spitting and use of shocking vocabulary are also characteristic. Speech is hampered by athetoid dysarthria. Whereas most patients have IQ's around 60–70, some display normal intelligence. Approximately 50% of the patients have seizures. Soon or later they form uric acid stones. Mothers of Lesch-Nyhan patients have reported the finding of orange crystals on their affected son's diapers during the first few weeks after birth. Untreated, the uric acid nephrolithiasis progresses to obstructive uropathy and renal failure during the first decade of life. The latter clinical picture may, exceptionally, also be observed in early infancy.

Partial HPRT deficiencies without self-injurious behaviour are now also named attenuated variants of Lesch-Nyhan disease [63]. All patients, sooner or later, have evidence of overproduction of uric acid. Whereas most patients with Lesch-Nyhan disease do not develop gouty arthritis, this finding is common in partial HPRT deficiency. Evaluation of a cohort of 46 patients has led to the recognition of the spectrum of neurological features associated with HPRT deficiency. At one end is the full Lesch-Nyhan disease; on the other end are very rare patients with isolated overproduction of uric acid but no neurological or behavioural abnormalities. In between are milder phenotypes who present with neurological rather than uric acid problems. These include motor abnormalities ranging from subtle clumsiness to severe disabling generalized dystonia, and varying degrees of cognitive or behavioural abnormalities. Noteworthy, several females have been reported with the Lesch-Nyhan syndrome.

Metabolic Derangement

The overproduction of uric acid results from the acceleration of its *de novo* synthesis, caused by the increased availability of PRPP, which is not recycled by HPRT. The pathogenesis of the neurological symptoms is still not satisfactorily explained. Autopsies have not revealed overt morphological changes [62]. A number of studies point to dopaminergic dysfunction, involving 60–90% decreases of the concentration of dopamine and of the activity of the enzymes required for its synthesis,

although dopaminergic drugs are not useful [64]. Recent studies indicate that HPRT deficiency impairs the function of several transcription factors that play a key role in the development and function of dopaminergic neurons [65].

Genetics

Both the Lesch-Nyhan syndrome and the partial deficiencies of HPRT are transmitted in a X-linked recessive manner. The frequency of the disorder is estimated at 1:380,000 [66]. Presently, over 250 mutations of the HPRT gene have been described, ranging from point mutations to extensive deletions resulting in suppression of enzyme synthesis [66][67]. Molecular studies have led to precise prenatal diagnosis and efficient carrier testing of at-risk females [68]. In females, the Lesch-Nyhan syndrome is due to non-random or skewed inactivation of the X-chromosome [66].

Diagnostic Tests

Patients excrete excessive amounts of uric acid, ranging from 25 to 140 mg (0.15 to 0.85 mmol)/kg of body weight per 24 h, as compared to an upper limit of 18 mg (0.1 mmol)/kg per 24 h in normal children. Determination of the ratio of uric acid to creatinine (mg/mg) in morning samples of urine provides a screening test. This ratio is much higher in HPRT deficiency than the normal upper limits of 2.5, 2.0, 1.0 and 0.6 for infants, 2 years, 10 years and adults, respectively [69]. Increased ratios are also found in other disorders with uric acid overproduction, such as PRPP synthetase superactivity, glycogenosis type I, or lymphoproliferative diseases (see hyperuricemias in ▶ Chapter 1). The overproduction of uric acid is as a rule accompanied by an increase of serum urate, which may reach concentrations as high as 18 mg/dl (1 mmol/L), and by an increased urinary excretion of hypoxanthine and xanthine. Occasionally, however, particularly before puberty, uricemia may be in the normal or high normal range. Female carriers may have elevated uric acid excretion.

Patients with the Lesch-Nyhan syndrome display nearly undetectable HPRT activity in red blood cells [70]. In partial deficiencies, similar low or higher values may be found. Rates of incorporation of hypoxanthine into the adenine nucleotides of intact fibroblasts correlate better with the clinical symptomatology than HPRT activities in erythrocytes [71].

Treatment and Prognosis

Allopurinol is indicated to prevent urate nephropathy. Allopurinol, even when given from birth or in combination with adenine has, however, no effect on the neurological symptoms [72], which have so far been resistant to all therapeutics attempts. Patients should be made more comfortable by appropriate restraints, including elbow splints, lip guards and even tooth extraction, to diminish self-mutilation. Diazepam, haloperidol and barbiturates may sometimes improve choreoathetosis.

In a 22-year-old patient, bone marrow transplantation restored erythrocyte HGPRT activity to normal, but did not change neurological symptoms [73]. In a single patient, disappearance of self-mutilation was obtained by chronic stimulation of the globus pallidus [74]. In others it was unsuccessful and even led to a death [66]. More recently, a number of patients have been treated with oral S-adenosylmethionine with erratic results: some clearly benefited [75] but the majority experienced worsened self-injurious behavior [76].

35.1.13 Adenine Phosphoribosyltransferase Deficiency

Clinical Picture

The deficiency may become clinically manifest in childhood [77], even from birth [78], but also remain silent for several decades [79][80]. Symptoms include urinary passage of gravel, small stones and crystals, frequently accompanied by abdominal colic, dysuria, hematuria and urinary tract infection. Some patients may even present with acute anuric renal failure [81]. The urinary precipitates are composed of 2,8-dihydroxyadenine, radiotranslucent, and undistinguishable from uric acid stones by routine chemical testing.

Metabolic Derangement

The deficiency results in suppression of the salvage of adenine (◘ Fig. 35.1), provided by food and by the polyamine pathway. Consequently, adenine is oxidized by xanthine oxidase into 2,8-dihydroxyadenine, a very poorly soluble compound. The deficiency can be complete or partial. The partial deficiency (type II) is only found in the Japanese, among whom it is quite common [82].

Genetics

APRT deficiency is inherited as an autosomal recessive trait. All the type II Japanese patients carry the same c.2069T-> C substitution in exon 5. In Caucasians, more than 30 mutations have been identified, some of which seem more common, also suggesting founder effects [79][83].

Diagnostic Tests

Identification of 2,8-dihydroxyadenine requires complex analyses, including UV and infrared spectrography, mass spectrometry and X-ray cristallography [78]. It is therefore usually easier to measure APRT activity in red blood cells.

Treatment and Prognosis

In patients with symptoms, allopurinol should be given to inhibit the formation of 2,8-dihydroxyadenine. Both in patients with stones and in those without symptoms, dietary purine restriction and high fluid intake are recommended. Alkalinization of the urine is, however, not advised: unlike that of uric acid, the solubility of 2,8-dihydroxyadenine does not increase up to pH 9.

Ultimate prognosis depends on renal function at the time of diagnosis. Of note is that kidney transplantation has been reported to be followed by recurrence of microcrystalline deposits and subsequent loss of graft function [79][84].

35.1.14 Adenylate Kinase 1 Deficiency

Adenylate kinase (AK), also called myokinase, plays an important role in energy metabolism by rephosphorylating ADP into ATP and AMP (Fig. 35.3). Two isoenzymes which are expressed in most tissues exist. Deficiency of AK1, a cytosolic enzyme, results in chronic nonspherocytic haemolytic anaemia associated with hepatosplenomegaly and psychomotor retardation [85].

35.1.15 Adenylate Kinase 2 Deficiency

Mutations of AK2, a mitochondrial enzyme which is uniquely expressed in neutrophils, T lymphocytes and the stria vascularis of the inner ear, cause reticular dysgenesis, a form of severe combined immunodeficiency (SCID) [86]. AK2 deficiency has also been associated with severe haematopoietic defects and sensorineural deafness [87]. AK2 has been shown to be critical in the control of energy metabolism [88].

35.1.16 Adenosine Kinase Deficiency

Deficiency of this enzyme, first diagnosed in 6 children [89], and more recently in 11 additional patients [90], was shown to cause severe progressive developmental delay and liver dysfunction. All patients have shown frontal bossing, psychomotor retardation and muscular hypotonia. Almost all had neonatal onset liver dysfunction of variable severity, with microvesicular steatosis on histology. Other features have included sparse hair, slender hands and feet, epilepsy and hyperinsulinaemic hypoglycaemia.

Biochemical analysis revealed increased plasma levels of methionine, S-adenosylmethionine (AdoMet), and S-adenosylhomocysteine (AdoHcy) but normal or mildly elevated homocysteine (Hcy) levels, indicating a block in the methionine cycle (▶ Chapter 20). Involvement of adenosine kinase was identified by increased urinary adenosine excretion and exome sequencing. Mutation analysis is recommended to confirm the diagnosis as the enzyme analyses are too complicated for use in a diagnostic setting. Homozygous missense mutations were found in the patients. Recent work has shown that additional substrates of adenosine kinase, namely AICA riboside and succinyl AICA riboside, accumulate in the disorder [91]. A methionine restricted diet led to improved liver function in four patients but only one patient showed neurological improvement. In two patients, the hyperinsulinism responded to diazoxide.

35.1.17 Adenylate Cyclase 5 Mutations

Recently, gain of function mutations of this enzyme, which converts ATP into cyclic AMP, have been found by exome sequencing in sporadic and inherited cases of autosomal dominant infantile and adult familial dyskinesia with facial myokymia [92][93]. Functional studies show an increased accumulation of cyclic AMP upon stimulation by beta-receptor agonists. The mutations cause childhood onset involuntary paroxysmal choreiform and dystonic movements. Mutations in *ADCY5* are not rare and cause a wide range of hyperkinetic abnormal movements with variable ages of onset and genotype/phenotype correlation. Mosaicism plays an important role in the phenotypic variability. Recurrent mutations suggest a particular functional importance of residues 418 and 726 in the pathogenesis of ADCY5-related dyskinesia [94].

35.1.18 IMP Dehydrogenase Mutations

IMP Dehydrogenase catalyzes the first step in the conversion of IMP into GMP and hence into the guanine nucleotides (Fig. 35.1). Humans have two IMPDH genes. Mutations in the gene encoding IMPDH1 have been found in autosomal dominant retinitis pigmentosa and in some cases of Leber congenital amaurosis [95][96].

35.1.19 Deoxyguanosine Kinase Deficiency

Two forms of deoxyguanosine kinase (DGUOK) deficiency, identified in approximately 100 patients [97], are known. The majority have the hepatocerebral form, a multisystemic mitochondrial DNA depletion syndrome (see also ▶ Chapter 14), characterised within weeks of birth by cholestasis and progressive liver failure, neurological abnormalities (severe hypotonia, developmental regression, rotary nystagmus evolving into opsoclonus), hypoglycemia, and increased lactate. A minority of patients present in infancy or childhood with isolated, cholestatic hepatic disease, which may be accompanied by renal disease. Deficiency of mitochondrial deoxyguanosine kinase underlies both forms [97][98]. This enzyme phosphorylates the deoxy counterpart of guanosine (Fig. 35.1) into dGMP and plays an essential role in the supply of precursors of mitochondrial DNA, particularly in liver and brain that lack a cytosolic form of the enzyme. The deficiency is autosomal recessive. A single nucleotide deletion in the mitochondrial *DGUOK* gene segregated with the disease in 19 patients in 3 kindreds [98]. Since then, other mutations have been identified, including a cause of juvenile onset mitochondrial myopathy [99]. Noteworthy, both the hepatocerebral and the isolated hepatic disease have been observed in families harboring the same mutations [98]. Progressive hepatic disease is the most frequent cause of death in both forms. Orthotopic liver transplantation is of no avail in the hepatocerebral form but has been successful in several children with isolated hepatic or hepatorenal disease [100]. Administration of deoxynucleotides might become a therapeutic option in view of its favourable effect *in vitro* [101].

35.1.20 Thiopurine Methyltransferase Deficiency

Thiopurine S-methyltransferase (TPMT, not shown ◘ Fig. 35.1) catalyzes the S-methylation of a number of synthetic pharmacologic purine analogs which contain a thiol group such as 6-mercaptopurine (Purinethol), 6-thioguanine (Lanvis), and azathioprine (Imuran] that is converted to 6-mercaptopurine in vivo. These drugs are used to treat various diseases, including cancers, rheumatoid arthritis, Crohn's disease, and other autoimmune disorders, and also as immunosupressants after organ transplantation. They are converted via phosphoribosylation by HPRT into active thionucleotides which exert their therapeutic action by incorporation into DNA and RNA. Their oxidation by xanthine oxidase, and S-methylation by TPMT, results in inactivation.

The wide variations in therapeutic response and occurrence of toxic side effects in individual patients receiving thiopurines, led to the identification of TPMT as a determining factor in this variability (reviewed in [102][103]). Approximately 90% of individuals in various ethnic populations have high TPMT activity, about 10% have intermediate activity, and 1 in 300 lack activity, explained in 85% of the cases by a variant allele, TPMT*3A [104]. Patients with no or less efficient methylation of thiopurines have more extensive conversion to active thionucleotides that leads to severe, potentially fatal myelosuppression. Determination of the TPMT status (genotyping or phenotyping) prior to treatment with thiopurines and follow-up by 6-thioguanine nucleotide measurement is therefore now considered mandatory [105].

35.1.21 Inosine Triphosphatase Deficiency

Inosine triphosphate pyrophosphohydrolase (ITPase) catalyzes the conversion of inosine triphosphate (ITP) into IMP and pyrophosphate (not shown in ◘ Fig. 35.1). Its activity is variably decreased, owing to genetic polymorphisms, in approximately 5% of the Caucasian population. Reduced ITPase activity results in accumulation of ITP in RBC. This used to be considered benign. Nevertheless, it should be taken into account that ITPase intervenes in the degradation of thio-ITP formed from pharmacologic thiopurines, and hence that its deficiency might be implicated in their accrued toxicity in some patients [103].

35.2 Inborn Errors of Pyrimidine Metabolism

▶ Pyrimidine Metabolism.

Inborn errors of pyrimidine metabolism comprise defects of:

- *pyrimidine synthesis*: CAD, UMP synthase deficiency and Miller syndrome;
- *pyrimidine catabolism*: deficiencies of dihydropyrimidine dehydrogenase (DPD) dihydropyrimidinase (DHP), ureidopropionase, thymidine phosphorylase (a mitochondrial disorder, see also Chapter 14), pyrimidine 5'-nucleotidase and cytidine deaminase, and superactivity of cytosolic 5'-nucleotidase;
- *pyrimidine salvage*: thymidine kinase 2 deficiency (a mitochondrial disease, see also ▶ Chapter 14).

35.2.1 CAD (Carbamoylphosphate Synthetase II, Aspartate Transcarbamylase, Dihydroorotase) Deficiency

CAD is a trifunctional enzyme containing carbamoyl-phosphate synthetase 2 (CPS2), aspartate transcarbamylase (ATCase) and dihydroorotase (DHOase) activities. These are the first three of six reactions required for de novo pyrimidine biosynthesis.

In one patient presenting with developmental delay, intestinal disaccharidase deficiency and dyserythropoietic anaemia, mutations in CAD gene resulted in impaired de novo pyrimidine synthesis and decreased glycosylation precursors. These anomalies were partially rescued in vitro by uridine supplementation suggesting a potential therapy for this new glycosylation disorder [106]. Note added in proofs: Patients with CAD deficiency presenting with an uridine-responsive epileptic encephalopathy have recently been described [148].

35.2.2 UMP Synthase Deficiency (Hereditary Orotic Aciduria)

Clinical Presentation

Megaloblastic anaemia, which appears a few weeks or months after birth, is usually the first manifestation [107][108]. Peripheral blood smears often show anisocytosis, poikilocytosis, and moderate hypochromia. Bone marrow examination reveals erythroid hyperplasia and numerous megaloblastic erythroid precursors. Characteristically, the anemia does not respond to iron, folic acid or vitamin B_{12}. Unrecognized, the disorder leads to failure to thrive and to retardation of growth and psychomotor development.

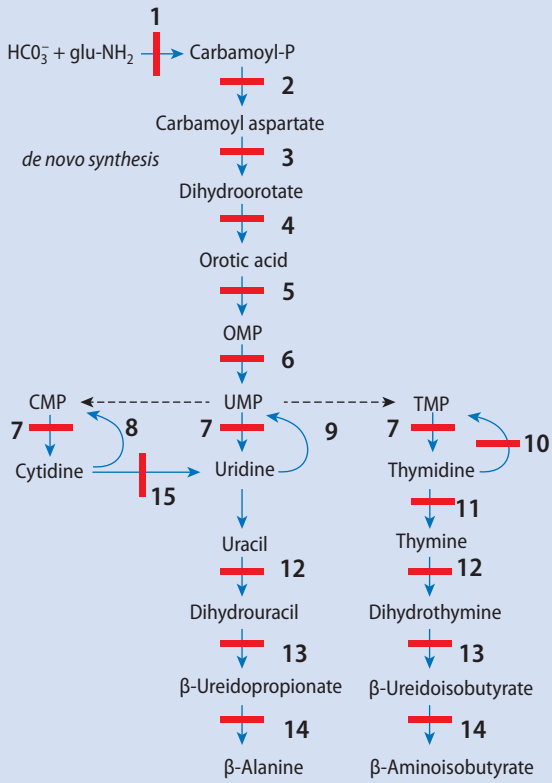
Metabolic Derangement

Uridine monophosphate (UMP) synthase is a bifunctional enzyme of the *de novo* synthesis of pyrimidines (◘ Fig. 35.3). A first reaction, orotate phosphoribosyltransferase (OPRT), converts orotic acid into OMP, and a second, orotidine-5'-monophosphate decarboxylase (ODC), decarboxylates OMP into UMP. In most patients, termed type I, OPRT activity was claimed selectively defective, but later studies showed that both activities are deficient [109]. The defect provokes a massive overproduction of orotic acid, attributed to the ensuing decrease of the feedback inhibition exerted by the pyrimidine nucleotides on the first enzyme of their *de novo* synthesis, cytosolic carbamoyl phosphate synthetase 2 (◘ Fig. 35.3), and a deficiency of pyrimidine nucleotides [108]. The deficiency of pyrimidine nucleotides leads to impairment

Pyrimidine Metabolism

Similarly to that of the purine nucleotides, the metabolism of the pyrimidine nucleotides can be divided into three pathways (▣ Fig. 35.3):

- The biosynthetic, *de novo* pathway starts with the formation of carbamoylphosphate by cytosolic carbamoylphosphate synthetase (CPS II), which is different from the mitochondrial CPS I which catalyzes the first step of ureogenesis (▶ Chapter 20; ▶ Fig. 20.1). This is followed by the synthesis of UMP, and hence of CMP and TMP.
- The catabolic pathway starts from CMP, UMP and TMP, and yields β-alanine and β-aminoisobutyrate which are converted into intermediates of the citric acid cycle.
- The salvage pathway, composed of kinases, converts the pyrimidine nucleosides, cytidine, uridine, and thymidine, into the corresponding nucleotides, CMP, UMP, and TMP. It also converts several pharmacological anticancer and antiviral nucleoside analogs into their active forms.



▣ **Fig. 35.3 Pathways of pyrimidine metabolism**. *CMP*, cytidine monophosphate; *glu-NH2*, glutamine; *OMP*, orotidine monophosphate; *PRPP*, phosphoribosylpyrophosphate; *TMP*, thymidine monophosphate; *UMP*, uridine monophosphate. *1*, carbamoylphosphate synthetase II; *2*, aspartate transcarbamylase; *3*, dihydroorotase (1 to 3 form CAD); *4*, dihydroorotate dehydrogenase; *5*, orotate phosphoribosyltransferase; *6*, orotidine decarboxylase (5 and 6 form UMP synthase); *7*, pyrimidine (cytosolic) 5′-nucleotidase; *8*, cytidine kinase; *9*, uridine kinase; *10*, thymidine kinase; *11*, thymidine phosphorylase; *12*, dihydropyrimidine dehydrogenase; *13*, dihydropyrimidinase; *14*, ureidopropionase; *15*, cytidine deaminase. Enzyme defects are indicated by **solid bars** across the arrows.

of cell division, which results in megaloblastic anemia and in retardation of growth and development. In two patients who did not display megaloblastic anemia, a mutation in the ODC domain was found [109][110].

Genetics

Hereditary orotic aciduria is inherited as an autosomal recessive trait. UMPS is a relatively small gene containing 6 exons. The 5’ end of the gene encodes the N-terminal domain of the bifuctional protein, carrying the OPRT activity, and the 3’ end encodes the C-terminal domain, carrying the ODC activity [111].

The genetic lesion results in synthesis of an enzyme with reduced stability or activity [110][111][112].

Diagnostic Tests

Urinary analysis reveals a massive over excretion of orotic acid, reaching, in infants, 200- to 1000-fold the normal adult value of 1–1.5 mg per 24 h. Occasionally, orotic acid crystalluria is noted, particularly upon dehydration. In most patients, the ratio of the excretion of orotic acid to that of the dephosphorylation product of OMP, orotidine, is above 10. In contrast, in the two patients with a postulated defect of ODC, the ratio of both compounds is approximately 1 [109]. Enzymatic diagnosis can be performed on red blood cells.

Treatment and Prognosis

The enzyme defect can be by-passed by the administration of uridine, which is converted into UMP by uridine kinase (Fig. 35.3). An initial dose of 100–150 mg/kg, divided over the day, induces prompt hematologic response and acceleration of growth. Further dosage should be adapted to obtain the lowest possible output of orotic acid. In some cases normal psychomotor development was achieved, but not in others, possibly owing to delayed onset of therapy.

35.2.3 Miller syndrome (Dihydroorotate Dehydrogenase Deficiency)

Miller Syndrome, also named Genee-Wiedemann syndrome, is a very rare genetic condition also referred to as »postaxial acrofacial dysostosis«, characterized by distinctive craniofacial malformations associated with limb abnormalities. It is the first mendelian disorder identified by exome sequencing allowing identification in several patients of mutations of *DHODH*, the gene encoding dihydroorotate dehydrogenase (Fig. 35.3), which catalyses the fourth step of *de novo* pyrimidine synthesis [113]. Interestingly, the malformations in Miller syndrome resemble those of fetal exposure to methotrexate, an inhibitor of *de novo* purine synthesis. Defects of purine and pyrimidine biosynthesis might thus cause similar birth defects. The mechanism by which mutations of *DHODH* cause malformations remains to be elucidated but may involve disturbed fibroblast growth factor signalling [114]. In theory, dietary supplementation with orotic acid or uridine could bypass the metabolic bloc. However, as the principal damage occurs in utero, this therapy is unlikely to be effective.

35.2.4 Dihydropyrimidine Dehydrogenase Deficiency

Clinical Picture

Two forms occur. The first is found in children, most of whom display epilepsy, motor and mental retardation, often accompanied by generalized hypertonia, hyperreflexia, growth delay, dysmorphic features including microcephaly, and autistic features [115]. In these patients, the deficiency of dihydropyrimidine dehydrogenase (DPD) is complete or near-complete. The marked clinical heterogeneity justifies systematic screening for the deficiency in unexplained, profound as well as mild psychomotor retardation, in neurological disease with convulsions and/or hypotonia, and in individuals with autistic behaviour. The second clinical picture is found in adults who receive the pyrimidine analog, 5-fluorouracil, a classic treatment of various cancers including breast, ovary or colon [116][117]. It is characterised by severe toxicity, manifested by profound neutropenia, stomatitis, diarrhea and neurologic symptoms, including ataxia, paralysis and stupor. In these patients, DPD deficiency is as a rule partial, and only revealed by 5-fluorouracil therapy.

Metabolic Derangement

The deficiency of DPD, which catalyzes the catabolism of uracil and thymine into dihydrouracil and dihydrothymine, respectively (Fig. 35.3), leads to the accumulation of the former compounds [115]. How the defect leads to neurological symptoms remains elusive, but reduction of the concentration of β-alanine, a neurotransmitter, may play a role. The deficiency might also potentiate the toxicity of the anticancer drug 5-fluorouracil.

Genetics

The infantile form of DPD deficiency is inherited as an autosomal recessive trait. More than 40 mutations have been identified. Most frequent are a splice site mutation (IVS14+1G>A), and a D949V mutation [115][117][118]. Strikingly, patients who carry the same mutation may display widely variable clinical symptoms. In the adult form of DPD deficiency, characterized by 5'-fluorouracil toxicity, approximately 25 % of patients are heterozygotes for the IVS14+1G>A mutation [117]. Large genomic deletions and deep intronic mutations affecting pre-mRNA splicing have also been identified in severely affected patients [119][120].

Diagnostic Tests

Patients excrete high amounts of uracil (56–683 mmol/mol creatinine), and of thymine (7–439 mmol/mol creatinine. Excretion of both compounds may also be less elevated in patients with high residual DPD activity. The pyrimidine catabolites can be detected by HPLC, LCMS-MS [19][20], and analysis of amino acids in urine before and after acid hydrolysis [121].

The enzyme defect can be demonstrated in the patients' fibroblasts, liver and blood cells, with the exception of erythrocytes [115][117]. In the pediatric patients, DPD deficiency is complete or near-complete; in the adult cancer patients experiencing acute 5-fluorouracil toxicity it is partial, with residual enzyme activities ranging from 3 to 30%. Evaluation of several tests for screening for DPD deficiency is presently under way [122][123].

Treatment and Prognosis

No treatment is available for pediatric patients. Symptoms usually remain the same, but death in early infancy of a more severely affected child has been reported. In the adult cancer patients, discontinuation of 5-fluorouracil results in slow resolution of the toxic symptoms [116][117].

35.2.5 Dihydropyrimidinase Deficiency

Clinical Picture

Approximately 30 patients with this disorder have been diagnosed [115][124]. As in DPD deficiency, the non specific clinical picture varies from severe psychomotor retardation with epilepsy, dysmorphic features or microcephaly, to completely asymptomatic. Nearly half of the patients present with gastrointestinal problems such as feeding difficulties, cyclic vomiting, gastroesophageal reflux and malabsorption.

Metabolic Derangement

Dihydropyrimidinase (DHP) catalyzes the cleavage of dihydrouracil and dihydrothymine into, repectively, β-ureidopropionate and β-ureidoisobutyrate (Fig. 35.3). Consequently, considerable quantities of dihydrouracil and dihydrothymine, which are normally found in small amounts, are excreted in urine [115][124]. There is also a moderate elevation of uracil and thymine excretion. Increased sensitivity to 5-fluorouracil, leading to severe toxicity has also been reported [125].

Genetics

The defect is inherited as an autosomal recessive trait. A variety of mutations in *DPYS* have been identified both in symptomatic and asymptomatic individuals [124][126] Enzyme expression showed no significant difference in residual activity between the mutations of the symptomatic and the asymptomatic individuals.

Diagnostic Tests

Elevation of urinary dihydrouracil and dihydrothymine, not UV-detectable, can be measured by LC-MSMS [19][20]. Enzyme assay requires liver biopsy, since more accessible tissues do not possess DHP activity.

Treatment and Prognosis

There is no therapy and prognosis seems unpredictable [127].

35.2.6 Ureidopropionase Deficiency

Beta-ureidopropionase (also termed β-alanine synthase) catalyzes the last step of the pyrimidine degradative pathway, the conversion of θ-ureidopropionate and β-ureidoisobutyrate into β-alanine and β-aminoisobutyrate, respectively (Fig. 35.3). The enzyme has been found deficient in 16 patients presenting with non specific clinical pictures varying from early-onset psychomotor retardation with severely delayed myelination, optic atrophy, pigmentary retinopathy, cerebellar hypoplasia, epilepsy, dysmorphic features, or urogenital and colorectal anomalies, to completely asymptomatic [128][129][130][131]. Large subdural hematomas and global supratentorial atrophy were found in a patient with an acute life-threatening status epilepticus [132]. Recently, 13 individuals with highly variable phenotypes, ranging from asymptomatic to severe neurological involvement, were described. Ten of these were found after urine screening of 24,000 neonates in Japan [133].

The deficiency provokes increased urinary excretion of ureidopropionic acid (also called N-carbamyl-β-alanine) and ureidoisobutyric acid (also called N-carbamyl-β-aminoisobutyric acid) which may act as neurotoxins [134]. Dihydrouracil, dihydrothymine, uracil and thymine are only moderately increased.

35.2.7 Pyrimidine 5′-Nucleotidase Deficiency

Pyrimidine 5'-nucleotidase (P5'N-1) or cytosolic 5-nucleotidase III (NT5C3) deficiency also called Uridine monophosphate hydrolase (UMPH-1) deficiency is restricted to erythrocytes. It leads to accumulation of pyrimidine nucleotides resulting in basophilic stippling and chronic non spherocytic hemolytic anemia. It is the third most common cause of haemolytic anemia. This clinical presentation is similar to lead intoxication, because of its inhibitory effect on P5'N-1. Several mutations have been identified but the mechanism by which the increased pyrimidine nucleotides cause hemolysis remains unknown [135].

35.2.8 Cytosolic 5′-Nucleotidase Superactivity

Four unrelated children have been described with a syndrome including developmental delay, growth retardation, seizures, ataxia, recurrent infections, autistic features and hypouricosuria [136]. Studies in the patients' fibroblasts showed 6- to 20-fold elevations of the activity of cytosolic 5'-nucleotidase, measured either with a pyrimidine (UMP) or a purine (AMP) as substrate. Based on the possibility that this increased catabolism might cause a deficiency of pyrimidine nucleotides, the patients were treated with uridine at the dose of 1 g/kg per day. Remarkable developmental improvement and a decrease in frequency of seizures and infections were recorded. Existence of the disease is however not established, since no other patients and no gene alterations have been described up to now.

35.2.9 Thymidine Phosphorylase Deficiency

Thymidine phosphorylase deficiency is the most frequent cause of mitochondrial neurogastrointestinal encephalomyopathy (MNGIE), an autosomal recessive disease associated with multiple deletions of skeletal muscle mitochondrial DNA (▶ Chapter 14). A variety of mutations have been described [137]. The enzyme deficiency results in marked accumulation of urinary and plasma thymidine and deoxyuridine, which most likely provokes imbalance of the mitochondrial nucleotides, and hence compromises the replication of mitochondrial DNA. Allogenic hematopoietic stem-cell transplantation has been performed in a number of patients with varying suc-

cess [138][139]. More recently, enzyme replacement therapy using recombinant Escherichia coli thymidine phosphorylase has resulted in significant biochemical and clinical improvement [140].

35.2.10 Cytidine Deaminase Deficiency

Cytidine deaminase catalyzes the conversion of cytidine into uridine along the pyrimidine catabolic pathway (Fig. 35.3). It plays a key role in the inactivation of gemcitabine, a cytidine analog with activity against solid tumors. Cytidine deaminase deficiency, linked with a number of genetic polymorphisms and found in 7% of adult patients receiving the drug, is associated with the risk of developing severe toxicity [141], and constitutes another example of predictive pharmacogenetics.

Cytidine deaminase is part of a superfamily which also includes activation-induced cytidine deaminase. This RNA editing enzyme is specifically expressed in B-lymphocytes in which it is required for the terminal differentiation necessary for efficient antibody response. Its deficiency causes autosomal recessive type II hyper-IgM syndrome [142], presenting with recurrent infections, lymphoid hyperplasia, autoimmune and related inflammatory disorders. Early initiation of intravenous immunoglobulin [143] and antibiotic prophylaxis [144] most often drastically improve the symptoms.

35.2.11 Thymidine Kinase 2 Deficiency

Initially described in four independent patients with very severe, isolated myopathy, motor regression and early death [145], the clinical spectrum of this disorder has now been expanded to include spinal muscular atrophy type 3-like presentation, rigid spine syndrome, and a milder myopathic phenotype without motor regression and with longer survival [146]. The deficiency results in depletion of muscular mitochondrial DNA (▶ Chapter 14), and is caused by mutations of the gene encoding thymidine kinase-2, the mitochondrial form of this thymidine salvage enzyme [145][147]. As in the deficiencies of deoxyguanosine kinase and thymidine phosphorylase, the defect likely produces imbalance of the mitochondrial nucleotides which disturbs the replication of mitochondrial DNA.

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Disorders of Haem Biosynthesis

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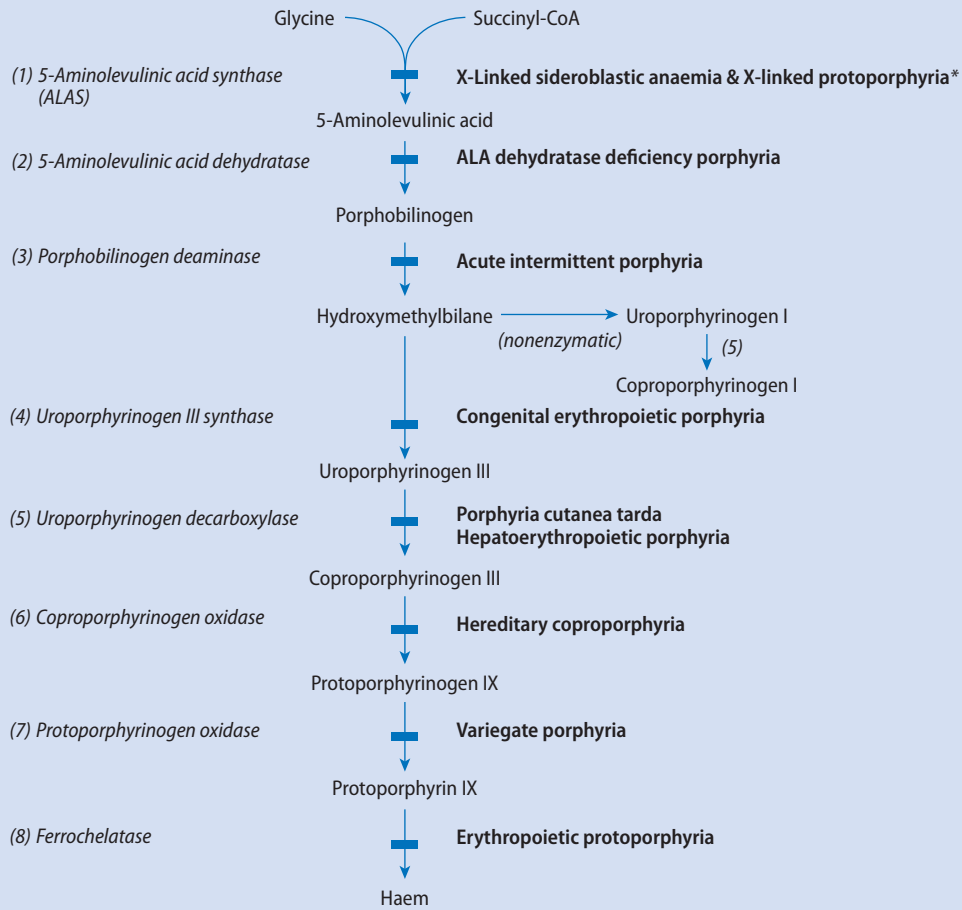
The Haem Biosynthetic Pathway

Haem (iron protoporphyrin) is a metalloporphyrin with iron as the central metal atom, and is the prosthetic group for many haemoproteins. The largest amounts of haem are produced in the bone marrow, for formation of haemoglobin, and in the liver, primarily for cytochrome P450 enzymes. The pathway for haem synthesis (◘ Fig. 36.1) consists of eight enzymes and their substrates and products. The first and last three enzymes are located in mitochondria and the other four in the cytosol.

The pathway is regulated differently in bone marrow and liver. The first enzyme of the pathway, 5-aminolevulinic acid synthase (ALAS), also known as δ-aminolevulinic acid synthase, is the only enzyme in this pathway for which erythroid and housekeeping forms of the enzyme are encoded by separate genes. The housekeeping enzyme (termed ALAS1) is rate limiting in the liver, is subject to negative feedback by haem, which represses its synthesis and its import into mitochondria, and is induced by a variety of drugs, steroids and other chemicals that also induce cytochrome P450 enzymes. By contrast, the erythroid-specific enzyme (ALAS2), which is encoded by a separate gene located on the X chromosome, is induced by haem and iron, providing for erythroid-specific regulation of haem synthesis [1].



◘ **Fig. 36.1 Pathway of haem biosynthesis**. Intermediates and enzymes of the haem biosynthetic pathway are listed. *ALA*, 5-aminolevulinic acid; *CoA*, coenzyme A. Diseases caused by the various enzyme alterations (indicated by solid bars across the arrows) are given in bold. *Gain of function mutations cause X-linked protoporphyria

X-Linked sideroblastic anaemia is often due to loss of function mutations of *ALAS2*. Characteristics of the disease include adult-onset anaemia, ineffective erythropoiesis with formation of ring sideroblasts, iron accumulation and pyridoxine responsiveness. *Porphyrias* are metabolic disorders that are due to altered activity of enzymes of this pathway, and are associated with striking accumulations and excess excretion of haem pathway intermediates and their oxidised products.

The three most common porphyrias, are *porphyria cutanea tarda, acute intermittent porphyria* and *erythropoietic protoporphyria*. Acute intermittent porphyria presents with acute neurovisceral symptoms and can be aggravated by certain drugs,

hormones and nutritional changes, and is treated with intravenous haemin and carbohydrate loading. The skin is affected in the other two, but quite differently. Erythropoietic porphyrias usually present in childhood and hepatic porphyrias during adult life. All porphyrias are inherited, with the exception of porphyria cutanea tarda, which is mostly due to an acquired enzyme deficiency in the liver (▶ The Haem Biosynthetic Pathway).

36.1 X-Linked Sideroblastic Anaemia

Clinical Presentation

Sideroblastic anaemia can be either acquired or inherited, and result from defects in mitochondrial haem synthesis, iron-sulfur cluster biogenesis and mitochondrial protein synthesis. Its presence is suggested by hypochromic anaemia in the presence of increases in serum iron concentration and transferrin saturation. The bone marrow contains nucleated erythrocyte precursors with iron-laden mitochondria surrounding the nucleus (ring sideroblasts). Progressive iron accumulation may result from ineffective erythropoiesis, leading to organ damage [2].

Metabolic Derangement

These features reflect a deficiency of haem synthesis, which at least in the inherited form is due to a deficiency of ALAS2. Acquired forms have been attributed to alcohol, chemotherapy and the early stages of a myelodysplastic syndrome, which might affect one or more steps in protoporphyrin and haem synthesis. However, mutations of *ALAS2* or other mediators of mitochondrial iron metabolism have not been excluded in many of these cases.

Genetics

X-Linked sideroblastic anaemia due to loss-of-function mutations of *ALAS2* is the most common cause of congenital sideroblastic anemia [2][3][4], and its genotypes and phenotypes are heterogeneous [5][6]. More than 60 different *ALAS2* mutations have been reported in 120 families with X-linked sideroblastic anaemia [7]. Some point mutations occur in the pyridoxine binding site of the enzyme, and in such cases enzyme activity may be at least partially restored and anaemia corrected by high doses of this vitamin. Cases of sideroblastic anaemia due to mutations of *SLC25A38*, a mitochondrial transporter [8], and of *TRNT1*, which is involved in maturation of nuclear and mitochondrial transfer RNA, have also been described [9] (▶ Chapter 14).

Diagnostic Tests

Hypochromic anaemia with evidence of iron overload suggests this diagnosis. Ring sideroblasts in the bone marrow and pyridoxine responsiveness is further evidence. Detection of an *ALAS2* mutation and demonstration of its X-linked inheritance is important for a definite diagnosis. Patients who also have haemochromatosis (*HFE*) mutations are at greater risk for iron accumulation.

Treatment and Prognosis

Treatment consists in administration of pyridoxine and folic acid. The starting dose of pyridoxine is 100–300 mg/day, which is followed by a maintenance dose of 100 mg/day. Phlebotomy to remove excess iron not only prevents organ damage, which is the primary cause of morbidity in this disease, but also may increase responsiveness to pyridoxine [2].

36.2 The Porphyrias

Porphyrias result from altered activity of haem synthetic pathway enzymes and are characterised by accumulation and excess excretion of pathway intermediates and their oxidised products. The photosensitising effects of excess porphyrins cause cutaneous manifestations. Neurological effects are poorly explained, but are associated with increases in the porphyrin precursors, 5-aminolevulinic acid (5-ALA) (also known as δ-aminolevulinic acid) and porphobilinogen.

The complex patterns of excess intermediates in these disorders are characteristic for each type of porphyria. 5-ALA and porphobilinogen are water soluble, colourless and nonfluorescent and are excreted almost entirely in urine, as are porphyrins with a large number of carboxylic side chains (e.g. uroporphyrin, an octacarboxylic porphyrin). Protoporphyrin (a dicarboxylic porphyrin) is not soluble in water and is excreted entirely in bile and faeces. Coproporphyrin (a tetracarboxylic porphyrin) is excreted in both urine and bile, and its urinary excretion increases when hepatobiliary function is impaired. Most of the porphyrin intermediates are porphyrinogens (reduced porphyrins), and these undergo autooxidation if they leave the intracellular environment and are then excreted primarily as the corresponding porphyrins. Porphyrinogens are colourless and nonfluorescent but porphyrins are reddish and display red fluorescent when exposed to ultraviolet light.

36.2.1 Classification and Diagnosis

The porphyrias are classified according to the tissue where intermediates initially accumulate (the liver in hepatic porphyrias and the bone marrow in erythropoietic porphyrias), or by their clinical presentation (acute neurovisceral or cutaneous porphyrias) (◘ Table 36.1).

The four acute porphyrias are associated with striking increases in 5-ALA, and three of these with increases in porphobilinogen. The diverse symptoms of these acute porphyrias (e.g. abdominal pain, neuropathy, and mental disturbances) can be mimicked by many other more common disorders. Porphyrias accompanied by skin manifestations are termed cutaneous porphyrias. Excitation of excess porphyrins in the skin by light with a wavelength near 400 nm, which is within the lower part of the visible range, leads to generation of singlet oxygen and cell damage [10].

Table 36.1 Diseases associated with alterations in enzymes in the haem biosynthetic pathway and the classification of porphyrias based on the major tissue site of overproduction of haem pathway intermediates (hepatic vs erythropoietic) or the type of major symptoms (acute neurovisceral vs cutaneous). X-Linked protoporphyria is due to gain of function, and the others to loss of function mutations. Porphyria cutanea tarda results mostly from an acquired enzyme deficiency in the liver

Disease	Enzyme	Gene	Porphyria classification				
			Inheritance	Hepatic	Erythropoietic	Acute	Cutaneous
X-Linked sideroblastic anaemia	5-Aminolevulinic acid synthase, erythroid form	*ALAS2*	XL	NA	NA	NA	NA
X-Linked protoporphyria			XL		X		X
5-Aminolevulinic acid dehydratase porphyria	5-Aminolevulinic acid dehydratase	*ALAD*	AR	X		X	
Acute intermittent porphyria	Porphobilinogen deaminase[1]	*HMBS*	AD	X		X	
Congenital erythropoietic porphyria	Uroporphyrinogen III synthase	*UROS*	AR		X		X
Porphyria cutanea tarda	Uroporphyrinogen decarboxylase[2]	*UROD*	AD (but mostly acquired)	X			X
Hepatoerythropoietic porphyria	Uroporphyrinogen decarboxylase[2]		AR	X	X		X
Hereditary coproporphyria	Coproporphyrinogen oxidase	*CPOX*	AD	X		X	X
Variegate porphyria	Protoporphyrinogen oxidase	*PPOX*	AD	X		X	X
Erythropoietic protoporphyria	Ferrochelatase	*FECH*	AR		X		X

NA, not applicable
[1] The enzyme is also known as hydroxymethylbilane synthase, and formerly as uroporphyrinogen I synthase
[2] Inherited deficiency of uroporphyrinogen decarboxylase is partially responsible for familial (type 2) porphyria cutanea tarda

A diagnosis of porphyria should be considered in patients with unexplained neurovisceral symptoms or cutaneous photosensitivity. In contrast to the nonspecific nature of symptoms, laboratory tests can be both sensitive and specific, if properly chosen and interpreted [11][12]. However, some tests, particularly urinary porphyrin measurements, may be abnormal in other diseases. Measurements of deficient enzymes and especially DNA studies are important for diagnostic confirmation, genetic counselling and screening of family members.

The clinical presentation determines the type of initial laboratory testing (Table 36.2). It is very important to confirm or exclude acute hepatic porphyrias promptly, because treatment is more successful if started soon after the onset of symptoms. Acute hepatic porphyrias are often clinically latent with no excess accumulation of pathway intermediates even throughout life, and the family history is often negative. Diagnosis of active cases is based on measurement of porphyrin precursors and porphyrins in urine, blood and faeces. To avoid delay, a random urine sample rather than a 24-h collection is preferred. Current recommendations are that all major medical centres should have capabilities for rapid screening of spot urine samples for excess porphobilinogen, and that 5-ALA and total porphyrins be measured later on the same sample [11]. Finding normal levels of these compounds excludes all acute porphyrias as potential causes of current symptoms.

Total urine and plasma porphyrins are increased and should be measured in all patients with blistering skin lesions due to porphyrias. A fluorescence emission scan of diluted plasma at neutral pH is useful for differentiating several cutaneous porphyrias [13]. Measurement of erythrocyte protoporphyrin is necessary for the diagnosis of protoporphyrias, which cause nonblistering photosensitivity [12]. If a screening test is positive, further testing will be needed to establish the type of porphyria.

Table 36.2 First-line laboratory tests for screening for porphyrias and second-line tests for further evaluation when initial testing is positive

Testing	Acute neurovisceral symptoms	Cutaneous photosensitivity
First-line	Urinary 5-aminolevulinic acid, porphobilinogen and total porphyrins [1] (quantitative; random or 24-h urine)	Blistering skin lesions: Total plasma porphyrins [2] Nonblistering: Erythrocyte porphyrins [3]
Second-line	Urinary 5-aminolevulinic acid, porphobilinogen and total porphyrins [1] Total faecal porphyrins [1] Erythrocyte porphobilinogen deaminase Total plasma porphyrins [2] Mutation analysis	Erythrocyte porphyrins Urinary 5-aminolevulinic acid, porphobilinogen and total porphyrins [1] Total faecal porphyrins [1] Mutation analysis

[1] Fractionation of urinary and faecal porphyrins is usually not helpful unless the total is increased
[2] The preferred method is direct fluorescence spectrophotometry.
[3] Erythrocyte porphyrins are generally expressed as protoporphyrin; however, the method detects other porphyrins as well. This test lacks specificity, because erythrocyte protoporphyrin is increased in many erythrocytic disorders. Testing should include measurement of erythrocyte total protoporphyrin and, if elevated, metal-free and zinc protoporphyrin [12]

36.3 5-Aminolevulinic Acid Dehydratase Porphyria

Clinical Presentation

Only six cases of this porphyria are documented in the literature by molecular methods [14]. Symptoms resemble those of acute intermittent porphyria, including abdominal pain and neuropathy, usually beginning soon after puberty. In one severe case disease onset was in early childhood, with failure to thrive and anaemia. An adult-onset case was associated with polycythaemia vera.

Metabolic Derangement

The disorder is due to markedly deficient activity (<5% of normal) of 5-ALA dehydratase, the second enzyme in the haem biosynthetic pathway [14] (Fig. 36.1; Table 36.1). This porphyria is commonly classified as hepatic, along with the other acute porphyrias, but the marrow contributes at least to an increase in erythrocyte zinc protoporphyrin.

Lead poisoning can be distinguished by showing reversal of inhibition of 5-ALA dehydratase activity in erythrocytes by dithiothreitol added *in vitro*. Hereditary tyrosinaemia type 1, resulting from a deficiency of fumarylacetoacetase, leads to accumulation of succinylacetone (2,3-dioxoheptanoic acid), a potent inhibitor of 5-ALA dehydratase (▶ Chapter 17). Other heavy metals and styrene can also inhibit this enzyme.

Genetics

This is an autosomal recessive disorder with severe enzyme deficiency in affected individuals and half normal activity in both parents. Five of the cases so far described had compound heterozygous mutations, and the sixth had a myeloproliferative disorder and a heterozygous mutation. Immunological studies to date have indicated that most mutant alleles produce a defective enzyme protein.

Diagnostic Tests

Characteristic findings include elevations in urinary 5-ALA and coproporphyrin III and erythrocyte zinc protoporphyrin, normal or only slightly increased urinary porphobilinogen, and a marked decrease in erythrocyte 5-ALA dehydratase activity. Excess coproporphyrin III probably results from metabolism of 5-ALA via the haem biosynthetic pathway in a tissue other than the site of its initial accumulation. Erythrocyte zinc protoporphyrin content is also increased, as in other homozygous types of porphyria. The diagnosis of this porphyria should be confirmed by DNA studies [11][14].

Treatment and Prognosis

In general, the approach is the same as in acute intermittent porphyria. Haemin therapy is generally more effective than glucose loading. It is prudent to avoid drugs that are harmful in other acute porphyrias.

36.4 Acute Intermittent Porphyria (AIP)

Clinical Presentation

This autosomal dominant condition is the most common of the acute porphyrias. Most heterozygotes remain clinically asymptomatic for all or most of their lives. Factors that contribute to clinical expression include certain drugs, steroid hormones and nutrition. Symptoms are very rare in children. Acute attacks of neurovisceral symptoms and signs are the most common presentation, although subacute and chronic manifestations can also occur [15]. Symptoms are more common in women than in men. Attacks usually last for several

days or longer, often require hospitalisation and are usually followed by complete recovery. Severe attacks are sometimes fatal, especially if the diagnosis is delayed. Abdominal pain, the most common symptom, is usually steady and poorly localised, but is sometimes crampy. Tachycardia, hypertension, restlessness, fine tremors and excess sweating reflect sympathetic overactivity. Other common manifestations may include nausea, vomiting, constipation, pain in the limbs, head, neck or chest, muscle weakness and sensory loss. Dysuria and bladder dysfunction as well as ileus, with abdominal distension and decreased bowel sounds, may accompany an attack. However, increased bowel sounds and diarrhoea may occur. Tenderness, fever and leukocytosis are mild or absent. A peripheral neuropathy that is primarily motor can develop, and is manifested by muscle weakness that most often begins proximally in the upper extremities. It may progress to involve all extremities and the respiratory muscles, and even lead to bulbar paralysis. Tendon reflexes are usually decreased or absent with advanced neuropathy. Muscle weakness is sometimes focal and asymmetrical. Cranial and sensory nerves can be affected. Advanced motor neuropathy and death are rare. Seizures may occur as a result of hyponatraemia, or as a neurological manifestation of porphyria. Hyponatraemia can be due to electrolyte depletion from vomiting or diarrhoea, poor intake, renal sodium loss, or inappropriate antidiuretic hormone secretion. In some patients, MRI findings have resembled the reversible posterior leukoencephalopathy syndrome [16]. Other severe complications may include acute kidney failure and rhabdomyolysis [17].

Persistent hypertension and impaired renal function may develop over the long term [18]. Chronic abnormalities in liver function tests, particularly transaminases, are common, although few patients develop significant hepatic impairment. The risk of hepatocellular carcinoma is increased in this and other acute porphyrias, and also in porphyria cutanea tarda [18][19].

Metabolic Derangement

AIP is due to reduced activity of porphobilinogen deaminase (PBGD) (also known as hydroxymethylbilane synthase (HMBS) and formerly as uroporphyrinogen I synthase) (Fig. 36.1, Table 36.1). Most heterozygotes remain asymptomatic with normal levels of urinary porphyrin precursors. Clinical expression of the disease is accompanied by an initial accumulation of haem pathway intermediates in liver, followed by excretion primarily in urine.

The deficient enzyme can become limiting for haem synthesis when certain drugs, hormones, or nutritional factors increase the demand for hepatic haem by increasing the synthesis of cytochrome P450 enzymes and ALAS1 in the liver, and 5-ALA and porphobilinogen accumulate. Excess porphyrins originate non enzymatically from porphobilinogen, and perhaps enzymatically from 5-ALA transported to tissues other than the liver [1].

Genetics

Inheritance is autosomal dominant; more than 300 different mutations of the PBGD gene (*HMBS*) have been identified in unrelated families [1]. Two forms of this enzyme, an erythroid-specific and a housekeeping form are derived from the same gene. A deficiency of the housekeeping form in the liver is essential for causing AIP. Mutations located in or near the first of the 15 exons in this gene can cause AIP by impairing the synthesis of the housekeeping form but not of the erythroid-specific form of PBGD. Homozygous cases of AIP are extremely rare, but should be suspected in early childhood cases [20].

Diagnostic Tests

The finding of a substantial increase in urinary porphobilinogen is a sensitive and specific indication that a patient has either AIP, hereditary coproporphyria or variegate porphyria (Table 36.2) [11][21]. Porphobilinogen usually remains increased between attacks of AIP unless there have been no symptoms for a prolonged period. Faecal total porphyrins are generally normal or minimally increased in AIP, and markedly increased in active cases of hereditary coproporphyria and variegate porphyria. Variegate porphyria is also characterised by increased total plasma porphyrins, as discussed later, whereas this is not characteristic of AIP. Urinary coproporphyrin is generally more increased in hereditary coproporphyria and variegate porphyria than in AIP. Urinary uroporphyrin can be increased in all these disorders, especially when porphobilinogen is increased. Other biomarkers to predict greater activity are sought, and increased glycosylation of circulating vitamin D binding protein was suggested as a candidate [22].

Decreased erythrocyte PBGD helps to confirm a diagnosis of AIP and diagnose clinically latent disease. Limitations include a wide normal range (up to 3-fold) that overlaps the range of patients with AIP, and falsely low activity with suboptimal sample processing or storage. Also, the erythroid enzyme is not affected if AIP is caused by an *HMBS* exon 1 mutation. Therefore, DNA studies are preferred [11][23].

Treatment and Prognosis

Intravenous haemin (haem arginate or haematin, 3–4 mg/kg body weight, infused i.v. once daily for 4 days or longer) is considered specific therapy for acute attacks of porphyria, because it represses hepatic ALAS1 and markedly reduces levels of 5-ALA and porphobilinogen [11][21]. Carbohydrate loading, by intravenous administration of 10% glucose during acute attacks, also represses ALAS1, but is much less effective than haemin. Glucose may be started initially until haemin is obtained. Haem arginate is the preferred form of haemin for intravenous administration. Haematin (haem hydroxide) commonly causes phlebitis at the site of infusion and has a transient anticoagulant effect. In countries where haem arginate is not available, haematin can be reconstituted with human albumin, which stabilises the haem as haem albumin and confers some of the advantages of haem arginate [24].

Repeated haemin infusions can lead to iron overload [25]. Serum ferritin concentration should be followed and small

volume phlebotomies instituted if the ferritin becomes elevated. Overdoses of haemin can cause acute renal failure and acute liver failure [26].

Most acute attacks are severe enough to require hospitalisation for administration of intravenous haemin or glucose and observation for neurological complications, respiratory impairment and electrolyte imbalances. Narcotic analgesics are commonly required for abdominal, back or extremity pain, and small doses of a phenothiazine are useful in the short term for nausea, vomiting, anxiety and restlessness. Ondansetron is also a safe antiemetic, whereas metoclopramide is unsafe. Chloral hydrate can be administered for insomnia. Benzodiazepines in low doses are safe if a minor tranquilliser is required. Bladder distension may require catheterization.

Intravenous glucose can be tried instead of haemin for mild attacks or until haemin becomes available. At least 300 g daily is recommended, and >500 g daily may be more effective. If tolerated, oral carbohydrate loading is also an option [11].

With resolution of an attack abdominal pain may disappear within hours, and paresis begins to improve within days. After a prolonged attack with severe motor neuropathy, muscle weakness may resolve gradually and completely but there may be some residual weakness.

Treatment of seizures is problematic, because almost all anticonvulsants can exacerbate acute porphyrias. Bromides, gabapentin, levetiracetam and vigabatrin can be given safely. β-Adrenergic blocking agents may control tachycardia and hypertension in acute attacks of porphyria. Progressive renal disease may develop and may be accompanied by increased plasma porphyrins and blistering photosensitivity. Renal failure may be treated by haemodialysis or renal transplantation [18][27].

Allogeneic liver transplantation for patients with frequent attacks refractory to haemin is effective if accomplished before there is advanced motor paralysis [27][28][29]. A higher than expected rate of hepatic artery thrombosis has been reported [30]. Some patients with renal failure have undergone combined liver and kidney transplantation [27]. Domino transplantation, whereby the explanted AIP liver is used as a donor liver, leads to development of AIP in the recipient [31]. Investigational therapies include RNA interferance targetting ALAS1 [32] and gene therapy to correct porphobilinogen deaminase deficiency in hepatocytes [33].

Precipitating factors such as harmful drugs, dietary indiscretions, smoking, endogenous or exogenous hormones (particularly progesterone and progestins) and intercurrent infections must be addressed. Frequent attacks during the luteal phase of the cycle (when progesterone levels are highest) can be prevented by administration of a gonadotropin-releasing hormone analogue to prevent ovulation [34].

With prompt treatment and precautions to prevent further attacks, the outlook for patients with acute porphyrias is usually excellent [11][18]. However, some patients continue to have attacks in the absence of identifiable precipitating factors. Some develop chronic pain and may become narcotic dependent. Such patients need to be followed closely because there is often coexisting depression and an increased risk of suicide.

36.5 Congenital Erythropoietic Porphyria (CEP) (Gunther Disease)

Clinical Presentation

This is usually a severe disease with manifestations noted soon after birth, or even in utero. Rarely, symptoms first appear during adult life. Cutaneous features resemble those in porphyria cutanea tarda, but are much more severe in most cases. Lesions include bullae and vesicles on sun-exposed skin, hypo- or hyperpigmented areas, hypertrichosis and scarring. Digits and facial features may be lost due to infection and scarring. The teeth are reddish brown (erythrodontia) because of porphyrin deposition, and fluoresce when exposed to long-wave ultraviolet light. Porphyrins are also deposited in bone. Haemolysis is almost invariably present, resulting from ineffective erythropoiesis and the markedly increased erythrocyte porphyrin levels, and is accompanied by splenomegaly. Life expectancy is often shortened by infections or haematological complications. There are no neurological manifestations [35].

CEP can present in utero as non immune hydrops [36]. When this is recognised, intrauterine transfusion is possible, and after birth severe photosensitivity can be prevented by avoiding phototherapy for hyperbilirubinaemia.

Metabolic Derangement

This rare disorder is due to a severe deficiency of uroporphyrinogen III synthase (also known as cosynthase) (UROS) (Fig. 36.1, Table 36.1). There is considerable accumulation of hydroxymethylbilane (the substrate of the deficient enzyme), which is converted nonenzymatically to uroporphyrinogen I, a nonphysiological intermediate, which cannot be metabolised to haem. Uroporphyrinogen I is metabolized to coproporphyrinogen I, and the corresponding porphyrins (uroporphyrin I and coproporphyrin I) accumulate in bone marrow, plasma, urine and faeces. Porphyrin accumulation in erythroid cells results in intramedullary and intravascular haemolysis. To compensate, erythropoiesis and haem synthesis are actually increased in spite of the severe enzyme deficiency. Although the porphyrins that accumulate in this disease are primarily type I porphyrin isomers, type III isomers are also increased.

Adult-onset cases are likely to be associated with a myeloproliferative disorder and clonal expansion of erythroblasts with UROS deficiency [37].

Genetics

CEP is an autosomal recessive disorder. Patients have either homozygous or compound heterozygous *UROS* mutations. Like other porphyrias, this disease is genetically heterogeneous, and at least 39 different *UROS* mutations and an X-linked *GATA-1* mutation have been identified [38][39]. Parents and other heterozygotes are asymptomatic and display half-normal enzyme activity.

Diagnostic Tests

Erythrocyte, plasma and urine porphyrins are markedly increased. Uroporphyrin I, coproporphyrin I and even zinc

protoporphyrin may be increased in erythrocytes. Porphyrins in urine are primarily uroporphyrin I and coproporphyrin I, and in faeces mostly coproporphyrin I. Porphyrin precursors are not increased. UROS activity is markedly deficient, but this enzyme assay is not widely available. The diagnosis should be confirmed by mutation analysis. The disease can be diagnosed in utero by porphyrin measurements and DNA studies.

Treatment and Prognosis

A multidisciplinary approach is emphasized [40]. Protection of the skin from sunlight is essential. Minor trauma, which can lead to denudation of fragile skin, should be avoided and secondary bacterial infections treated promptly to prevent scarring and mutilation. Haemolysis may improve after splenectomy. Oral charcoal may be helpful by increasing faecal excretion of porphyrins. High-level blood transfusions and hydroxyurea may be effective by suppressing erythropoiesis and porphyrin synthesis [41][42]. Improvement of symptoms with iron restriction was reported in one patient [43].

Stem cell transplantation is the most effective current therapy [40], and gene therapy may eventually be possible [44]. Proteasome inhibitors may become applicable for missense mutations causing premature degradationof the UROS enzyme [45].

36.6 Porphyria Cutanea Tarda (PCT)

Clinical Presentation

This is the most common and readily treated form of porphyria and causes chronic, blistering skin lesions, especially on the dorsal hands, forearms, face and (in women) the dorsal feet. Neurological effects are not observed. Sun-exposed skin becomes friable, and minor trauma may precede the formation of bullae or cause denudation of the skin. Small white plaques (›milia‹) may precede or follow vesicle formation. Hypertrichosis and hyperpigmentation are also noted. Thickening, scarring and calcification of affected skin may be striking, and is referred to as ›pseudoscleroderma‹. Skin lesions are indistinguishable clinically from those in all other cutaneous porphyrias, except for erythropoietic and X-linked protoporphyria (▶ see later discussion).

A normal or increased amount of hepatic iron is required to develop the disease [46]. Acquired and inherited susceptibility factors include moderate or heavy alcohol intake, hepatitis C and less commonly HIV infection, oestrogen use, smoking, *HFE* (hemochromatosis) mutations, uroporphyrinogen decarboxylase (*UROD*) mutations and low levels of ascorbic acid and carotenoids [47]. There are geographic differences in the association with hepatitis C; in some locations up to 80% of patients are infected with this virus.

PCT has developed after ingestion of seed wheat treated with hexachlorobenzene as a fungicide and after occupational exposure to related chemicals. However, such toxic exposures are seldom evident in isolated cases of porphyria cutanea tarda [46][47].

Metabolic Derangement

This porphyria is caused by a profound deficiency of hepatic UROD (Fig. 36.1, Table 36.1). A specific inhibitor of hepatic UROD has been characterised as a uroporphomethene [48].

Patterns of excess porphyrins in this disease are complex and characteristic. Uroporphyrinogen and hepta-, hexa- and pentacarboxylic porphyrinogens accumulate in liver, and pentacarboxyl porphyrinogen can be metabolised by coproporphyrinogen oxidase (the next enzyme in the pathway) to a tetracarboxyl porphyrinogen termed isocoproporphyrinogen, which is further metabolized by gut bacteria. All these compounds appear as the oxidised porphyrins in plasma and are excreted in urine, bile and faeces. Successful treatment may require some time before the massive porphyrin accumulations in liver are cleared.

Genetics

PCT is classified as types 1–3 based on the presence or absence of *UROD* mutations and a family history of the disease [46]. These types have few clinically important differences, and hepatic UROD is inhibited in all overt cases. In type 1 there are no *UROD* mutations and the amount of hepatic enzyme protein remains normal if measured immunochemically. In type 2, which accounts for approximately 20% of patients, erythrocyte UROD is approximately 50% of normal due to a heterozygous mutation inherited as an autosomal dominant trait with low penetrance. More than 100 mutations have been identified in type 2 disease. Type 2 becomes clinically manifest when the remaining hepatic enzyme becomes profoundly inhibited, as in type 1. In type 2 there may be earlier onset and occasionally a family history of manifest disease. Cases classified as type 3 are rare, have no *UROD* mutations, but one or more relatives also have the disease. Type 3 cases in the same family may have shared genetic (e.g. *HFE* mutations), infectious or environmental susceptibility factors.

Diagnostic Tests

The characteristic blistering skin lesions and skin histopathology are not specific, and the diagnosis is established by laboratory testing before instituting therapy. Plasma and urine porphyrins are increased in all porphyrias that cause blistering skin lesions. PCT is confirmed by increased total urinary or plasma porphyrins with a predominance of highly carboxylated porphyrins, especially uroporphyrin and heptacarboxyl porphyrin. In contrast to erythropoietic porphyrias, erythrocyte porphyrins are normal or only modestly elevated.

The fluorescence spectrum of plasma porphyrins can rapidly distinguish variegate porphyria from PCT (Table 36.2) [13]. Cases of so-called pseudoporphyria have skin lesions resembling PCT but no significant increases in porphyrins. Sometimes a photosensitising drug is implicated.

Treatment and Prognosis

Iron depletion by phlebotomy is standard treatment at most centres, although low-dose hydroxychloroquine (or chloroquine) is also effective [49]. Patients are also advised to discontinue alcohol, oestrogens, iron supplements and other

contributing factors. Repeated phlebotomy stimulates erythropoiesis and utilisation of storage iron for haemoglobin formation, and gradually reduces the serum ferritin to a target range of 15–20 ng/ml. This can usually be achieved by removal of only 5–6 units (450 ml each) of blood at 2-week intervals. Further iron depletion is of no additional benefit and may cause anaemia and associated symptoms. Many more phlebotomies may be needed in patients who have marked iron overload. Plasma and urine porphyrin levels, which fall more slowly than ferritin, may not yet be normal when the target ferritin level is reached but will continue to decrease. Hepatic UROD activity gradually increases to its genetically determined level with treatment.

After remission, ferritin may increase without recurrence, in most cases. Postmenopausal women who have been treated for PCT can usually resume oestrogen replacement if needed. The disease recurs especially in patients who resume alcohol intake, but they are expected to respond to another course of phlebotomies. The serum ferritin should be maintained below about 100 ng/ml in patients who also have haemochromatosis, and perhaps in other patients who experience multiple relapses.

A low dose of hydroxychloroquine (100 mg twice weekly) or chloroquine (125 mg twice weekly) gradually removes excess porphyrins from the liver. This is a suitable alternative when phlebotomy is contraindicated or difficult, and is the preferred treatment in some centres. Time to remission is equivalent to treatment by phlebotomy [49]. Standard doses of these 4-aminoquinolines exacerbate photosensitivity and cause hepatocellular damage, and should not be used. These drugs may produce retinal damage, although this risk is very low, and may be lower with hydroxychloroquine than chloroquine. The mechanism by which these drugs remove porphyrins from the liver in this condition is not understood, and they are not effective in other porphyrias.

36.7 Hepatoerythropoietic Porphyria

Clinical Presentation

This rare disease is clinically similar to CEP and usually presents with blistering skin lesions shortly after birth. Mild cases may present later in life and more closely resemble PCT. Concurrent conditions, such as viral hepatitis, may accentuate porphyrin accumulation.

Metabolic Derangement

Hepatoerythropoietic porphyria is the homozygous form of familial (type 2) PCT and is due to a substantial deficiency of UROD [46][50]. Intermediate deficiencies of the enzyme are found in the parents (Fig. 36.1, Table 36.1). The disease has features of both hepatic and erythropoietic porphyrias. Although it is usually a more severe disease than PCT, there are reports of mild and atypical forms of the disease [51].

Diagnostic Tests

The excess porphyrins found in urine, plasma and faeces in this condition are similar to those in PCT. In addition, erythrocyte zinc protoporphyrin is substantially increased, as in a number of other autosomal recessive porphyrias. This finding probably reflects an earlier accumulation of uroporphyrinogen in erythroblasts, which after completion of haemoglobin synthesis is metabolised to protoporphyrin and chelated with zinc by ferrochelatase. Erythrocyte porphyrins in CEP are usually mostly uroporphyrin I and coproporphyrin I, but in some cases there is a predominance of zinc protoporphyrin. It is important to document the diagnosis by molecular methods.

Genetics

This porphyria results from homozygous or compound heterozygous *UROD* mutations. The disease is genetically heterogeneous. *UROD* mutations found in this disease generally result in marked decreases in enzyme activity, but some activity remains, so haem formation can occur [46].

Treatment and Prognosis

Therapeutic options are essentially the same as in CEP.

36.8 Hereditary Coproporphyria and Variegate Porphyria

Clinical Presentation

These disorders are classified as acute hepatic porphyrias because they can present with acute attacks that are identical to those in AIP. However, unlike the latter disease, they are also cutaneous porphyrias, because they may cause blistering skin lesions that are indistinguishable from those of PCT. Factors that exacerbate AIP are important in both of these porphyrias. Homozygous cases of hereditary coproporphyria and variegate porphyria have been described, and in such cases clinical manifestations may begin in childhood. Symptoms in heterozygotes almost never occur before puberty. Variegate porphyria is particularly common in South Africa.

Metabolic Derangement

Hereditary coproporphyria and variegate porphyria result from deficiencies of coproporphyrinogen oxidase and of protoporphyrinogen oxidase, respectively, (Fig. 36.1, Table 36.1). Heterozygotes have approximately 50% deficiencies of these enzymes. In hereditary coproporphyria there is marked accumulation of coproporphyrin III (derived from autooxidation of coproporphyrinogen III), and urinary porphyrin precursors and uroporphyrin are increased particularly in association with acute attacks. Similar abnormalities are seen in variegate porphyria, but in addition protoporphyrin (derived from autooxidation of protoporphyrinogen) is increased in faeces and plasma porphyrins are increased. A close association of coproporphyrinogen oxidase and protoporphyrinogen oxidase in the mitochondrial membrane may explain the accumulation of both coproporphyrinogen and

protoporphyrinogen in variegate porphyria. Protoporphyrinogen and coproporphyrinogen have been shown to inhibit PBGD, which along with induction of hepatic ALAS1, may account for the increase in porphyrin precursors during acute attacks [52].

Genetics

Both of these porphyrias are autosomal dominant conditions in which affected individuals and latent carriers have approximately 50% activity of the affected enzyme. Genetic heterogeneity is a feature of both. As expected, a single mutation (R59W) accounts for the many descendants with variegate porphyria in South Africa, which is an example of the founder effect [52]. Homozygous cases are rare.

Diagnostic Tests

Urinary 5-ALA and porphobilinogen are increased during acute attacks of these porphyrias, although the increases may be smaller and more transient than in AIP. Urinary coproporphyrin increases may be more prominent and prolonged than in AIP, but it is a highly nonspecific finding that can also be observed in many other conditions, especially with hepatic or bone marrow dysfunction.

A marked, isolated increase in faecal coproporphyrin III is distinctive for hereditary coproporphyria. Faecal coproporphyrin and protoporphyrin are about equally and markedly increased in variegate porphyria. Plasma porphyrins are commonly increased in variegate porphyria, and the fluorescence spectrum of plasma porphyrins is characteristic and very useful for rapidly distinguishing this disease from other porphyrias [13][53].

Reliable assays for protoporphyrinogen oxidase and coproporphyrinogen oxidase in cultured fibroblasts or lymphocytes are available only in a few research laboratories. Erythrocytes cannot be used to measure these mitochondrial enzymes, because mature erythrocytes do not contain mitochondria. Identification of the familial mutation confirms the diagnosis and enables screening of family members [11][23].

Treatment and Prognosis

Attacks of neurological symptoms are treated as in AIP (see ▶ above). Cutaneous symptoms are more difficult to treat, and therapies that are effective for PCT (phlebotomy and low-dose hydroxychloroquine) are not effective in these conditions. Protection from sunlight is important. As in other acute porphyrias, screening of active cases for hepatocellular carcinoma is recommended [11].

36.9 Erythropoietic Protoporphyria and X-Linked Protoporphyria

Clinical Presentation

Erythropoietic protoporphyria is the third most common porphyria, and the most common in children. X-linked protoporphyria is less common and has the same phenotype. Cutaneous symptoms begin in early childhood, and are generally much more prominent than objective changes by examination. Pain can affect sun-exposed areas within minutes of exposure, and if exposure is prolonged be followed by diffuse oedema that may resemble angioneurotic oedema. Chronic subtle skin changes may include lichenification, leathery pseudovesicles, labial grooving and nail changes, but are absent in patients who avoid sunlight. In contrast to other cutaneous porphyrias, blistering, milia, friability, scarring and hypertrichosis are not prominent. There is no fluorescence of the teeth and, in the absence of hepatic failure (see ▶ below), no neuropathic manifestations. Mild anaemia with hypochromia and microcytosis is common, and is poorly understood [54][55][56].

The severity of the symptoms is generally stable over time. Patients adjust their lifestyles and occupations in order to avoid sunlight. However, the disease has a substantial effect on quality of life [55]. Adjustment is especially difficult in children with unexplained symptoms before diagnosis, which is often much delayed. Drugs that exacerbate hepatic porphyrias are not known to worsen this disease. Gallstones containing protoporphyrin may also develop. Some patients develop liver disease, referred to as protoporphyric hepatopathy, which can progress rapidly to death from hepatic failure. Operating room lights have produced severe skin and peritoneal burns in some patients with protoporphyric hepatopathy. A motor neuropathy may further complicate the course of liver decompensation in this disease, and is unexplained [57].

Metabolic Derangement

Erythropoietic protoporphyria is due to an inherited deficiency of ferrochelatase, the eighth and last enzyme in the haem biosynthetic pathway, (◘ Fig. 36.1, ◘ Table 36.1). Ferrochelatase, deficiency is substantial (10–30% of normal) in erythropoietic protoporphyria, leading to increases in protoporphyrin in bone marrow, circulating erythrocytes, plasma, bile and faeces. Bone marrow reticulocytes are the primary source of the excess protoporphyrin. Circulating erythrocytes, which are no longer synthesising haem, contribute smaller amounts. Excess protoporphyrin is transported in plasma and excreted in bile and faeces. Because zinc protoporphyrin is also a product of ferrochelatase activity, the excess protoporphyrin found in erythrocytes in erythropoietic protoporphyria is mostly metal free. Young circulating erythrocytes appear fluorescent when a blood smear from a patient with this condition is examined by fluorescence microscopy.

X-linked protoporphyria, which has the same phenotype, results from gain-of-function mutations affecting ALAS2, the erythroid form of the first enzyme in the pathway [58]. *ALAS2* is located on the X chromosome. Because ferrochelatase is not deficient, the proportion of zinc protoporphyrin in erythrocytes is greater (15~50% of the total) than in erythropoietic protoporphyria (0~15%). On average porphyrin levels are higher and liver disease may be more common in X-linked protoporphyria.

Protoporphyrin is excreted in bile in both diseases and may undergo enterohepatic circulation. Large amounts of

protoporphyrin originating from the bone marrow can cause cholestasis and hepatocellular damage.

Genetics

At least 125 ferrochelatase mutations have been identified in erythropoietic protoporphyria [59][60], and most mutant alleles express little or no ferrochelatase (disabling or null mutations). Most patients with manifest disease have inherited a severe ferrochelatase mutation from one parent and a weak, or hypomorphic, ferrochelatase allele from the other [61]. This allele is found in ~10% of normal Caucasians, and has no consequence unless trans to a severe ferrochelatase mutation. This explains why ferrochelatase activity is only 10–30% of normal in patients with manifest disease. This pattern of inheritance, which is found in the great majority of cases, is best described as autosomal recessive. Rarely, families have two disabling mutations, where at least one of the two mutant alleles expresses some ferrochelatase activity in those who are compound heterozygotes [23][54][62]. For unknown reasons, seasonal palmar keratoderma occurs in some of these families [62]. Late-onset protoporphyria may develop in the presence of myeloproliferative disorders, with clonal expansion of erythropoietic cells with a ferrochelatase gene mutation [63].

In families with X-linked protoporphyria, the disease is likely to be more common and severe in males and more variable in severity in females, presumably reflecting the degree of X chromosome inactivation.

Diagnostic Tests

The most sensitive screening test for both protoporphyrias is a determination of total erythrocyte protoporphyrin. Although this test lacks specificity, fractionation to discern whether the protoporphyrin is predominantly metal-free or complexed with zinc establishes or excludes the diagnosis [12]. Metal-free protoporphyrin comprises 85~100% of the total in erythropoietic protoporphyria, 50–85% in X-linked protoporphyria, and less than ~50% in other conditions (e.g., iron deficiency, anaemia of chronic disease and lead poisoning).

The plasma porphyrin concentration is increased in almost all cases. Moreover, the excess protoporphyrin found in plasma in this condition is particularly sensitive to light exposure, which may increase the chance of a falsely normal measurement [64].

Total faecal porphyrins may be normal or increased in protoporphyria, with a predominance of protoporphyrin. Urinary porphyrins and porphyrin precursors are normal. Urinary coproporphyrin is elevated with protoporphyric hepatopathy, as with other liver diseases.

Treatment and Prognosis

Photosensitivity is managed by avoidance of sunlight. Oral β-carotene or cysteine improve tolerance to sunlight in some patients, perhaps by quenching singlet oxygen or free radicals. Narrow-band ultraviolet light therapy can increase skin pigmentation and sunlight tolerance. Afamelanotide, a synthetic agonistic analogue of α-melanocyte-stimulating hormone, increases sunlight tolerance by increasing melanin formation and darkening the skin [65]. Cholestyramine may reduce protoporphyrin levels by interrupting its enterohepatic circulation, but has not been extensively studied. Iron deficiency, caloric restriction, and drugs or hormone preparations that impair hepatic excretory function should be avoided. Mild iron deficiency is said to be beneficial [66], but correction of iron deficiency has been beneficial in some patients [67][68]. Vitamin D and calcium supplements and vaccinations for hepatitis A and B are also recommended. Gene therapy is being studied in this and other erythropoietic porphyrias [69].

Treatment of liver complications is difficult. Transfusions and intravenous haemin may suppress erythroid protoporphyrin production. Plasma exchange, cholestyramine, ursodeoxycholic acid and vitamin E are also administered. Liver transplantation is sometimes required [28]. Sequential bone marrow transplantation can prevent recurrence of hepatopathy [70][71].

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Section IX Disorders of Metal Transport and Metabolism

Disorders in the Transport of Copper, Iron, Magnesium, Manganese, Selenium and Zinc

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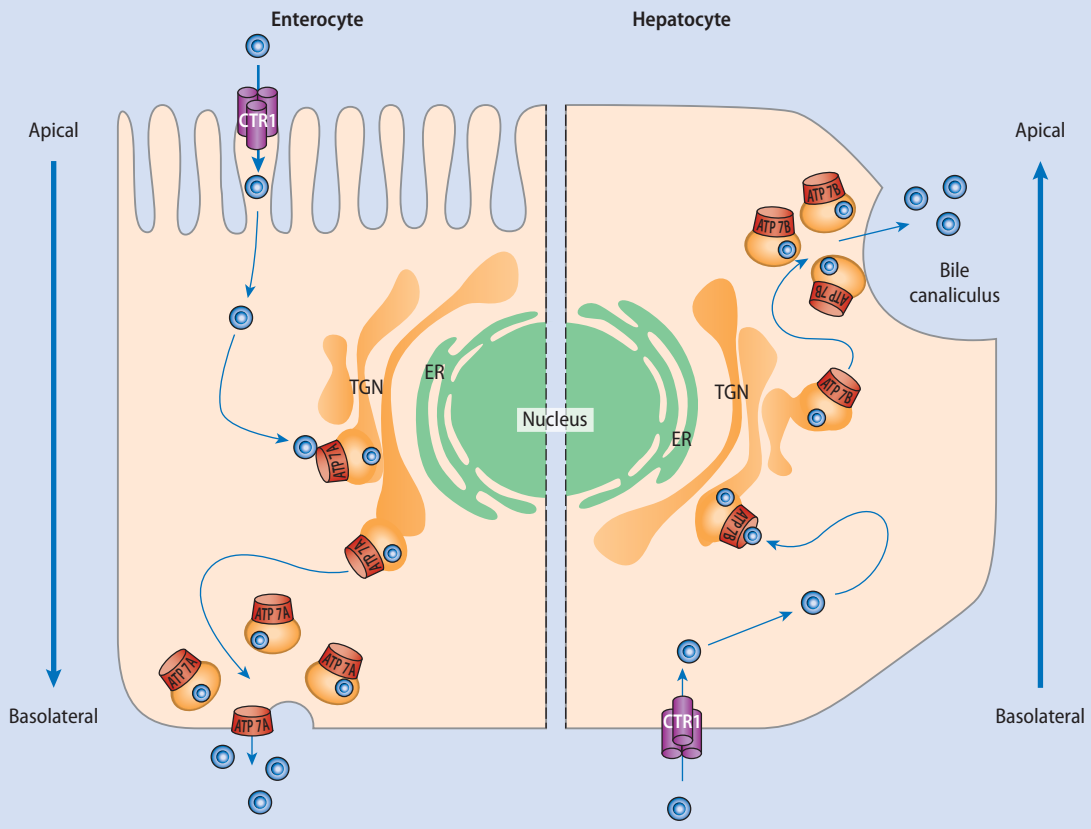
Metals are indispensable elements of cell biology.They function as cofactors for many specific proteins and are involved in all major metabolic pathways. The number of recognised IEM involving the absorption, transport,or metabolism of metals is rapidly growing. Clinical presentations can involve all organs and systems including the liver and the central nervous system.

Deficiency of metals results mostly in loss of function of metal-dependent proteins while excess can result in unregulated oxidation of proteins, lipids and other cellular components. Treatments rely on daily supplementation of the deficient metal at pharmacological doses and on chelating drugs where there is excess.

37.1 Copper

Copper Metabolism

Each day approximately 2 mg copper is absorbed from the intestine, which is subsequently removed from the portal circulation by the hepatocytes. Excretion of copper by the liver into the bile is the only mechanism of copper elimination and in physiological conditions the amount of copper excreted into the bile is equivalent to that absorbed from the intestine. The uptake of copper, both by enterocytes and hepatocytes, is done through the copper transporter CTR1, a protein residing in the plasma membrane. Excretion in these two cell types is done by two closely related ATP-ases, ATP7A and ATP7B (◘ Fig. 37.1). As copper is essential for cellular metabolism, but also potentially toxic, it is bound within the cell to small proteins, copper chaperones, which direct this metal to specific proteins, such as superoxide dismutase. In the circulation over 90% of plasma copper is bound to serum ceruloplasmin.



◘ **Fig. 37.1 Cellular copper metabolism.** In both the enterocyte (*left panel*) and the hepatocyte (*right panel*) copper enters the cell through CTR1, the main copper transporter, and is then transported to ATP7A (in the enterocyte) or ATP7B (in the hepatocyte). These proteins are synthesized in the endoplasmatic reticulum (*ER*), but subsequently reside in the Trans Golgi Network (*TGN*). Upon a rising intracellular copper concentration they relocalise to the cell periphery (ATP7A and ATP7B), and also the plasma membrane (ATP7A), which process is essential for the excretion of copper. Through these proteins copper is excreted by the enterocyte to the blood (ATP7A), while in the hepatocyte copper is excreted to the bile (ATP7B). Figure modified from [1]

Copper balance is disturbed in two inborn errors: Wilson disease and Menkes disease. Wilson disease, or hepatolenticular degeneration is due to mutations in *ATP7B*, and is characterized by a gradual accumulation of copper in the liver and, secondarily, in other organs, such as brain, kidney and cornea. Clinical symptoms result from the copper accumulation in the liver and/or the brain. Early treatment with copper chelators or zinc is generally effective.

Menkes disease is an X-linked disorder due to mutations in the *ATP7A* gene. The disorder is characterized by a general copper deficiency. Patients manifest progressive neurodegeneration, which is usually fatal in infancy or childhood. Early therapy with copper histidine can be effective in selected patients. Occipital horn syndrome and a rare phenotype, X-linked distal hereditary motor neuropathy, are also due to *ATP7A* mutations, and can be observed in older children or adults.

Indian Childhood Cirrhosis (ICC), also known as Idiopathic Copper Toxicosis (ICT), is a rare copper storage disease seen in infants susceptible to high oral copper intake. Several other rare disorders are recognized with a low serum copper and ceruloplasmin: MEDNIK syndrome and AT-1 deficiency, both primarily characterized by severe mental retardation. Also some Congenital Disorders of Glycosylation (CDG) can present with a low serum ceruloplasmin and copper as well as mild liver dysfunction. In some patients this could be attributed to PGM-1 mutations, but in most no cause has been found (CDG-X).

37.1.1 Wilson Disease

Clinical Presentation

The overwhelming majority of cases display hepatic and/or neurological symptoms, and the disease should be suspected in any patient with liver disease without obvious cause or with a movement disorder [2]. In addition, the diagnosis is often made when siblings of a patient are screened. Occasionally, Wilson disease presents with isolated raised transaminases, Kayser-Fleischer rings or haemolysis.

Patients with hepatic symptoms generally present between 8 and 20 years of age, but may be as young as 3 or over 50. The presentation can be acute and severe with hepatitis, jaundice and impending liver failure. Transaminases, although raised, generally are much lower than in autoimmune or viral hepatitis. While liver disease is rapidly progressive in some patients, in others jaundice can persist for months without progression to liver failure, or even subside. These patients ultimately develop liver cirrhosis and may present several years later with neurological disease.

Neurological symptoms usually develop in the second or third decade, although patients may be as young as 8 years of age. Symptoms include dysarthria and diminished control of movements, accompanied in a later stage by tremors, rigidity and drooling in combination with swallowing problems. A frequent early sign is a deterioration in the quality of handwriting. In some patients psychiatric symptoms predominate, ranging from behavioural disturbances, often characterized by impulsivity and irritability, to frank psychosis.

Most patients have aminoaciduria in combination with excessive loss of bicarbonate, calcium and phosphate, and may develop renal stones or osteoporosis. Haemolytic anaemia, leading to gall-stones, may be present. Cardiomyopathy has also been described.

The greenish brown Kayser-Fleischer ring, located in the membrane of Descemet at the limbus of the cornea, can be seen with the naked eye in the majority of patients with full-blown neurological disease. Careful slit lamp examination will reveal this ring in almost all these patients. In contrast, in a substantial proportion of the patients presenting with liver disease and in most pre-symptomatic patients, the Kayser-Fleischer ring is absent. Conversely, a Kayser-Fleischer ring is occasionally found in patients with cholestatic liver disease. Its absence thus does not rule out Wilson disease, while its presence does not confirm the disorder.

Metabolic Derangement

Wilson disease is caused by reduced excretion of copper into bile, resulting in a gradual accumulation of copper in the liver and, secondarily, in the brain, kidneys and eye. A number of patients exhibit severe liver disease, while others redistribute copper to the brain, especially the basal ganglia, causing neurological disease. Copper excess exerts its hepatic toxicity by generating free radicals that oxidize the mitochondrial membranes, resulting in their swelling and loss of oxidative phosphorylation capacity. The characteristic Kayser-Fleischer ring is a deposit of copper and sulphur. The renal dysfunction is a consequence of copper accumulation in the renal tubules. The increased urinary copper excretion, characteristic for Wilson disease, is due to the loss of unbound, dialysable copper through the kidneys. This unbound copper can cause haemolysis in some patients.

The primary defect in Wilson disease is a lesion of a protein localized in the trans-Golgi network, ATP7B, an adenosine triphosphatase (ATPase), which is responsible for the excretion of copper [1] and for the incorporation of copper into ceruloplasmin. Owing to the reduced half-life of ceruloplasmin without copper, the concentration of serum ceruloplasmin is subnormal in Wilson disease. Some rare patients, although unable to excrete copper into bile, can incorporate copper into ceruloplasmin and have normal serum ceruloplasmin [3].

Genetics

Wilson disease in an autosomal recessive condition caused by mutations in *ATP7B* [4]. Its transcript, ATP7B, has six copper binding domains and is expressed predominantly in liver and kidney. ATP7B is highly homologous to ATP7A, the protein defective in Menkes disease.

Over 500 mutations in *ATP7B* have been described so far. The distribution of mutations within various racial groups is quite different, with the R778L mutation being common amongst Asian patients [5], the H1069Q mutation amongst European patients [6], and still other mutations being prevalent elsewhere. Most patients are compound heterozygotes. Mutations that completely destroy the function of the protein

Table 37.1 Laboratory results in Wilson disease and controls

	Wilson disease	Normal
Serum ceruloplasmin (mg/l)	0–200	200–400
Serum copper (μmol/l)	<11	11–24
Urinary copper (μmol/24 h)	>1.6	<0.6
Liver copper (μg/g dry weight)	>250	<50

are generally found in patients who present early, while residual function is associated with late presentation. For example, patients homozygous for the non-functional R778L mutation tend to present earlier, with hepatic manifestations [5], whereas those homozygous for the H1069Q mutation present relatively late (around 21 years of age), with neurological symptoms, indicative of a relative slow build up of copper [6].

Diagnostic Tests

Wilson disease is characterized by low serum ceruloplasmin and serum copper, elevated urinary copper, and increased liver copper (Table 37.1). These laboratory results should only be interpreted in combination, because each individual parameter can be abnormal in situations other than Wilson disease [2]. For example, liver copper is raised in liver cirrhosis, whereas serum ceruloplasmin is low in a substantial proportion of heterozygotes for Wilson disease, and in patients with hereditary aceruloplasminemia. Conversely, serum ceruloplasmin is normal in a small proportion of patients with Wilson disease. Given these diagnostic difficulties a scoring system has been developed [2].

Since over 90% of serum copper is normally bound to ceruloplasmin, it is generally low when serum ceruloplasmin is low, as is the case in Wilson disease. Characteristically the fraction of serum copper not bound to ceruloplasmin, called free serum copper, is raised. This sensitive parameter can be calculated with the knowledge that each mg of ceruloplasmin contains 3.4 μg of copper, provided the laboratory can reliably measure ceruloplasmin concentrations in the subnormal ranges, i.e. <200 mg/l.

Urinary copper excretion is determined in a 24 h collection, but is sensitive to contamination. Excretion is always increased in symptomatic patients, but may be normal or only borderline elevated in presymptomatic individuals.

When Wilson disease is diagnosed in a family, siblings should be investigated. Genetic analysis is more reliable than laboratory investigations of copper metabolism which cannot always distinguish between carriers and young patients who still have a low copper load.

Treatment and Prognosis

The prognosis is excellent for patients who start treatment before severe tissue damage has occurred, i.e. when presymptomatic or diagnosed at an early stage [2][7]. The prognosis can still be good for those with more advanced disease, provided aggressive decoppering treatment is instituted immediately after diagnosis. Several therapeutic agents are available: penicillamine, trien and zinc.

The largest experience is with penicillamine, the first agent to be introduced. Penicillamine chelates copper by forming a stable complex that is subsequently excreted in urine. Maintenance dose for adults is 750–1500 mg/day, divided in 2–3 doses, together with 25 mg/day of pyridoxine. Dosing in children is 20 mg/kg/day. With this therapy the majority of patients with liver disease will recover, although liver transplantation cannot be avoided in all [2][7]. Of patients with neurological disease 80% will recover, but the majority will have some residual disabilities. In the remainder no improvement is seen, or there may even be a deterioration. In this group mortality is not uncommon [7]. A significant proportion of patients with neurological disease will have initial worsening of symptoms after starting penicillamine therapy. In addition, side effects and toxic reactions are seen in up to 25% of the patients treated with penicillamine and therapy has to be stopped in half of them [2][7]. Tolerability of penicillamine might, however, be better when starting with a lower dose, i.e. 250 mg/day, with 250 mg increments each 4–7 days. Given this suboptimal safety profile, alternatives for penicillamine have been sought, with trien (trientine) being the first to be introduced. This agent is also a copper chelator, with an efficacy that seems to be similar to penicillamine, however with less side effects [8]. The starting dose is 900–2500 mg/day in adults, divided in 2 to 3 doses, initially in the lower end of this range, with a maintenance therapy of 900–1500 mg/day [2].

Oral zinc has been used in the treatment of Wilson disease for more than 25 years. It induces metallothionein synthesis in the small intestinal epithelium. Since metallothionein binds copper preferentially over zinc, copper balance will become negative through faecal excretion, as villus cells are lost into the intestinal lumen. As compared to penicillamine, zinc does not have any serious side effects, although some patients experience gastric complaints on zinc sulphate. This can generally be solved by switching to zinc gluconate or zinc acetate. Given its favourable side effect profile, zinc seems the agent of choice in presymptomatic individuals. In patients with symptomatic disease (particularly with neurological symptoms) a small non-randomized, non-blinded trial showed similar outcomes for zinc and penicillamine [9]. Given the side effects of penicillamine and the frequency of initial deterioration in patients with neurological disease, zinc should be seriously considered in this group [2][7]. In patients with hepatic disease, which can evolve rapidly, zinc seems less appropriate because it may have a slower effect on copper overload. The initial dose of zinc sulphate for adults is 600 mg/day, divided in 3 doses; this dose can be doubled if insufficient effect is obtained. Urinary copper excretion should be followed: it should fall rapidly initially, and more slowly there-

after. A reasonable goal is to achieve an excretion below 2 µmol/day. Copper depletion should be avoided: in the maintenance phase, 300 mg/day of zinc sulphate or even less can be sufficient.

In patients presenting with severe liver disease, sufficient experience is only available for penicillamine. In this group the revised King's score will predict who will recover and who will need a liver transplant [10]. As penicillamine therapy will fail in a substantial proportion of these patients, other treatment modalities have been tried, such as the combination of zinc and penicillamine (or trien), in which case the administration of both drugs should be separated in time, as the chelator will bind zinc. Anecdotal evidence is available for tetrathiomolybdate, which might be highly effective [11]. There is also some evidence that amitriptyline might inhibit the apoptosis that is characteristic for acute liver failure in Wilson disease [12]. However, none of these interventions have been thoroughly investigated in patients.

37.1.2 Menkes Disease

Clinical Presentation

Symptoms are generally noted in male infants at the age of 2–3 months, when neurodegeneration provoked by the disease becomes manifest with seizures, hypotonia and loss of developmental milestones [13]. Usually, nonspecific signs are already present at birth, including prematurity, large cephalhaematomas, skin laxity and hypothermia. The hair, if present, breaks easily in areas exposed to the mild pressure of lying down and has a sandpaper feel. It can already exhibit the characteristic pili torti, which will appear later on in all cases. A typical facial appearance, with sagging cheeks, gradually becomes prominent. Over time, hypotonia is replaced by spasticity. Feeding difficulties, vomiting and/or chronic diarrhoea are common. Weight gain is generally insufficient while linear growth is relatively well preserved. The loose skin, which is particularly prominent at the back of the neck and on the trunk, is a consequence of defective collagen crosslinking, as are the vascular tortuosity and bladder diverticula, which are present in virtually all patients. The latter are a frequent source of infection. Umbilical or inguinal hernias and/or a pectus excavatum are also commonly encountered.

Attenuated forms occur in 10–15% of the patients. Of these, *occipital horn syndrome* is characterised by connective tissue abnormalities much like those in Menkes disease, with minimal effects on neurodevelopment [14]. Exostoses, particularly at the occipital insertion of the paraspinal muscles (hence its name) are characteristic. Skin and joint laxity are common, as are urinary tract diverticuli, but pili torti are usually absent. Patients have orthostatic hypotension and chronic diarrhoea, likely due to autonomic neuropathy.

A third and rare phenotype, *X-linked distal hereditary motor neuropathy,* presents with symptoms of distal muscular atrophy and weakness in older children or adults [15]. Motor neurons are particularly sensitive to minor perturbations in copper homeostasis, which, however, take years to develop. These symptoms are reminiscent of those observed in acquired copper deficiency.

Metabolic Derangement

In Menkes disease, cellular copper uptake is normal but copper cannot be exported from cells due to a defect in the ATP7A protein [13]. Copper efflux from the intestinal cells into the circulation is severely reduced, and insufficient copper is available for incorporation into the ~20 cuproenzymes for proper functioning. Affected copper-requiring enzymes include lysyloxidase, a critical enzyme in collagen crosslinking, and tyrosinase, which is necessary for melanin formation. Copper-requiring enzymes in the brain are dopamine β-hydroxylase, which is essential for catecholamine biosynthesis, peptidyl glycine monooxygenase, which is involved in the processing of neuropeptide precursors, and cytochrome-c-oxidase, involved in the respiratory chain. Deficient activity of these enzymes is probably responsible for a significant part of the cerebral pathology in Menkes disease.

Genetics

Menkes disease is a rare condition with an incidence of approximately 1:250,000 [13]. Although inherited as an X-linked recessive trait it should be noted that approximately one third of patients have *de novo* mutations. The disease is caused by mutations in *ATP7A* and expressed in all tissues, except liver. The mutation spectrum in Menkes disease is wide, ranging from single base-pair changes to intragenic deletions encompassing one or more exons. Chromosomal abnormalities, mostly X-autosome translocations have also been reported [16]. The vast majority of these changes are predicted to result in a truncated, nonfunctional protein. Splice site mutations that potentially permit small amounts of *ATP7A* to be transcribed have been described in most patients with occipital horn syndrome [17]. In patients with X linked distal *hereditary motor neuropathy* a small set of missense mutations have been reported that are associated with even higher residual ATP7A activity [15].

Diagnostic Tests

A level of serum copper (<11 µmol/l) and serum ceruloplasmin (<200 mg/l) below the usual range supports the diagnosis of Menkes disease, but is not specific in the first 3 months of life as these low levels are normal in this age category. Abnormal levels of catecholamines and their metabolites, however, are quite specific, especially the ratio between dopamine and norepinephrine and the ratio of dihydroxyphenylacetic acid to dihydroxyphenylglycol in plasma [18]. The copper retention characteristic of Menkes disease can be demonstrated by measuring the increased accumulation and reduced efflux of radiocopper in cultured fibroblasts [19]. The final diagnosis requires identification of the mutation. Larger deletions, responsible for approximately 15% of Menkes cases, may be missed by mutation analysis and require MLPA analysis. Prenatal diagnosis is preferably made by mutation analysis. Carrier detection should be undertaken by DNA analysis, especially as biochemical studies of copper accumulation in

fibroblasts can give false-negative results due to random inactivation of the X-chromosome.

Treatment and Prognosis

Classically, most patients die before 3 years of age due to infections or vascular complications, although with current medical care, especially improved feeding techniques, longer survival is not uncommon. However, parenteral treatment with copper histidine can theoretically bypass the intestinal block, making copper available for incorporation into cuproenzymes. Initial results of this therapy, which is given by daily or twice-daily subcutaneous injection, were generally disappointing, but in the majority of the patients treatment was only started after the 3rd month [18]. When treatment is started early, i.e. in the first weeks of life, and continued, survival beyond 3 years of age is the rule. Nevertheless, normal or near-normal intellectual and motor development seems only possible when some residual activity of the ATP7A protein is present [18] or in those rare patients with mutations selectively affecting copper export, without affecting the ability to incorporate copper into cuproenzymes [20].

37.1.3 Other Copper Storage Disorders

Indian Childhood Cirrhosis (ICC) is characterized by an abnormal serum ceruloplasmin and an extremely high liver copper (800–6500 μg/g dry weight) [21]. It is seen solely in young children. The usual outcome is liver failure, although this can be prevented by early decoppering therapy. The disorder is caused by an increased dietary copper intake in genetically susceptible individuals, due to the use of copper utensils when cooking milk. Eliminating this practice has virtually eradicated ICC. Although the disease is confined to India (hence its name) a similar disease has been seen in Tyrol (*Endemic Tyrolean Infantile Cirrhosis,* ETIC), which is also caused by using copper vessels when preparing milk [22]. Sporadic cases from all over Europe and Northern America have been described (generally labelled *Idiopathic Copper Toxicosis,* ICT), mostly associated with a high copper content of water in certain wells. Given the similarities in clinical and biochemical characteristics it seems possible that all three entities are in fact the same disease. Also many of these patients are from consanguineous families, so a genetic cause of this disorder is likely.

37.1.4 Other Disturbances of Copper Metabolism with a Low Serum Copper

Every disorder in which the main serum copper binding protein, ceruloplasmin, is low, secondarily also displays a low serum copper. Conversely a disturbed intracellular transport of copper, resulting in a secretion defect, will result in a lowered serum ceruloplasmin. Several of these disorders have been described: *MEDNIK syndrome*, *SLC33A1 deficiency*, *aceruloplasminemia* and a disturbance of glycosylation (*CDG-X*), which could be identified as *PGM-1 deficiency* in some (▶ Chapter 41).

MEDNIK syndrome is caused by mutations in *AP1S1*, which encodes the small subunit σ1A of the adaptor protein-1 (AP1) complex. When deficient a low serum copper and ceruloplasmin, in combination with mental retardation, variable intestinal pseudo-obstruction, deafness, ichthyosis and raised transaminases is seen [23]. The AP-1 complex is necessary for the intracellular trafficking of ATP7A and possibly other membrane proteins such as ATP7B, through its involvement in clathrin coated vesicle assembly. When disturbed this secondarily results in abnormal intracellular copper metabolism with aspects of both Menkes and Wilson disease. Zinc-acetate therapy may improve clinical and biochemical abnormalities [23].

AT-1 deficiency is a lethal autosomal recessive disorder, caused by mutations in *SLC33A1,* which encodes this highly conserved acetyl-CoA transporter. Clinical symptomatology, most notable a severe psychomotor retardation with cerebellar and cerebral atrophy, hearing loss and congenital cataracts, may be primarily due to hypoacetylation of proteins crucial for normal brain development, with the hypoceruloplasminemia, resulting in a low serum copper, being a secondary effect of insufficient acetylation [24]. A single patient with this deficiency also displayed a mutation in CCS, the copper chaperone for superoxide dismutase [25]. Some minor clinical differences with the patients deficient for AT-1 were found.

Aceruloplasminemia is characterized by a very low serum ceruloplasmin and serum copper [26]. It primarily causes a disturbance of iron metabolism and is discussed there (▶ Section 37.2.3).

CDG-X, an uncharacterized disorder of protein glycosylation, has been described in patients with a low serum ceruloplasmin, low serum copper, disturbed transaminases, and normal or slightly elevated liver copper and urinary copper excretion, resembling Wilson disease, but not fulfilling the relevant diagnostic criteria [2]. In some of these patients a mutation in phosphoglucomutase-1 (*PGM-1*) has been identified [27] (▶ Chapter 41).

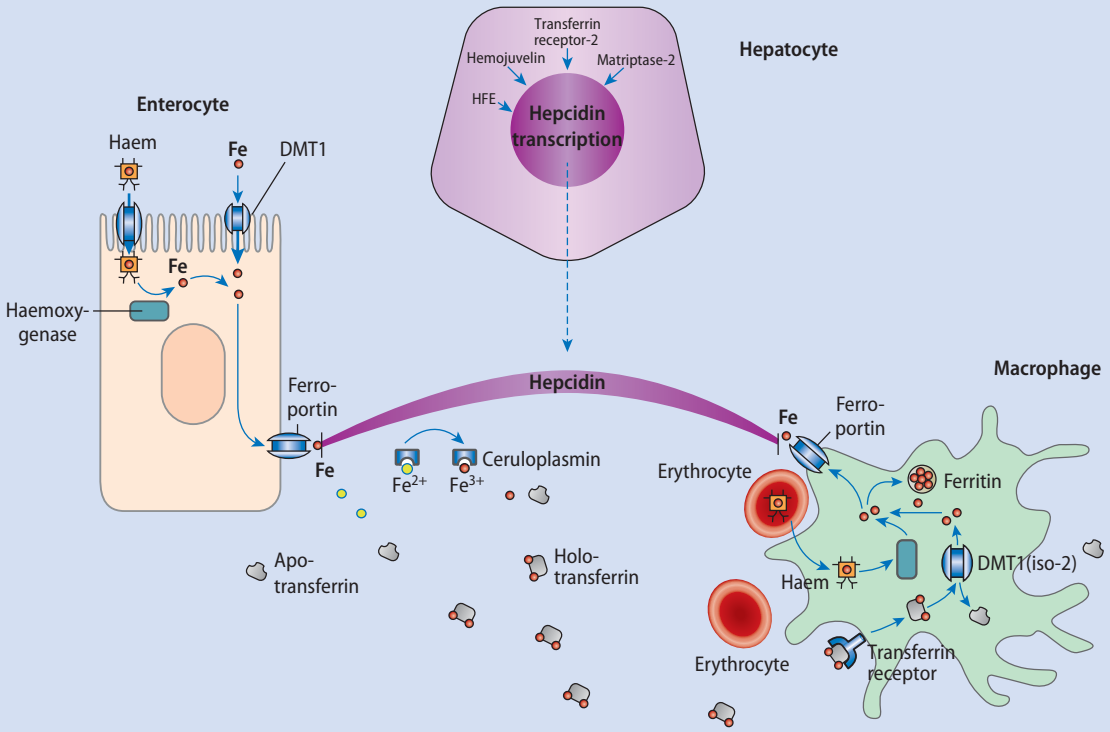
37.2 Iron

Iron Metabolism

Iron is essential for the synthesis of haem and other metalloproteins. Among these metalloproteins, the iron sulfur protein cluster is especially important, as it plays a crucial role in mitochondrial metabolism, evidenced by the recent description of a number of inherited defects of Fe-S protein biosynthesis (▶ Chapter 14). For all these functions more than 20 mg of iron per day is required, with only 1–2 mg derived from intestinal absorption, the remainder being re-used.

Absorption of iron occurs primarily in the duodenum (◘ Fig. 37.2) through the divalent-metal transporter DMT1, which is encoded by *SLC11A2*. The major recycling route for iron is its removal from erythrocytic haem by haemoxygenase, both in macrophages and enterocytes. In the circulation free iron is bound to apo-transferrin forming holo-transferrin. Transferrin can only bind iron in the ferric state (Fe^{3+}); ceruloplasmin optimises binding of iron to transferrin by allowing the oxidization of ferrous iron (Fe^{2+}) into ferric iron. The transferrin receptor mediates the uptake of transferrin. Subsequently, iron is released from intracellular transferrin by a specific isoform of DMT1. This is, amongst others, important to meet the iron requirement for haem production in erythroid cells. In several cell types, including macrophages, iron can be stored bound to ferritin until needed.

As iron, especially when unbound, is toxic its homeostasis is strictly regulated. Ferroportin, encoded by *SLC40A1*, is responsible for the export of iron into the circulation, both from enterocytes and macrophages. Hepcidin, the key regulator of circulating iron levels encoded by *HAMP*, inhibits iron release by ferroportin when high, and facilitates iron export into the circulation when low. The synthesis of hepcidin in turn is regulated by other proteins, including HFE encoded by *HFE1*, matriptase-2 encoded by *TMPRSS6*, hemojuvelin encoded by *HJV*, and transferrin receptor 2 encoded by *TRF2* [28].



◘ **Fig. 37.2 Cellular iron metabolism.** Iron is imported into the enterocytes through the divalent metal transporter, DMT1. Iron can also be acquired through direct uptake of haem, from which it is released through haemoxygenase. Export of iron into the circulation is accomplished by ferroportin. This process is controlled through hepcidin, a protein synthesized in the hepatocytes. Hepcidin transcription in turn is regulated by the following proteins: HFE, hemojuvelin, matriptase-2 and transferrin receptor-2. In the circulation, iron in the ferric state (Fe^{2+}) is bound to apo-transferrin, forming holo-transferrin. Plasma iron in the ferrous state is oxidized to the ferric state by ceruloplasmin to allow binding. The transferrin receptor enables the uptake of transferrin in target tissues, e.g. the erythrocyte. Iron is subsequently set free from transferrin by a specific DMT1 isoform. In several cell types, such as the macrophage, excess iron can be bound to ferritin, and stored until needed

Iron cannot be actively secreted from the body. An excess of iron leads to an increased concentration of circulating free iron, which is primarily taken up by the liver, the pancreas and the heart [29]. This is why syndromes associated with iron overload – or haemochromatosis – when fully developed, manifest with a triad of cirrhosis, diabetes and cardiomyopathy. The archetypal hereditary haemochromatosis (type 1) is caused by mutations in *HFE*, causing hepcidin deficiency and resulting in systemic iron overload which only becomes manifest during the 4th or 5th decade, if at all. In juvenile haemochromatosis (type 2), which is caused by mutations in *HJV* or in *HAMP*, hepcidin deficiency is more prominent and patients present already in young adulthood with symptoms of systemic iron overload. In TFR2-related haemochromatosis (type 3), caused by mutations in *TFR2*, symptoms are virtually identical to those of type 1 haemochromatosis. While the first three subtypes are autosomal recessively inherited, ferroportin disease (type 4) is autosomal dominant. Ferroportin disease form A is due to loss of function mutations in *SLC40A1* which hamper the export of iron, causing iron accumulation in macrophages along with borderline microcytic anemia. Ferroportin disease form B is caused by gain of function mutations in *SLC40A1* as a consequence of which ferroportin – and thus iron export – is no longer inhibited by hepcidin. Clinically, this gives rise to a more classical hemochromatosis phenotype [30].

In 4 disorders, iron deficiency anemia is a prominent sign. Iron-refractory iron deficiency anaemia (IRIDA) is caused by mutations in *TMPRSS6*. It results in high hepcidin levels, which prevent the release of iron from enterocytes and macrophages, causing microcytic anaemia. DMT1 deficiency is caused by mutations in *SLC11A2*, hampering the uptake of iron by erythroblasts and resulting in microcytic anaemia. Patients also exhibit liver iron overload. Patients with mutations in *STEAP3* present with a similar phenotype [31]. Mutations in *TFRC*, encoding transferrin receptor 1, cause combined immunodeficiency with intermittent leukopenia/thrombocytopenia and moderate anemia [32].

Ten disorders are presently known to provoke iron accumulation in the brain, particularly the basal ganglia and neurodegeneration. Of these, only two have a clear link to iron homeostasis [33]. Aceruloplasminaemia is caused by mutations in *CP*. Patients accumulate iron in the brain, especially the basal ganglia, and in the islets of Langerhans, and present with extrapyramidal symptoms and/or diabetes. Neuroferritinopathy is caused by mutations in *FTL*, resulting in deposition of iron and ferritin in the basal ganglia. In the remaining 8 gene deficiencies, iron accumulation is poorly understood, may be variable and appears to be only partially responsible for the symptomatology. Of these, Pantothenate kinase deficiency, caused by mutations in *PANK2*, and Coenzyme A synthetase deficiency, encoded by *COASY*, are involved in the synthesis of Coenzyme A, an important player in lipid metabolism. The product of at least 2 other genes, *PLA2G6* and *FA2H*, mutations in which are responsible for infantile neuroaxonal dystrophy and fatty acid hydroxylase associated neurodegeneration respectively, also involve lipid metabolism. Mutations in *C19ORF12*, which encodes a mitochondrial membrane protein, may also affect lipid metabolism [34]. Other disorders which are associated with iron accumulation in the brain are Woodhouse-Sakati syndrome, caused by mutations in *DCAF17*, Kufor-Rakeb syndrome, caused by mutations in *ATP13A2*, and Static Encephalopathy of childhood with Neuro Degeneration in Adulthood (SENDA), an X-linked dominant disorder caused by mutations in *WDR45*. The latter two genes appear to be involved in autophagosome pathways [35].

37.2.1 Systemic Iron Overload Syndromes (Haemochromatosis)

Classic Hereditary Haemochromatosis (Type 1)

■ Clinical Presentation

Classic hereditary haemochromatosis, also called type 1 or HFE related a haemochromatosis, is an autosomal recessive disorder, characterized by a slow but progressive accumulation of iron in various organs, which becomes clinically apparent during the fourth or fifth decade of life [28][30][36]. The initial symptoms are nonspecific and include fatigue, weakness, abdominal pain, weight loss and arthralgia. Given the increased awareness of this condition, and the improved diagnostic possibilities, the classic symptoms of full-blown haemochromatosis, such as liver fibrosis and cirrhosis, diabetes, cardiomyopathy, hypogonadotrophic hypogonadism, arthropathy and skin pigmentation are now seen only rarely [28][30][36].

■ Metabolic Derangement

Classic hereditary haemochromatosis is caused by a disturbance in iron homeostasis associated with hepcidin deficiency and systemic accumulation of iron. The exact role of the gene known to be mutated in classic haemochromatosis, *HFE*, and its product HFE, is unclear at present. Most probably it is essential for sensing iron levels and thus indirectly for regulating hepcidin synthesis [28][30].

■ Genetics

As many as 0.5% of the Northern European population are homozygous for the C282Y mutation in *HFE*, yet only 5% of male and <1% of female C282Y homozygotes eventually develop liver fibrosis or cirrhosis [30][37]. Other mutations in *HFE* are also described, e.g. H63D, with compound heterozygosity for H63D and C282Y being associated with iron overload [30][37].

■ Diagnostic Tests

In patients with haemochromatosis, transferrin saturation initially increases, followed by serum ferritin, reflecting the increasing body iron overload. When transferrin saturation is elevated (above 45%) and serum ferritin is too (>200 ng/ml in adult females and >300 ng/ml in adult males) genetic testing of *HFE* should be performed [30][37].

Treatment and Prognosis

At least half of all male and female C282Y homozygotes have normal serum ferritin levels and may never require therapy [30][37]. In addition, many more will only have moderately elevated serum ferritin levels (200–1000 ng/ml), and it is unclear at present whether all should have regular phlebotomies to reduce systemic iron load [37]. However, with serum ferritin levels exceeding 1000 ng/ml a phlebotomy regimen is clearly necessary. In adults, initially 500 ml blood is removed weekly or bi-weekly. Phlebotomy frequency is usually reduced to once every 3-6 months when serum ferritin levels are below 50 ng/ml [30][36][37].

Juvenile Hereditary Haemochromatosis (Type 2)

Juvenile hereditary haemochromatosis, also called type 2 haemochromatosis, is the most severe type of hereditary haemochromatosis, probably because hepcidin deficiency is more pronounced. Patients present in the 2nd and 3rd decades, mostly with hypogonadotropic hypogonadism and cardiomyopathy as a result of iron overload. Type 2A is caused by mutations in the *HJV* gene encoding for hemojuvelin, which is necessary for proper hepcidin synthesis, and type 2B from mutations in the *HAMP* gene encoding hepcidin. In juvenile hereditary haemochromatosis serum ferritin is high and transferrin iron saturation elevated, as in classic HFE-related haemochromatosis. A final diagnosis is made by mutation analysis. Phlebotomy is the treatment of choice and may prevent organ damage if initiated early [30][36][37].

TFR2-Related Hereditary Haemochromatosis Type 3)

The transferrin receptor 2, encoded by *TFR2*, is thought to be important for sensing the intracellular iron status, particularly in erythroid cells [38]. Mutations in this gene result in an iron overload phenotype which resembles classic, HFE-related haemochromatosis, although patients are generally somewhat younger [30]. Low hepcidin levels along with elevated transferrin iron saturation, elevated ferritin and high liver iron content are present. Mutation analysis leads to the correct diagnosis in the absence of the classic haemochromatosis genotype. Phlebotomy is the treatment of choice.

Ferroportin Related Hereditary Haemochromatosis (Type 4)

Haemochromatosis type 4, ferroportin disease, differs in several aspects from the other three subtypes of haemochromatosis. It is autosomal-dominantly inherited and caused by mutations in *SLC40A1*, encoding ferroportin, which is not only expressed at the enterocyte, but also at the cellular membrane of the macrophages. Loss of function mutations impair the export of iron from macrophages causing an iron deficiency in erythrocytic precursors. Patients present with a combination of mild microcytic anaemia with low transferrin saturation, yet with an iron overload predominantly in macrophages. In this subtype tolerance to phlebotomy is limited by the concurrent anaemia. In contrast, gain of function mutations cause resistance to feedback inhibition by hepcidin. These patients present with a more classic hepatic iron overload haemochromatosis phenotype [28][30].

Neonatal Haemochromatosis

Neonatal haemochromatosis (NH), once thought to be an autosomal recessive inherited disorder, is now recognized as being acquired, and an example of the principle that any disease state that chronically prevents the synthesis or activity of hepcidin will lead to haemochromatosis [30]. Patients present in the first few weeks of life with severe liver failure. It is caused by a maternal alloimmune reaction to the infant liver, which already starts in utero. Liver injury leads to a decrease in hepcidin which manifests as severe siderosis of both liver and extrahepatic organs. The diagnosis is made in any child with neonatal liver failure in combination with high serum ferritin and extrahepatic siderosis, as evidenced by MRI and/or oral mucosal biopsy, which will demonstrate iron deposits in minor salivary glands in patients with NH. Therapy is by exchange transfusion in combination with intravenous immunoglobulins (IVIGs) to remove/bind maternally derived IgG, which is responsible for ongoing liver injury [39]. There might be an additional role for simultaneous antioxidant therapy. The risk of recurrence in a subsequent pregnancy from a mother who has given birth to an affected child is as high as 90%. However, therapy with IVIGs during pregnancy will reduce this risk substantially [40].

37.2.2 Iron Deficiency and Distribution Disorders

Iron-Refractory Iron Deficiency Anaemia (IRIDA)

This disease is caused by a deficiency of matriptase-2, which is encoded by *TMPRSS6*. If a mutation in both copies of this gene is present the normal cleavage of haemojuvelin is interrupted, resulting in high hepcidin levels [28][30]. This will result in iron deficiency, low transferrin saturation (<10%) and microcytic anaemia at a young age [41]. Oral iron supplementation is not effective, as high hepcidin levels will prevent iron release from the enterocytes, necessitating intravenous iron therapy.

Atransferrinaemia

Although first described in 1961, very few cases of atransferrinaemia have thus far been described. Patients with this autosomal recessive disorder, caused by mutations in *TF*, present with moderate to severe hypochromic microcytic anaemia and growth retardation along with signs of haemochromatosis. Serum transferrin levels are very low, serum ferritin levels are elevated. Plasma infusions to increase the transferrin pool, represent an effective treatment [42].

Hypochromic Microcytic Anaemia with Iron Overload Type 1

Hypochromic microcytic anaemia with iron overload type 1 is caused by mutations in *SLC11A2*, encoding DMT1. One of the isoforms of DMT1 is responsible for removing iron from

absorbed transferrin in erythroid cells. Consequently these patients present at a young age with microcytic anaemia in combination with mild hepatic iron overload. Both transferrin saturation and serum ferritin levels are elevated. With erythropoietin (EPO) treatment regular transfusions can often be avoided [43].

Hypochromic Microcytic Anaemia with Iron Overload Type 2

This subtype is caused by mutations in *STEAP3*. The encoded protein, STEAP3, is an endosomal ferrireductase which facilitates the transferrin mediated uptake of iron. In the 3 siblings reported thus far, anaemia was present from early childhood, while patients became transfusion dependent several years later, usually in late childhood. High ferritin levels, together with low transferrin and increased transferrin saturation were found. Although the degree of liver iron overload varied, all 3 had hypogonadism [31].

37.2.3 Neurodegeneration with Brain Iron Accumulation (NBIA)

Aceruloplasminaemia

Aceruloplasminaemia is an autosomal recessive disorder characterised by accumulation of iron in the liver, islets of Langerhans and brain, in particular the basal ganglia and the retina [26]. Clinically the disease consists of adult-onset neurological disease (chorea, cerebellar ataxia, dystonia, parkinsonism and psychiatric signs), retinal degeneration and diabetes mellitus. The major isoform of ceruloplasmin in the brain is necessary for iron import through its ferroxidase activity. More than 30 aceruloplasminaemia-causing mutations in the ceruloplasmin (*CP*) gene have been identified. The diagnosis is made by a combination of clinical symptoms, iron overload in liver and brain, and a nondetectable level of serum ceruloplasmin. In addition, serum iron is low (<45 µg/dl) while there is a high serum ferritin concentration (850–4000 ng/ml). Desferrioxamine, a high-affinity iron chelator, reduces body iron stores and may therefore ameliorate diabetes as well as hepatic and neurological symptoms [26].

Neuroferritinopathy

Neuroferritinopathy is an autosomal dominant disease characterised by accumulation of deposits of iron and ferritin in the brain, most prominently in the basal ganglia, where it can even result in cavitation. Most patients present their first symptoms in early adulthood and over decades develop the full clinical picture, consisting of chorea, ataxia, rigidity and dystonia, as well as mixed complaints of cognitive dysfunction. Thus far, all mutations that have been described in *FTL* in patients with this disease affect the last part of the light chain of ferritin. Biochemical indicators of iron metabolism are normal, with the exception of serum ferritin, which is in the low to low-normal range. There is currently no effective treatment [33].

Pantothenate Kinase-Associated Neurodegeneration (PKAN)

In typical patients this disease presents before the age of 6 years with dystonia, rigidity and chorea-athetosis. Symptoms are slowly progressive, with involvement of the corticospinal tract and development of spasticity. Affected children lose the ability to walk within 10–15 years [44]. In atypical patients the onset is later and progression is slower. On MRI iron accumulation in the basal ganglia can be seen, showing up as areas of hypointensity in the globus pallidus, with bilateral areas of hyperintensity (›eye of the tiger‹ sign). This autosomal recessive disease is caused by mutations in *PANK2* encoding pantothenate kinase 2, which is a key enzyme in the biosynthesis of coenzyme A [33][34][44][45]. A dysfunction of this enzyme will hinder the beta oxidation of fatty acids, giving oxidative stress and probably resulting in pathological changes at the sites that are most vulnerable, i.e. the basal ganglia. Diagnosis is made by MRI and genetic testing in a child presenting with extrapyramidal symptoms. Treatment is symptomatic.

Coenzyme A Synthetase Deficiency

This is an autosomal recessive disease which, based on the few cases reported, causes a very similar clinical picture as observed in PKAN. It is caused by mutations in *COASY*, which encodes Coenzyme A synthetase, a bifunctional enzyme that catalyzes the last two steps in Coenzyme A synthesis [33][34].

Infantile Neuroaxonal Dystrophy (INAD)

INAD is an autosomal recessive disorder caused by mutations in *PLA2G6* encoding the calcium independent phospholipase enzyme iPLA2-VI, which catalyses the hydrolysis of glycerophospholipids. Most patients with INAD present in infancy to early childhood with motor regression and hypotonia. On MRI there is iron deposition in the globus pallidus and substantia nigra [33][34][46].

Fatty Acid Hydroxylase Associated Neurodegeneration

Mutations in *FA2H* may also lead to iron accumulation in the brain. The encoded protein, fatty acid hydroxylase, is involved in the modification of the ceramide moiety of sphingolipids. Patients present in early childhood with gait difficulties, due to progressive spastic paraparesis and dysmetria. Cognitive decline is noted in their teens. Optic atrophy and xeroderma were also reported. On neuroimaging, profound pontocerebellar atrophy, confluent periventricular white matter abnormalities were found along with evidence of iron accumulation [33][34] (see ▶ Chapter 38).

Mitochondrial Protein Associated Neurodegeneration

Mutations in *C19ORF12*, encoding a mitochondrial protein which is involved in mitochondrial magnesium homeostasis appear to be a frequent cause of iron deposition in the basal ganglia [33][34][47]. Psychiatric signs, optic atrophy, motor axonal neuropathy are common additional findings. Compared with PKAN, patients present at an older age and exhibit a more protracted course (see ▶ Chapter 14).

Woodhouse-Sakati Syndrome

Patients with this syndrome, an autosomal recessive disorder caused by mutations in *DCAF17*, the product of which is thought to play a role in ubiquitinylation, present with hypogonadism, diabetes mellitus, partial alopecia along with varying degrees of mental retardation. Brain MRI in the minority of patients who subsequently developed an extrapyramidal movement disorder revealed profound white matter abnormalities along with basal ganglia abnormalities consistent with iron deposition [33].

Static Encephalopathy of Childhood with Neurodegeneration in Adulthood (SENDA)

SENDA is an X linked dominant disorder caused by *de novo* heterozygous or hemizygous mutations in *WDR45*. *WDR45* encodes a protein with a 7-bladed propeller structure that offers a platform for reversible protein-protein and phospholipid interactions. It has an important role in autophagy [35]. Patients exhibit global developmental delay throughout childhood, and usually only develop extrapyramidal signs along with dementia in early adulthood. Iron accumulation in the globus pallidus and substantia nigra is noted in the later stages of the disease [33].

Kufor-Rabek Syndrome

This autosomal recessive disorder characterized by juvenile onset Parkinson disease is caused by mutations in *ATP13A2*. Supranuclear gaze palsy, pyramidal signs and dementia are additional features. Evidence of basal ganglia iron deposition is present in some [33].

37.3 Magnesium

Magnesium Metabolism

Magnesium is the second most abundant intracellular cation and plays an essential role in many biochemical processes as well as neuromuscular excitability. Normal serum magnesium concentration (0.75–1.4 mmol/l) is maintained by adapting the urinary magnesium excretion to the uptake in the small intestine.

Magnesium absorption in the small bowel is mainly done through active uptake by the TRPM6 magnesium channel, which is expressed at the brush border of the epithelial cells, although magnesium can also be imported from the gut through paracellular diffusion. Urinary excretion of magnesium is carefully regulated by modulating the reabsorption of the huge quantities of magnesium that are filtered each day. While most magnesium is passively reabsorbed in the loop of Henle, the active reabsorption that takes place in the distal convoluted tubule determines the actual magnesium balance.

Primary hypomagnesemia with secondary hypocalcemia generally presents in the first months of life with increased neuromuscular irritability or even frank convulsions. It is caused by mutations in *TRPM6*, reducing uptake of magnesium from the gut. Magnesium supplementation is highly effective.

Hypomagnesaemia with hypercalciuria and nephrocalcinosis can be caused by mutations in *CLDN16* or in *CLDN19*, together encoding a tight junction complex in the ascending limb of the loop of Henle. Deficiency of either protein provokes calcium deposition in the kidney, leading to renal failure, with few symptoms of hypomagnesaemia. Magnesium supplements do not prevent the development of end stage renal disease.

Isolated dominant hypomagnesaemia can provoke generalized convulsions and is associated with mutations in either *FXYD2, CNNM2* or *KCNA1*.

Isolated autosomal recessive hypomagnesaemia has no other symptoms and is caused by mutations in *EGF*.

37.3.1 Primary Hypomagnesaemia with Secondary Hypocalcaemia

■ Clinical Presentation

Primary hypomagnesaemia with secondary hypocalcaemia (HSH) is a rare autosomal recessive disorder. It was first recognized in 1965 and since then more than 70 infants from all over the world have been described [48]. Patients commonly present in the first months of life with generalized seizures or other symptoms of increased neuromuscular excitability such as irritability, poor sleeping, muscle spasms and/or tetany.

■ Metabolic Derangement

Primary hypomagnesaemia is caused by impaired magnesium uptake from the gut [49]. A lowered renal threshold for magnesium, causing a significant renal magnesium leak, is a contributing factor and also generally prevents serum magnesium from completely normalizing during supplementation [50]. The disease is caused by a defect of a protein, TRPM6, a member of the long transient receptor potential channel (TRPM) family, which complexes with its closest homolog, TRPM7, to form an ion-channel for magnesium at the cell surface. Genetic lesions of TPRM6 prevent assembly of a functional complex and hence impair magnesium transport [51].

Severe hypomagnaesaemia blocks synthesis and/or release of parathormone (PTH). In addition, when hypomagnesaemia is present, the administration of PTH fails to induce a rise in serum calcium. The hypocalcaemia in HSH is thus secondary to low PTH levels in combination with some form of end organ resistance.

Genetics

Primary hypomagnesaemia is an autosomal recessive disorder that is caused by mutations in *TRPM6* [52][53]. This gene is expressed in the small and large intestine as well as in the cells lining the distal tubules. To date almost 40 mutations have been identified [51].

Diagnostic Tests

Primary hypomagnesaemia is characterized by a very low serum magnesium (0.24 ± 0.11 mmol/l; normal 0.65–1.20 mmol/l) in combination with a low serum calcium (1.64 ± 0.41 mmol/l; normal 2.12–2.70 mmol/l). In the presence of serum hypomagnesaemia, the urinary excretion of magnesium is reduced, and PTH levels are inappropriately low. No evidence for malabsorption of other nutrients is found, and renal function is not otherwise compromised.

Treatment and Prognosis

Untreated, the disorder will result in permanent neurological damage or death. However magnesium supplementation corrects all clinical symptoms. During the initial stage this should be given intravenously, with concurrent parenteral supplementation of calcium. After stabilization, magnesium therapy can be continued orally in an amount that is adjusted to the clinical response. The individual dosage varies greatly between patients (between 0.4 and 3.9 mmol/kg/day of elemental magnesium) [48][50]. With this regimen, serum calcium normalizes, but serum magnesium will generally remain just below normal [48][50]. Dividing oral magnesium supplementation in three to five doses will reduce fluctuations of serum magnesium and will prevent the development of chronic diarrhoea in many, but not all patients.

The prognosis of primary hypomagnesaemia is good if the diagnosis is made early; with treatment both growth and development are normal. However, patients who have frequent hypomagnesaemia/hypocalcaemia-induced convulsions, either before or after the diagnosis is made, are at risk for developing psychomotor retardation.

37.3.2 Hypomagnesaemia with Hypercalciuria and Nephrocalcinosis

Clinical Presentation

More than a 100 patients with familial hypomagnesaemia with hypercalciuria and nephrocalcinosis (FHHNC) have been reported [54][55]. Patients usually present during childhood with recurrent urinary tract infections, polyuria/polydipsia, nephrolithiasis and/or failure to thrive. At presentation less than half of the patients will have nephrocalcinosis; however ultimately this will be present in all. Clinical signs of hypomagnesaemia such as seizures are less common, in line with only moderately depressed serum magnesium levels [56]. A subset of patients has severe ocular defects.

Metabolic Derangement

In most patients FHHNC is caused by a defect of claudin-16, while in a minority of patients claudin-19 is defective [54][55][56][57]. In the thick ascending limb of Henle's loop these two proteins form a complex at the tight junction [55]. Mutations in either component of this complex will change ion selectivity at the tight junction, resulting in excessive renal wasting of magnesium, with secondary development of hypercalciuria, nephrocalcinosis and ultimately renal failure.

Genetics

Mutations in *CLDN16* or *CLDN19* can cause FHHNC. To date 56 distinct mutations in *CLDN16* have been identified, and 17 mutations in *CLDN19* [57]. Although some patients with *CLDN16* mutations have mild ocular pathology (strabismus, myopia), all patients with *CLDN19* mutations have severe ocular abnormalities (macular colobomata, nystagmus). Therefore this subtype is now generally referred to as FHHNC with severe ocular involvement [54][55][57].

Diagnostic Tests

Serum magnesium is low, with a median level of 0.49 mmol/l (IQR 0.39–0.60 mmol/l) in patients with *CLDN16* mutations and 0.59 mmol/l (IQR 0.50–0.60 mmol/l) in patients with *CLDN19* mutations [54]. At diagnosis, glomerular filtration rate is already reduced in the majority of patients, and subsequently deteriorates further. Renal sonography shows nephrocalcinosis, with its characteristic medullary distribution, early in the course of the disease.

Treatment and Prognosis

Oral magnesium salts are used to supplement renal loss, while thiazide diuretics are given to reduce calcium excretion rates in an effort to prevent the progression of nephrocalcinosis, which correlates with development of renal failure. However, these strategies do not seem to significantly influence the progression of renal failure [56]. End stage renal disease will develop during puberty or early adulthood in the majority, with the renal disease in patients with *CLDN19* mutations being more progressive [54].

37.3.3 Isolated Dominant Hypomagnesemia

Autosomal dominant hypomagnesaemia is caused by a reduced tubular threshold for magnesium and may be associated with a lowered urinary calcium excretion [58][59][60]. Patients have hypomagnesaemia (0.35–0.56 mmol/l; normal 0.65–1.20 mmol/l) and may display symptoms associated with a low serum magnesium such as generalized convulsions, muscle cramps or weakness, headaches, or may have no symptoms at all.

The disorder can be caused by mutations in any of the following genes: *CNNM2* [58], *FXYD2* [59] or *KCNA1* [60], with only one or a few families described for each defect. *CNNM2* encodes cyclin M2 (CNNM2), and is present in the basolateral membrane of the distal convoluted tubules, where it is essential for magnesium transport, although the exact

mechanism has still to be elucidated [58]. *FXYD2* encodes the γ-subunit of Na^+K^+-ATPase, which is expressed both in the proximal and distal tubules [59]. For this defect too the exact mechanism that causes the hypomagnesaemia is unclear. *KCNA1* encodes the potassium channel Kv1.1, localised at the distal tubules [60]. Obviously potassium secretion and magnesium reabsorption are coupled, causing secondary hypomagnesaemia upon Kv1.1 deficiency.

37.3.4 Isolated Autosomal Recessive Hypomagnesaemia

Isolated autosomal recessive hypomagnesaemia has been described in two children from a consanguineous family [61]. Apart from the hypomagnesaemia, no biochemical abnormality is present, and specifically calcium excretion in the urine is normal. This disorder is caused by a mutation in *EGF*, leading to insufficient activation of kidney TRPM6, which is essential for renal tubular magnesium reabsorption [62].

37.4 Manganese

Manganese Metabolism

Manganese is an essential trace element and a co-factor for enzymes involved in peptide and amino acid metabolism (prolidase, arginase, glutamine synthetase), protein O-glycosylation, carbohydrate metabolism, production of cellular energy, superoxide elimination as well as others. As manganese is also potentially toxic its concentration must be carefully controlled. This process is however not fully understood at present. For the intestinal uptake of manganese DMT-1 and transferrin receptor (TfR) seem to be necessary. SLC30A10 is a key player in its excretion.

With manganese being amply available in the diet no manganese deficiency syndromes have been described in humans. However, manganese toxicity can be seen in situations where intake escapes normal control mechanisms, such as exposure to manganese dust in adults working in the mining or welding industry, or in patients on prolonged TPN, especially neonates. These patients present with extrapyramidal motor symptoms such as dystonia and rigidity due to deposits of manganese in the basal ganglia, which can be seen on an MRI of the brain. Manganese neurotoxicity has been attributed variously to impaired dopaminergic, glutamatergic and GABAergic neurotransmission, mitochondrial dysfunction, oxidative stress, and neuroinflammation.

37.4.1 Inherited Manganism Due to Mutations in *SLC30A10*

Patients with inherited manganism due to mutations in *SLC30A10* also present with a high serum manganese and similar neurological symptoms to patients who have had high exposure to manganese. In addition they exhibit polycythaemia and chronic liver disease leading to cirrhosis. Treatment is by a combination of chelation and iron supplementation. The latter competively inhibits manganese uptake.

▪ Clinical Presentation

Inherited manganism is a rare autosomal recessive disorder [63][64][65]. The neurological disease can present in childhood as a four-limb dystonia with a »cock-walk« gait, dysarthria, fine tremor and bradykinesia or occasionally spastic paraplegia, or in adulthood as parkinsonism (shuffling gait, rigidity, bradykinesia, hypomimia and monotone speech). An MRI shows the typical appearances of manganese accumulation and whole blood manganese level is markedly elevated. Hepatomegaly is usually present and a liver biopsy shows a variable degree of fibrosis / cirrhosis. Most patients have polycythaemia and this can be the presenting feature at a young age. The liver disease can progress to liver failure and patients can occasionally present with liver failure.

▪ Metabolic Derangement

The hypermanganesaemia in this syndrome is caused by a deficiency of SLC30A10. SLC30A10 is believed to be important in excretion of manganese by the liver. Mutations in *SLC30A10* lead to accumulation of Mn in the liver and secondarily in the blood and brain; the dopaminergic cells in the basal ganglia are particularly vulnerable. Manganese accumulation in hepatocytes and neurons leads to inflammation and cellular dysfunction, giving the characteristic symptoms of this disease. The polycythaemia is thought to be the result of an induction of erythropoietin gene expression.

▪ Genetics

Inherited manganism is an autosomal recessive disease that is caused by mutations in *SLC30A10*. This gene is highly expressed in the liver and brain, especially in the basal ganglia. Most described patients have homozygous mutations, including missense mutations, nonsense mutations, small deletions, frameshift mutations and one family with a large deletion including exons 1 and 2. The patients with adult onset parkinsonism had a mutation leading to a protein that was only truncated by the last 49 amino acids.

▪ Diagnostic Tests

Whole-blood manganese concentrations often exceed 2000 nmol/L (normal: <320 nmol/L). Typical findings on T1-weighted MRI of the brain include bilateral hyperintense sig-

nal in the basal ganglia, the midbrain and cerebellar nuclei and the cerebral and cerebellar white matter. In addition almost all patients exhibit polycythaemia as well signs of liver disease, i.e. hepatomegaly and raised transaminases; a biopsy often shows cirrhosis.

Treatment and Prognosis

Iron supplementation combined with chelation therapy is the treatment of choice. The chelation with intravenous disodium calcium edetate induces excretion of manganese in the urine, while oral iron supplementation competitively inhibits manganese uptake in the intestine. Treatment with i/v $Na_2CaEDTA$ (20mg/kg/dose twice daily for 5 days a month), particularly when combined with oral iron, can result in improvement of neurological symptoms and MRI abnormalities, and normalization of serum manganese levels and may interrupt cirrhosis progression. Treatment should be monitored by measurement of electrolytes, calcium, phosphate and magnesium, renal and liver function tests and plasma concentrations of manganese, iron, zinc, copper and selenium. Some patients have required zinc supplementation because of a fall in plasma zinc or a reduction in iron supplementation because of a high serum iron level.

37.4.2 Manganese Transporter Defect

Mutations in *SLC39A8* which encodes a cationic transporter for Mn and Zn have recently been found to be associated with a disorder characterised by low levels of plasma manganese and a defect in glycosylation. The disorder which is potentially treatable is discussed in ▶ Chapter 41, ▶ Section 41.5.8. Note added in proofs: A cohort of patients with a novel autosomal recessive manganese transporter defect caused by mutations in *SLC39A14* and presenting with childhood-onset parkinsonism–dystonia have recently been identified [84].

37.5 Selenium

Selenium Metabolism

Selenium is an essential micronutrient. Its biological role is mainly mediated through selenocysteine, which is incorporated into the 25 known human selenoproteins, such as the glutathione peroxidases, mediating the removal of cellular reactive oxygen species, and deiodinases, which are involved in thyroxine metabolism. Selenocysteine is not encoded in the DNA itself, but its incorporation into a selenoprotein is mediated through a cotranslational process involving a specialised SElenoCysteine Insertion Sequence, or SECIS element, in the 3′ untranslated region of the mRNA. Interaction of this element with proteins such as the SECIS-binding protein 2 encoded by *SECISBP2* directs a specific tRNA to incorporate selenocysteine at an UGA codon. The final step in the synthesis of this tRNA, i.e. switching a serine residue for a selenocysteine residue, is mediated through SepSecS, encoded by *SEPSECS*.

Two rare inborn errors of metabolism are identified within this system. The first, involving mutations in *SECISBP2*, gives a reduced synthesis of all selenoproteins and has a multisystem expression with oligospermia, myopathy and an increased dermal photosensitivity. At least part of this phenotype seems to be related to a reduced ROS defence. In addition, the deficiency of deiodinases gives an abnormal thyroid hormone profile with lowered serum T_3 and increased T_4 [66][67]. The second inborn error involves mutations in *SEPSECS*, giving progressive microcephaly, profound mental retardation and severe spasticity, an autosomal recessive clinical entity known as progressive cerebello-cerebral atrophy or pontocerebellar hypoplasia type 2D [68]. Some of these patients have axonal neuropathy in combination with an elevated blood lactate, which might falsely suggest a mitochondrial cause of the disease [69].

37.6 Zinc

Zinc Metabolism

Zinc is a cofactor for over 100 enzymes and, as such, is involved in all major metabolic pathways. It is also essential for nucleic acid metabolism and protein synthesis and their regulation through so called zinc-finger proteins. Zinc deficiency, either hereditary or acquired, has major detrimental effects, whereas high serum zinc has few, probably because of binding to albumin and a2-macroglobulin. Homeostasis of zinc is maintained through the coordinated action of two families of zinc transporters: SLC30 (ZnT) and SLC39 (Zip). These transporters have opposing roles in cellular zinc metabolism. The SLC30 family transporters decrease intracellular zinc concentration by promoting zinc efflux from cells (or to intracellular vesicles), while the SLC39 family increases intracellular zinc concentration by promoting zinc influx into cells (or the release of zinc from intracellular vesicles).

Acrodermatitis enteropathica is due to mutations in *SLC39A4*, encoding the major zinc importing carrier in the intestine. Symptoms typically start in infancy after the introduction of bottle feeding, and include periorificial and acral dermatitis, diarrhoea, infections, and growth retardation. Therapy with zinc is extremely effective.

Zinc deficiency in breast fed babies presents with the same dermatological symptoms as acrodermatitis enteropathica. It is caused by maternal heterozygous mutations in *SLC30A2*. Zinc therapy is highly effective.

Hyperzincemia with hypercalprotectinaemia is characterized by extremely elevated levels of calprotectin thought to cause uncontrolled, harmful inflammatory reactions.

Autosomal dominant hyperzincaemia without symptoms is most likely a non-disease.

37.6.1 Acrodermatitis Enteropathica

Clinical Presentation

Children with acrodermatitis enteropathica (AE) are healthy at birth, but develop symptoms some weeks after breast feeding has been stopped. The most striking clinical feature is a severe dermatitis, classically localized at the acral and periorificial sites [70][71]. At onset, these skin lesions are erythematous, while after the first year of life pustular and hyperkeratotic changes become more prominent. Secondary infection with *Candida albicans* and/or *Staphylococcus aureus* is not uncommon. In addition to the skin lesions, seen in almost all patients, intermittent diarrhoea can develop, which in more advanced stages can progress to intractable watery diarrhoea and failure to thrive. If untreated, a significant fraction of the patients will have a gradual downhill course, although the majority seems to be able to survive without treatment into adulthood. Mood changes are an early sign of zinc deficiency, presenting as apathy and irritability in infancy and later on as depression. Infections are also frequent, and can be life threatening. Other clinical features include alopecia and nail deformities, as well as ophthalmological symptoms such as blepharitis, conjunctivitis and photophobia.

Metabolic Derangement

AE is caused by insufficient intestinal absorption of zinc, as can be demonstrated in jejunal biopsies of patients [72]. This defect is due to deficiency of ZIP4. The reduced zinc absorption results in zinc deficiency with impairment of the function of many enzymes that have zinc as cofactor. Tissues with a high cellular turnover, such as skin, intestine, and lymphoid system are most severely affected.

Genetics

AE is an autosomal recessive disease caused by mutations in *SLC39A4* [73][74]. *SLC39A4* encodes a zinc transporter, ZIP4, with eight transmembrane domains, which probably form a zinc channel, and is expressed at the apical membrane of the enterocytes. Over 40 mutations have been identified so far, mainly in families from Europe, the Middle-East and North-Africa [75].

Diagnostic Tests

In most patients, serum zinc levels are lower (5.8±3.2 μmol/l) than normal (12.6±2.3 μmol/l), although values within the normal range can be found [76]. Measurements of zinc in other tissues, such as hair and red or white blood cells, do not seem to improve diagnostic accuracy. In addition, several conditions, such as chronic diarrhoea due to other causes, can present with low serum zinc. Therefore, the diagnosis of AE can never be based solely on serum zinc levels. Other tests may contribute to a certain extent: low urinary zinc excretion (reflecting a low serum zinc level), low serum alkaline phosphatase activity, changes in the serum fatty acid profile, hypobetalipoproteinemia, and a reduction of serum vitamin A. In many patients, both humoral and cell-mediated immunity are depressed [77]. Small bowel biopsy generally shows partial to subtotal villous atrophy and Paneth cell inclusions on electron microscopy.

The defect in active zinc transport can be proven in jejunal biopsies [72]. However, as this is not available in most settings, a practical approach is to start zinc therapy when the clinical diagnosis is suspected, and await the response, which should occur within one week. When the clinical signs of acrodermatitis were equivocal one may consider to temporarily withdraw zinc therapy after some time to provoke a relapse, and in this way differentiate between true AE (which will relapse quickly) and acquired zinc deficiency. Direct investigation of *SLC39A4* for mutations is theoretically superior. However, it may take several months before the existence of *SLC39A4* mutations are confirmed or disproved, and the condition of the patient generally warrants an earlier intervention.

Treatment and Prognosis

Before zinc supplementation was serendipitously found to correct the abnormalities in AE, patients were given breast milk and later on iodo-hydroxyquinolines. This generally resulted in partial or even total remission. Zinc therapy was introduced in 1975 [78], and is now used in all patient with AE. The usual dose is 150–400 mg zinc sulphate/day (equivalent to 35–90 mg elemental zinc/day), on which patients will start to show clinical improvement within days. Simultaneously, laboratory abnormalities such as serum zinc levels, urinary zinc excretion and alkaline phosphatase activity will normalise. Generally, the initial dose can be maintained throughout childhood, although some patients may need an increase during their growth spurt. After puberty, the requirements for zinc may be lower, but during pregnancy and lactation 400–500 mg zinc sulphate/day is needed. If the preparation causes gastric problems it may be encapsulated, or alternatively zinc gluconate or other zinc salts may be used. As zinc therapy will decopper patients it is necessary to monitor serum copper, and either reduce the dose of zinc or supplement copper if a deficiency is found. With zinc supplementation prognosis is excellent.

37.6.2 Zinc Deficiency in Breastfed Babies

Rarely, zinc deficiency with acrodermatitis can occur in breast-fed babies, especially in premature infants, as they have an increased zinc requirement in combination with a reduced capacity for zinc uptake in the gut [79]. Although this condition responds rapidly to oral zinc supplements, it is clearly different from AE, as it is seen exclusively during breast feeding and no impairment of intestinal zinc uptake can be found. The deficiency is caused by reduced levels of zinc in maternal milk, due to heterozygous mutations in *SLC30A2* [80].

37.6.3 Hyperzincaemia with Hypercalprotectinaemia

A very high serum zinc (77-200 µmol/l) can be encountered in combination with an extreme elevation of serum calprotectin (up to 1000 times the upper limit of normal) [81][82]. These patients present with recurrent infections, hepatosplenomegaly, arthritis, anaemia and persistently raised concentrations of C-reactive protein. It is speculated that the very high concentration of calprotectin, the major zinc binding protein of phagocytes, results in the uncontrolled and harmful inflammatory reactions which characterize this syndrome, while the hyperzincaemia is caused by the zinc capturing properties of calprotectin. Treatment with cyclosporine A or tacrolimus has been tried, however with varying results [82]. Inheritance of this syndrome is not clear yet.

37.6.4 Autosomal Dominant Hyperzincaemia without Symptoms

Elevated serum zinc (40–70 µmol/l) was described in seven family members from one large pedigree [83]. The condition seems to be inherited in an autosomal dominant fashion. Zinc concentrations in hair and erythocytes were normal, as was serum albumin, to which most of the excess zinc seemed to be bound. There were no clinical symptoms, nor additional biochemical abnormalities, so this condition appears to be benign.

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Section X Organelle-Related Disorders: Lysosomes, Peroxisomes, and Golgi and Pre-Golgi Systems

Disorders of Sphingolipid Synthesis, Sphingolipidoses, Niemann-Pick Disease Type C and Neuronal Ceroid Lipofuscinoses

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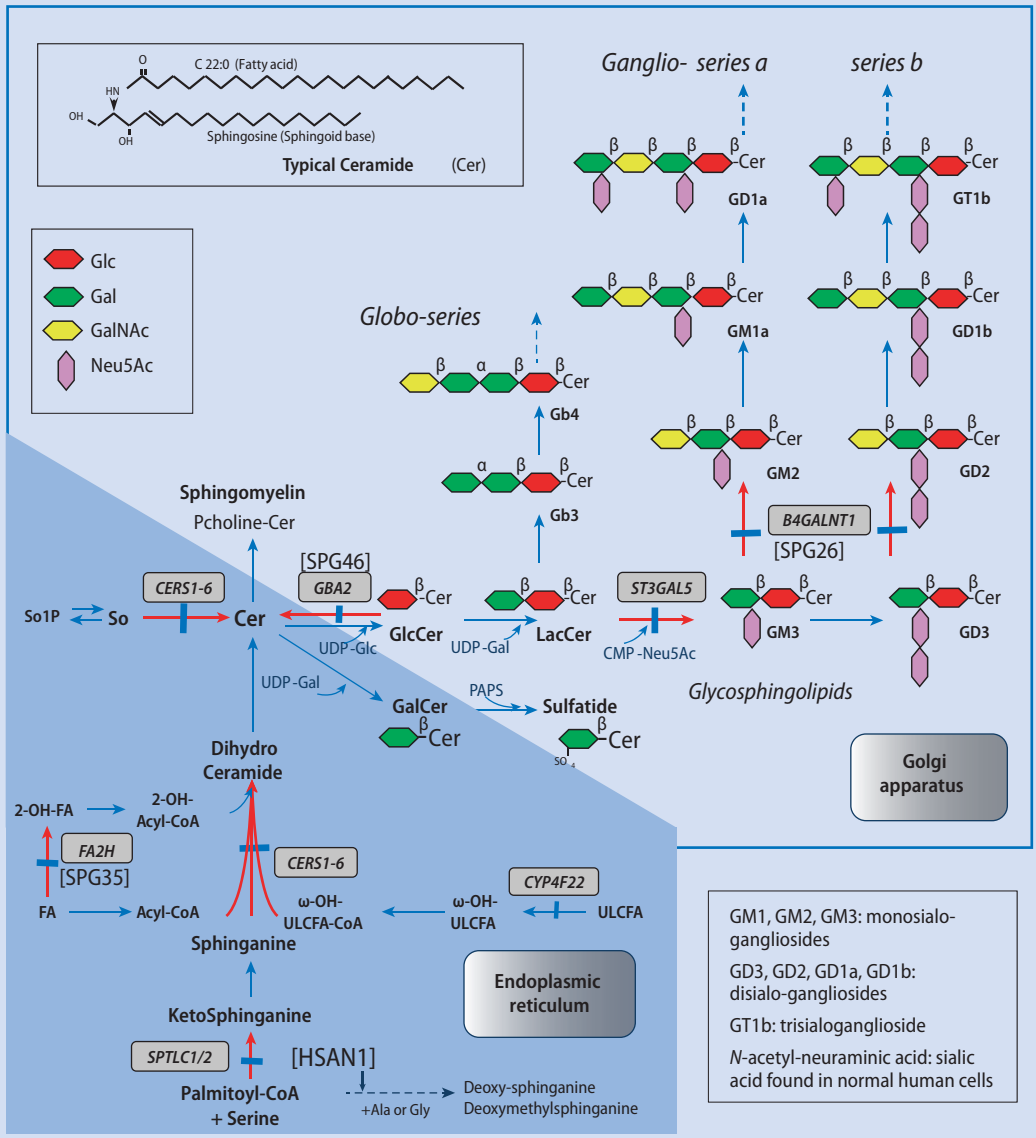


Fig. 38.1 Schematic representation of the structure of the main sphingolipids, and their biosynthetic pathways. Red arrows denote the defective pathways that are discussed in this chapter. The names of genes that are mutated are indicated. *Cer*, ceramide; *FA*, fatty acid; *Gal*, galactose; *GalCer*, galactosylceramide; *GalNAc*, N-acetyl-galactosamine; *Gb3*, globotriaosylceramide; *Gb4*, globotetraosylceramide (globoside); *Glc*, glucose; *GlcCer*, glucosylceramide; *LacCer*, lactosylceramide; *Neu5Ac*, N-acetyl-neuraminic acid; *2-OH-FA*, 2-hydroxy-fatty acid; *ω-OH-ULCFA*, ω-hydroxy ultra-long chain fatty acid; *PAPS*, 3′-phosphoadenosine-5′-phosphosulfate; *Pcholine*, phosphorylcholine; *So*, sphingosine; *So1P*, sphingosine-1-phosphate. Solid bars indicate metabolic blocks

Sphingolipid Structure and Metabolism

Sphingolipids are ubiquitous lipids found in all mammalian cell membranes and in plasma lipoproteins. Many exhibit dual functions, as key structural elements, but also as modulators of numerous biological/physiological functions. Their backbone is a long chain sphingoid base (sphingosine being the prototype) that can be N-acylated by a variety of fatty acids, forming ceramides (Cer) (Fig. 38.1). Depending on the type of hydrophilic head group linked to the 1-OH group of the sphingoid base, two main classes of sphingolipids are distinguished. Phosphosphingolipids contain phosphorylcholine (sphingomyelin), phosphorylethanolamine, or a phosphate group. Glycosphingolipids contain one (glucose or galactose) or several sugar residues, and can be very complex. Sialic acid (N-acetyl-neuraminic acid in humans)

containing glycosphingolipids are named gangliosides. Depending on the precise structure (sugar and linkage) of the oligosaccharide moiety, several glycosphingolipid lineages (ganglio-, globo- etc.) have been defined. ›Cerebroside‹ usually refers to the major myelin lipid galactosylceramide; ›sulfatide‹ to its sulfated derivative. Lysosphingolipids (e.g. psychosine) lack the fatty acid of ceramide. The main sphingolipids are depicted in ◘ Fig. 38.1 and ◘ Fig. 38.2. Sphingolipids are synthesised and degraded in different cellular compartments. A further aspect of sphingolipid homeostasis not discussed here is the recycling or salvage pathway.

Biosynthesis

◘ Fig. 38.1.

The *de novo* synthesis of ceramide occurs in the endoplasmic reticulum and starts by the condensation of serine and palmitoyl-CoA, a reaction catalyzed by serine palmitoyltransferase. The resulting ketosphinganine is reduced to sphinganine prior to N-acylation by ceramide synthases. Distinct fatty acids, including 2-hydroxylated long chain fatty acids and ω-hydroxylated ultra-long chain fatty acids, can be incorporated. Then, dihydroceramide is desaturated to produce ceramide. The sphingosine that is released by the action of ceramidases on the ceramide derived from the degradation of complex sphingolipids can also be N-acylated by ceramide synthases. Subsequent steps of sphingolipid synthesis (except galactosylceramide) occur in the Golgi apparatus, where ceramide is processed to sphingomyelin or to glucosylceramide; stepwise addition of further monosaccharides, catalysed by specific glycosyltransferases, leads to the formation of more complex neutral glycosphingolipids and gangliosides. Sphingolipids are then transported and inserted in various membranes.

Degradation

◘ Fig. 38.2.

After transport by the endolysosomal pathway to the lysosome, sphingolipid degradation proceeds by stepwise hydrolysis by specific acid sphingohydrolases, some of which may need co-factors called sphingolipid activator proteins (also lysosomal) for their *in vivo* action.

Sphingolipidoses are a subgroup of lysosomal storage disorders in which sphingolipids accumulate in one or several organs as the result of a primary deficiency in enzymes or activator proteins. Niemann-Pick type C disease is currently considered as a lipid trafficking disorder, resulting in lysosomal accumulation of cholesterol and sphingolipids. This chapter also describes neuronal ceroid lipofuscinoses, now recognised as another subgroup of lysosomal storage diseases. Additionally, inherited defects of sphingolipid biosynthesis are presented (▶ Sphingolipid Structure and Metabolism).

38.1 Disorders of Sphingolipid Synthesis

Most of the genes encoding the enzymes, transporters and activators operating in the sphingolipid synthesis pathway have been characterised, and an increasing number of monogenic defects affecting some steps of the biosynthesis of sphingolipids have been delineated in recent years (◘ Table 38.1). A majority of disorders were first described as a component of a genetically heterogeneous clinical syndrome -e.g., hereditary sensory and autonomic neuropathies (HSAN), autosomal recessive hereditary spastic paraplegias… – before the function of the protein encoded by the mutated gene was recognised. Mechanisms underlying the pathophysiology of most sphingolipid synthesis disorders are still enigmatic. With the exception of HSAN1, the reported mutations result in a loss of function of the corresponding enzyme. Alterations in the sphingolipid profile of the diseased tissues have not been described in all conditions. In general, it is still unknown whether tissue dysfunction and symptoms are due to the lack (or insufficient production) of one or more sphingolipid species, and/or accumulation of a precursor molecule or a potentially toxic metabolite.

Regarding genetic transmission, with the exception of HSAN1 due to a defect in serine palmitoyltransferase 1 or 2, and possibly the defect in ceramide synthase 2, enzymatic deficiencies of sphingolipid synthesis are inherited as autosomal recessive traits. So far, their diagnosis relies on DNA analysis although biochemical testing in plasma is possible for detection of HSAN1. There is currently no effective specific therapy for this type of IEMs. Most of these conditions remain extremely rare. Their clinical spectrum is broadening with description of new cases and the field is likely to quickly evolve in the near future. For these reasons, and since comprehensive reviews on the subject with exhaustive referencing have been published very recently [1][2], only a brief outline of each disorder will be given in this chapter.

38.1.1 Serine Palmitoyltransferase (Subunit 1 or 2) Deficiency and HSAN1

A defect in the very first step of sphingolipid biosynthesis is the major cause underlying the dominant hereditary sensory and autonomic neuropathy (HSAN1). Other (unrelated) genes that have been linked to HSAN1 are *ATL1*, *RAB7A* and *DNMT1* [1]. This peripheral neuropathy is characterised by a late onset (between the 2nd and 4th decade), a slow disease progression, and primarily sensory deficits (loss of pain and temperature sensation spreading from the distal limbs). Painless ulcerations in the lower limbs are quite frequent, as well as spontaneous lancinating pain attacks. Hypohidrosis is also seen. Some patients exhibit a more severe phenotype, starting in early childhood, with motor involvement, global hypotrophy, and developmental retardation [1].

Table 38.1 Sphingolipid biosynthesis disorders

Enzyme	Gene	Metabolic disturbance	Main clinical features
Disorders with primarily nervous system involvement			
Serine palmitoyltransferase, subunit 1 or 2	*SPTLC1* or *SPTLC2*	Accumulation of 1-deoxysphingolipids (in plasma)	[HSAN1] - Peripheral sensory neuropathy, distal sensory loss, ulcerative mutilations
Ceramide synthase 1	*CERS1*	Possibly decreased C18-ceramide levels (in cultured cells)	Progressive myoclonic epilepsy and cognitive decline
Ceramide synthase 2	*CERS2*	Possibly decreased very-long chain ceramide levels (in cultured cells)	Progressive myoclonic epilepsy
Fatty acid 2-hydroxylase	*FA2H*	Possibly decreased hydroxylated sphingomyelin levels (in cultured cells)	Spastic paraplegia [SPG35], dystonia, dysarthria, ataxia
Non-lysosomal β-glucosidase	*GBA2*	Unknown	Spastic paraplegia [SPG46], cerebellar ataxia
GM3 synthase	*ST3GAL5* (*SIAT9*)	Lack of GM3, GD3 and higher gangliosides, and increased lactosylceramide and Gb4 levels (in plasma and cultured cells)	[Amish infantile epilepsy] Epilepsy, intellectual disability, »salt and pepper« syndrome
GM2/GD2 synthase	*B4GALNT1*	Decreased GM2 and increased GM3 levels (in cultured cells)	Spastic paraplegia [SPG26], ataxia
Disorders with primarily skin involvement			
Ceramide synthase 3	*CERS3*	Lack of ceramides with very-long chain fatty acids (in cultured cells)	Ichthyosis
(Ultra-long chain) fatty acid ω-hydroxylase	*CYP4F22*	Decreased ultra-long acylceramide levels (in skin and cultured cells)	Ichthyosis

Current evidence related to the metabolic derangement points to the accumulation of abnormal sphingoid bases as the main pathogenic mechanism. Specific mutations of *SPTLC1* or *SPTLC2* encoding subunits 1 or 2 of serine palmitoyltransferase, the first and rate-limiting step in the *de novo* synthesis of sphingolipids, alter its substrate specificity. Instead of using L-serine as a substrate (Fig. 38.1), the mutant enzyme preferentially uses L-alanine or L-glycine. The resulting 1-deoxysphinganine and 1-deoxymethyl-sphinganine, and 1-deoxyceramides (or some other derivatives), which cannot be converted to complex sphingolipids, appear to account for the observed neurotoxicity. Of note is the fact that only several missense mutations in the *SPTLC1* or *SPTLC2* genes cause the autosomal dominant disorder HSAN1. Substitution of Ser331 in the subunit 1 of serine palmitoyltransferase seems to result in an early-onset and more severe phenotype.

When a hereditary sensory neuropathy is suspected, elevated plasma levels of 1-deoxy-sphinganine and 1-deoxymethyl-sphinganine, as determined by liquid chromatography coupled to mass spectrometry, provide a strong biochemical argument in favour of a SPTLC1/2 defect. Moreover, plasma 1-deoxy-sphingolipid levels seem to correlate with disease severity.

There is currently no effective specific therapy. However, a 10-week pilot study on patients affected with HSAN1 showed that, like in a mouse model for this disease, L-serine supplementation (200 or 400 mg/kg/day) could reduce the plasma levels of 1-deoxysphingolipids [3]. Whether such a supplementation can ameliorate the sensory deficits requires further investigation.

38.1.2 Defects in Ceramide Synthases 1 and 2 and Myoclonic Epilepsy

Six human ceramide synthases, encoded by *CERS* genes, have been characterised. They display distinct tissue-specificities as well as acyl-CoA substrate specificities, which can explain the neurological (CERS1 and 2) or dermatologic (CERS3, see 38.1.7) expression in case of a defect in one of them.

Very recently, a homozygous missense mutation in *CERS1* has been identified in 4 siblings of an Algerian family showing progressive myoclonic epilepsy and cognitive decline/dementia. The mutation was associated with decreased C18-ceramides levels in cultured fibroblasts. It has also been proposed that progressive myoclonic epilepsy since age 10 in an adult patient (associated with ataxia, dysarthria and photosensitivity) was due to a heterozygous deletion of *CERS2* together with decreased very-long chain ceramides in fibroblasts [1].

38.1.3 Fatty Acid 2-Hydroxylase Deficiency (SPG35/FAHN)

Mutations in *FA2H* encoding fatty acid 2-hydroxylase result in a complex hereditary spastic paraplegia, SPG35, also called fatty acid hydroxylase-associated neurodegeneration (FAHN). To date, 38 patients have been reported, with varied ethnicity. Most patients present in childhood and develop slowly progressive lower and then upper limb spasticity, dysarthria and mild cognitive decline. Dystonia is another common neurologic feature. MRI shows signs of leukodystrophy and diffuse cortical and pontocerebellar atrophy. Neurodegeneration with brain iron accumulation (NBIA), mostly located in the globus pallidus (T2 hypodensity, but no »eye of the tiger« sign), can occur, although not in all patients. The clinical spectrum of SPG35 is widening, with later onset patients and more clinical variability.

The underlying abnormality is likely the insufficient production of 2-hydroxy-galactosphingolipids. Indeed, 2-hydroxylated long chain and very-long chain fatty acids are essentially found in galactosylceramides and sulfatides from myelin, and their proportion relative to non-hydroxylated fatty acids is known to increase with brain development and myelin maturation. Not unexpectedly, in *Fa2h*-deficient mice, brain galactosylceramides were found to contain almost exclusively non-hydroxylated fatty acids.

38.1.4 GM3 Synthase Deficiency and Amish Epilepsy Syndrome

The deficiency of GM3 synthase resulting from *ST3GAL5 (SIAT9)* mutations causes an autosomal recessive infantile-onset symptomatic epilepsy, also called Amish epilepsy syndrome. During the first 3 months of life, affected children show irritability and failure to thrive. Then, within the first year of life, generalized tonic-clonic seizures as well as other seizure types develop, along with a profound developmental stagnation and regression. In some patients, brain MRI shows occipital white matter abnormalities and atrophy in the visual cortex. The severity of the disease varies significantly, some patients suffering from visual loss and deafness. A majority of patients exhibit hyperpigmented maculae on the dorsal part of hands and feet, but also in other locations. Some patients also show patches of skin depigmentation. These skin changes are not associated with the severity of the neurologic disease. The combination of hyper and hypo-pigmented skin maculae, facial dysmorphism, scoliosis, intellectual disability, seizures, choreoathetosis, and spasticity has been described under the term »salt and pepper« syndrome. Associated biochemical features in plasma and cultured cells are the lack of GM3, GD3 and higher gangliosides, and increased lactosylceramide and Gb4 levels.

38.1.5 GM2/GD2 Synthase Deficiency (SPG26)

Mutations of *B4GALNT1* resulting in a defect of GM2/GD2 synthase are associated with SPG26, a slowly progressive complex hereditary spastic paraplegia with mild to moderate cognitive impairment. Ten multiplex families from various ethnic origins have so far been described. The clinical picture is a progressive weakness, with spastic gait and lower limb spasticity. EMG shows an axonal sensorimotor neuropathy in many patients. The disease can be accompanied by cerebellar symptoms, dysarthria, and dysphagia [4]. Studies in cultured fibroblasts of patients have shown decreased GM2 levels with an increase of its precursor, GM3.

38.1.6 Nonlysosomal β-Glucosidase GBA2 Deficiency: SPG46 and Ataxia

GBA2 is a membrane-associated protein localised at the endoplasmic reticulum and Golgi, most likely facing the cytosol. This enzyme can hydrolyse glucosylceramide to ceramide and glucose. While acting on the same substrate but in a different subcellular location, GBA2 is very distinct from the lysosomal acid β-glucosidase GBA1 deficient in Gaucher disease (▶ Section 38.2.1). The formed ceramide re-enters the biosynthetic pathway (◘ Fig. 38.1), or could play a role as a bioactive lipid in case of excessive formation.

Since 2013, several studies have shown that mutations in *GBA2* should be added to the heterogeneous group of ARCA (autosomal recessive cerebellar ataxias), and also underlie the hereditary (complex) spastic paraplegia locus SPG46. Most patients with GBA2 deficiency develop since childhood a marked spasticity in lower extremities with progressive gait disturbances and later, ataxia and other cerebellar signs. Variable additional symptoms have been reported, such as hearing loss or cognitive impairment. Some patients presented testicular hypotrophy associated with spermatozoid head abnormalities [1]. Besides DNA sequencing, diagnosis can be achieved by determination of enzyme activity using a specific method.

Potential interactions between GBA1 and GBA2 may play a role in Gaucher disease. The paradoxical clinical amelioration reported in mouse models of Gaucher and Niemann-Pick C (▶ Section 38.3) diseases after GBA2 inhibition remains an intriguing observation [5][6].

38.1.7 Ceramide Synthase 3 and Ultra-Long Chain Fatty Acid ω-Hydroxylase (CYP4F22) Deficiencies: Autosomal Recessive Congenital Ichthyosis (ARCI)

Autosomal recessive congenital ichthyosis (ARCI) represents a heterogeneous group of disorders of epidermal cornification, in which 9 causative genes have to date been identified. Two of those, *CERS3* and *CYP4F22* encode proteins involved in ceramide synthesis (◘ Fig. 38.1). Specific ceramides are

particularly abundant in the stratum corneum of the skin, where they play a crucial role in maintaining skin barrier homeostasis, preventing water loss and protecting against microbial infections. These ceramides may in particular contain α- or ω-hydroxylated fatty acids and ultra-long chain fatty acids (ULCFAs) (C26 or longer). Acylceramides are unique, very hydrophobic ceramide species present in the epidermis, which contain C28-C36 ω-hydroxylated ULCFAs and are further esterified with linoleic acid.

Ceramide synthase 3 (*CERS3*), which is markedly expressed in the skin, generates epidermis-specific ceramides by N-acylating dihydrosphingosine with ULCFA-CoAs (and likely ω-hydroxylated ULCFA-CoAs). Indeed, functional analysis of a skin sample and *in vitro* differentiated keratinocytes from a patient with a *CERS3* missense mutation severely affecting enzyme activity demonstrated an impairment in the synthesis of ceramides with non-hydroxylated and ω-hydroxylated ULCFA moieties, disturbing epidermal differentiation and leading to premature keratinisation. *CYP4F22* encodes the fatty acid ω-hydroxylase required for acylceramide synthesis, using ULCFAs as substrates [7].

Acylceramides in the stratum corneum have been shown to play a key role in the formation and stabilization of cornified envelopes through covalent binding to corneocyte proteins, and ultimately in skin permeability barrier. It is therefore logical that defective synthesis of these lipids will manifest as severe skin disorders. For the above two defects, the ARCI clinical phenotype has been quite variable in different patients, often including lamellar ichthyosis and palmo-plantar hyperlinearity, but also in some cases collodion membrane at birth (that may be self-improving) and more or less severe congenital ichthyosiform erythroderma. For ichthyotic manifestations due to these defects, topical application of some specific ceramides could be envisioned.

38.1.8 Mutations in Ceramide Kinase-Like (CERKL) Gene and Retinal Dystrophy

Mutations in *CERKL* have been associated with a group of inherited retinal dystrophies presenting as retinitis pigmentosa or cone rod dystrophy. The name ceramide kinase-like was given because of 29% identity and 50% similarity with the human ceramide kinase that converts ceramide into ceramide 1-phosphate, but neither the substrate nor the function of the CERKL protein are still known. At the current stage of knowledge, this disorder thus does not belong to sphingolipid synthesis disorders. It has been listed here by default due to its name, as it cannot yet be classified from a metabolic viewpoint (see [1] for more details).

38.1.9 Alkaline Ceramidase 3 (ACER3) Deficiency: Infantile Leukodystrophy

Similarly, deficiency of ACER3 is reported here because it is not a lysosomal storage disease; whether it represents a true sphingolipid synthesis or a remodelling defect remains to be determined. A homozygous missense mutation in *ACER3*, coding for alkaline ceramidase 3 (localised to both the Golgi complex and the ER), has recently been described in two siblings with leukodystrophy. They presented with neurological regression at 6–13 months of age, truncal hypotonia, appendicular spasticity, dystonia, optic disc pallor, peripheral neuropathy and neurogenic bladder. The *ACER3* mutation was associated with undetectable ACER3 catalytic activity towards natural and synthetic ACER3-specific substrates, and an accumulation in plasma of ACER3 substrates, C18:1- and C20:1-ceramides and dihydroceramides, as well as some complex sphingolipids, including monohexosylceramides and lactosylceramides [8].

38.2 Sphingolipidoses

Sphingolipidoses are a subgroup of lysosomal storage disorders in which sphingolipids accumulate in one or several organs as the result of a primary deficiency in enzymes or activator proteins involved in their degradative pathway. Except for Fabry disease (X-linked recessive), the mode of inheritance is autosomal recessive. The clinical presentation and course of the classic forms are often typical. With the help of relevant procedures (e.g. imaging, neurophysiology, ophthalmology), examination of the patient and perusal of the disease history (especially age at and type of first symptom) should lead to a provisional diagnosis and oriented laboratory tests. Late-onset forms, often less typical, have been overlooked in the past. No global biochemical screening procedure is available to date. In most sphingolipidoses the diagnosis is easily made by demonstration of the enzymatic defect, generally expressed in most cells and tissues (leukocytes represent the most widely used enzyme source, followed by dried blood spots). In a few instances, other biochemical tests and/or a molecular genetic assessment are necessary. Efforts should be made to genotype all patients. Specific therapies are well established for non-neuronopathic Gaucher and Fabry diseases, and under clinical trial for Niemann-Pick B. In spite of active research and ongoing preclinical and clinical trials, progress towards therapy of the neurological forms remains limited.

38.2.1 Gaucher Disease

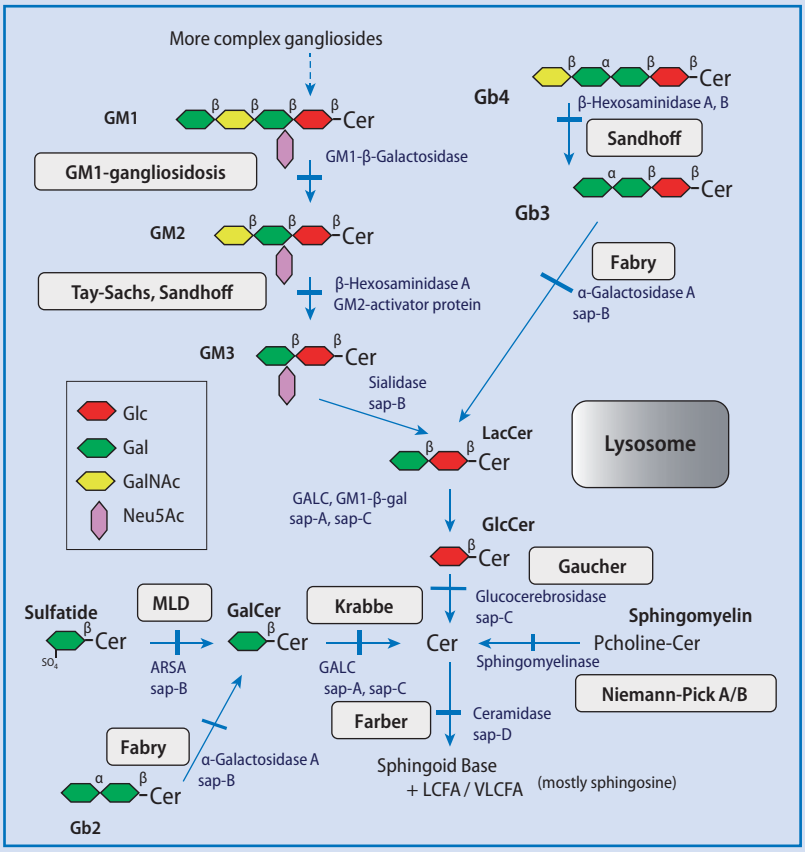
Clinical Presentation

Historically, three clinical phenotypes are recognised, but the full disease spectrum is a continuum. All types are panethnic, but type 1 has a particularly high prevalence in the Ashkenazi Jewish population (carrier frequency 1:13). The overall incidence is about 1 in 40,000 to 1 in 50,000 live births [9].

Type 1 It is defined by the lack of neurological symptoms, and accounts for about 90% of all cases. It can present at any age, but manifests in childhood in more than half of patients [10]. There is a wide variability in the pattern and severity of the

Sphingolipid Degradation and Sphingolipidoses

Only the main sphingolipids implicated in sphingolipidoses are depicted in ◘ Fig. 38.2. The scheme illustrates their degradation by stepwise hydrolysis, and shows enzymatic blocks leading to a disease. It also indicates which sphingohydrolase or sphingolipid activator protein (► Section 38.2.4 and ► Section 38.2.9) is implicated, as well as the name commonly used to designate the various disorders.



◘ **Fig. 38.2 Sphingolipid degradation.** *ARSA*, arylsulfatase A; *GALC*, galactocerebrosidase; *GalCer*, galactosylceramide (or galactocerebroside); *GalNAc*, N-acetyl-galactosamine; *Gb2*, galactobiosylceramide; *Gb3*, globotriaosylceramide; *Gb4*, globotetraosylceramide (globoside); *GlcCer*, glucosylceramide (or glucocerebroside); *GM1*, GM1 ganglioside; *GM2*, GM2 ganglioside; *GM3*, GM3 ganglioside; *LacCer*, lactosylceramide; *LCFA*, long chain fatty acids; *MLD*, metachromatic leukodystrophy; *Neu5Ac*, N-acetyl-neuraminic acid (sialic acid); *Pcholine*, phosphorylcholine; *sap*, saposin. *VLCFA*, very long chain fatty acids. Enzyme defects are indicated by solid bars across the arrows

symptoms, from extremely handicapping to asymptomatic forms, with most symptomatic patients having visceral, haematological and (more frequently in adults) skeletal disease [11]. Children often show severe splenomegaly, generally associated with hepatomegaly. The degree of visceromegaly is highly variable, in both children and adults. This may lead to anaemia, thrombocytopenia and, thus, a bleeding tendency. Leukopenia is less frequent. Children may show delayed growth and menarche. Subcapsular splenic infarctions may cause attacks of acute abdominal pain and medullary infarction of long bones, excruciating pain referred to as bone crises. Essentially in adult patients, bone involvement represents a major cause of morbidity. Aseptic necrosis of the femoral head and spontaneous fractures due to osteopenia are other common complications. Lung involvement with diffuse infiltration may occur. In adults, pulmonary hypertension has been described in rare, usually splenectomised, patients. Co-morbidities with close association to Gaucher disease have been identified, particularly non-Hodgkin's B-cell lymphoma and multiple myeloma, and Parkinson's disease [12][13][14]. Peripheral polyneuropathy was also reported more frequently than in a control population.

Type 2 (acute neuronopathic Gaucher disease) Classically, patients present early in infancy with brain stem dysfunction and pyramidal signs. Retroflexion of the neck, opisthotonus, feeding difficulties and squint are major early signs, apnoeas appear later, and trismus and stridor are less frequent. Splenomegaly is constant but may not be present initially [15][16][17]. Exceptional cases have manifested as haemophagocytic lymphohistiocytosis. The downhill clinical course is rapid, with pronounced spasticity, failure to thrive and cachexia, and few of these patients survive beyond the age of 2 years. Some other patients show strabismus, paucity of facial movements, less sign or none at all of pyramidal involvement, irritability or cognitive impairment and a slower course (some survive up to 5 years) [15][16][17].

The perinatal lethal form is associated with hepatosplenomegaly, pancytopenia and skin changes. Many of these cases are associated with hydrops fetalis, and some have been described as ›collodion babies‹. Arthrogryposis is seen in 40% of cases [17][18][19].

Type 3 (subacute or chronic neuronopathic Gaucher disease) This type is heterogeneous. The mean age at onset is 5 years (but between 5 months and 46 years), with a mean age of neurological onset around 8 years. The most common form consists in severe systemic involvement and supranuclear saccadic horizontal gaze palsy, with or without developmental delay, hearing impairment and other brain stem deficits [10][20][21]. The second most common phenotype shows a relatively mild systemic disease but progressive myoclonic encephalopathy, with seizures, dementia and death. There are also patients with severe systemic involvement and supranuclear gaze palsy who develop a progressive myoclonic encephalopathy [10][20][21]. Brain stem auditory evoked response (BAER) testing may reveal abnormal wave forms (III and IV). A particular presentation with cardiac involvement (heart valve and aortic calcification), supranuclear gaze palsy, mild hepatosplenomegaly and bone disease, has been associated with homozygosity for the D409H mutation. In neurological Gaucher disease, extrapyramidal involvement has also been observed.

Metabolic Derangement

The primary metabolic defect resides in a block of the lysosomal degradation of (β-)glucosylceramide (glucocerebroside) and glucosylsphingosine. In the vast majority of cases this is due to the deficient activity of acid β-glucosidase (glucocerebrosidase, glucosylceramidase) (Fig. 38.2). Exceedingly rare cases, presenting as type 3 or 1 (reviewed in [22]) are due to a deficiency of the saposin sap-C, which is required for the in vivo hydrolysis of glucosylceramide. Glucosylceramide accumulates massively in liver and spleen of patients in all types. Although elevated in cerebral grey matter of type 2 and type 3 patients, its concentration in brain remains low. Glucosylsphingosine also accumulates (but not in brain of type 1 patients). Pathophysiology of the disease is poorly understood [9]. Cytotoxicity due to glucosylsphingosine, disruption of cellular Ca^{2+} homeostasis, as well as inflammatory responses appear to be involved.

Genetics

The disease (except for sap-C deficiency) is caused by mutations in *GBA1* [9]. More than 430 mutations are known. N370S (now c.1226A>G; p.Asn409Ser), the most common one in Ashkenazim, is also very frequent in Caucasian populations. The finding of one single N370S is predictive of a non-neuronopathic phenotype. The severity can vary widely in Gaucher patients with the same genotype, including N370S homozygotes [23]. The second most frequent mutation, L444P (now c.1448T>C; p.Leu483Pro), first described in Norbottnian type 3, is more frequently associated with types 2 and 3. Complex alleles due to genetic rearrangements are more often associated with severe forms, including perinatal lethal forms [19]. A number of genotype-phenotype correlations have been made [10].

Diagnostic Tests

Bone marrow examination (not mandatory) may have revealed Gaucher cells (multinucleated reticuloendothelial cells with a vacuolated cytoplasm, with a »wrinkled tissue paper« appearance). Signs of haemophagocytosis with lymphohistiocytosis have been observed in exceptional cases. Several plasma biomarkers, e.g. chitotriosidase, the chemokine CCL18/PARK, and glucosylsphingosine [24] are typically very elevated, but they are above all used to monitor treated patients. The assay of glucocerebrosidase activity in peripheral blood lymphocytes/ leukocytes or dried blood spots (using fluorogenic or short-chain glucosylceramide substrates) constitutes the primary diagnostic test. Cultured cells have a much higher activity. DNA testing, complicated by the existence of a highly homologous pseudogene, is required for carrier detection and improves diagnostic accuracy for patients with high residual enzyme activity. In sap-C deficiency, glucocerebrosidase activity is normal; the findings of Gaucher cells and elevated levels of the above biomarkers should lead to *PSAP* gene sequencing [25]. In all cases, lipid studies in liver and/or spleen would reveal pathognomonic glucosylceramide storage.

Treatment and Prognosis

Two approaches are currently available for the specific treatment of type 1 (and to some extent type 3 [20]) patients: enzyme replacement therapy (ERT) and substrate reduction therapy (SRT) (see [26] for review). Splenectomy enhances the risk of progression of the disease at other sites, especially bone and lung, and should be avoided. Pregnancy is not contraindicated in untreated patients, but bleeding may become critical before and after birth, and there is now a good experience of ERT throughout pregnancy. Enzyme therapy, conducted with slow infusions of a recombinant enzyme exposing mannose groups (optimal uptake by macrophages), has largely proved safe and effective [9][27]. Imiglucerase has been used worldwide for over 20 years, and two other products, velaglucerase alfa [28][29] and taliglucerase alfa [30] have come onto the market. The natural history of type 1 can be dramatically improved. ERT prevents progressive manifestations and ameliorates Gaucher disease-associated anaemia, thrombocytopenia, organomegaly, bone pain and bone crises. However, the enzyme does not

cross the blood-brain barrier, and this treatment has no effect on the neurological manifestations of type 2. While ERT aims at restoring the degradation rate of the accumulated substrate, SRT tends to reduce the cell burden by slowing down the rate of synthesis of the substrate to a level where it can be slowly cleared by a deficient enzyme with some residual activity. This may be achieved by small molecules that can be administered orally. Two inhibitors of glucosylceramide synthase are currently approved for treatment of Gaucher type 1: the iminosugar miglustat [31][32] and, more recently, eliglustat [33]. Although pharmacological chaperones constitute an attractive approach, the only clinical trial so far did not reach phase III. Other potential strategies are still very experimental [26].

38.2.2 Acid Sphingomyelinase-Deficient Niemann-Pick Disease (Type A, Type B and Intermediate Forms)

Since the early 1980s, the heterogeneous group of »Niemann-Pick disease« has been divided in two separate entities: ›acid sphingomyelinase-deficient Niemann-Pick disease‹ (ASM-deficient NPD) [34], and Niemann-Pick disease type C (▶ below).

Clinical Presentation

ASM deficiencies have historically been categorised into a severe, acute neuronopathic form, or type A, and a non-neuronopathic form, or type B, but there appears to be a continuum ranging from mild to severe type B, and then from late-onset neurological forms toward severe classic type A. Type A has its highest prevalence in Ashkenazim and is rare in other ethnic groups. Type B does not have an Ashkenazi Jewish predilection, and appears to be more frequent in southern Europe, North Africa, Turkey and the Arabian Peninsula than in northern Europe.

Classic Niemann-Pick disease type A The neonatal period is usually normal, with vomiting or diarrhoea, or both, appearing in the first weeks of life. Failure to thrive often motivates the first consultation, leading to the discovery of a prominent and progressive hepatosplenomegaly and lymphadenopathy, in most cases before 3-4 months of age and sometimes earlier. Hypotrophy is observed in 70% of the cases [35]. Neurological examination is essentially normal until the age of 5-10 months, when the child shows hypotonia, progressive loss of acquired motor skills, lack of interest in the surroundings and reduction of spontaneous movements. Psychomotor retardation may at first be overlooked owing to the poor general condition. Initial axial hypotonia is later combined with bilateral pyramidal signs. A decrease of nerve conduction velocities is generally present. A cherry-red spot in the retina is a typical feature, but is often not present until an advanced stage. Severe cachexia is common. Loss of motor function and intellectual deterioration continue to the point where patients become spastic and rigid. Seizures are rare. Brownish-yellow discoloration and xanthomas may be detected in the skin. Death usually occurs between 1.5 and 3 years. Cases with a milder systemic involvement, slightly protracted onset of neurological symptoms and slower course are also seen [35].

Type B Type B is a chronic, non-neuronopathic disease, with a very variable degree of systemic involvement. Most typically, the presenting sign is splenomegaly or hepatosplenomegaly in late infancy or childhood [36], but discovery may occur at any age from birth until late adulthood. Bruising and epistaxis are frequent. Hypersplenism occurs in a small proportion of patients. Splenectomy, seldom necessary, should be avoided. The most constant associated signs are radiographic abnormalities of the lung (diffuse, reticulonodular infiltrations) and interstitial lung disease with variable impairment of pulmonary function [37]. In adults with a long follow-up, pulmonary involvement is often the main complaint, ranging from dyspnoea on exertion (frequent) to oxygen dependency. In children, retarded body growth is a common finding between the ages of 6 and 16 years. Skeletal age and puberty are often delayed [36]. Alterations of liver function are in general mild, but a few cases have been described with liver cirrhosis and liver failure. Hypercholesterolaemia with markedly decreased HDL cholesterol is common even in children. Other features associated with the disease are joint/limb pain, bruising, headache, abdominal pain and diarrhoea. True type B patients do not have neurological involvement and are intellectually intact, but ophthalmoscopic examination may reveal a retinal macular halo or cherry red maculae [36]. Although there are severe forms, the most frequent clinical phenotype is that of a moderately serious disorder compatible with an essentially normal life-span. In a longitudinal study the disease was characterised by hepatosplenomegaly with progressive hypersplenism, worsening atherogenic lipid profile, gradual deterioration in pulmonary function and stable liver dysfunction.

Intermediate forms of ASM-deficient NPD This is a heterogeneous category. Some patients are closer to type A with a late infantile, juvenile or adult neurological onset and a slowly progressive disease that may include cerebellar ataxia, extrapyramidal involvement or psychiatric disorders [38][39]. Some others are closer to type B, with minimal nervous system involvement (often peripheral neuropathy) and/or mild mental retardation [40].

Metabolic Derangement

A primary deficiency of the lysosomal (or acid) sphingomyelinase (◘ Fig. 38.2) resulting from mutations in *SMPD1* leads to the progressive accumulation of sphingomyelin in systemic organs in all types of the disease, and in brain in the neuronopathic forms [34]. Sphingomyelin storage is massive in liver and spleen in type A, slightly less so in type B. A significant increase of unesterified cholesterol occurs secondarily [41]. By in vitro measurements a marked ASM deficiency is observed in all patients, but hydrolysis of sphingomyelin in live cells demonstrates a significant level of residual activity in typical type B patients, suggesting this could be enough to limit accumulation and protect the brain. Sphingosylphosphorylcholine

(lysosphingomyelin) (increased in systemic organs of all types and in type A brain) may participate in the pathogenic cascade.

Genetics

More than 180 disease-causing mutations of *SMPD1* have been reported [42]. In Ashkenazi Jewish type A patients, 3 mutations (R496L, L302P, P330fs) [now p.Arg498Leu, p.Leu304Pro, p.Phe333fs*52 (c.996delC)] account for >90% of alleles. R608del (now p.Arg610del), highly prevalent in North African patients, is the most common type B mutation (20–30% of alleles) in many countries. So far, it has always been correlated with a type B phenotype whatever the nature of the second mutated allele. Q292K (p.Gln294Lys) is associated with late-onset neurological involvement. *SMPD1* appears to be paternally imprinted.

Diagnostic Tests

Bone marrow usually reveals the presence of (nonspecific) foamy histiocytes or sea-blue histiocytes. Among plasma biomarkers, the oxysterols cholestane-3β,5α,6β-triol and 7-ketocholesterol, as well as lysosphingomyelin and ›lyso-sphingomyelin-509‹, are elevated [43][44]. Chitotriosidase is moderately elevated. Note that these biomarkers are also elevated in Niemann-Pick type C (▶ below) and for oxysterols, in acid lipase deficiencies and some other conditions [45][46]. The diagnosis is made by demonstration of a deficiency in ASM activity in leukocytes (or lymphocytes) or in cultured cells (much higher level of activity). The choice of a specific substrate is critical. Radioactively labelled native sphingomyelin or a short-chain analogue with tandem mass spectrometry measurement [47] are best. The fluorogenic substrate [48] should be used with caution [38]. The *in vitro* assay does not reliably distinguish A from B phenotypes.

Treatment and Prognosis

No specific therapy is yet available. Limited experience of bone marrow transplantation (BMT) in type A patients has not appeared to improve symptoms. In type B, splenectomy may have a deleterious effect on the lung disease. Pregnancy is not contraindicated in type B patients, although monitoring for bleeding is advisable. Morbidity/mortality of type B has been studied [49]. Encouraging results of an ERT phase 1 clinical trial in adult type B patients using olipudase alpha [50] have led to a phase 2 trial in children and adults.

38.2.3 GM1 Gangliosidosis

Clinical Presentation

First descriptions of infantile GM1 gangliosidosis emphasised its characteristics of a neurovisceral lipidosis sharing features with both Tay-Sachs disease and Hurler disease. Forms with an almost exclusive neuronal storage were described later. Three clinical phenotypes are recognised [51].

In the **typical early infantile form** (or type 1), children are often hypotonic in the first days or weeks of life, with poor head control. The arrest in neurological development is observed at 3–6 months of age. Feeding difficulties and failure to thrive are common. Many infants have facial and peripheral oedema. In typical cases, dysmorphic features may be present very early or develop with time, with a puffy face, moderate macroglossia, hypertrophic gums, depressed nasal bridge and chipmunk face, but an increasing number of infantile patients have presented without dysmorphic expression. Hepatomegaly and later splenomegaly are almost always present. Dorsolumbar kyphoscoliosis is common. After a few months, signs of visual failure appear, often with a pendular nystagmus. A macular cherry-red spot is found in about 50% of cases, but seldom before 6 months of age. As time passes, hypotonia gives way to spasticity. Rapid neurological regression is usual after the 1st year of life, with generalised seizures, swallowing disorder, decerebrate posturing and death, often before age 2. Radiological signs in the long bones and spine are constant in clinically severe patients, but can be minimal in cases with only psychomotor deterioration. Subperiosteal bone formation can be present at birth. Widening of the diaphyses and tapering of the extremities appear later. At the age of 6 months, striking Hurler-like bone changes are seen, with vertebral beaking in the thoracolumbar zone, broadening of the shafts of the long bones with distal tapering and widening of the metacarpal shafts with proximal pinching of the four lateral metacarpals.

A severe **neonatal form** with cardiomyopathy has been described. GM1 gangliosidosis is also a cause of nonimmune *foetal hydrops*.

The **late infantile variant** (or type 2) usually begins between 12 and 18 months (but up to 3 years), with unsteadiness in sitting or standing, or difficulty in walking. Regression is rapid and severe, and a spastic quadriparesis develops, associated with pseudobulbar signs. Seizures are frequent and may become a major problem. The patients are not dysmorphic, and hepatosplenomegaly is not present. Vision is generally normal. Radiography of the spine reveals moderate but constant changes, with mild anterosuperior hypoplasia of the vertebral bodies at the thoracolumbar junction.

The term ›**adult form**‹ has been employed to designate the **chronic late-onset form** of GM1 gangliosidosis with onset in late childhood, adolescence or adulthood. Dysarthria and extrapyramidal signs, especially dystonia, are the most common signs [52][53]. Cognitive impairment is absent to moderate, and there are no ocular abnormalities. Bone changes are inconstant. The course of the disease is very slow.

Metabolic Derangement

GM1 gangliosidosis is due to a deficient activity of lysosomal acid β-galactosidase (◘ Fig. 38.2), which cleaves glycoconjugates containing a terminal β-galactosidic linkage and is necessary for the degradation of GM1 ganglioside, other galactose-containing glycosphingolipids or oligosaccharides, as well as keratan sulfates (▶ Chapter 39). Consequently, the most severe forms of the disease combine features of a neuronal lipidosis, a mucopolysaccharidosis and an oligosaccharidosis. Acid β-galactosidase functions in a multienzyme lysosomal complex with neuraminidase, the protective protein/cathep-

sin A (PPCA) and N-acetyl-galactosamine-6-sulfate sulfatase [54]. This explains the quite similar clinical phenotype of galactosialidosis, a distinct condition due to the deficiency of PPCA, which causes a combined secondary deficiency of acid β-galactosidase and acid sialidase (neuraminidase) (▶ Chapter 39). Finally, β-galactosidase deficiency can be associated with two clinically different diseases, GM1 gangliosidosis, with prominent features of a sphingolipidosis, and Morquio B disease (mucopolysaccharidosis type IVB), in which abnormalities of mucopolysaccharide metabolism prevail. In tissues from patients with GM1 gangliosidosis, three main groups of accumulated compounds have been identified: the sphingolipid GM1 ganglioside, glycoprotein-derived oligosaccharides and keratan sulfate. Massive storage of GM1 occurs in brain tissue. Increased levels of its lysocompound, potentially of pathogenic significance, have been reported. Galactose-containing oligosaccharides have been found in liver and urine. Keratan sulfate and other mucopolysaccharides accumulate in liver and spleen. Keratan sulfate excretion in urine is lesser in GM1 gangliosidosis than in Morquio B disease.

▪ Genetics

About 150 mutations of *GLB1* (3p21.33) have been described. Neither the type nor location of the mutation correlate well with a specific phenotype.

▪ Diagnostic Tests

Vacuolated lymphocytes may be found in peripheral blood, and foamy histiocytes in the bone marrow. Radiographic bone examination showing Hurler-like abnormalities (▶ above) may suggest the diagnosis. In the infantile form, brain computerised tomography (CT) and magnetic resonance imaging (MRI) usually give nonspecific results, with diffuse atrophy of the central nervous system (CNS) and features of myelin loss in the cerebral white matter. Lesions in the basal ganglia may be present in the adult form. Analysis of urinary oligosaccharides is a good orientation test. In the classic early infantile form excretion is massive, with a pathognomonic profile. Excretion can be much lower in forms with predominant neurodegenerative disease. Mucopolysaccharide analysis in urine usually shows increased levels of keratan sulfate. The diagnosis is established by demonstration of a deficient activity of acid β-galactosidase, which can be measured on leukocytes or dried blood spots using an artificial fluorogenic substrate. A subsequent study of neuraminidase (in leukocytes or cultured fibroblasts) should be systematically performed to exclude galactosialidosis.

▪ Treatment and Prognosis

No specific treatment is available to date. SRT or chaperones are potential approaches for clinical trials in late-onset forms [51][55].

38.2.4 GM2 Gangliosidoses

GM2 gangliosidoses are divided into three genetic and biochemical subtypes: **Tay-Sachs disease** (or B variant), **Sandhoff disease** (or 0 variant), and **GM2 activator deficiency** (AB variant). All are characterised by impaired lysosomal catabolism of GM2 ganglioside (◘ Fig. 38.2), which requires three gene products: the β-hexosaminidase α- and β-subunits and the GM2 activator protein. Tay-Sachs disease corresponds to a deficiency of the α-subunit and thus of β-hexosaminidase A (αβ-heterodimer), Sandhoff disease, to a deficiency of the β-subunit and thus of both β-hexosaminidases A and B (ββ-homodimer). Classic Tay-Sachs disease has a very high carrier rate (estimated to ~1:27) in the Ashkenazi Jewish population, and also in subjects of French Canadian descent. Infantile forms are most common, but juvenile and adult forms are also recognised. A particular enzymatic variant of Tay-Sachs disease (B1 variant) has a high incidence in northern Portugal [56] and is globally more frequent in southern Europe. Variant AB is exceedingly rare (<10 reported cases), albeit probably underdiagnosed.

▪ Clinical Presentation

The **infantile** forms of the three subtypes have a very similar presentation [57]. Around 4–6 months of age, motor weakness and hypotonia are the usual earliest signs, almost constantly associated with a typical startle response to sounds with extension of the arms (hyperacusis). Hypotonia progresses, with loss of acquired milestones. Loss of visual attentiveness is also seen early, and ophthalmoscopic examination almost invariably reveals a typical macular cherry-red spot in the retina. Blindness follows, and spasticity, swallowing disorder and seizures develop. Macrocephaly begins by 18 months of age. By year 3 the child is demented and decerebrate. Death often occurs, due to aspiration pneumonia. In Sandhoff disease, in spite of an additional accumulation of glycolipids and oligosaccharides in visceral organs, organomegaly and bony abnormalities are rarely observed.

Late infantile and **juvenile** forms [57][58] are mostly due to a deficiency of β-hexosaminidase A (often B1 variant). The onset of symptoms is usually between 2 and 10 years of age, with ataxia, incoordination and dysarthria, followed by progressive psychomotor deterioration, spasticity and seizures. Myoclonus can be prominent. Cherry red-spots are inconstant.

Chronic or **adult** forms can show variable presentations, with pyramidal and extrapyramidal signs, movement disorders (dystonia, athetosis, ataxia), psychosis (reported in 30–50% of adult-onset patients) and a syndrome of lower motor neuron and spinocerebellar dysfunction with supranuclear ophthalmoplegia [59][60]. Some patients show autonomic dysfunction.

▪ Metabolic Derangement

The normal catabolism of GM2 ganglioside requires the GM2 activator protein to extract GM2 from the plasma membrane before presenting it to hexosaminidase A (αβ-heterodimer). Hexosaminidase B (ββ-homodimer) hydrolyses other sub-

strates with a terminal hexosamine (glycoproteins and glycolipids), but not GM2 ganglioside. In Tay-Sachs disease (affecting the α-subunit), hexosaminidase A only is deficient. In Sandhoff disease (affecting the β-subunit) both hexosaminidases are inactive. In GM2 activator deficiency, the substrate is not made available to the otherwise normally functioning enzyme. All types are characterised by storage of GM2 ganglioside in neurons. This results in meganeurites, with aberrant neurite formation that may play a role in the pathophysiological mechanisms. GM2 storage is very pronounced in infantile forms, less so in juvenile forms, and even less in adult forms. Increased levels of lyso-GM2 have also been reported in infantile forms. In Sandhoff disease, asialo-GM2 also accumulates in brain, while other compounds – such as globoside and oligosaccharides – accumulate in liver and other visceral organs.

Genetics

More than 130 mutations of *HEXA* have been identified. Three of them – c.1274-1277dup, c.1421+1G>C in infantile cases, p.Gly269Ser in adult forms – account for>95% of the Ashkenazi Jewish alleles. A carrier screening programme initiated in the early 1970s has proven very successful to decrease incidence of the disease in this population. A 7.6 kb deletion is common in French Canadian patients. Mutations at codon 178 altering the three-dimensional structure of the enzyme, result in the enzymatic B1 variant presenting as a juvenile form in the homozygous state. Relatively good genotype-phenotype correlations have been reported. More than 40 mutations (including a common 16 kb deletion) in *HEXB* and 6 in the GM2 activator *GM2A* gene have been described.

Diagnostic Tests

In Tay-Sachs and Sandhoff diseases, the clinical diagnosis can easily be confirmed by enzyme testing on leukocytes or cultured fibroblasts. The assay for total hexosaminidases (A+B) using a synthetic fluorogenic substrate is straightforward and allows the diagnosis of Sandhoff disease. Differential assay of hexosaminidase A using heat or acid inactivation does not identify patients with the B1 variant; the direct assay of hexosaminidase A using the sulfated synthetic substrate (4-MU-6-sulfo-β-glucosaminide) specific for the α-subunit is the method of choice. A high residual activity is found in Sandhoff disease, owing to excess of hexosaminidase S (αα-dimer). In GM2 activator deficiency, hexosaminidase A activity measured in vitro is normal; electron microscopic examination of a skin or conjunctival biopsy may provide strong evidence in favour of the diagnosis by demonstrating concentric lamellated bodies in nerve endings. The CSF shows increased levels of GM2. The definitive diagnosis requires *GM2A* sequencing.

Treatment and Prognosis

Seizures are generally responsive to standard treatment. No effective curative treatment is currently available. Neither SRT (miglustat) [61][62], nor chaperone therapy (pyrimethamine) [63] trials led to measurable clinical improvement. A gene therapy clinical trial is underway in the United Kingdom and the United States.

38.2.5 Krabbe Disease

Clinical Presentation

Krabbe disease (or globoid cell leukodystrophy) leads to demyelination of the central and peripheral nervous system. Its estimated overall incidence is between 0.75 and 1 in 100,000 live births. It is more frequent in Scandinavia (but not in Finland). The classic early infantile form accounts for about 65% of diagnosed cases. Late onset cases appear to be more common in southern Europe, especially Italy and Sicily, and in Japan. The incidence of adult-onset cases has been underestimated.

Infantile forms Clinical presentation is quite uniform, usually very suggestive of the diagnosis.

In the **early infantile** form [64], the onset is from birth to 6 months of age (often 3–4 months) [65]. Initial symptoms include increasing irritability, crying, vomiting and other feeding problems, hyperesthesia, tonic spasms on light or noise stimulation, and signs of peripheral neuropathy. Bouts of unexplained fever are also common. This stage with hypertonic episodes is followed by permanent opisthotonic posturing with characteristic flexed upper extremities and extended lower extremities. Seizures may appear. Hyperpyrexia and hypersalivation are frequent. As the disease progresses blindness occurs, followed by loss of bulbar functions and hypotonia. Death occurs from hyperpyrexia, respiratory complications or aspiration, classically before the age of 2 years but in current practice not so rarely later.

In the **late infantile** phenotype (onset between 7–12 months, about 10% of cases), patients typically present with a loss of developmental milestones and poor feeding, crying and irritability being later signs [66].

Later onset forms Clinical recognition of these forms is more difficult.

The **juvenile** form [66] starts between the ages of 13 months and 10 years (in most cases before the age of 5 years). The first signs are often gait disturbances (spastic paraparesis or ataxia or both, sometimes spastic hemiplegia) in a previously normal or mildly retarded child. Visual failure with optic atrophy is also a common symptom in younger children [66][67]. At variance with the infantile form, peripheral neuropathy is only present in approximately half of the cases. Time of onset and severity of mental deterioration are variable. Seizures are infrequent; when present they can be a major therapeutic problem. The course of the disease is quite variable and unpredictable, even in siblings. Many patients show initial rapid deterioration followed by gradual progression lasting for years.

Most **adult** patients (reviewed in [68]) present with a gait disorder, showing a pyramidal syndrome with spastic paraparesis, with or without peripheral neuropathy. One third have cerebellar ataxia in addition. Usually they do not

show cognitive dysfunction. At MRI, hyperintensities along the pyramidal tracts are a characteristic and nearly constant sign.

Metabolic Derangement

Krabbe disease (Fig. 38.2) results from β-galactosylceramidase (or galactocerebrosidase, cerebroside β-galactosidase) deficiency, a lysosomal enzyme that catabolises (β-)galactosylceramide – a major lipid component of myelin – as well as lactosylceramide and galactosylsphingosine (psychosine). In vivo, galactosylceramide degradation further requires the saposin sap-A. Two cases due to sap-A deficiency are known [69]. Galactosylceramidase deficiency leads to an accumulation of galactosylceramide in the pathognomonic ›globoid cells‹ (multinucleated macrophages) seen in the demyelinating lesions of the white matter, and of a toxic metabolite, galactosylsphingosine (psychosine) in the oligodendrocytes and the Schwann cells. Psychosine, a highly apoptotic compound increased in the brain of infantile patients, is thought to play a major role in the pathogenesis of the disease and, more specifically, to underlie the early destruction of oligodendrocytes characteristic of the infantile form, and thus an arrest of myelin formation [70].

Genetics

More than 150 *GALC* mutations are known. The most frequent mutant allele (never found in Japanese patients) combines a large (30-kb) deletion and the polymorphism 502C>T (now c.550C>T); G270D (now p.Gly286Asp) is frequent among adult-onset patients [68][71][72]. Some common polymorphisms – in particular 1637T>C (now c.1685T>C) and 502C>T – influence enzyme activity and may be responsible for a pseudodeficiency state, particularly when in compound heterozygosity with a disease-causing allele [71]. Two unrelated infantile cases were assigned to a mutation in the sap-A domain of *PSAP*.

Diagnostic Tests

Motor nerve conduction velocities are consistently low in infantile and most late infantile cases, but only about 60% of juvenile or adult patients display signs of peripheral neuropathy. MRI shows areas of hyperintensity on T_2-weighted images that correlate well with areas of demyelination and globoid cell accumulation [73]. In late-onset cases, T_2-weighted images may show more localised areas of hyperintensity with less involvement of cerebellum and deep grey matter [74][75]. In adult-onset cases, typical T_2 hyperintensities along the pyramidal tracts involving optic radiations and corticospinal tracts are nearly constant [68]. In typical infantile cases, CT shows diffuse cerebral atrophy with hypodensity of the white matter. Calcifications may be observed in the thalamus, basal ganglia and periventricular white matter. Brain stem evoked potentials have also been studied [76]. Protein in CSF is usually elevated in infantile cases, but inconstantly in late-onset cases. The ultimate diagnosis is made by studying galactosylceramidase activity in leukocytes, dried blood spots or cultured fibroblasts. This assay is subject to pitfalls of either technical (substrate) or biological (pseudodeficiency) nature. Use of a natural radiolabelled substrate is the gold standard, and a short-chain analogue (final measurement by tandem mass spectrometry) has also shown good specificity [47][77]. Published experience remains limited regarding a less sensitive fluorogenic substrate. In Krabbe disease, like in metachromatic leukodystrophy (▶ below), a pseudodeficiency state is relatively common and can lead to misinterpretation of correct data. For this reason, study of both parents is particularly important. Genotyping of all patients is recommended as prenatal diagnosis using molecular genetics is today preferred to enzymatic studies. In the two known patients with sap-A deficiency, galactosylceramidase activity was deficient in leukocytes but not in cultured fibroblasts (sap-A may stabilise galactosylceramidase).

Treatment and Prognosis

In advanced disease, supportive analgesic treatment of the often severe pain that can result from radiculopathy is important, as is treatment of spasticity. Allogenic BMT or cord blood transplantation may be effective in preventing onset or halting progression of the disease in late-onset cases [78]. In symptomatic infantile cases BMT gives poor results, unless performed presymptomatically. Initial results with umbilical cord blood transplantation to 12- to 44-day-old babies were very promising [79], leading to newborn screening in 2 states in the USA [77]. However, long-term follow-up indicated that over time most children developed slowly progressive motor and language deterioration along with somatic growth failure and persistent cognitive deficits [80][81]. Diffusion tensor imaging tractography might help in predicting neurodevelopmental outcomes in neonates with the disease [82].

38.2.6 Metachromatic Leukodystrophy

Clinical Presentation

Metachromatic leukodystrophy (MLD) is panethnic, with reported incidences ranging between 1 in 40,000 and 1 in 170,000, except in specific ethnic groups with higher frequency.

The **late infantile** form [83][84] is the most common. First symptoms appear between the ages of 1 and 2 years (median onset 18 months): walking delay, progressive difficulty in locomotion around 14–16 months (weaker lower limbs and falls); 15% of children never walk independently. Examination usually shows hypotonia, reduced or absent deep tendon reflexes and extensor plantar responses. Walking and then standing soon become impossible. The child develops spastic quadriplegia, speech deterioration, gradual mental regression and optic atrophy leading to blindness, followed by a vegetative state and death.

The age at onset of the **juvenile** form [84] ranges between 2.5 and 14 years. Some authors differentiate early (onset between 2.5 and 3.5 years) and late juvenile forms. Failure in school, behavioural problems or disturbance of cognitive function may precede motor abnormalities, especially in patients with a later onset (>6 years). Progressive difficulties in walking,

with pyramidal signs and peripheral neuropathy, together with cerebellar ataxia constitute the most common presentation, but various other symptoms can occur, such as hemiplegia, dystonia and choreoathetosis. Seizures may also develop.

A severity scoring based on a gross motor function classification has been developed for late infantile and juvenile forms. Age of entry into the different stages and dynamics of decline of gross motor function have been reported [85], as well as natural course of language and cognition [86].

Two distinct types of **adult** MLD have been identified [87]. In the first group, patients have predominant motor disease, with pyramidal and cerebellar signs, dystonia and peripheral neuropathy, or isolated peripheral neuropathy. In the second group, behavioural and psychiatric problems (often confused with schizophrenia) are the presenting symptoms, followed by dementia and spastic paresis [88].

Metabolic Derangement

The primary metabolic defect is a block in lysosomal degradation of sulfatide (or galactosylceramide-sulfate) and other sulfated glycolipids (Fig. 38.2). *In vivo*, the sulfatide is presented to the enzyme arylsulfatase A (ASA) as a 1:1 complex with sap-B. A deficiency of either ASA or sap-B can cause MLD. Few cases with sap-B deficiency have been documented, most with a late infantile form. Sulfatide is a prominent lipid component of the myelin sheath. Its ratio to galactocerebroside plays a role in the stability and physiological properties of this membrane. Progressive accumulation of sulfatides (and likely lysosulfatide) in the central and peripheral nervous system will soon lead to disruption of the newly formed myelin and intense demyelination. In MLD, sulfatide also accumulates in the kidney, which is reflected in a highly abnormal excretion of sulfatide in urine sediment.

Genetics

About 200 different *ARSA* mutations are known [89]. The three more frequent alleles among European patients are c.465+1G>A (traditional denomination 459+1G>A) (severe phenotype), p.Pro428Leu (P426L) (mild phenotype) and p.Ile181Ser (I179S) (mild phenotype). There is a relatively good genotype-phenotype correlation [84]. Two very frequent *ARSA* polymorphisms, one leading to the loss of an *N*-glycosylation site and the second to the loss of a polyadenylation signal, result in reduction of the amount of enzyme and constitute the molecular basis of ASA pseudodeficiency [89]. They often occur jointly, but can also be found independently. In some countries, as many as 15% of the general population carry one allele with such a pseudodeficiency (*pd*) [84]. MLD due to sap-B deficiency is panethnic, but seems more frequent in Saudi Arabia, Turkey and North Africa. These patients have mutations (10 described to-date) in *PSAP*.

Diagnostic Tests

In most patients, motor nerve conduction velocities of peripheral nerves are decreased and sensory nerve action potentials have a diminished amplitude with a prolonged peak latency [90]. Decreased nerve conduction is not always present in adult MLD. MRI shows similar fairly characteristic symmetrical changes of the central white matter in all forms. A sheet-like area of abnormal T_2 signal hyperintensity initially envelops the frontal and parietal periventricular and central white matter regions, faint in mild disease and denser in moderate to severe disease. As severe disease develops, the sheet of white matter signal intensity abnormality also involves the inner half of the subcortical white matter, and a tigroid pattern emerges [91][92]. The late infantile form also involves cerebral atrophy. Abnormalities are also described by diffusion MRI and proton magnetic resonance spectroscopy (MRS). The CSF protein content is usually elevated in late infantile patients (although not at an early stage), inconstantly in the juvenile form and rarely in the adult form.

Determination of ASA activity in leukocytes (or cultured fibroblasts) using *p*-nitrocatechol-sulfate as a substrate constitutes the first biochemical test. Pseudodeficiency is a major pitfall [84]. Individuals homozygous for a *pd* allele (1–2% of the European population) or subjects compound heterozygotes for a disease-causing *mld* and a *pd* allele have about 5–15% of normal ASA activity but no detectable clinical abnormality or pathology. Deficient ASA activity is therefore not enough to conclude to the diagnosis of MLD. The study of sulfatides in the urinary sediment circumvents the problem. MLD (but also multiple sulfatase deficiency, see ▶ below) patients excrete massive (late infantile and juvenile patients) or significant (adult-onset type) amounts of sulfatides, while subjects with an ASA pseudodeficiency have levels within or slightly above the normal range. ASA pseudodeficiency also poses problems in genetic counselling. In a newly diagnosed family, it is important to measure enzyme activity in both parents. Full genotyping of the index case and study of parental DNA are highly recommended. Prenatal testing of MLD by DNA analysis is now the preferred strategy in many laboratories.

Another cause of erroneous interpretation of an ASA deficiency is **multiple sulfatase deficiency** (MSD), due to a deficiency in the formylglycine-generating enzyme (FGE) encoded by *SUMF1*. Whenever a deficiency of one sulfatase is found, it is mandatory to systematically measure the activity of another one (here, arylsulfatase B or iduronate-2-sulfatase) to exclude MSD, as the clinical picture can be misleading.

In MLD patients with sap-B deficiency, the in vitro ASA assay will not show a deficiency. Studies of sulfatides and globotriaosylceramide (Gb3) excretion in urine are essential. Both lipids are elevated (combined MLD and Fabry pattern). The definitive diagnosis will require *PSAP* molecular genetics study.

Treatment and Prognosis

Symptomatic treatment of spasticity and of pain resulting from radiculopathy is important. Allogenic HSCT has been performed in a number of cases. It is generally considered that adult-onset and juvenile-onset patients benefit, with slowing of the disease progression and improvement of cognitive functions, but challenging reports have appeared [84][93][94][95][96]. Whether HSCT is indicated in the late infantile form remains controversial [84]. Symptomatic patients are not can-

didates; a few presymptomatic affected siblings have received HSCT, with significant difference in survival and CNS involvement compared with untransplanted siblings, but no effect on the peripheral neuropathy. More recently, three presymptomatic patients received lentiviral hematopoietic stem cell gene therapy with good results at evaluations up to 18 or 24 months post-treatment [97]. In late infantile patients, a phase 1/2 clinical trial with intrathecal administration of rhASA is ongoing, as well as a phase 1/2 trial with intracranial administration of AAV10-rhASA.

38.2.7 Fabry Disease

Clinical Presentation

Fabry disease, the only X-linked sphingolipidosis, is associated with severe multiorgan dysfunction [98][99][100]. Its incidence is estimated at 1:40,000 to 1:60,000 live births for males, but from neonatal screening studies [101], late onset phenotypes appear largely undiagnosed. Of note, many heterozygous females are symptomatic. Males with the classic form have a disease onset during the 1st decade, typically with crises of severe pain in the extremities (acroparesthesia) provoked by exertion or temperature changes, that may last hours to days. Unexplained bouts of fever and hypohidrosis, heat, cold and exercise intolerance, gastrointestinal problems and corneal dystrophy (cornea verticillata) not affecting vision, are other manifestations. At this stage, renal function, urinary protein excretion and cardiac function and structure are generally still normal [102]. Characteristic skin lesions, angiokeratomas, appear on the lower part of the abdomen, buttocks and scrotum in 80% of patients. Progressive renal involvement, which may result in end-stage renal disease and require dialysis or transplantation, occurs in adulthood. Cardiac manifestations include left ventricular hypertrophy, valvular disease (mitral insufficiency), ascending aortic dilatation, coronary artery disease and conduction abnormalities leading to congestive heart failure, arrhythmias and myocardial infarction. Cerebrovascular manifestations include early stroke, transient ischaemic attacks, white matter lesions, hemiparesis, vertigo or dizziness, and complications of vascular disease, in particular hearing loss. A recent survey indicates that symptoms such as acroparesthesias, neuropathic pain, gastrointestinal problems can occur even in early childhood (before 5 years of age) [103]. Clinical manifestations in heterozygous females range from asymptomatic to full-blown disease, as severe as in affected males but with globally a later onset and slower progression. A growing number of patients with atypical cardiac, renal or cerebrovascular disease variants with a milder, later onset phenotype or single organ involvement have been described (for a comprehensive review see [98]). Screenings have been conducted in »high-risk« populations [104].

Metabolic Derangement

The primary defect is a deficient activity of the lysosomal enzyme α-galactosidase A, which releases galactose from ceramide trihexoside (globotriaosylceramide, Gb3) and elevated glycosphingolipids (especially galabiosylceramide, Gb2), due to mutations of the *GLA* gene (◘ Fig. 38.2). This results in progressive accumulation of Gb3 in vascular endothelial cells, perithelial and smooth muscle cells, leading to ischaemia and infarction especially in the kidney, heart and brain. Early and substantial deposition of Gb3 occurs in podocytes, leading to proteinuria, and with age, in cardiomyocytes, causing cardiac hypertrophy and conduction abnormalities. Small-fibre polyneuropathy is the cause of pain and anhidrosis. Lysosomal storage and cellular dysfunction are believed to trigger a cascade of events resulting in tissue ischaemia and development of irreversible cardiac and renal tissue fibrosis [98].

Genetics

Fabry disease has an X-linked recessive transmission. Adequate genetic counselling in the family, including female carrier detection, is therefore essential. Nearly 1000 variations of *GLA* are known, and defining their pathogenicity remains a crucial problem, especially in screening programmes [105]. Many mutations are private; a number are recurrent in specific countries. The p.Asn215Ser mutation seems to be associated with the cardiac variant. De novo mutations are rare. In females, the X-chromosome inactivation pattern seems more contributive to disease expression than the mutation itself [106].

Diagnostic Tests

In affected males with the classic or variant phenotype, the disease is readily diagnosed by showing a deficient α-galactosidase A activity in leukocytes. Dried blood spots are better suited to large-scale screening, but subsequent confirmation in leukocytes is essential. Heterozygous females show normal to low levels of activity; enzyme assay is thus not reliable for carrier detection, and studying the family mutation is the test of choice in subjects related to a patient with definite diagnosis. Results of *GLA* sequencing alone can often be difficult to interprete in cases of suspected Fabry disease [107]. In urinary sediment, Gb3 and Gb2 are excreted in large amounts by untreated male hemizygotes (except those with a renal graft or with a cardiac variant), and in smaller amounts by 90% of heterozygous females, symptomatic or not. Plasmatic lyso-Gb3 [105][108] is recognized as a sensitive and useful biomarker. Definite diagnosis of Fabry disease should combine several biological and clinical criteria; in atypical – particularly cardiac – variants, electron microscopic study of the target organ may be necessary [107].

Treatment and Prognosis

The disease results in a significant reduction in life expectancy due to renal disease and cardiovascular or cerebrovascular complications [98]. There is also the psychosocial burden of a rare, chronic and progressive disease. Alleviation of pain and treatment of the renal and cardiac disease are important issues. Dialysis or renal transplantation may be necessary for patients with end-stage renal failure. There is now a long-term experience of ERT with recombinant α-galactosidase A products (agalsidase alpha or agalsidase beta). The oral pharmacological chaperone migalastat has recently been approved in the

EU for treatment of patients aged 16 years or older with an amenable mutation. ERT leads to improvement for many of the symptoms and appears efficient in reducing left ventricular hypertrophy, with lesser effect on renal function. But in long-term studies, it did not prevent progression [109][110][111]. A European consortium has proposed recommendations for initiation and cessation of ERT [111].

38.2.8 Farber Disease / Acid Ceramidase Deficiency

Clinical Presentation

The very rare »Farber lipogranulomatosis« is clinically heterogeneous. It often presents during infancy causing death within the 1st year, but later onset cases (up to an adult age) have been described, as well as foetal forms [112]. The most frequent signs are painful joint swelling, deformation and contractures, periarticular subcutaneous nodules and hoarseness due to laryngeal involvement. The presentation of some patients mimics juvenile idiopathic arthritis [113]. Hepatomegaly and a macular cherry-red spot may be present. Neurological manifestations are of variable severity (from mild to psychomotor deterioration and epilepsy); juvenile-onset patients may show neurological involvement only. A distinct form of acid ceramidase deficiency showing spinal muscular atrophy and progressive myoclonic epilepsy (SMA-PME) has been delineated [114], with more cases being recently described [115].

Metabolic Derangement and Genetics

The deficiency of acid ceramidase activity leads to the storage of ceramides in various organs [116]. More than 30 mutations of *ASAH1* have already been described [117], including a large deletion.

Diagnostic Tests

Electron microscopy of an excised nodule or of a skin biopsy may reveal inclusions with typical curvilinear bodies in histiocytes, and ›banana bodies‹ in Schwann cells. In vitro measurement of ceramidase activity requires a specific substrate available in only few laboratories [118]; so are ceramide precursors loading tests in living fibroblasts or ceramide levels determinations. It is therefore often easier and quicker to directly sequence *ASAH1*.

Treatment and Prognosis

Currently there is no specific therapy. Good results of BMT have been reported only in patients without CNS involvement [119]. Development of ERT and gene therapy is being facilitated by the recent availability of a suitable mouse model.

38.2.9 Prosaposin Deficiency

Clinical Presentation

The eight published cases have shown almost the same course, with severe neurovisceral storage disease manifesting immediately after birth with rapidly fatal course and death between 4 and 17 weeks of age. The patients have hepatosplenomegaly, hypotonia, massive myoclonic bursts, abnormal ocular movements, dystonia and seizures [120].

Metabolic Derangement and Genetics

Sphingolipid activator proteins are small glycoproteins that are required as cofactors for the lysosomal degradation of sphingoglycolipids with short hydrophilic head groups and ceramide. They act either by solubilising the substrate or by mediating enzyme binding to the membrane or modifying the enzyme conformation. *PSAP* encodes the prosaposin protein, which is transported to the lysosome where it is processed to four homologous proteins. Sap-A is a cofactor for degradation of galactosyl- and lactosylceramide; its deficiency causes a Krabbe disease variant (2 cases known); sap-B is involved in the in vivo degradation of sulfatides and Gb3, and its deficiency causes an MLD variant (>25 cases known); sap-C is necessary for hydrolysis of glucosylceramide, and its deficiency causes a Gaucher disease variant (5 cases known). Although no patient has been described with sap-D deficiency, this factor is implicated in ceramide degradation. Prosaposin deficiency is due to the combined lack of all four sap- factors, explaining tissue storage of all the lipids cited above. The disorder is autosomal recessive. Mutations identified in patients explain abolished production of the prosaposin precursor and thus of all four factors.

Diagnostic Tests

Gaucher-like cells are found in bone marrow. Study of glycolipids in urine sediment shows a pattern close to that described for sap-B deficiency. Galactocerebrosidase activity has been reported to be deficient in leukocytes and fibroblasts. Lipid studies in liver tissue revealed a combined increase of glucosylceramide, lactosylceramide and ceramide. The loading test in living fibroblasts described for Farber disease shows a severe block in ceramide hydrolysis. In practice, the abnormal typical profile of urinary glycolipids and/or elevated plasma lyso-Gb3 and lyso-glucosylceramide should lead to complete *PSAP* sequencing.

38.3 Niemann-Pick Disease Type C

38.3.1 Clinical Presentation

Niemann-Pick disease type C (NP-C) is panethnic, with an estimated incidence around 1 in 100,000 [121][122]. The clinical course is extremely heterogeneous and age at presentation varies from the perinatal period to late adulthood. Visceral involvement (liver, spleen and lung) and neurological or psychiatric manifestations arise at different times, and they follow an independent course. Systemic disease, when present, always precedes the onset of neurological symptoms; the systemic component may decrease with time, be minimal, or absent. Apart from a small subset of patients who die in the perinatal period and exceptional adult cases, all patients

ultimately develop a progressive and fatal neurological disease. For periods other than perinatal, some patients show only systemic signs, while others start to show neurological symptoms. A classification by neurological form (rather than by age at disease onset) is widely used, because a correlation between age at neurological onset and following course of disease and life-span has been established [122].

▪ Perinatal Presentations

Foetal period Foetal hydrops or foetal ascites (often with splenomegaly) can occur.

Neonatal period In early life, liver involvement is often present. About one third of NP-C patients show a *prolonged neonatal cholestatic icterus* with hepatosplenomegaly. In most patients, the icterus resolves spontaneously and only hepatosplenomegaly remains [122]. Such patients may later develop any neurological form, although rarely an adult onset form [123]. In a few infants, the liver disease worsens and they die from hepatic failure before 6 months of age, defining a **neonatal, cholestatic rapidly fatal form**. Isolated *hepatosplenomegaly or splenomegaly* can also start at this period. A few infants develop a severe respiratory insufficiency [122].

▪ Period with Isolated Systemic Symptoms

Isolated splenomegaly or hepatosplenomegaly can be the first sign of disease, and be detected at any age. Onset of neurological symptoms may be protracted by many years. NP-C at this stage is one of the critical differential diagnoses of Niemann-Pick type B. A handful of adults up to 60 years of age have been described with systemic disease only [122]. More such cases may stay undiagnosed [121].

▪ Neurological Forms

Early infantile form In the severe early infantile neurological onset form, infants with a pre-existing hepatosplenomegaly (often with a history of neonatal cholestatic jaundice) show an early delay in motor milestones that becomes evident between the ages of 9 months and 2 years, and hypotonia. Most never learn to walk. The mental status is less severely affected. A loss of acquired motor skills is followed by spasticity with pyramidal tract involvement and mental regression. Signs of white matter involvement are present. Survival rarely exceeds 6 years [122].

Late-infantile- and juvenile-onset neurological forms (classic NP-C, 60–70% of cases) In the late infantile form, hepatosplenomegaly has generally been present for a varying period, but may be absent. Language delay is frequent. At the age of 3 to 5 years, the first obvious neurological signs are gait problems and clumsiness, due to ataxia. The motor problems worsen, cognitive dysfunction appears. In the juvenile form, onset of neurological disease is between 5–6 and 12 years, with more insidious and variable symptoms. Splenomegaly is variable. School problems, with difficulty in writing and impaired attention, are common and may lead to misdiagnosis. The child becomes clumsier with increasing learning disabilities, and obvious ataxia. In both forms, vertical supranuclear gaze palsy, with an increased latency of initiation of vertical saccades, is constant when correctly assessed and a characteristic sign. Gelastic cataplexy occurs in about 20% of patients and can be the presenting symptom. As ataxia progresses, dysphagia, dysarthria and dementia develop. Action dystonia is also frequent. About half of the patients develop seizures, which may become difficult to treat. In a later stage, the patients develop pyramidal signs and spasticity and severe swallowing problems. Most require gastrostomy. Death usually occurs between 7 and 12–14 years of age in late-infantile-onset patients, and is very variable in the juvenile form, some patients being still alive by age 30 or more [122][123].

Adolescent/adult onset form Age at diagnosis varies between 15 and 60 years or more. In adult-onset patients, presentation is even more insidious and diagnosis seldom made at an early stage. Atypical signs may in retrospect have been present since adolescence. Major signs are ataxia, dystonia and dysarthria, movement disorders, with variable cognitive dysfunction; psychiatric symptoms and dementia are dominant in certain patients [123][124]. In recent cohorts, vertical gaze palsy was a nearly constant sign. Epilepsy is rare in adult NP-C. Splenomegaly is inconstant.

38.3.2 Metabolic Derangement

NPC2 is a small soluble lysosomal protein which binds cholesterol with a high affinity; NPC1 is a large transmembrane protein with a main late endosomal localisation. When either protein is non functional, the cellular trafficking of endocytosed LDL-derived cholesterol is impaired, resulting in accumulation of unesterified cholesterol in the endosomal/lysosomal system and delay in homeostatic reactions. This specific abnormality constitutes the basis for biological diagnosis of NP-C. The two proteins act in sequence. In the prevailing »hand-off« model, cholesterol first binds to NPC2. NPC2 transfers it to NPC1, which facilitates its lysosomal egress by a still unknown mechanism [41][122][125]. However, the complete functions of NPC2, and above all of NPC1, remain unclear. In extraneural organs, the lipid storage pattern includes, besides unesterified cholesterol, sphingomyelin, several glycolipids, sphingosine and bis(monoacylglycero)phosphate, with no prevailing compound. Sphingolipid accumulation could be secondary to cholesterol storage [41]. In brain, neurons store significant amounts of glycolipids, particularly GM2 and GM3 gangliosides; but, despite clearly abnormal filipin staining in neurons, there is no quantitative increase of cholesterol (nor of sphingomyelin) in grey matter [41][125]. Main pathologic changes in brain, besides neuronal storage, are a prominent loss of Purkinje cells, neuroaxonal dystrophy, neurofibrillary tangles, meganeurite formation and ectopic dendritogenesis. Signs of delay in myelination and severe myelin loss are only prominent in the early infantile neurological form. The role of the more recently described block in Ca^{2+} release from acidic compartments in the pathogenic cascade is not well understood [41][122][125].

38.3.3 Genetics

Approximately 95% of patients harbour mutations in *NPC1*, the remainder in *NPC2*. More than 350 disease-causing *NPC1* mutations are already known, as are >100 polymorphisms. The most frequent mutant allele in patients of western European descent is p.Ile1061Thr, followed by p.Pro1007Ala. Some 50 families are known with *NPC2* mutations. Studies in multiplex families indicate that mutations correlate with the global neurological form rather than with the systemic manifestations. Certain mutations (e.g. p.Pro1007Ala) are associated with a lesser block in cholesterol trafficking (›variant‹ filipin test, see diagnostic tests section) [122][126].

38.3.4 Diagnostic Tests

Neuroimaging is generally not contributive to the diagnosis. Foamy and sea-blue histiocytes may (not always) be found in bone marrow aspirates. In plasma, the oxysterols cholestane-3β,5α,6β-triol and 7-ketocholesterol as well as ›lysosphingomyelin-509‹ are elevated in most cases [43][127] (as in ASM deficiencies), while lysosphingomyelin (also high in ASM deficiencies) shows a modest or no elevation [128]. The bile acid derivative 3β,5α,6β-trihydroxy-cholanoyl-glycine is also increased in NPC and ASM deficiencies. Note that oxysterols are also elevated in acid lipase deficiencies and some other diseases including neonatal cholestasis [45][46]. The definitive biochemical diagnosis requires live cultured fibroblasts. After culture in an LDL-enriched medium, pathognomonic accumulation of free cholesterol in lysosomes can be visualised by fluorescence microscopy after staining with filipin [122]. This »filipin test« will give unequivocal results in about 85% of patients, while interpretation is more difficult in the remainder, described as ›variants‹. In the latter, cholesterol accumulation is less prominent and not present in all cells, and complementary gene sequencing may be necessary to conclude. Today, positive biomarker testing is usually directly followed by molecular genetic analysis. The filipin study can be helpful to define the pathogenic nature of new gene variations or if mutations remain unidentified. Characterization of mutations may require more than DNA sequencing (large deletions, deep intronic mutations). Genotyping all patients is essential, as only the molecular genetics approach is now used for prenatal diagnosis [122][129]. For review see [130].

38.3.5 Treatment and Prognosis

Cataplectic attacks can be treated by clomipramine or CNS stimulants. Management of epilepsy, when present, is essential. With progression, most patients will require tube feeding or gastrostomy [129][131]. To date, miglustat is the only treatment specifically approved for neurological manifestations of NP-C, in the EU and many other countries (but not in the USA). Indications, clinical utility and monitoring have been discussed [129][131]. Initial data indicating stabilisation of patients for one year or more [132][133] and a slower rate of progression of the disease after treatment [134] have been confirmed; patients with later onset forms appear as better responders [135]. There is a rationale for HSCT in NP-C2 patients (at variance with NP-C1), but (early) follow-up is known in only one patient. Following encouraging preclinical studies in the *Npc1* feline model [136], a phase 1/2 trial with intrathecal administration of 2-hydroxypropyl-β-cyclodextrin is being completed; a phase 2b/3 is starting. Another clinical trial with oral administration of arimoclomol, a heat shock protein (hsp70 and hsp40) enhancer, is also underway.

38.4 Neuronal Ceroid Lipofuscinoses

Neuronal ceroid lipofuscinoses (NCLs) are a group of inherited progressive neurodegenerative diseases, among the most frequent in childhood. The term NCL is widely used in Europe, but the generic term »Batten disease« is common in the USA. The first description dates back to 1826, and since then, many clinical forms have been reported in the literature, strongly suggesting a large heterogeneity of the disease. The past 20 years have seen major advances in the field and the clinical diversity has now been linked to a wide genetic heterogeneity, with already 13 different genes identified, and probably more to come. Five of them encode soluble proteins, the others encode transmembrane proteins whose function and possible interactions still remain incompletely understood. NCLs are now considered as lysosomal storage diseases, due to the lysosomal accumulation of lipopigments, and above all, the more recent localisation of several NCL proteins to the lysosome.

38.4.1 Clinical Presentation

NCLs are usually characterised by progressive psychomotor retardation, seizures, visual loss and early death. Four main clinical forms have been described according to the age of onset and the order of appearance of clinical signs : infantile, late infantile (the most common in South Europe), juvenile (common in Anglo-Saxon countries) and adult (rare) [137]. However, numerous other clinical variants have been reported. This clinical heterogeneity is related to the diversity of the genes involved and to the variable severity of mutations. Therefore, the first classification based on the clinical forms has now been replaced by a new one using the genetic loci and including various forms with different ages of onset even if one form is usually predominant for each gene [138] (◘ Table 38.2).

- **Classic Infantile Neuronal Ceroid Lipofuscinosis (INCL, Santavuori-Haltia) Linked to *PPT1* (*CLN1*)**

Its incidence is high in Finland (1 in 20,000). Children with INCL are normal at birth. Symptoms usually begin between 6 and 24 months. They include delayed development, hypotonia, deceleration of head growth, seizures and jerks. Sleep disturbances are seen in most children. Rapid visual impairment occurs due to optic atrophy and macular degeneration. Stereo-

Table 38.2 Classification of NCLs with the corresponding genes, proteins and clinical forms. The different loci are organized by age of onset of the main clinical form (indicated in bold). The non-NCL phenotypes are given in italics

Gene	Protein	Clinical forms
CTSD (or ***CLN10***)	Cathepsin D	**Congenital** Late infantile, juvenile, adult
PPT1 (or ***CLN1***)	Palmitoyl protein thioesterase 1 (PPT1)	**Classic infantile** Late infantile, juvenile, adult
KCTD7 (or ***CLN14***)	Potassium channel tetramerization domain-containing protein 7 (KCTD7)	**Infantile** *Progressive myoclonic epilepsy* *Opsoclonus-myoclonus ataxia-like syndrome*
TPPI (or ***CLN2***)	Tripeptidyl peptidase 1 (TPP1)	**Classic late infantile** Juvenile, protracted, *SCAR7*
CLN5	CLN5 protein	**Late infantile** Juvenile, protracted, adult
CLN6	CLN6 protein	**Late infantile** Protracted **Adult type A Kufs** (recessive)
MFSD8 (or ***CLN7***)	MFSD8	**Late infantile** Protracted
CLN8	CLN8 protein	**Late infantile** Protracted **Northern epilepsy** (EPMR)
CLN3	CLN3 protein	**Classic juvenile** Protracted, *retinitis pigmentosa, autophagic vacuolar myopathy*
ATP13A2 (or ***CLN12***)	ATPase	**Juvenile** *Kufor-Rakeb syndrome*
DNAJC5 (or ***CLN4***)	Cysteine-string protein alpha (CSPα)	**Adult type A Kufs (dominant)**
CTSF (or ***CLN13***)	Cathepsin F	**Adult type B Kufs (recessive)**
GRN (or ***CLN11***)	Progranulin	**Adult** *Frontotemporal lobar dementia (heterozygous)*

typed hand movements may be present. Death takes place in the first decade of life. Even though mutations in *PPT1* are mainly responsible for this classic infantile NCL, later-onset forms (juvenile, adult) have also been described, probably due to less severe mutations.

Variants due to another gene More recently, mutations have been reported in *KCTD7* (now *CLN14*) in patients with infantile-onset progressive myoclonic epilepsy (PME), vision loss, cognitive and motor regression and premature death. *KCTD7* mutations have also been described in another phenotype called opsoclonus myoclonus ataxia syndrome (OMS) associating acute onset of myoclonus and ataxia with abnormal opsoclonus-like eye movements [139].

Classic Late Infantile Neuronal Ceroid Lipofuscinosis (LINCL, Jansky-Bielschowsky) Linked to *TPP1* (*CLN2*)

Children may be initially referred for delayed speech. Seizures, which may be of any type (partial, generalized tonic-clonic, absences), occur between 2 and 4 years of age. Ataxia, myoclonus and developmental regression become apparent, followed by a gradual decline of visual ability culminating in blindness by 5 or 6 years. Death happens in middle childhood after a bedridden stage. Besides this classic LINCL, mutations in *TPP1* have also been involved in atypical phenotypes with delayed onset and slower progression. Moreover, mutations in *TPP1* have been reported in autosomal recessive spinocerebellar ataxia 7 (SCAR7). Patients showed ataxia, but neither visual abnormalities, nor epilepsy and the disease is slowly progressive until old age.

Variants due to other genes Variants with similar or later onset, or delayed evolution compared to the classic late infantile form have been described. The Northern epilepsy or progressive epilepsy with mental retardation (EPMR) linked to *CLN8* is characterised by tonic-clonic seizures occurring between 5 and 10 years. Mental deterioration is observed 2 to 5 years after the onset of epilepsy. Vision problems are rare.

Some patients are living well over 40 years. Mutations in *CLN8* have also been reported in a subset of late-infantile patients from Turkish consanguineous families. A variant involving *CLN5* is common in Finland even if other patients have now been described in other countries. It usually begins around 4.5–6 years by clumsiness and difficulties in concentration. Visual impairment, ataxia and epilepsy appear a few years later. Life expectancy is between 13 and 35 years. Two additional genes are commonly involved in late-infantile variants presenting a clinical pattern close to the CLN2 disease. Mutations in *CLN6* are mainly seen in patients originating from South Europe, Indian subcontinent and South America. *CLN7* (*MFSD8)* has been initially involved in Turkish patients with LINCL, but abnormalities in this gene have now been reported in patients from different countries [139].

■ Classic Juvenile Neuronal Ceroid Lipofuscinosis (JNCL, Batten or Spielmeyer-Vogt) Linked to *CLN3*

The onset is between 4 and 10 years. Visual failure is usually the first clinical sign and it results in total blindness in 2–3 years. Seizures appear between 5 and 18 years, and predominant types are generalized tonic-clonic, myoclonic or partial seizures. Speech becomes dysarthric and echolalia is frequent. Many patients develop signs of parkinsonism. Mental capacity is progressively altered and dementia becomes evident in several years. Behavioural problems with aggressiveness may occur. Most patients live until the late teens or early/late 20s. A protracted atypical phenotype has recently been reported in patients showing a rapid visual failure followed 20 years later by seizures, hypertrophic cardiomyopathy, the presence of autophagic vacuoles in muscle biopsy and only mild cognitive impairment after 40 years of evolution.

Variant due to another gene Mutations in *ATP13A2* (now *CLN12*) have been associated with a NCL juvenile variant showing learning difficulties around 8 years, followed by unsteady gait, myoclonus, mood disturbance, and extrapyramidal signs such as akinesia, rigidity and dysarthric speech. It is important to note that *ATP13A2* is a known cause of Kufor-Rakeb syndrome which is a rare parkinsonian syndrome with juvenile onset marked by dementia, supranuclear palsy and pyramidal signs [139].

■ Classic Adult Neuronal Ceroid Lipofuscinosis (ANCL, Kufs)

Symptoms usually start around age 30 years, but onset during adolescence or late adulthood has been reported. Kufs disease is usually inherited as an autosomal recessive trait, but a rare dominant form (sometimes called Parry disease) also exists. Classically, two major forms of Kufs disease have been delineated. Type A is characterised by progressive myoclonic epilepsy while type B is marked by dementia and a diversity of motor signs. Retinal vision is generally preserved. During years, the genes involved in these forms have remained uncharacterised even though mutations have been found in *PPT1* in some patients. More recently, *CLN6* has been reported to be a major gene in recessive type A Kufs disease and the dominant form has been linked to *DNAJC5* (now called *CLN4*) encoding cysteine-string protein alpha (CSPα). Causal abnormalities have also been found in *CTSF* (or *CLN13*) encoding cathepsin F in patients with type B Kufs disease.

Variant due to another gene Mutations have been reported in *GRN* (or *CLN11*) encoding progranulin in siblings with rapidly progressive visual failure around 20 years, myoclonic seizures, cerebellar ataxia and early cognitive deterioration. Unexpectedly, these patients were homozygous for a *GRN* mutation, while heterozygous mutations in the same gene are a major cause of frontotemporal lobar dementia. These two diseases significantly differ by their age of onset and neuropathology [139].

■ Congenital Form

This rare form presents with microcephaly and seizures at birth, resulting in death within the first days of life. Mutations in *CTSD* (or *CLN10*) have been found in some patients, but other causative genes probably remain to be characterised [139].

38.4.2 Metabolic Derangement

Ceroid lipofuscinoses are characterised by the accumulation of autofluorescent ceroid lipopigments, mainly in neural tissues. They show different ultrastructural patterns, such as granular, curvilinear or fingerprint profiles [140]. The main components of this storage material are either saposins A and D in infantile forms, or subunit c of mitochondrial ATP synthase (SCMAS) in late infantile and juvenile forms. They are probably not disease-specific substrates, but secondary markers. NCL proteins are mainly localized in the lysosome (CLN1, CLN2, CLN3, CLN5, CLN7, CLN10, CLN12, CLN13), but also in the endoplasmic reticulum (CLN6, CLN8) or in the cytosol in association with vesicular membranes (CLN4, CLN14). Five of them are soluble proteins: palmitoyl protein thioesterase 1 (CLN1), tripeptidyl peptidase 1 (CLN2), cathepsin D (CTSD), cathepsin F (CTSF) and CLN5. Others are transmembrane proteins (CLN3, CLN7, CLN12), the function of which is badly understood. For a review, see [141]. Briefly, CLN1 is involved in the degradation of S-fatty acylated proteins and it possibly regulates exo- and endocytosis in the neuronal presynaptic area. CLN2 is a serine protease which removes tripeptides from proteins facilitating their degradation in lysosomes. It is likely involved in macroautophagy and TNFα-induced apoptosis. Cathepsin D is an aspartyl endopeptidase which has been related to apoptosis and autophagy while cathepsin F is a cysteine protease recently associated to proteasome degradation and autophagy. Function of CLN5 is still unclear. CLN3 was shown to affect lysosomal pH regulation, autophagy, proliferation and apoptosis. CLN6 has been associated with autophagy, regulation of pH, endocytosis, and biometal metabolism. CLN8 (member of a superfamily including TRAM and Lag1) could participate in regulation of sphingolipid metabolism. CLN7/MFSD8 belongs to the major

facilitator superfamily (MFS), and is therefore likely to transport small (still unidentified) molecules across membranes. Progranulin (CLN11) plays important roles in inflammation or tumorigenesis, and could be involved in autophagy and lysosomal homeostasis. ATP13A2 (CLN12) is a lysosomal transmembrane protein which belongs to the P-type ATPases family; its possible role is to protect against α-synuclein toxicity. CLN14 (KCTD7) probably affects neuronal excitability by regulating K+ conductance in neurons. CSPα, altered in the rare dominant adult form, is a molecular co-chaperone with Hsc70, potentially involved in synaptic vesicle exocytosis and endocytosis.

38.4.3 Genetics

NCLs are usually inherited in an autosomal recessive manner (except an adult form which is dominantly transmitted). They result from mutations in the thirteen known genes encoding the various NCL proteins [139] (Table 38.2). Numerous *PPT1* (*CLN1*) mutations have been reported, but p.Arg122Trp and p.Arg151* are common in Finnish and non-Finnish patients, respectively. Two mutations are common in *TPPI* (*CLN2*): c.509-1G>C and p.Arg208*, but more than 100 private mutations have now been described. For *CLN3*, a 1 kb deletion (c.461-280_677+382del966) is particularly frequent (80-90% of alleles). Concerning *CLN5*, p.Tyr392* is frequent in the Finnish population, but different mutations have been found in other countries. Northern epilepsy is mainly due to the p.Arg24Gly mutation, but other *CLN8* abnormalities have been described in patients presenting with late infantile variants. Numerous mutations have been reported in the other NCL genes characterised to date and the majority of them are private. Details are given in the NCL Mutation Database (http://www.ucl.ac.uk/ncl/).

38.4.4 Diagnostic Tests

Electrophysiological studies are helpful to establish the diagnosis of NCLs. Electroretinogram (ERG) is generally diminished at presentation and it becomes rapidly extinguished. In INCL, the first abnormality in the electroencephalogram (EEG) is the disappearance of eye opening/closing reaction, followed by a loss of sleep spindles. Then, EEG becomes rapidly flat. In LINCL, an occipital photosensitive response to photic stimulation at 1–2 Hz with eyes open is present. MRI shows progressive brain atrophy, particularly severe in INCL, sometimes beginning on cerebellum in other forms.

Vacuolated lymphocytes are only present in the juvenile form (*CLN3*). Electron microscopy (EM) on tissue biopsies – usually skin – shows the presence of pathological inclusions. Granular osmiophilic deposits (GROD) are mainly found in early forms involving *CLN1* or *CLN10/CTSD*. Curvilinear (CV) profiles are present in the classic LINCL (*CLN2*) and in the variant form linked to *CLN7*, while fingerprints (FP) are common in JNCL (*CLN3*). Mixed inclusions diversely associating GROD, CV and FP are found in LINCL variants (*CLN5, CLN6, CLN7, CLN8*) or in adult forms. EM is essential to confirm the diagnosis of NCL [140].

For the *CLN1* and *CLN2* loci, diagnosis is established by measuring respectively the palmitoyl protein thioesterase 1 and the tripeptidyl peptidase 1 activities, either on leukocytes or cultured fibroblasts, using specific artificial fluorogenic substrates. The disease-causing mutations are then characterised by complete sequencing of the corresponding genes [142]. The diagnosis strategy is similar for *CTSD* encoding cathepsin D (rare cases). For all the other genes, complete sequencing is performed directly, except for *CLN3* where it can be preceded by the rapid detection of the common 1 kb deletion [143]. Prenatal diagnosis is possible using specific enzymatic assay and/or detection of the mutation(s) previously determined in the index case. It is important to note that diagnosis of NCLs will soon benefit from the development of next generation sequencing (NGS). They will be diagnosed more rapidly by using gene panels specifically dedicated to these diseases or focused on pathologies (such as myoclonic epilepsies, ….) sharing clinical features with NCLs.

38.4.5 Treatment and Prognosis

Among symptomatic treatments, antiepileptic drugs need to be selected with caution. Lamotrigine is usually efficient on seizures. Levetiracetam may also be beneficial, but carbamazepine and phenytoin can worsen the symptoms. Diazepines should be useful on seizures, anxiety, and sleep disturbances. Gastrostomy is used to maintain nutritional status in the late stages of the disease. Specific therapies are in development for NCL and some of them are entering the clinic (http://clinicaltrials.gov/) [144]. In CLN2, two clinical trials are ongoing: ERT with intrathecal administration of recombinant TPP1, following demonstration of its efficacy in murine and canine models; and gene transfer with direct intracerebral administration of an AAV10 encoding *TPP1*. In CLN1 patients, a treatment combining cysteamine bitartrate and N-acetylcysteine was able to deplete GROD in peripheral leukocytes, but did not significantly change the course of the disease. As autoantibodies were found in the brain of juvenile NCL, a treatment based on mycofenolate mofetil was tested in mice, and demonstrated its capacity to decrease neuroinflammation and to protect neurons. A clinical trial is now ongoing in patients with a juvenile form involving *CLN3*. Several other experimental approaches, including neural stem cells, are under investigation [145].

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Mucopolysaccharidoses, Oligosaccharidoses and Sialic Acid Disorders

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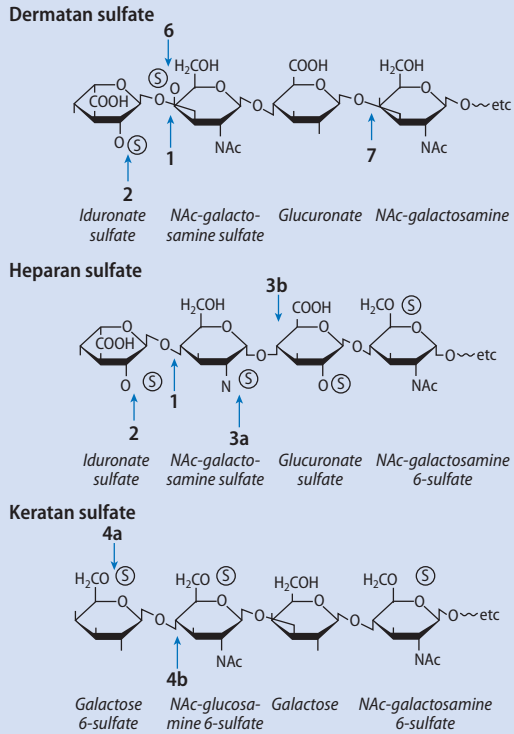
Mucopolysaccharides

Mucopolysaccharides (now preferentially termed glycosaminoglycans, GAGs) are essential constituents of connective tissue, including cartilage and vessel walls. They are composed of long sugar chains, containing highly sulfated, alternating uronic acid and hexosamine residues, assembled into repeating units. The polysaccharide chains are bound to specific core proteins within complex macromolecules called proteoglycans. Depending on the composition of the repeating units, several mucopolysaccharides are known (■ Fig. 39.1). Their degradation takes place inside the lysosomes and requires several acid hydrolases. Deficiencies of specific degradative enzymes have been found to be the cause of a variety of eponymous disorders, collectively termed mucopolysaccharidoses.



■ **Fig. 39.1 Main repeating units in mucopolysaccarides and location of the enzyme defects in the mucopolysaccharidoses (MPS).** *NAc*, N-acetyl; *S*, sulfate; *1*, α-iduronidase (MPS I: Hurler and Scheie disease); *2*, iduronate sulfatase (MPS II: Hunter disease); *3a*, heparan N-sulfatase (MPS IIIa: Sanfilippo A disease); *3b*, α-N-actylglucosamindase (MPS IIIb: Sanfilippo B disease); *4a*, N-acetyl-galactosamine-6-sulfatase (MPS Iva: Morquio A disease); *4b*, β-galactosidase (MPS IVb: Morquio B disease); *6*, NAc-galactosamine-4sulfatase (MPS VI: Maroteaux-Lamy disease); *7*, β-glucuronidase (MPS VII: Sly disease)

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Genetic defects in enzymes that are involved in the lysosomal degradation of the mucopolysaccharides (glycosaminoglycans, GAGs) (► Mucopolysaccharides; ■ Fig. 39.1) and the oligosaccharide chains of glycoproteins (■ Fig. 39.6) lead to chronic and invariably progressive disorders. Although these disorders share many clinical features, the presentation can be highly variable and the spectrum of phenotypic severity is extremely broad. Signs and symptoms include bone dysplasia (dysostosis multiplex), hepatosplenomegaly, neurological abnormalities, cardiac disease and, in some of the disorders, developmental regression. Life expectancy is generally reduced at the severe end of the clinical spectrum. Mucopolysaccharidoses (MPS) and oligosaccharidoses are transmitted in an autosomal recessive manner, except for the X-linked MPS II (Hunter syndrome). Diagnosis of these disorders is initially by detecting increased concentrations of (partially degraded) GAGs or oligosaccharides in urine, confirmed by specific enzyme assays in serum, leukocytes or skin fibroblasts followed by mutational analysis.

Over recent years important advances have been made in the disease modifying treatment of a number of the mucopolysaccharidosis, including enzyme replacement therapy (ERT) and hematopoietic stem cell transplantation (HSCT) and many more treatment options, including gene therapy, are currently under study. While these treatments may result in improvement of a number of clinically relevant symptoms there is generally significant residual disease, especially involving the musculoskeletal system. Prenatal diagnosis is possible for all the MPSs and oligosaccharidoses. Although not discussed further in this chapter a number of inherited disorders of GAG synthesis are now recognised [79], including a disorder recently described in 9 individuals with infantile-onset severe developmental delay and skeletal dysplasia due to mutations in *NANS*, the gene encoding the synthase for N-acetylneuraminic acid (NeuNAc; sialic acid) [80].

39.1 Mucopolysaccharidoses

39.1.1 Clinical Presentation

Seven separate types of MPS are distinguished: MPS I, II, III, IV, VI, VII and IX (Table 39.1). In addition, differences in phenotypic severity has led to separation of MPS I into different subtypes (Hurler, Hurler/Scheie and Scheie phenotypes). Furthermore, MPS III is subdivided into 4 types (A to D), depending on the deficient enzyme and MPS IV into 2 subtypes (A and B), also depending on the specific enzyme deficiency.

All MPS are rare and information about their birth prevalence is relatively limited. Although there are differences between regions, MPS I and MPS III are the most prevalent disorders, except for the United Arab Emirates where MPS VI is most frequent.

MPS are all chronic, progressive and multisystem disorders. Although patients generally appear normal at birth, accumulation of GAGs already starts before birth and may lead to very early symptoms such as hydrops fetalis and intrauterine death (MPS VII) or the presence of skeletal deformities such as thoracolumbar kyphosis at birth (MPS IH). In general, MPS disorders characteristically present with one of the following:

1. Dysmorphic features from an early age in combination with growth failure, umbilical and/or inguinal hernia, protruding abdomen and musculoskeletal disease; all frequent in MPS I (the more severe MPS IH and MPS IH-S phenotypes), MPS II, MPS VI and MPS VII.
2. Primarily skeletal disease (skeletal dysplasia) with growth failure and relatively minor dysmorphic features (MPS IV).
3. Learning difficulties with behavioural problems followed by cognitive decline in combination with (mild) coarse facial features (MPS III).

It is important to note that the wide phenotypic spectrum observed in all MPS subtypes can lead to an atypical, pauci-symptomatic, presentation which may easily lead to misdiagnosis, for instance, of a skeletal dysplasia.

Important signs and symptoms in MPS include:

- Dysostosis multiplex is a term used to describe the skeletal disease associated with MPS and consists of a collection of radiographic abnormalities resulting from defective endochondral and membranous growth throughout the body. Typically, the growth of the long bones is stunted, vertebral bodies are hypoplastic and abnormally shaped, which may result in kyphosis with or without scoliosis (Fig. 39.2), and the knees are in the valgus position. Hip abnormalities, due to failure of ossification of the lateral acetabular roof, medial proximal epiphyseal growth failure of the femur and coxa valga, lead to a form of hip dysplasia often accompanied by deformation leading to subluxation or dislocation of the femoral head (Fig. 39.3).
- Other radiologic findings include bullet-shaped metacarpals and phalanges, an enlarged and thickened skull, broad clavicles and broad oar-shaped ribs (Fig. 39.2). The pathophysiology of the dysostosis multiplex is complex and not fully understood. Intra- and extracellular deposition of GAGs and GAG fragments leads to impaired cell-to-cell signalling, altered mechanical properties and upregulated inflammatory pathways, all of which are believed to affect the growth plate, osteoclasts and osteoblasts, thus contributing to the skeletal dysplasia. Dysostosis multiplex can be observed in all MPS, but is most pronounced in MPS I, II, IVA, VI and VII (Table 39.1).
- Facial dysmorphism, with progressive coarsening of facial features with flat face, depressed nasal bridge, bulging forehead, thickening of the tongue and lips, thick and often abundant hair (hirsutism), is a feature in MPS I, VI and VII and to a lesser extent in MPS II, III and IVA (Fig. 39.4 and Fig. 39.5). The cause of the

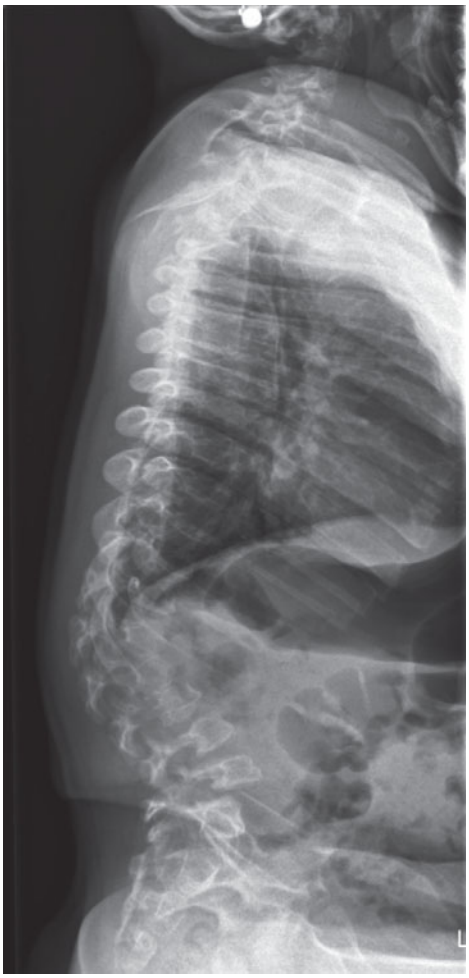


Fig. 39.2 Lateral X-ray of the spinal column of a 12 year old MPS I Hurler patient who had a successful hematopoietic stem cell transplantation at the age of 1 year. Abnormally shaped dysplastic vertebral bodies with thoracolumbar kyphosis. In addition, broad oar shaped ribs can be observed

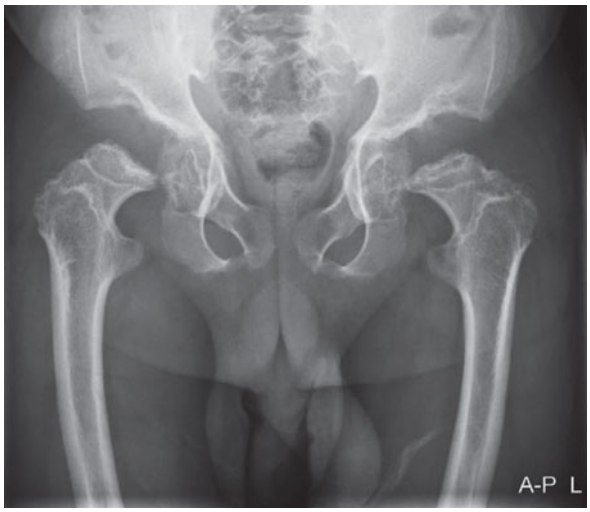


Fig. 39.3 Femoral and acetabular pathology due to dysostosis multiplex in a 15 year old boy with MPS IVA

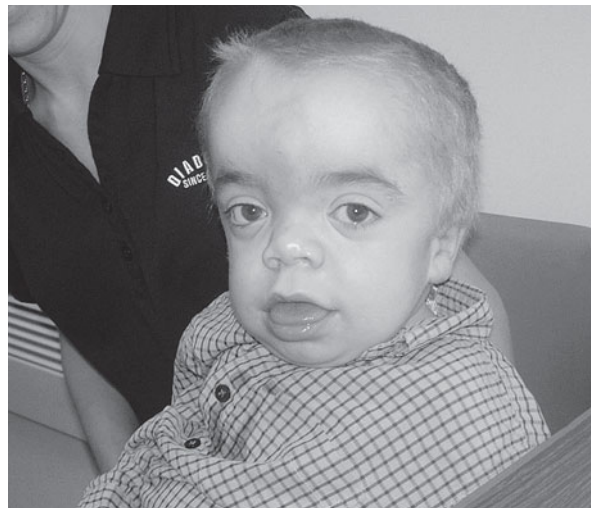


Fig. 39.4 Facial features of Hurler syndrome (MPS IH)

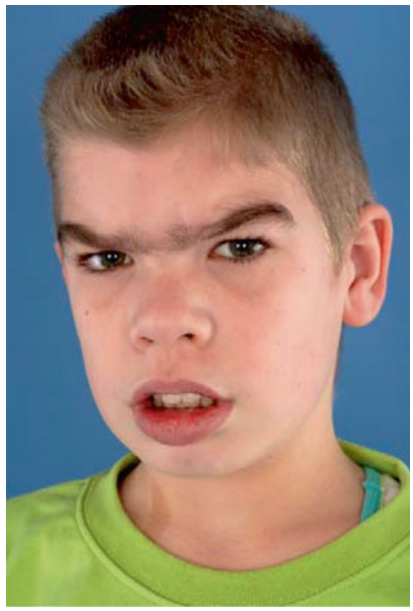


Fig. 39.5 Classical facial features in a 10 year old boy with Sanfilippo syndrome

dysmorphism is the combination of dysostosis multiplex in facial and cranial bones and subcutaneous storage of GAGs.

- Corneal clouding, probably as a result of accumulation of GAGs in keratocytes, is a common feature in MPS I, VI and VII but can also be detected in patients with III and IVA. In addition, glaucoma and optic nerve disease are common in MPS [1].
- Cardiac valve disease with thickening of valves leading to dysfunction (insufficiency and/or stenosis) is most common in MPS with accumulation of dermatan sulfate (MPS I, II, VI and VII), which is the most abundant GAG in heart valves [2]. A generally milder valvular disease also occurs in MPS IVA. In addition, cardiomyopathy and coronary artery stenosis has been reported in a number of MPS.
- Hepatosplenomegaly, which is often and erroneously, considered as a clinical hallmark of lysosomal storage disorders, is a common symptom in MPS I, II, VI and VII but can be minimal or absent in the more attenuated, slowly progressive phenotypes. In addition, hepatosplenomegaly is usually absent in MPS IVA (and probably IX) and often less prominent in MPS III.
- Central nervous system (CNS) disease with progressive cognitive impairment occurs in the severe, rapid progressive, phenotypes of MPS I and II and in MPS VII, and is a key feature of MPS III; all MPS in which heparan sulfate is one of the accumulating GAGs. Other disorders of the CNS include compression of the spinal cord due to stenosis of the spinal canal (MPS I, II and VI), atlanto-occipital instability (MPS IVA) and communicating hydrocephalus (MPS I, II and VI). Compression of the spinal cord in MPS I, II and VI is caused by dural thickening (pachymeningitis cervicalis) and thickening of the transverse ligaments even if the craniocervical junction. This often presents insidiously with loss of endurance before more obvious signs of ascending paralysis become apparent.
- Recurrent inguinal and umbilical hernia are a frequent finding in MPS I, II and VI and are probably related to abnormalities in connective tissue due to accumulation of dermatan sulfate in combination with increased intra-abdominal pressure as a result of hepatosplenomegaly.

Table 39.1 The 7 different MPS, with the most important clinical signs and symptoms

MPS Type	Disease name	Dysostosis multiplex	Valvular heart disease	Progressive cognitive impairment	Spinal cord compression	Enzyme deficiency	Gene	Main GAG accumulating (Urine screening)	Diagnostic enzyme assay
MPS IH	Hurler	+++	+++	+++	++	Alpha-L-iduronidase	*IDUA*	HS, DS	WBC
MPS IH-S	Hurler-Scheie	++	++	++	++				
MPS IS	Scheie	++	++	-	++				
MPS II	Hunter, neuronopathic phenotype	++	++	+++	++	Iduronate-2-sulfatase	*IDS*	HS, DS	Plasma
	Hunter, attenuated phenotype	++	++	-/+	++				
MPS IIIA	Sanfilippo A	+	+/-	+++	-	Heparan-*N*-sulfatase	*SGSH*	HS	WBC
MPS IIIB	Sanfilippo B	+	+/-	+++	-	*N*-acetyl glucosaminidase	*NAGLU*		Plasma
MPS IIIC	Sanfilippo C	+	+/-	+++	-	Acetyl CoA glucosamine *N*-acetyltransferase	*HGSNAT*		WBC
MPS IIID	Sanfilippo D	?	?	+++	-	*N*-acetyl-glucosamine-6-sulfatase	*GNS*		WBC
MPS IVA	Morquio A	+++	+	-	+++	*N*-acetylgalactosamine-6-sulfatase	*GALNS*	KS, CS	WBC
MPS IVB	Morquio B	+++	+	-	+++	β-galactosidase	*GLB1*	KS	WBC
MPS VI	Maroteaux-Lamy	+++	+++	-	+++	*N*-acetylgalactosamine-4-sulfatase	*ARSB*	DS	WBC
MPS VII	Sly	+++	++	+++	+	β-glucuronidase	*GUSB*	DS, HS, CS	WBC
MPS IX	-	?	?	?	?	Hyaluronidase	*HYALI*	HA	Cultured cells
Multiple sulfatase deficiency	Austin	++	+	+++	?	Formylglycine-generating enzyme	*SUMF1*	HS, DS	WBC

Accumulating GAGs: *HS*, heparan sulfate; *DS*, dermatan sulfate; *KS*, keratan sulfate; *CS*, chondroitin sulfate; HA, hyaluronic acid
Presence of signs and symptoms: -: never reported; +/-: vary rare; +: can be present; ++: often present; +++: almost always present; ?: not known (only very few patients reported)

MPS I: Hurler Syndrome (MPS IH), Hurler-Scheie Syndrome (MPS IH-S) and Scheie Syndrome (MPS IS)

Patients with MPS I have deficiency of the enzyme α-l-iduronidase (Fig. 39.1) and accumulate the GAGs dermatan and heparan sulfate (DS, HS). Infants with severe disease (MPS 1H, Hurler syndrome) are usually diagnosed in the 1st year of life [3]. Upper airway obstruction and frequent ear, nose and throat infections dominate the clinical picture at an early stage. The full clinical picture of short stature, hepatosplenomegaly, increasing facial dysmorphism (Fig. 39.4), cardiac disease, progressive learning difficulties and corneal clouding evolves over the 2nd and 3rd years of life. Signs and symptoms of dysostosis multiplex, leading to severe spinal and hip disease, are particularly pronounced in MPS IH. Patients with severe MPS I usually die before the age of 10 years as a result of cardiorespiratory disease. The severe MPS IH phenotype is much more prevalent than the more attenuated forms of the disease. At the other end of this clinical spectrum patients with Scheie syndrome (MPS IS) are intellectually normal, often reach an almost normal height and can live a normal life span, although many patients become disabled as a result of progressive joint disease, corneal opacity and cardiac valve lesions. The symptoms of patients between these two extremes can be extremely variable (Hurler-Scheie syndrome, MPS IH-S) and can include short stature, coarse facies, corneal clouding, joint stiffness, deafness and valvular heart disease. The onset of symptoms in MPS IH-S is observed between ages 3 and 8 years, and there is usually variable intellectual dysfunction. Untreated, the condition usually leads to death from cardiac or respiratory disease during the 2nd or 3rd decade of life.

Hunter Syndrome (MPS II)

MPS II (Hunter syndrome) differs from other MPS in that its inheritance is X-linked recessive and manifesting female heterozygotes are exceptionally rare. Like MPS I, this disorder is a spectrum with severely affected patients sharing many of the clinical signs and symptoms of patients with the severe form of MPS I, with the exception that the cornea remains clear in MPS II [4]. Severe patients appear to be more prevalent than mild, non-neuronopathic patients. Prominent Mongolian blue spots and a characteristic papular rash are other features that are prominent in severe MPS II. Patients with the more attenuated form of MPS II can live well into adult life, and a number have gone on to have their own families.

Sanfilippo Syndrome (MPS III)

There is a defect in the degradation of heparan sulfate in all of the four subtypes of MPS III (A, B, C and D, Sanfilippo syndrome). This results in a disorder which primarily affects the central nervous system, whereas somatic abnormalities are relatively mild [5], often leading to a considerable delay in diagnosis. The condition has three phases. The first phase, usually before diagnosis, consists of developmental delay alone, often primarily affecting speech. Some patients have ear disease and will fail hearing tests, which is the usual reason given, initially, for the speech delay. In the second phase (age 3-10 years) the illness is dominated by a severe behavioural disturbance, characterized by hyperactivity, challenging behaviour, and profound sleep disturbances. Abundant hair and hirsutism are frequently noted. The third phase of the illness (usually after the first decade) is associated with continuing loss of skills and motor functions, epilepsy and slow deterioration into a vegetative state, death usually occurring early in the 3rd decade. There are no absolute differences in clinical signs and symptoms between the MPS III subtypes. As in all the other MPS there is considerable heterogeneity, and not all patients will follow the same rapid progression of neurocognitive deterioration and patients with attenuated phenotypes have been reported [6][7]. Somatic manifestations in MPS III include mild dysostosis multiplex, ENT problems and, sometimes, hepatomegaly.

Morquio Disease (MPS IV)

MPS IV (Morquio disease) is caused by a defect in the degradation of keratan sulfate. In classic Morquio type A (MPS IVA, galactose-6-sulfatase deficiency) the patients are affected by a very severe skeletal dysplasia characterised by vertebral platyspondyly, hip dysplasia (Fig. 39.3) and genu valgum [8]. Intellectual impairment does not occur in MPS IVA, but the height prognosis is very poor, with affected adults ranging from 95 to 105 cm when fully grown. Odontoid hypoplasia is often associated with a high risk for atlanto-occipital subluxation which renders the patients vulnerable to acute or chronic cervical cord compression. In Morquio B (MPS IVB, β-galactosidase deficiency, GM1-gangliosidosis) the skeletal involvement is similar, but often not as pronounced, but patients may have central nervous system disease and a slowly progressive neurodegenerative course.

Maroteaux-Lamy Syndrome (MPS VI)

Patients with MPS VI (Maroteaux-Lamy syndrome) have somatic features resembling MPS I, but without neurological impairment [9]. As in all MPS, MPS VI shows a wide phenotypic spectrum of symptoms. The characteristic skeletal dysplasia includes short stature, dysostosis multiplex and degenerative joint disease. In addition, patients may have cardiac valve disease, hearing loss, obstructive sleep apnoea, corneal clouding, carpal tunnel disease, and inguinal or umbilical hernia. Cervical cord compression, communicating hydrocephalus, optic nerve atrophy and blindness may occur.

Sly Syndrome (MPS VII)

MPS VII (β-glucuronidase deficiency, Sly syndrome) is a very rare and variable disorder, which has hydrops fetalis as its most common presentation. Patients who survive pregnancy have a clinical disease similar to MPS I, including the same degree of clinical heterogeneity. In patients who survive the prenatal and neonatal presentation with hydrops, the hydrops may resolve followed by the typical MPS I like presentation.

MPS IX

MPS IX (hyaluronidase deficiency) appears to be extremely rare. This disorder was first reported by Natowicz et al. [10],

and has only been reported in 4 patients from 2 families and is clinically characterized by short stature and periarticular soft masses and symptoms resembling familial juvenal idiopathic arthritis.

- **Multiple Sulfatase Deficiency (Austin Disease)**

Multiple sulfatase deficiency is caused by mutations in the sulfatase modifying factor 1 (*SUMF1*) gene, leading to a deficiency of the FGE (formylglycine-generating enzyme) protein. As FGE is involved in the posttranslational activation of all sulfatases in the endoplasmatic reticulum, its deficiency leads to a deficiency of 17 different sulfatases, including all lysomal sulftatases. Multiple sulfatase deficiency is very rare and clinical signs and symptoms are variable, with progressive psychomotor retardation invariably present [11]. The course of the disease varies from rapid progressive to a slower evolution. Urinary GAG concentrations can be in the normal range, necessitating direct enzymatic assay of multiple sulfatases in the diagnostic workup.

39.1.2 Metabolic Derangement

MPS comprise a group of lysosomal storage disorders caused by a deficiency in one of the lysosomal enzymes (hydrolases) involved in the degradation of GAGs (Table 39.1). GAGs are linear polysaccharides ubiquitously distributed in extracellular matrices and on cell surfaces throughout the body and have many structural and complex biological functions. The classification of these polysaccharides is based on the repeating structural units in the molecule (Fig. 39.1). GAGs display a very high degree of heterogeneity with regards to molecular mass, disaccharide construction, and the degree of sulfation, important for the biological functions.

The GAGs dermatan sulfate, heparan sulfate, chondroitin sulfate and keratan sulfate (DS, HS, CS and KS) are long polysaccharides, generally covalently attached to specific core proteins that form proteoglycans. The GAG hyaluronan (HA) is not sulfated or protein-linked. There are important differences in the abundance of the different GAGs between different tissues, which appear to be partially related to the signs and symptoms of the different MPSs.

39.1.3 Genetics

All MPS are inherited as autosomal recessive traits except for MPS II, which is X-linked. All genes have been located and sequenced and genetic testing is available for all disorders for confirmation and for carrier detection, allowing genetic counselling and family planning. Genotype – phenotype correlation is generally poor in MPS. However, several mutations, including two nonsense mutations, can predict the severe Hurler phenotype in MPS I [11]. With the exception of only a few predictive mutations in MPS II, IIIA and MPS VI [6][13][14][15][16][17], there is only poor predictive value of genotyping in the other MPSs.

39.1.4 Diagnostic Tests

Diagnosis of MPS is generally performed by quantification of urinary GAGs by a dimethylmethylene blue dye binding assay (DMB) [18], followed by two-dimensional electrophoresis for qualification of the type of excreted GAGs. A positive screening is followed by analysis of the relevant enzyme activity in leucocytes or cultured skin fibroblasts. The Berry spot test lacks sufficient sensitivity and is generally regarded as obsolete for screening for MPS. However, false-negative results have also been reported using the DMB test, especially in MPS IVA and older MPS patients with a more attenuated course. Therefore, if there is sufficient clinical suspicion, a negative DMB test should be repeated and/or appropriate enzymatic studies should be done. Recent studies show that quantification of specific GAG derived disaccharides by liquid chromatography tandem-mass spectrometry (LC-MS/MS) in urine may provide a much more sensitive diagnostic screening method [19][20][21].

Enzymatic studies are the gold standard necessary to establish a final diagnosis. Subsequent mutational analysis will identify the mutations, which can sometimes be predictive of the phenotype in some MPSs, and may be used for genetic counselling of involved families. In case of a sulfatase deficiency, it is necessary to measure at least one other sulfatase, in order to exclude multiple sulfatase deficiency as the cause of the disease.

Pilot studies have shown the feasibility of newborn screening (NBS) via dried bloodspots for several MPS, applying high-throughput multiplexed enzyme assays [22][23][24]. As early initiation of treatment may lead to improved outcomes and because early diagnosis of MPS is generally difficult due to the rarity of the disorders and the unspecific initial signs and symptoms, all leading to a long diagnostic delay, diagnosis through NBS is probably the best approach for several of the MPS. Indeed, NBS is, or will soon be, introduced for MPS I in several countries.

39.1.5 Treatment and Prognosis

Multi-disciplinary symptomatic care remains the most important aspect of the management of patients with MPS. As MPS are all multi-systemic disorders, multi-disciplinary teams preferably should involve at least orthopedic surgeons, neurologists, neurosurgeons, ear, nose and throat surgeons, cardiologists, physical therapists, rehabilitation specialists and ophthalmologists. Metabolic paediatricians, internists and clinical geneticists are often essential in such teams to guarantee the necessary holistic approach. Expert opinion based guidelines for the management of MPS I, II and IVA have been published [25][26][27][28]. In addition, guidelines on the management of several clinical symptoms and procedures, including spinal cord compression in MPS IVA [29] and MPS VI [30]; orthopaedic management of extremities in MSP IVA [31]; thoracolumbar kyphosis in MPS I [32]; hip dysplasia in MSP I [33]; anaesthesia and airway management, including obstructive sleep apnoea syndrome, in MPS [34][35]; cardiac disease [2]

and eye disorders [1][36][37] may help to optimize treatment. Pain is a very common symptom in MPS [38] and this should be addressed separately. Severe behavioural problems and sleep disturbances, generally present in patients with MPS III, often require a tailored pharmacological treatment.

It is important to note that anaesthesia in patients with MPS needs special attention as it carries a high risk due to the upper airway obstruction resulting from anatomical changes due to the dysostosis multiplex and GAG deposition in soft tissues, restrictive pulmonary disease, cardiovascular disease and potential instability of the atlanto-occipital joint [34]. Therefore, anaesthesia should be performed by an experienced team and after full information about the clinical signs and symptoms of the individual patient has been acquired.

In addition, patients with MPS may have an increased risk for peri- or post-surgical development of spinal cord lesions, remote from the site of surgery, leading to paraplegia [39][40][41]. This may be caused by a combination of low mean arterial pressure in conjunction with potentially compromised arterial spinal circulation, the duration of the surgery and the position on the operating table. Stringent preoperative evaluation, careful positioning on the operation table, perioperative monitoring of motor-evoked potentials and somatosensory-evoked potentials and prevention of low blood pressure during the operation are probably essential to prevent these complications. As a result of the complexity of the disorders, necessitating the presence for a dedicated multi-disciplinary team, patients with MPS are best managed at specialized treatment centres.

Disease modifying treatment options are available for several of the MPS and consist of hematopoietic stem cell transplantation (HSCT) and intravenous enzyme replacement therapy (ERT). Clinical studies on the efficacy of intravenous ERT in MPS VII as well as other approaches, including gene therapy, intra-thecal enzyme therapy and small molecule therapy are currently ongoing, and may lead to a significant improvement of the clinical outcome of MPS over the next years.

Hematopoietic Stem Cell Transplantation

HSCT for an MPS was first performed in the UK over 30 years ago in a patient with MPS I Hurler phenotype [42] and this procedure is now the preferred treatment strategy for these patients, if diagnosed before the age of approximately 2.5 years [43]. The success of HSCT has dramatically improved over the last decades, with a marked decrease in morbidity and mortality and an improved rate of engraftment, due to changes in chemotherapeutic conditioning, supportive care and stem cell source [44]. A recent large multi-centre study showed that the long-term outcome of Hurler patients after HSCT improves after early referral for HSCT, using noncarrier donors and regimens designed to achieve full-donor chimerism [45]. However, there is still considerable residual disease burden in many patients, often related to the musculoskeletal system which apparently responds less well to HSCT. Early diagnosis of MPS I Hurler patients through NBS may lead to early transplantation, thus improving the outcome of HSCT. HSCT is also performed in patients with the severe neuropathic phenotype of MPS II [46], and in patients with MPS VI [47]. However, as only relatively small patient series have been studied, the exact place of HSCT in the treatment of MPS II and VI needs to be further delineated. HSCT has been shown to be ineffective in MPS III [48] and IVA [49].

Enzyme Replacement Therapy

Intravenous ERT for MPSs was first approved for clinical use in MPS I [50] and subsequently for MPS II [51], VI [52] and IVA [53]. These pivotal trials, in combination with long-term follow up studies, have demonstrated that ERT can effectively treat a number of symptoms resulting in improvement on the 6 minute walk test, improved joint mobility and pulmonary function with a reduction of liver and spleen size, improved growth and a decrease in urinary GAG excretion, which may all lead to improved survival [54][55]. However, all studies show that there is generally still significant residual disease burden despite long-term ERT. Studies comparing the effects of ERT in sibships, which include older siblings treated with ERT after the development of significant clinical symptoms, and younger siblings treated before the onset of significant symptomatology, have demonstrated that an early start of ERT, i.e. before irreversible changes have occurred, leads to a much more favourable outcome [57][58][59][60]. This again highlights the importance of an early diagnosis, allowing early start of treatment. As intravenously administered enzyme does not cross the blood–brain barrier, at least not in sufficient amounts at the labelled doses, intravenous ERT will not result in neurocognitive benefits, which limits its effectiveness in MPS I Hurler and Hurler-Scheie, as well as in the severe, neuronopathic, form of MPS II.

The formation of alloantibodies against the recombinant enzyme has been demonstrated to occur in most patients during ERT and these antibodies may interfere with the enzymatic activity and/or with cellular uptake. Studies in MPS I and MPS II indeed demonstrated interference of antibodies with biochemical and clinical effects of the infused enzyme [21][61][62].

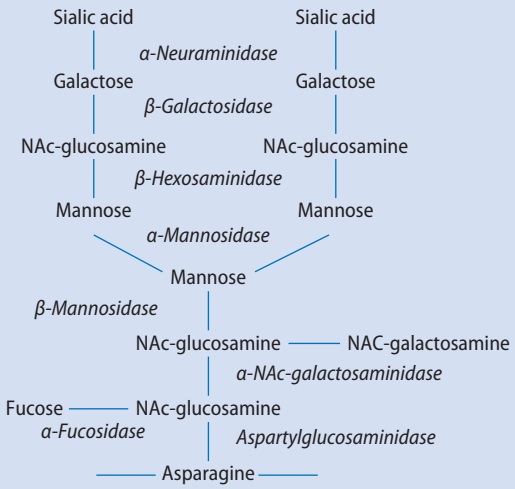
The extremely high costs involved with long-term ERT has led to discussions with reimbursing authorities on criteria for cost-effectiveness. In some countries, decisions on reimbursement of drugs depend on the incremental costs per quality-adjusted life year (QALY). It is clear, however, that for ultra-rare diseases such as MPS other economic models need to be used [63][64]. Evaluation of long-term efficacy of ERT, using robust and clinically relevant outcome measures, will be essential to demonstrate the efficacy of these treatments in the ›real world‹ i.e. outside the domain of trials, and this will only be feasible through international cooperation.

39.2 Oligosaccharidoses and Mucolipidoses

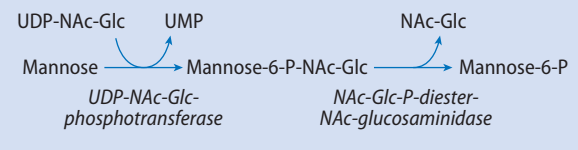
▶ Oligosaccharides/Glycoproteins.

Oligosaccharides/Glycoproteins

Almost all the secreted and membrane-associated proteins of the body are glycosylated, as well as numerous intracellular proteins, including the lysosomal acid hydrolases. A great variety of oligosaccharide chains are attached to the protein backbone via the hydroxyl group of serine or threonine (*O*-linked), or via the amide group of asparagine (*N*-linked), to form tree-like structures (◘ Fig. 39.6). The chains usually have a core composed of *N*-acetylglucosamine and mannose, often contain galactose, fucose and *N*-acetylgalactosamine, and frequently possess terminal sialic acids (*N*-acetylneuraminic acid). Oligosaccharide chains with a terminal mannose-6-phosphate are involved in the targeting of lysosomal enzymes to lysosomes. This recognition marker is synthesised in two steps from UDP-*N*-acetylglucosamine (◘ Fig. 39.7). Deficiencies of the enzymes required for the degradation of the oligosaccharide chains cause oligosaccharidoses (glycoprotein storage diseases). Defects of the synthesis of the mannose-6-phosphate recognition marker result in the mislocalisation of lysosomal enzymes. Defects of the synthesis of the oligosaccharide chains are discussed in ▶ Chapter 38.



◘ **Fig. 39.6** General composite example of a glycoprotein oligosaccharide chain. *NAc*, N-acetyl. Degradative enzymes are listed in *italics*



◘ **Fig. 39.7** Synthesis of mannose-6-phosphate recognition marker. *NAc-Glc*, N-acetylglucosamine; *UDP*, uridine diphosphate; *UMD*, uridine monophosphate. Enzymes are listed in *italics*

Oligosaccharidoses or glycoprotein storage disorders share many features in common with MPS disorders, but the urine GAG screen is normal or only shows nonspecific abnormalities. For convenience, the mucolipidoses (ML), disorders that combine clinical features of MPS and sphingolipidoses, are also considered here. These include sialidosis I (ML I), which is caused by α-neuraminidase deficiency, and mucolipidosis II (ML II) and its milder allelic variant mucolipidosis III (ML III), both of which are caused by the deficiency of UDP-*N*-acetylglucosamine-1-phosphotransferase, an enzyme not involved in lysosomal degradation but in the synthesis of a recognition marker.

39.2.1 Clinical Presentation

▪ Mannosidosis

A deficiency of α-mannosidase gives rise to the extremely variable disorder α-mannosidosis. A mild Hurler phenotype, associated with variable learning difficulties, hepatosplenomegaly, deafness and skeletal dysplasia, is complicated by an immune deficiency which can dominate the clinical progression of the disease [65]. β-Mannosidosis, which is due to a deficiency of β-mannosidase, is much less prevalent than α-mannosidosis and is very variable, but severe learning difficulties, challenging behaviour, deafness and frequent infections are relatively common [66].

▪ Fucosidosis

Patients with fucosidosis lack the typical facial dysmorphism seen in the other disorders described in this chapter. Deficiency of α-fucosidase activity leads to a variable neurodegenerative disorder, often with seizures and mild dysostosis. Affected patients often exhibit prominent and widespread angiokeratomas, which often progress with age [67].

▪ Galactosialidosis

Galactosialidosis is caused by combined deficiency of the lysosomal enzymes β-galactosidase and α-neuraminidase. The combined deficiency has been found to result from a defect in protective protein/cathepsin A (PPCA), an intralysosomal protein which protects these enzymes from premature pro-

teolytic processing. The clinical features of affected patients includes hydrops fetalis [68] as well as a more slowly progressive disorder associated with learning difficulties, dysostosis multiplex and corneal opacity.

Transport Defects

The allelic disorders Salla disease (Finnish type sialuria) and infantile free sialic acid storage disease (ISSD) result from mutations in *SLC17A5* coding for sialin, a lysosomal membrane protein that transports sialic acid out of lysosomes. ISSD has a severe phenotype with infantile onset (including severe visceral involvement, cardiomyopathy, skeletal dysplasia and learning difficulties), while the Finnish variant, Salla disease, has a milder phenotype with later onset. Both disorders cause learning difficulties, but ISSD is generally fatal in early childhood whilst patients with Salla disease often survive into middle age [69]. Mutations in *SLC17A5* have also been reported in patients with mental retardation and hypomyelination [70] but while free sialic acid was increased in CSF, the urinary excretion of free sialic acid was normal. Finally, cerebellar ataxia with elevated CSF free sialic acid (CAFSA) has been reported in five patients who in addition to the ataxia had peripheral neuropathy and cognitive decline [71]. The molecular basis of this new disease is yet to be established.

Sialuria (French type)

Sialuria is caused by mutations in *GNE,* encoding uridinediphosphate-N-acetylglucosamine 2-epimerase (UDP-GlcNAc 2-epimerase). As a result of a failure of feedback inhibition there is excessive free sialic acid synthesised. Only 5 patients with the disorder have been described; the inheritance is thought to be autsomal dominant. Clinical features include hepatosplenomegaly, coarse facial features, and varying degrees of developmental delay. The disorder differs from sialidoses in that there is accumulation and excretion of free sialic acid and neuraminidase activity is normal or increased [72].

Aspartylglucosaminuria

This disorder is due to a deficiency of aspartylglucosaminidase and has a higher prevalence in Finland but is rare in other countries. A characteristic facial dysmorphism has been described and there is slowly progressive psychomotor retardation, with death in middle age [73].

Schindler Disease

This disease, due to α-*N*-acetylgalactosaminidase deficiency, is a rare, clinically heterogeneous disorder with a wide spectrum, including an early-onset neuroaxonal dystrophy and a late-onset form characterised by abundant angiokeratoma. It is unclear how many of the manifestations seen in the reported early onset patients are related to α-*N*-acetylgalactosaminidase [74].

Sialidosis (α-Neuraminidase Deficiency, ML I)

This disorder is characterised by the progressive lysosomal storage of sialic acid-rich glycopeptides and oligosaccharides caused by a deficiency of the enzyme α-neuraminidase. It has also been termed Mucolipidosis type 1 (ML I) or cherry red spot myoclonus syndrome. The sialidoses are distinct from the sialurias (infantile sialic acid storage disease, ISSD and Salla disease), in which there is storage and excretion of free sialic acid rather than bound sialic acid. The clinical spectrum in sialidosis ranges widely, from a presentation with hydrops fetalis to slowly progressive seizure disorders with myoclonus.

I-Cell Disease (ML II) and Pseudo-Hurler (ML III)

ML II and ML III are caused by deficiency of UDP-*N*-acetylglucosamine-1-phosphotransferase. Patients with ML II have a Hurler-like phenotype often presenting in the newborn period or even prenatally with fractures. There is often a very severe skeletal dysplasia, and patients often have a small head circumference owing to premature sutural synostosis. Cardiomyopathy and severe coronary artery disease can be present. Most children die of respiratory failure in the 1st decade. ML III is extremely variable, and many patients survive into adult life with little or no learning difficulty. Skeletal dysplasia, including an erosive arthropathy affecting ball-and-socket joints (shoulder and hips), can be extremely disabling in adults with ML III.

Mucolipidosis type IV (ML IV)

ML IV is caused by mutations in *MCOLN1* coding for mucolipin-1, a protein found in the membrane of lysosomes and endosomes and which is involved in trafficking of lipids and proteins. The disorder is characterised by developmental delay and progressive visual impairment. Of patients described with ML IV, over 80 have been Ashkenazi Jews [75].

Psychomotor delay is moderate to severe and usually becomes apparent during the first year of life. Affected individuals have intellectual disability, limited or absent speech, difficulty chewing and swallowing, hypotonia that gradually turns into spasticity, and problems controlling hand movements. Most patients are unable to walk independently. In about 15% of affected individuals, the psychomotor problems worsen over time. Vision may be normal at birth in people with typical ML IV, but it becomes increasingly impaired during the first decade of life with corneal clouding and progressive retinal dystrophy. By their early teens, affected individuals have severe vision loss or blindness. There is no treatment available.

Pycnodysostosis

Cathepsin K is a lysosomal cysteine proteinase abundant in osteoclasts, where it plays a vital role in the resorption and remodelling of bone. A deficiency of this enzyme was shown to be associated with the skeletal dysplasia pycnodysostosis, the disorder thought to be the cause of Toulouse-Lautrec's disability [76]. In addition to short stature (150–160 cm), affected individuals have a generalised increase in bone density and wormian bones of the skull with open fontanelles, partial absence of the distal phalanges and bone fragility. Dental abnormalities and life threatening upper airway obstruction are also common.

Table 39.2 The oligosaccharidoses and mucolipidoses with their most important features

Disease name	Dysostosis multiplex	Progressive cognitive impairment	Angio-keratoma	Hepato-spleno-megaly	Enzyme/protein deficiency	Gene	Diagnostic test
α-Mannosidase	++	+	-	+	α-Mannosidase	*MAN2B1*	WBC
β-Mannosidase	+	+++	-	+	β-Mannosidase	*MANBA*	WBC
Fucosidosis	+	++	+++	-	Fucosidase	*FUCA1*	WBC
Galactosialidosis	++	++	-	+	PPCA	*CTSA*	Fibro + DNA
Salla disease	+	++	-	+	Sialin	*SLC17A5*	Fibro +DNA
ISSD	+++	+++	-	+++	Sialin	*SLC17A5*	Fibro +DNA
Sialuria (French type)	+	+	-	+	UDP-GlcNAc 2-epimerase	*GNE*	DNA
Aspartylglucos-aminuria	+	++	-	-	Aspartylglucosaminidase	*AGA*	WBC
Schindler disease	-	?+	++	-	α-N-acetylgalactos-aminidase	*NAGA*	WBC
Sialidosis (MLI)	++	++	-	+	α-Neuraminidase	*NEU1*	Fibro
Mucolipidosis II and III	+++	+	-	-	UDP-N-acetylglucos-amine-1-phosphotrans-ferase	*GNPTAB*, *GNPTAG*	Plasma
Mucolipidosis type IV	-	++	-	-	Mucolipin-1	*MCOLN1*	DNA
Pycnodysostosis	+++	-	-	+	Cathepsin K	*CTSK*	DNA

MLI, mucolipidosis type I; *ISSD*, infantile sialic acid storage disease; *PPCA*, protective protein/Cathepsin A; *WBC*, white blood cell enzyme assay; *Fibro*, fibroblast enzyme assay; *D*NA, mutation analysis; *Plasma*, plasma enzyme assay.

39.2.2 Metabolic Derangements

The vast majority of disorders are defects of single enzymes involved in the degradation of oligosaccharides (Fig. 39.5). The exceptions are ML II (and III), the transport defects (ISSD and Salla disease) and galactosialidosis. ML II and III share the same post-translational modification defect due to the absence of UDP-*N*-acetylglucosamine-1-phosphotransferase (Fig. 39.6), the enzyme necessary for synthesis of the marker required to target newly formed lysosomal enzymes to the lysosomes. As a result, the enzymes are mistrafficked to the extracellular space. In the transport defects the gene encoding the lysosomal membrane protein sialin is defective. Urinary excretion of free sialic acid is considerably elevated in these conditions. The combined defect of neuraminidase and β-galactosidase (galactosialidosis) is caused by a lack of the protective protein cathepsin A (PPCA), which is responsible for stabilisation of the enzyme complex within the lysosomes and their protection from rapid proteolytic degradation. PPCA may also have a role in the protection of elastin-binding protein (EBP) at the cell surface [77].

What is not clear is how the metabolic derangement leads to the clinical and functional defects seen in the patients, especially those affecting the central nervous system. The clinical phenotype partially depends on the type and amount of storage substance, but the pathogenic cascades leading to disease in the brain remain poorly understood.

39.2.3 Genetics

The disorders in this group are all inherited in an autosomal recessive manner. Genetic diagnosis is possible for all the oligosaccharidoses and in some cases (eg pycnodysostosis) is the only means to conclusively diagnose these cases. All families should be referred for genetic counselling and for carrier detection where available and necessary, and also given information on the availability of prenatal diagnosis and preimplantation genetic diagnosis.

39.2.4 Diagnostic Tests

The diagnosis of most oligosaccharidoses is based on clinical suspicion, supported by appropriate clinical and radiological examinations followed by urinary examination for oligosaccharide excretion and then specific enzyme assay, usually on white blood cells (■ Table 39.2). Thin-layer chromatography is used to detect abnormal urinary excretion of oligosaccharides and sialic acid. All urinary screening tests can give false-negative results, especially in older patients with an attenuated clinical course, and most have normal urine GAGs. Urinary sialic acid testing is less widely available and cases may be missed. Urine screening will not identify all oligosaccharidosis patients and so a combination of urine screening in a reliable laboratory and white cell enzyme screens is the optimal approach. Pycnodysostosis can only be diagnosed by mutation analysis currently. Patients with ML II and III are often missed on urinary testing, and therefore the definitive diagnosis should be confirmed by the finding of significant elevations in a number of plasma lysosomal enzyme levels.

39.2.5 Treatment and Prognosis

Palliative and supportive care remains an important aspect of the holistic management of patients with these disorders. For most conditions it is the only available therapy. All the disorders described in this section (perhaps with the exception of some patients with Schindler disease) have a progressive course, some very life-limiting. Multidisciplinary management is essential, and patients are best managed in specialist centres with access to a comprehensive range of clinical and supporting services. Ear, nose and throat surgery, orthopaedic review and neurosurgical intervention may all be indicated at some stage in affected patients. The anaesthetic considerations must not be forgotten, as the facial dysmorphism, skeletal dysplasia and upper airways obstruction present in many of these patients can be a challenge, although usually less severe than in the MPS diseases.

Attempts at disease modifying treatment for this group of disorders currently include HSCT and ERT. HSCT has been attempted in a number of cases of α-mannosidosis, aspartylglucosaminuria and in fucosidosis, with evidence of at least partial correction of the biochemical defect, and some evidence in mannosidosis for neurodevelopmental benefit (especially if transplanted early [78]). The role of HSCT in this group still remains unclear. ERT is in development for α- mannosidosis, but as so many of these disorders involve neurological manifestations, it is unclear how successful ERT can be for this group of disorders.

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Inborn Errors of Non-Mitochondrial Fatty Acid Metabolism Including Peroxisomal Disorders

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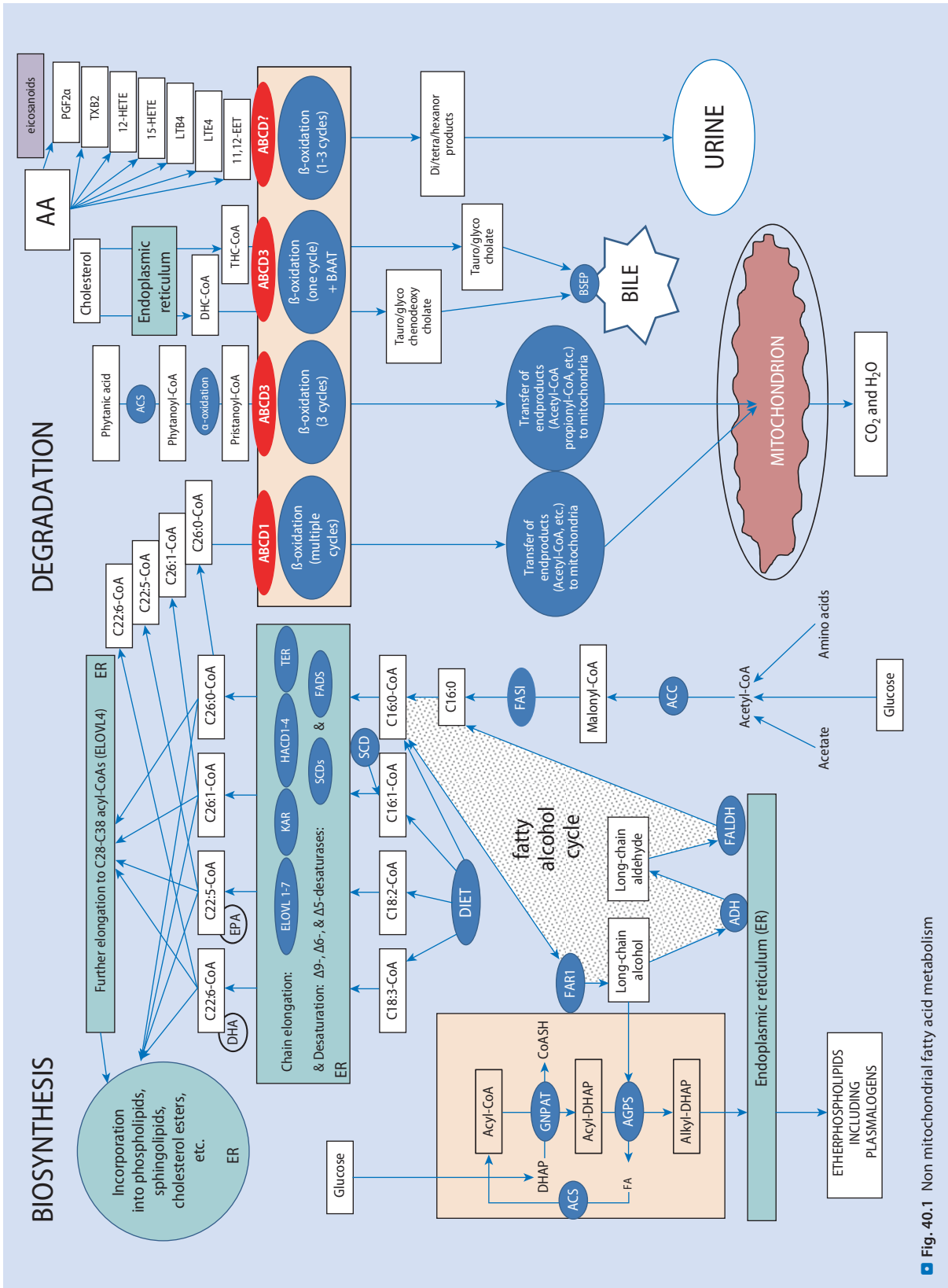


Fig. 40.1 Non mitochondrial fatty acid metabolism

Fatty Acids

Fatty acids are hydrocarbon chains which terminate in a carboxylic acid group (R-COOH) and may vary in length, degree of unsaturation, and number and types of side-chain substitutions (methyl-fatty acids, hydroxy-fatty acids, etc.). In principle, mammals including humans, can synthesize fatty acids *de novo* from acetyl-CoA via the FAS (fatty acid synthetase)-complex of which there are two: one in the cytosol (FASI) and one in mitochondria leading to lipoic acid (FASII) (▶ Chapter 23). The end product at least of cytosolic FAS (FASI) is palmitic acid (C16:0), a saturated fatty acid which can then be converted into longer-chain saturated fatty acids via chain elongation (see Fig.40.1). Chain elongation occurs by cycling through a four-step process including: condensation, reduction, dehydration and reduction again. In fact, fatty acid chain elongation is chemically identical to fatty acid beta-oxidation except that the two four-step pathways operate in reverse directions. The first step in the fatty acid chain elongation pathway located in the endoplasmic reticulum is mediated by one of seven different elongases (ELOVL1 – ELOVL7) each with a different substrate specificity. Subsequent steps are catalysed by one 3-ketoacyl-CoA reductase (KAR), four 3-hydroxyacyl-CoA dehydratases (HACD1-4) and one 2,3-*trans*-enoyl-CoA reductase (TER). The same system is also responsible for the formation of very-long-chain mono- and polyunsaturated fatty acids with C24:1 (nervonic acid) and C26:1, C22:5 (docosapentaenoic acid), and C22:6 (docosahexaenoic acid) as main end products derived from C18:1 (oleic acid), C18:2 (linoleic acid), and C18:3 (linolenic acid), respectively. Oleic acid can be synthesized *de novo* from stearic acid (C18:0) by a delta 9-desaturase (SCD). However, linoleic acid and linolenic acid cannot be synthesized endogenously and are thus essential fatty acids since mammals, including humans, unlike plants, do not possess delta 12- and delta 15-desaturases. The end products of fatty acid chain elongation are incorporated into a variety of other lipids including phospholipids (chapter 34) and cholesterol esters, but also undergo degradation as a natural part of their homeostatis, notably by beta-oxidation in peroxisomes (■ Fig. 40.1). Fatty acids can also be converted into the corresponding alcohols. The enzymes involved are (1) localized in the peroxisomal membrane with the catalytic (enzymatic) site facing the cytosol (■ Fig. 40.1), (2) react with different acyl-CoAs rather than with the free fatty acids and (3) use NADPH to drive synthesis of the alcohols. In mammals, two such enzymes called fatty acyl-CoA reductases (FAR1 and FAR2) have been identified. Since the peroxisomal membrane is freely permeable to low molecular weight metabolites up to 300-400 Dalton, the long-chain fatty alcohols produced by FAR1 and FAR2 as required for the synthesis of etherphospholipids (EPLs) can cross the peroxisomal membrane unhindered to enter the EPL-biosynthetic pathway at the level of the peroxisomal enzyme alkyl glycerone 3-phosphate synthase (AGPS; formerly known as ADHAPS) (■ Fig. 40.1). The importance, at least of FAR1, for etherphospholipid biosynthesis is exemplified by the recent identification of patients with FAR1 deficiency, in whom etherphospholipid biosynthesis was found to be completely deficient (see main text). Long-chain fatty alcohols also undergo retro conversion back to the corresponding fatty acids via an ill-defined alcohol dehydrogenase, followed by the enzyme fatty aldehyde dehydrogenase (FALDH/ALDH3A2) localized in the endoplasmic reticulum and deficient in Sjögren Larsson syndrome (SLS). Activation of the fatty acids produced by FALDH completes this cyclic process named the »fatty-alcohol cycle«. Importantly, the peroxisomal beta-oxidation system is not only involved in the degradation of the different very-long-chain fatty acids (VLCFAs; C≥22) albeit saturated, mono- or polyunsaturated, but also plays an essential role in the formation of bile acids from cholesterol by catalysing the chain-shortening of dihydroxycholestanoic acid (DHCA) and trihydroxycholestanoic acid (THCA), and the degradation of pristanic acid as derived from phytanic acid by alpha-oxidation. Moreover, the peroxisomal beta-oxidation system also catalyses the degradation of a host of other substrates including a variety of different eicosanoids as derived from arachidonic acid via one of three different pathways described in detail in ■ Fig. 40.2. The eicosanoids which are beta-oxidized in peroxisomes and thereby undergo inactivation followed by excretion into urine, include: PGF2-alpha, 8-iso-PGF2-alpha, thromboxane B2, 12-HETE, 15-HETE, LTB4, LTE4, and 11,12-EET (see main text for detailed information).

Degradation of all these metabolites in peroxisomes is mediated by two different acyl-CoA oxidases (first step), two bifunctional proteins (second and third step) and two thiolases (last step) (▶ Section 40.2). Furthermore, import of all these fatty acids and fatty acid derivatives into peroxisomes is mediated by 3 distinct half-ABC-transporters (ABCD1-3) of which ABCD1 catalyses the import of the CoA-esters of VLCFAs whereas ABCD3 (PMP70) mediates the uptake of pristanic-, phytanic-, and di- and trihydroxycholestanoic acid-CoA into peroxisomes.

Inherited defects in each of the metabolic pathways (▶ Fatty acids) depicted in Fig. 40.1 have been identified through the years. These include: (1) the etherphospholipid biosynthesis deficiencies; (2) the disorders of peroxisomal fatty acid alpha- and beta-oxidation; (3) the defects in chain elongation; (4) the disorders of eicosanoid metabolism; and (5) a few individual deficiencies including Sjögren Larsson syndrome (FALDH/ALDH3A2-deficiency), and bile acid-CoA: amino acid N-acyltransferase (BAAT) deficiency. The clinical signs and symptoms of each of the different (groups of) disorders is very diverse which explains why we will deal with each of them separately.

40.1 Disorders of Etherphospholipid Biosynthesis

The classical phenotype of a patient affected by a defect in the biosynthesis of etherphospholipids is Rhizomelic Chondrodysplasia Punctata (RCDP). Patients with classical RCDP have skeletal dysplasia characterized by rhizomelia, chondrodysplasia punctata (stippled calcification in epiphyseal cartilage), bone abnormalities, profound growth retardation and limited joint mobility, congenital cataracts and facial abnormalities including a high forehead, flat midface and small upturned nose. Until very recently only three different disorders of EPL-biosynthesis were known, including: (1) PEX7 deficiency; (2) glycerone 3-phosphate: acyltransferase (GNPAT) deficiency, and (3) alkylglycerone 3-phosphate synthase (AGPS) deficiency but in the last two years two additional disorders of EPL biosynthesis have been identified including FAR1 deficiency [1] and PEX5L deficiency [2].

40.1.1 Peroxin 7 (PEX7) Deficiency (RCDP Type 1)

RCDP type 1 as caused by mutations in *PEX7* is most frequent among the cohort of RCDP patients investigated until now (> 150 patients). *PEX7* codes for one of two peroxisomal so-called cycling receptors and targets a subgroup of proteins equipped with a PTS2-signal, made up of a stretch of nine conserved amino acids, to the peroxisome. Only three PTS2-containing peroxisomal enzymes are known so far and as a consequence of the functional deficiency of the PEX7 protein, peroxisomal 3-keto acyl-CoA thiolase, AGPS and phytanoyl-CoA hydroxylase are not imported into peroxisomes which results in defects in plasmalogen biosynthesis and alpha oxidation, respectively. The deficiency of peroxisomal 3-keto acyl-CoA thiolase 1 has no functional consequences for VLCFA degradation because the second peroxisomal thiolase (SCPx) can also handle 3-keto-VLCFAs. The clinical phenotype associated with mutations in *PEX7* has turned out to be markedly heterogeneous, ranging from the classical phenotype as described above to much milder phenotypes including RCDP without rhizomelia [3], bone dysplasia with only mild intellectual deficiency [4] to a Refsum-like phenotype [5].

40.1.2 Glycerone 3-Phosphate Acyltransferase (GNPAT) Deficiency (RCDP type 2)

RCDP type 2 is caused by mutations in *GNPAT* which codes for one of the two intraperoxisomal enzymes involved in EPL biosynthesis, i.e. GNPAT (DHAPAT). Some ten patients with this subtype have been reported in literature, all of whom presented with the characteristic severe clinical phenotype of RCDP with most patients dying in the first decade of life.

40.1.3 Alkylglycerone 3-Phosphate Synthase (AGPS) Deficiency (RCDP Type 3)

RCDP type 3 is caused by mutations in *AGPS* which codes for the second intraperoxisomal enzyme involved in EPL-biosynthesis, i.e. AGPS (ADHAPS). This enzyme catalyses the formation of the characteristic ether bond in etherphospholipids. RCDP type 3 is even less frequent when compared to type 2; five patients have been reported in literature, all of whom showed the severe lethal RCDP phenotype [6].

40.1.4 PEX5L-Deficiency (RCDP Type 4)

RCDP type 4 was only recently reported in four patients from two independent families. The defect is caused by a frame shift mutation located in the *PEX5L*-specific exon 9 which results in loss of only the PEX5L isoform whereas the PEX5S isoform is still expressed. Since both PEX5S and PEX5L recognize PTS1-targeted proteins, PTS1 protein import is normal. However, PEX5L also acts as a co-receptor for PTS2-targeted proteins by binding to PEX7 which explains why the loss of PEX5L results in deficient PEX7-mediated import of PTS2 targeted proteins. This also explains why the clinical and biochemical characteristics of PEX5L-deficient patients are identical to those observed in RCDP type 1 [2].

Metabolic Derangement

In all four types of RCDP EPL-biosynthesis is deficient which leads to a deficiency of plasmalogens in all tissues including erythrocytes albeit not in mildly affected patients. In types 1 and 4 there is also accumulation of phytanic acid which is age and diet dependent because of the deficiency of phytanoyl-CoA hydroxylase, one of the other PTS2 proteins.

Genetics

All types of RCDP are autosomal recessive disorders and the molecular defect in each of the different types of RCDP have been established. This includes two large series of RCDP type 1 patients [7][8].

Diagnostic Tests

Erythrocyte plasmalogen analysis is the single most important diagnostic test to ascertain whether a patient is affected by one of the types of RCDP or not. In the majority of patients erythrocyte plasmalogen levels are markedly decreased although in more mildly affected patients levels are only partially reduced and may even be normal. An additional diagnostic metabolite is phytanic acid, although in patients with milder phenotypes levels may be normal [5]. If erythrocyte plasmalogen levels are deficient, studies in fibroblasts need to be performed to discriminate between the different types of RCDP, followed by sequence analysis of the relevant gene.

Treatment and Prognosis

At present, there is no realistic therapy for either form of RCDP although studies in mouse models have shown that

plasmalogens may be corrected by alkylglycerol supplementation but only in peripheral tissues and not in brain [9].

40.1.5 Fatty Acyl-CoA Reductase 1 (FAR1) Deficiency

FAR1 deficiency has only been described very recently in three patients including two sibs at five years of age, and one patient 19 years of age, who all presented with severe intellectual disability, cataracts, epilepsy and growth retardation [1]. The gene involved was identified by exome sequencing. Interestingly, despite the marked reduction in erythrocyte plasmalogen levels no rhizomelia nor calcific stippling was observed in any of the patients.

40.2 Disorders of Peroxisomal β-Oxidation

As shown in Fig. 40.1 the peroxisomal beta-oxidation system plays an indispensable role in the oxidation of a range of fatty acids including: (1) very-long-chain fatty acids; (2) pristanic acid; (3) the bile acid intermediates di- and trihydroxycholestanoic acid, and (4) a range of eicosanoids, including PGF2-alpha, 8-iso-PGF2-alpha, thromboxane B2, 12-HETE, 15-HETE, LTB4, LTE4, and 11,12-EET. In patients with a defect in the biogenesis of peroxisomes as in Zellweger syndrome, the oxidation of all these fatty acids is impaired whereas in patients with a single enzyme or transport protein defect abnormalities are restricted to some fatty acids only. Oxidation of all these carboxylic acids is mediated by two distinct acyl-CoA oxidases (ACOX1 & 2), two bifunctional proteins (LBP & DBP) and two thiolases (ACAA1 & SCPx). Peroxisomes are unable to degrade fatty acids to completion with the number of beta-oxidation cycles differing per carboxylic acid – for instance: one cycle of beta-oxidation for di- and trihydroxycholestanoic acid, three cycles of beta-oxidation for pristanic acid, and one, two, or three cycles of beta-oxidation for several of the eicosanoids to produce the corresponding dinor-, tetranor-, and hexanor-derivatives (Fig. 40.1). The peroxisomal fatty acid beta-oxidation disorders can be subdivided into two groups, including: (1) the single peroxisomal enzyme or transport protein deficiencies, and (2) the generalized peroxisomal beta-oxidation deficiencies. X-linked adrenoleukodystrophy (X-ALD) is the prototype of the first group of disorders whereas Zellweger syndrome is the prototypic disorder of the second group.

40.2.1 X-Linked Adrenoleukodystrophy

Clinical Presentation

This is the most common peroxisomal disorder (1/20,000 males and females). The clinical picture can vary considerably even within the same family [10]. The phenotype correlates with neither the genotype nor the biochemical abnormality in plasma or fibroblasts (accumulation of saturated VLCFA). The childhood cerebral form is the most severe (35% of all X-ALD phenotypes in males), with onset of neurological symptoms between 4 and 12 years of age, leading to a vegetative state and death in a few years. Affected males may present with school failure, an attention deficit disorder or behavioural changes (due to visuospatial deficits and/or central hearing loss) as the first manifestations, followed by severe visual and hearing impairment, quadriplegia and cerebellar ataxia. Seizures or signs of intracranial hypertension are not uncommon. Hypoglycaemic and/or salt-losing episodes with increased skin pigmentation reflect adrenal insufficiency, which may precede, coincide with or follow the onset of neurological involvement. Most childhood patients show characteristic symmetrical cerebral lesions involving the white matter in the parietal and occipital lobes on computerised tomography (CT) or MRI. Following intravenous injection of contrast medium, a garland-like contrast enhancement adjacent to demyelinating lesions reflects an inflammatory reaction that coincides with rapid neurological deterioration. The initial topography of demyelinating lesions markedly influences the progression of the disease. The occipital forms progress much more rapidly than the frontal forms, which are more frequently observed in adolescents.

Adrenomyeloneuropathy (AMN) affects 65% of adult X-ALD male patients (20–50 years) and up to 88% of heterozygous women (>40 years) [11][12]. The presentation in both sexes is with progressive spastic paraparesis and sensory ataxia. Subsequently, 63% of men with AMN develop cerebral demyelination, which ultimately has the same fatal prognosis in at least 50% of the patients, although disease progression is initially slower than in boys [13]. Women with AMN do not develop cerebral demyelination.

Metabolic Derangement

In X-ALD patients the oxidation of VLCFAs is deficient due to the absence or functional deficiency of the peroxisomal ABC half-transporter ALDP. This leads to elevated VLCFA-CoA levels in the cytosol and their subsequent incorporation into a variety of lipids including cholesterol esters, phospholipids, and sphingolipids. As a consequence VLCFAs accumulate in virtually all tissues, including erythrocytes, white blood cells and plasma.

Genetics

X-ALD is caused by mutations in *ABCD1*. Hundreds of different mutations have been identified including point mutations, deletions, insertions and splice site mutations without any correlation with clinical phenotype (see www.x-ald.nl). The percentage of de novo mutations is less than 4%, and consequently it is important to undertake genetic counselling and screening in X-ALD families in order to detect individuals at risk, including heterozygous women, boys and adults with adrenal insufficiency and those who are asymptomatic neurologically with normal neuroimaging.

Diagnostic Tests

The single most important test for X-ALD is the analysis of VLCFAs, including C22:0, C24:0, and C26:0 in plasma after

alkaline and acid hydrolysis to release the VLCFAs from all lipid species. Work by Moser and co-workers [14] has shown that VLCFA analysis in plasma is highly reliable for diagnosis with virtually no false-negatives in males with X-ALD. False-positive results, however, have been reported in patients on a ketogenic diet [15] and especially in patients taking peanut butter [16]. Heterozygote detection by biochemical analysis is not as straightforward. Plasma VLCFAs have been found to be normal in about 5-15% of obligate heterozygotes. For the detection of heterozygous women, we advocate performing molecular studies and VLCFA analysis at the same time in families in which the molecular defect has been established in the index patient. When there is no family history of X-ALD, plasma VLCFA analysis must be performed first in males with clinical and/or neuroradiological abnormalities evoking X-ALD, followed by molecular studies of *ABCD1*. Neonatal screening for X-ALD based on the demonstration of increased levels of C26:0-lysophosphatidylcholine (LPC) in blood spots is currently performed in several US states and will soon be implemented in the Netherlands.

▪ Treatment and Prognosis

Risk of developing disease manifestations: A boy born with X-ALD has a 35% risk of developing cerebral ALD between the age of 4–12 years and 100% risk of developing AMN between the age of 25-50 years. Among adult males with AMN, at least 35% are at risk of developing a severe form of cerebral ALD between the age of 25–50 years. Furthermore, a boy born with X-ALD has at least a 65% risk to develop clinical symptoms of adrenal insufficiency (Addison's disease) during his life. A girl born with an *ABCD1* mutation on one of her X-chromosomes (and therefore heterozygous for X-ALD) has no risk of developing cerebral X-ALD but an 88% risk of developing signs of AMN by the age of 60 years. Addison's disease is very rare in heterozygous females.

Cerebral X-ALD can be treated in boys and adult males, but only at a very early stage of the disease, in practice when patients start to develop cerebral demyelination on brain MRI but have no or minimal neurologic symptoms. Therapeutic intervention in boys and adults with progressive inflammatory cerebral X-ALD relies on allogeneic haematopoietic cell transplantation (HCT) which can arrest the cerebral demyelination when the procedure is performed at a very early stage [17]. This treatment is however associated with a mortality risk of 15% in boys and 25% in adults. More recently, autologous haematopoietic stem cell (HSC) gene therapy with lentiviral vector has proven a valuable alternative with no mortality in 4 patients [18]. A Phase II-III trial is currently being conducted in a larger number of boys with cerebral X-ALD. If successful, autologous haematopoietic stem cell gene therapy will replace conventional HCT in the near future. However, allogeneic HCT or autologous HSC gene therapy is ineffective in patients in an advanced stage of cerebral X-ALD with obvious neurologic or cognitive deficits. It is therefore crucial to detect the first signs of cerebral demyelination in all asymptomatic X-ALD boys and adolescents (with or without Addison's disease) and importantly also in all men with AMN. In the absence of biomarkers that can predict the onset of cerebral X-ALD, the only strategy is to perform brain MRI every 6 months from the age of 4 to 12 years, and then once a year up to the age of at least 50 years. All other therapeutic approaches, including aggressive immunosuppression, have failed to modify the natural course of the disease.

There is yet no specific treatment for AMN. However active physical training and rehabilitation improve the burden of motor disability. 4-aminopyridine (Fampridine) also improves the speed of walking, the duration of walking without rest in 70% of men or women with AMN. Treatment of bladder dysfunction and neuropathic pains also improves the quality of life of AMN patients. Several pharmacological trials are ongoing in AMN. In the future, one may envisage a gene therapy approach correcting the *ABCD1* defect in the spinal cord of AMN patients. Finally, Lorenzo's oil (a mixture of oleic and erucic acid) allows the normalisation of plasma VLCFA but unfortunately has no curative or preventive effects in X-ALD patients.

40.2.2 D-Bifunctional Protein (DBP) Deficiency

▪ Clinical Presentation

DBP deficiency was first described as pseudo-Zellweger syndrome. Detailed analysis of more than 110 patients with proven DBP deficiency has revealed that, at the severe end of the clinical spectrum, the signs and symptoms, which can include neonatal hypotonia, seizures, cranial facial dysmorphia and liver disease, can be indistinguishable from patients affected by a Zellweger spectrum disorder (ZSD). Moreover, the MRI-features in DBP deficiency resemble those seen in ZSD patients [19]. In recent years a number of atypical and much milder presentations of DBP deficiency have been identified mostly through exome sequencing. Indeed, DBP deficiency has been described in two sisters with ovarian dysgenesis, hearing loss and ataxia resembling Perrault syndrome [20] and in two brothers aged 16.5 and 14 years characterized by normal early childhood development, followed by sensory neural hearing loss, progressive cerebellar and sensory ataxia and subclinical retinitis pigmentosa [21]. Additional mild cases of DBP deficiency have been described [22]. Recently, three adult siblings have been reported with a slowly progressive, juvenile-onset phenotype, comprised of cerebellar atrophy and ataxia, intellectual decline, hearing loss, hypogonadism, hyperreflexia, demyelinating sensory motor neuropathy, and in two of the three probands, supratentorial white matter changes [23]. Mutations in *HSD17B4* were found by whole exome sequencing and subsequent studies in fibroblasts confirmed DBP deficiency.

▪ Metabolic Derangement

DBP plays an essential role in the degradation of VLCFAs, pristanic acid and di- and trihydroxycholestanoic acid (Fig. 40.1) which explains why, in typical DBP deficient patients, all these three metabolites accumulate. However, since

pristanic acid is only derived from dietary sources, it may be deceptively normal in certain cases, particularly in neonates. Additionally, a number of patients with DBP deficiency have been described with metabolite profiles in plasma that are atypical or even normal, which complicates correct diagnosis (see ▶ Diagnostic Tests).

Genetics

DBP deficiency is an autosomal recessive disorder caused by mutations in *HSD17B4.* A large number of private mutations as well as one more frequent mutation have been described [24][25].

Diagnosis Tests

Plasma VLCFA analysis is in general a good initial diagnostic test for patients suspected to suffer from DBP deficiency, especially since phytanic acid and pristanic acid can be determined in the same assay. If abnormal, this should be followed up by analysis of the bile acid intermediates, but, more importantly, in order to discriminate between DBP deficiency and ZSD, the analysis of plasmalogens in erythrocytes. However, since plasmalogen levels may be entirely normal in patients with mild ZSD, further studies are essential to delineate whether the patient has a single peroxisomal beta-oxidation defect at the level of D-bifunctional protein or otherwise, or a peroxisome biogenesis disorder. In this situation catalase-staining of peroxisomes using immunofluorescence microscopy analysis is a powerful tool to assess the presence, absence and morphology of peroxisomes in cultured cells, notably fibroblasts. DBP can be assayed in fibroblasts and also in lymphocytes where it is highly expressed (authors' unpublished observations). Enzymatically proven DBP deficiency should always be followed up by molecular analysis of *HSD17B4.*

Treatment and Prognosis

At present there are no realistic options for curative treatment. Furthermore, the prognosis, particularly at the severe end of the clinical spectrum, is poor with most patients dying in the first three years of life [24][25].

40.2.3 Acyl-CoA Oxidase (ACOX) Deficiency

Clinical Presentation

The clinical presentation of ACOX deficiency mimics that of ZSD in many respects, most notably neonatal adrenoleukodystrophy (NALD) [26]. Most patients have had neonatal-onset hypotonia, seizures, failure to thrive, psychomotor retardation, sensory deafness, hepatomegaly, absent reflexes, and visual loss with retinopathy and extinguished electroretinograms. In approximately half, dysmorphic features have resembled mild ZSD. Patients may show some early motor development with a typical regression by 2–3 years of age. More recently a late onset presentation of ACOX deficiency has been described with development of progressive neurological symptoms in later childhood [27].

Metabolic Derangement

Since ACOX is involved in the oxidation of VLCFA only, VLCFA are increased without raised pristanic and di- and trihydroxycholestanoic acid levels.

Genetics

ACOX deficiency is an autosomal recessive disorder caused by mutations in *ACOX1*. The genetic basis has been determined in most patients published and is very heterogeneous [26].

Diagnostic Tests

VLCFA analysis in plasma after acid and alkaline hydrolysis is the method of choice as an initial biochemical test in any patient suspected to suffer from a peroxisomal beta-oxidation deficiency, including ACOX deficiency. No false-negatives have been reported in literature. The finding of elevated VLCFAs with normal levels of pristanic acid and the bile acid intermediates DHCA and THCA may point to ACOX deficiency although the same combination is also observed in X-ALD. Detailed studies in fibroblasts which include catalase staining by immunofluorescence microscopy analysis (normal in X-ALD cells; abnormal »giant« peroxisomes in ACOX deficiency) can easily resolve this issue.

Treatment and Prognosis

No treatment options have been described and the prognosis of affected patients is poor with early death at 5 years of age with a range between 4–10 years [24][25].

40.2.4 Methyl Acyl-CoA Racemase (AMACR) Deficiency

Clinical Presentation

Different phenotypes have been described for AMACR-deficiency. The first involves a relatively mild form of the disease mimicking Refsum disease, whereas the other is dominated by early-onset severe liver abnormalities. In recent years yet other variants have been described including tremor and deep white matter changes [28], relapsing encephalopathy [29], relapsing rhabdomyolysis [30], and in two patients a complex adult-onset phenotype with peripheral neuropathy, epilepsy, relapsing encephalopathy, bilateral thalamic lesions, cataract, pigmentary retinopathy and tremor [31].

Metabolic Derangement

The enzyme 2-methyl acyl-CoA racemase encoded by *AMACR* is targeted both to peroxisomes and mitochondria. In peroxisomes AMACR is involved in the oxidation of *2R*-methyl branched chain fatty acids which includes pristanic acid and the bile acids intermediates di- and trihydroxycholestanoic acid.

Genetics

AMACR deficiency is an autosomal recessive disorder caused by mutations in *AMACR*. In the few patients described in lit-

erature the molecular basis has been established and different mutations identified.

Diagnostic Tests

The diagnosis requires analysis of the bile acid intermediates di- and trihydroxycholestanoic acid, since plasma VLCFAs are not increased and pristanic acid levels may be deceptively normal in view of its dietary origin.

Treatment and Prognosis

No therapeutic options for AMACR deficiency have been described although patients may possibly benefit from a diet low in phytanic acid and from treatment with cholic acid thereby reducing the levels of di- and trihydroxycholestanoic acid which are harmful to the liver.

40.2.5 Sterol Carrier Protein-2 (SCPx) Deficiency

So far only a single case of SCPx deficiency has been described. The patient presented with torticollis and dystonic head tremor, slight cerebellar signs with intention tremor, nystagmus, hyposmia and azoospermia. Homozygous mutations were identified in *SCP2*, the gene encoding peroxisomal sterol carrier protein X (SCPx), also called sterol carrier protein 2. Since SCPx plays a central role in the peroxisomal degradation of 2-methyl branched chain fatty acids, the diagnosis requires analysis of bile acids, especially bile alcohols [24][25].

40.2.6 PMP70 Deficiency

PMP70 deficiency due to mutations in *ABCD3* has only recently been identified in a single patient who died at the age of 4 years after complications following liver transplantation for severe liver disease [32]. Plasma THCA and DHCA levels were raised and follow-up fibroblast studies revealed enlarged peroxisomes and a reduced capacity to oxidize pristanic acid, whereas beta-oxidation of VLCFA was normal. Subsequent studies revealed the absence of PMP70 due to homozygous truncating mutations in *ABCD3*. As with SCPx deficiency, bile acid analysis is required to identify PMP70 deficient patients. The patient involved was treated with chenodeoxycholic acid without success.

40.2.7 Contiguous *ABCD1, DXS1357A*-Deletion Syndrome (CADDS)

Three newborn boys were described in 2002 with clinical signs and symptoms and initial biochemical results (elevated VLCFA, deficient erythrocyte plasmalogens) consistent with a ZSD [33]. Fibroblast studies, however, showed the normal presence of peroxisomes. Molecular analysis revealed small deletions in the Xq28 region that extended into the promoter region of *ABCD1* and the neighbouring gene *DXS1357A* which codes for BAP31, an abundant endoplasmic reticulum protein of unresolved function. Only a few additional patients have subsequently been identified [22] (see [34] for review). Interestingly, isolated BAP31-deficiency due to mutations in *BCAP31* has recently been described [35]. The three patients reported suffered from motor and intellectual disabilities, dystonia, sensory neural deafness, and white matter changes.

40.2.8 Zellweger Spectrum Disorders (ZSD)

Clinical Presentation

The prototype of the ZSD is Zellweger syndrome (ZS) which is dominated by (1) the typical cranial facial dysmorphia including a high forehead, large interior fontanelle, hypoplastic supraorbital ridges, epicanthal folds, flat nasal bridge, and deformed ear lobes, and (2) profound neurological abnormalities. ZS children show severe psychomotor retardation, profound hypotonia, neonatal seizures, glaucoma, retinal degeneration, and sensory neural deafness. There is usually calcific stippling of the epiphyses and small renal cysts. Brain abnormalities in ZS arise in part from defective neuronal migration and include not only cortical dysplasia and neuronal heterotopias with polymicrogyria or pachygyria in the perisylvian regions but also white matter changes characterized by dysmyelination rather than demyelination [36]. Liver dysfunction with cholestasis and hepatomegaly is common. Infants with ZS usually die before one year of age from neurologic and respiratory complications. Later two additional phenotypes were described including neonatal adrenoleukodystrophy (NALD) presenting with predominant neurological signs and infantile Refsum disease (IRD) presenting with predominant gastrointestinal signs including failure to thrive, cholestasis and hypocholesterolemia. It is now clear, however, that these three historically defined clinical entities are members of a large spectrum of disorders with overlapping signs and symptoms. For this reason the name 'Zellweger spectrum disorders' has been suggested and is now generally used. The clinical spectrum associated with these disorders is constantly expanding, in part because of the introduction of whole exome and whole genome sequencing and has led to the recognition of very mild forms of ZSD [37][38]. This includes patients with cerebellar ataxia only [39][40]. Furthermore, Heimler syndrome, a rare recessive disorder characterized by sensory neural hearing loss, amelogenesis imperfecta, nail abnormalities, and occasional or late-onset retinal pigmentation but normal intelligence, was recently identified as a mild form of a peroxisome biogenesis disorder. Recently the history of patients with a Zellweger spectrum disorder surviving into adulthood has been described [41]. Seven of the nineteen patients aged 16–35 years had a progressive disease course while twelve remained clinically stable during follow-up. Disease progression usually manifested in adolescence as a gait disorder, caused by central and/or peripheral nervous system involvement. Nine patients were capable of living a partly independent life with supported employment. A normal MRI was found in five patients whereas only four patients had liver

cirrhosis and/or fibrosis. Seventeen of the nineteen patients had a typical biochemical phenotype in blood at the time of diagnosis although abnormalities tended to disappear with age [41].

Metabolic Derangement

As a consequence of the absence or marked deficiency of peroxisomes, as assessed by catalase-staining in fibroblasts using immunofluorescence microscopy analysis, in principle all peroxisomal functions are impaired including peroxisomal fatty acid beta-oxidation. In classical ZS this is reflected in a large series of abnormalities including the accumulation of VLCFAs, pristanic acid, di- and trihydroxycholestanoic acid, and pipecolic acid and deficient plasmalogens in erythrocytes [42].

Genetics

The genetic basis of the ZSD is markedly heterogeneous with so far biallelic mutations identified in *PEX1*, *PEX2*, *PEX3*, *PEX5*, *PEX6*, *PEX10*, *PEX12*, *PEX13*, *PEX14*, *PEX16*, *PEX19*, and *PEX26* [43]. All disorders are autosomal recessive.

Diagnostic Tests

VLCFA analysis remains a good initial biochemical test in patients suspected to suffer from any peroxisomal disorder in which peroxisomal beta-oxidation is impaired which includes the ZSD. If abnormal, VLCFA analysis should be followed up by analysis of the full set of peroxisomal metabolites in a blood sample which includes erythrocyte plasmalogens. A small subset of patients has been found by the unexpected finding of pipecolic acid upon amino acid analysis. Subsequently, detailed studies in fibroblasts should be undertaken, including complementation analysis to pinpoint the underlying defect in peroxisome biogenesis followed by molecular analysis of the relevant gene. An alternative which is likely to gain wider acceptance in the future would include mutation analysis using a DNA-panel containing all *PEX* genes or all genes coding for peroxisomal proteins.

Treatment and Prognosis

Currently there is no realistic curative option available for patients with ZSD. The claim that supplementation with docosahexaenoic acid (DHA) might be beneficial for patients has been disproven [44]. Currently, we and others are investigating the potential usefulness of a treatment based on cholic acid supplementation in order to reduce formation of the toxic bile acid intermediates di- and trihydroxycholestanoic acid (trial number: controlled-trials.com: ISRCTN96480891).

40.3 Disorders of Peroxisomal Fatty Acid Alpha-Oxidation

Although most fatty acids can be broken down by beta-oxidation, fatty acids with a methyl-group at the 3-position first need to undergo alpha-oxidation to generate a 2-methyl fatty acid which can then be beta-oxidized. Phytanic acid is the best-known 3-methyl fatty acid in humans which upon alpha-oxidation in peroxisomes generates pristanic acid plus CO_2. The enzymology of the phytanic acid alpha-oxidation pathway has been resolved in recent years and includes five consecutive enzyme reactions [45][46]. So far only a single defect in the alpha-oxidation pathway has been identified at the level of the enzyme phytanoyl-CoA hydroxylase which causes Adult Refsum Disease (ARD).

40.3.1 Adult Refsum Disease (ARD)

Clinical Presentation

Patients destined to develop ARD are perfectly normal as infants, show no obvious defects in growth and development and usually present in late childhood with progressive loss of night vision, a decline in visual capacity and anosmia. After 10–15 years or more, patients may develop additional abnormalities including deafness, ataxia, polyneuropathy, ichthyosis, fatigue, and cardiac conduction disturbances [47]. Short metacarpals and/or metatarsals are found in around 30% of patients. The full constellation of typical features defined by Refsum in the 1940s which includes retinitis pigmentosa, cerebellar ataxia and chronic polyneuropathy, is rarely seen in single patients with ARD. Retinitis pigmentosa may be the only recognized abnormality in adults. Polyneuropathy is of a mixed motor and sensory type that is asymmetrical, chronic and progressive in untreated ARD. Initially symptoms often wax and wane. Later the distal lower limbs are affected with muscular atrophy and weakness. Over the course of years muscular weakness can become wide-spread with disability involving not only the limbs but also the trunk. Almost without exception patients have peripheral sensory disturbances, most often impairment of deep sensation, particularly perception of vibration and position/motion in the distal legs. Some patients develop cardiomyopathy which can be lethal in the absence of cardiac transplantation.

Metabolic Derangement

Phytanoyl-CoA hydroxylase, the enzyme deficient in ARD, plays a crucial role in the alpha-oxidation of phytanic acid and explains the accumulation of phytanic acid in this disorder.

Genetics

ARD is an autosomal recessive disorder caused by mutations in *PHYH*. A large number of often private mutations has been identified [48].

Diagnostic Tests

Since phytanic acid is the only peroxisomal metabolite which is abnormal in ARD, laboratory diagnosis should start with its analysis in plasma. In all patients so far reported plasma phytanic acid levels were found to be markedly increased. Of note, it is the finding of a moderate phytanic acid accumulation that led to the description of the index patients so called infantile Refsum disease subsequently found to be affected by a peroxisomal biogenesis defect.

Treatment and Prognosis

Dietary restriction of phytanic acid is the mainstay of therapy in ARD patients and is critical to minimize ongoing tissue accumulation. The largest sources of phytanic acid and its metabolic precursor phytol are dairy products, meats and certain fishes and must be eliminated or at least severely restricted from the diet. Green leaves and vegetables do not need to be restricted since the phytanic acid they contain cannot be released from the chlorophyll molecule. Hearing impairment can be treated with aids and cochlear implants. Patients should be cautioned to avoid rapid weight loss since this may mobilize phytanic acid stores from adipose tissue and lead to acute symptomatic crises. With respect to the prognosis, there is a halt in the progression of symptoms and some functional recovery if the disease is recognized early and dietary restriction and regular lipid apheresis are maintained life-long. Once therapy is started, plasma phytanic acid levels may decrease rapidly and a more favourable long-term outcome can be expected. The peripheral neuropathy, ataxia, ichthyosis and cardiac abnormalities may stabilize or even improve, whereas the retinitis pigmentosa, deafness and anosmia seem more refractory.

40.4 The Fatty Acid Chain Elongation Disorders

As shown in Fig. 40.1 fatty acids either derived from dietary sources or synthesized *de novo* via the FAS complex, can be converted into longer-chain fatty acids either saturated, mono- or polyunsaturated. This is achieved through the consecutive action of the chain elongation system localized in the endoplasmic reticulum (ER) and different desaturases which can introduce double-bonds at specific positions. Chain elongation allows the stepwise extension of fatty acids by two carbon atoms and involves a four-step pathway mediated by ELOVL 1-7 (condensation), KAR (first reduction), HACD 1-4 (hydratation) and TER (second and final reduction) to produce the corresponding (n+2) acyl-CoA. The introduction of double-bonds at specific positions is mediated by so-called desaturases. Mammals only express delta9, delta6 and delta5 desaturase activities and the enzymes involved belong to two distinct families referred to as stearoyl-CoA desaturases (SCDs) [49] and fatty acid desaturases (FADS) [50]. The SCDs introduce a double-bond at position delta9 and several isoforms of SCD have been identified. Delta6 and delta5 desaturation is mediated by different FADS enzymes.

40.4.1 ELOVL4 Deficiency

Clinical Presentation

ELOVL4 deficiency is associated with different clinical phenotypes and is inherited in both an autosomal recessive as well as autosomal dominant form. Indeed, in juvenile onset, autosomal dominant Stargardt-like macular dystrophy (STGD3) a series of mutations have been identified in the last exon of *ELOVL4* which appear to be causative for the loss of central vision with progressive degeneration of the macula and peripheral retina in patients. Similar to the autosomal recessive form of Stargardt disease (STGD1) the onset of loss of vision in patients ranges from 3–50 years with a mean age of 14 years. Over decades, the macular lesion enlarges and visual acuity decreases to 20/300 to 20/800. The typical phenotype usually seen in patients is a well-circumscribed homogeneous atrophy of the retinal pigment epithelium (RPE) and choriocapillaris in the macula surrounded by yellow flecks and temporal optic nerve pallor. All of the mutations identified cause a frameshift that results in the introduction of a premature stop codon in the ELOVL4 message and hence premature termination of the protein with a resultant loss of the C-terminal endoplasmic reticulum retention/retrieval signal in the mutant protein. Current evidence holds that haploinsufficiency is not the key mechanism of pathogenesis [51] but rather the dominant negative effect exerted by mutant ELOVL4 which recruits the wild-type ELOVL4 protein into perinuclear cytoplasmic inclusions that resemble aggresomes. Interestingly, Aldahmesh and co-workers [52] identified bi-allelic recessive mutations in *ELOVL4* in patients with a completely different phenotype characterized by ichthyosis, seizures, mental retardation and spasticity which is a constellation that resembles Sjögren Larsson syndrome although the neurologic phenotype is more severe. Recently, the clinical phenotype associated with ELOVL4 deficiency has been expanded even further thanks to the finding of mutations in *ELOVL4* in a French-Canadian family with autosomal dominant spino-cerebellar ataxia and erythrokeratodermia [53].

Metabolic Derangement

Since ELOVL4 is specifically involved in the chain elongation of very-long-chain polyunsaturated fatty acids, including C26:6n3, C26:5n3, C26:4n6, and C26:5n6, the metabolic derangement caused by ELOVL4 deficiency probably involves the decreased formation of very-long-chain PUFAs although definitive information on this point is still missing.

Genetics

As already eluded to above the genetics of ELOVL4 deficiency may vary from autosomal dominant to autosomal recessive which is explained by the different mechanisms involved.

Diagnostic Tests

Diagnosis of ELOVL4 deficiency is problematic since ELOVL4 is only expressed in certain tissues including human retina but also in the brain, testis, skin and lens. However, ELOVL4 is not expressed in liver, kidney, lung or spleen which suggests that the presumed abnormalities at the level of the VLC-PUFAs will probably not be expressed in plasma.

Treatment and Prognosis

There are currently no effective treatments to combat ELOVL4 deficiency.

40.4.2 ELOVL5 Deficiency

ELOVL5 deficiency was identified only very recently in an Italian family with a pure form of spinocerebellar ataxia (SCA) which is a genetically heterogeneous group of autosomal dominant neurodegenerative disorders involving the cerebellum. Following the identification of a single missense mutation in *ELOVL5* in this family, 456 independent SCA-affected individuals were screened which led to the identification of the same mutation in two further unrelated families. Haplotype analysis showed that at least two of the three families shared a common ancestor. Furthermore, one further missense variant was found in a French family. All missense mutations were predicted to be damaging. Since *ELOVL5* encodes for an elongase involved in the synthesis of polyunsaturated fatty acids of the omega3 and omega6 series, which includes arachidonic acid and docosahexaenoic acid, the levels of these fatty acids were determined in the serum of affected individuals which revealed reduced levels of C20:4 and C22:6 amounting to 30 to 40% of normal. It remains to be established whether measurement of polyunsaturated fatty acids in serum could be used to diagnose these patients.

40.4.3 Trans-2,3-Enoyl-CoA Reductase (TER) Deficiency

So far only a single report on trans-2,3-enoyl-CoA reductase (TER) deficiency has been published which involves a consanguineous family with five adult children with non-syndromic mental retardation. Growth parameters were normal and there were no dysmorphic features other than a narrow palate in all siblings. Muscle bulk and tone, cranial nerves and peripheral reflexes were normal. Three patients had an intention tremor and slow rapid finger movements. There were no resting tremors, signs of ataxia or other abnormal movements. Exome sequencing led to the identification of a single variant segregating with the phenotype causing a substitution of a leucine for a highly conserved proline at amino acid position 182 in the trans-2,3-enoyl-CoA reductase enzyme. The P182L mutant enzyme was not only more unstable compared to the wild-type protein but also exhibited lower reductase activity. Furthermore, the levels of C24:1 sphingomyelin and C24:0 ceramide were lower in cells expressing the mutant reductase [54].

40.4.4 3-Hydroxyacyl-CoA Dehydratase1 (HACD1) Deficiency

So far HACD1 deficiency has only been described in a single family with six affected individuals all showing a severe myopathic phenotype at birth that gradually improved over time. Pregnancies were uneventful with uncomplicated births. Severe hypotonia was detected in all patients during the neonatal period with head lag, absent deep tendon reflexes and reduced newborn reflexes. Severe facial weakness was noted in all patients with drooling and reduced sucking reflexes in 5/6 patients. One patient required a gastrostomy and recurrent aspiration and pneumonia occurred in two patients. A marked delay in achieving motor milestones was detected in all patients who interestingly improved with age in all cases. Cognition however was within normal limits. Electrocardiograms were repeatedly normal. Laboratory investigations in affected individuals, including CPK, liver enzymes, amino acids, lactate, pyruvate, carnitine, ammonia, urinary organic acid, acylcarnitines, VLCFAs, pristanic and phytanic acid all were normal. Candidate genes associated with congenital myopathy showed no abnormalities. Homozygosity mapping followed by exome sequencing revealed a nonsense mutation in *HACD1* which codes for the 3-hydroxyacyl-CoA dehydratase protein1 involved in chain elongation. *HACD1* mainly expresses in skeletal, muscle and heart, whereas *HACD2* and *HACD3* are ubiquitously expressed. The nonsense mutation identified (Y248stop) revealed a full loss of activity. No lipid analyses were performed. It is speculated that the improvement with age detected in all patients is the result of temporal upregulation resulting in HACD2 and/or HACD3 taking over the role of HACD1 as patients mature.

40.5 Disorders of Eicosanoid Metabolism

▶ Eicosanoid metabolism

The eicosanoids constitute a large variety of biologically active molecules derived from arachidonic acid after liberation from cellular membranes by phospholipase A2 (PLA2) through three main pathways: the cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome P_{450} monooxygenase pathways. The COX pathway generates the different prostaglandins PGE2, PGD2, PGF2Alpha, PGI2, and TXA2 whereas the LOX-pathway generates the different HETEs (5-,8-,12-, and 15-HETE) plus the leukotrienes LTA4 (unstable), LTB4, LTC4, LTD4, and LTE4 (Fig. 40.2). Finally, the P_{450} pathway produces HETEs, HPETEs and EETs. So far only a few defects in eicosanoid metabolism have been identified as described below.

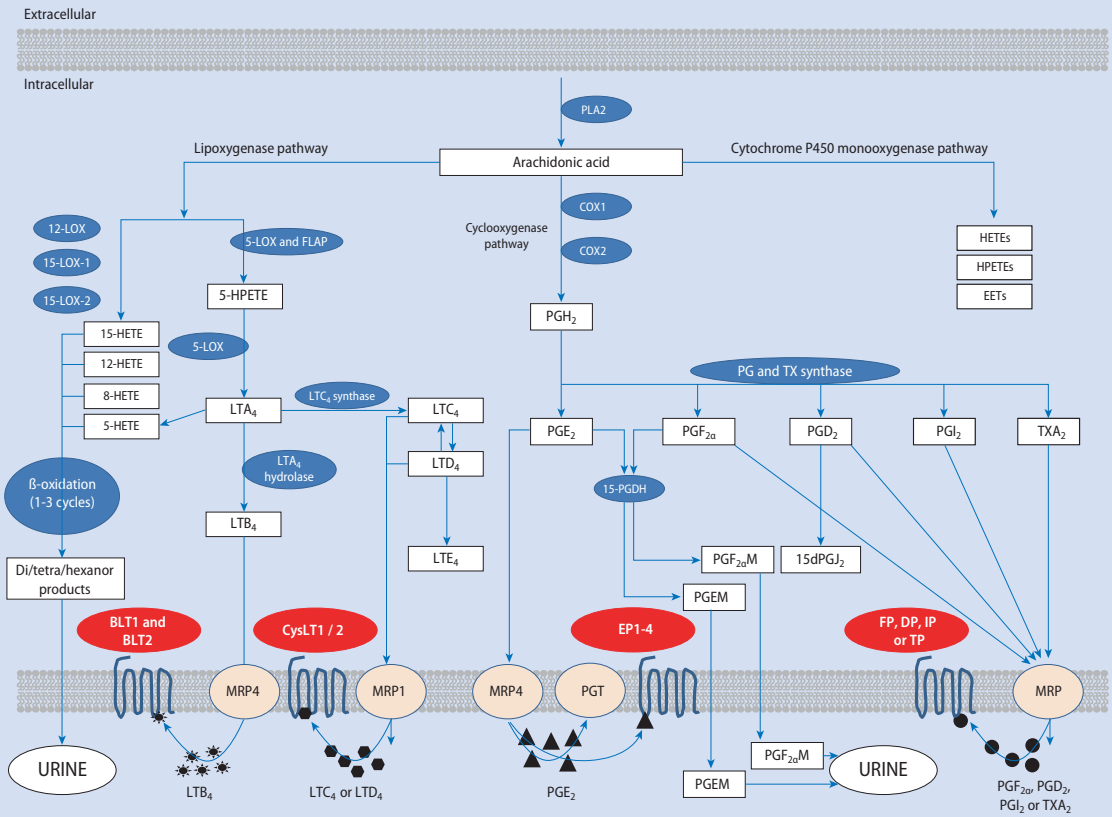
40.5.1 Primary Hypertrophic Osteoarthropathy Type 1 (PHOAR1): 15-Hydroxy Prostaglandin Dehydrogenase (PGDH) Deficiency and Type 2 (PHOAR2): Prostaglandin Transporter (PGT) Deficiency

Clinical Presentation

Hypertrophic Osteoarthropathy (PHO) is a disorder characterized by changes to the skin and bones, and occurs either in a rare familial, primary form (PHO: primary hypertrophic osteoarthropathy), also called pachydermoperiostosis (PDP) or, more commonly, secondary to other pathologies. Key features include digital clubbing, periostosis with bone and joint enlargement, and skin changes, such as pachydermia, abnor-

Eicosanoid Metabolism

Arachidonic acid present in the phospholipid bilayer of membranes is liberated by cytoplasmic phospholipase A2 (PLA2) (► Chapter 34) and can subsequently be metabolized to different eicosanoids through three major pathways (■ Fig. 40.2): (1) the cyclooxygenase (COX) pathway; (2) the lipoxygenase (LOX) pathway, and (3) the cytochrome P450 monooxygenase pathway. In the COX pathway the intermediate PGH2 is sequentially metabolized to prostanoids including prostaglandins (PGs) and thromboxanes (TXs) by specific prostaglandin and thromboxane synthases. In the LOX pathway arachidonic acid is converted into different leukotrienes and hydroxyeicosatetraenoic acids (HETEs) whereas the P450 mediated pathway metabolizes arachidonic acid into epoxyeicosatrienoic acid (EETs), HETEs and hydroperoxyeicosatetraenoic acid (HPETEs). In the 5-LOX pathway arachidonic acid is metabolized into the intermediate 5-HPETE which is further metabolized to form the unstable leukotriene A4 (LTA4). LTA4 is then converted into 5-HETE, LTB4, LTC4, LTD4, and LTE4. Members of the multidrug resistance-associated protein (MRP) gene family catalyze the efflux of several of the prostaglandins and leukotrienes including LTB4 by MRP4, LTC4 and LTD4 by MRP1, PGE2 via MRP4 and PGF2alpha, PGD2,, PGI2, and TXA2 by different MRPs. The eicosanoids exert their biological effects in an autocrine or paracrine manner by binding to their cognate cell surface receptors which belong to the G-protein-coupled receptor family. These receptors are designated DP and GPR44 for prostaglandin D2 (PGD2); EP1, EP2, EP3, and EP4 for PGE2; FP for PGF2alpha ; IP for PGI2, and TP for TXA2. LTB4 can bind two receptors, including BLT1 with high affinity and BLT2 with low affinity whereas the leukotrienes LTC4 and LTD4 can bind to at least two distinct receptors, CysLT1 and CysLT2 Abbreviations used: PGEM = 13,14-dihydro-15-keto-PGE2; PGFM = 13,14-dehydro-15-keto-PGF2alpha; 15-PGDH = 15-hydroxyprostaglandin dehydrogenase.



■ **Fig. 40.2** An overview of eicosanoid metabolism. (Modified from Fig. 1 in [55]).

mal furrowing, seborrhea, and hyperhidrosis. Specific developmental abnormalities have been found in some patients with PHO, such as wide cranial sutures, Wormian bones, and *patent ductus arteriosus*. In adults, when all major clinical features are present, PHO is relatively easy to diagnose. However, for the paediatrician, who often is presented with an incomplete clinical presentation, diagnosis may be a challenge. In young children with swollen and painful joints and a history of *patent ductus arteriosus*, delayed closure of the cranial sutures and fontanelles or Wormian bones should be investigated immediately for PHO because clubbing, periostosis and skin changes might be absent.

Genetics

Two genetic causes for PHO/PDP have been identified at the level of 15-hydroxyprostaglandin dehydrogenase (15-PGDH) and the prostaglandin transporter (PGT, encoded by SLCO2A1) named PHOAR1 and PHOAR2, respectively [56]. PHO expression is influenced by gender. This is true for patients with PHOAR2 due to *SLCO2A1* mutations, but not for PHOAR1 patients due to 15-PGDH deficiency [57].

Diagnostic Tests

Diagnosis of PHO due to mutations in *HPGD* or *SLCO2A1* can be done via urinary analysis of prostaglandins. In both genetic forms of PHO PGE2 levels in urine are markedly increased whereas the PGE2 metabolite PGEM (13,14-dihydro-15-keto-PGE2) which is the product of the 15-PGDH reaction, is elevated in patients with a defect in *SLCO2A1* whereas PGEM levels are undetectable in patients with 15-PGDH deficiency. Importantly, levels tend to decrease with age and may even normalize in the third to fourth decade of life. For these reasons molecular analysis is often advocated as the primary diagnostic test.

Treatment and Prognosis

Importantly, myelofibrosis is a life-threatening complication. Consequently, when germline *SLCO2A1* mutations have been identified the patient should be followed up periodically for myelofibrosis. The use of steroids can produce haematological improvement but has no effects on skin or digital clubbing and long-term treatment may help in stabilization of symptoms. NSAIDs appear to be the best option for treatment [57].

40.5.2 LTC4-Synthase Deficiency

In literature only a single patient has been identified with LTC4-synthase deficiency by Mayatepek and Flock [58]. The patient involved presented with hypotonia, psychomotor retardation, failure to thrive and microcephaly. The disease was rapidly progressive and the infant died after six months. In plasma and cerebrospinal fluid elevated levels of LTB4 were found whereas the levels of LTC4, LTD4, and LTE4 were below the detection limit. Furthermore, formation of LTC4 in monocytes of the patient turned out to be fully deficient. Unfortunately, no follow-up studies have been performed due to the lack of material from this patient which implies that the genetic defect has not been identified. Human LTC4-synthase has been purified and characterized as a protein of 18 kDa and has been cloned and localized on chromosome 5 [59][60].

40.6 Remaining Disorders of Fatty Acid Homeostasis

40.6.1 Sjögren Larsson Syndrome (SLS)

Clinical Presentation

The classical tetrad of abnormalities in SLS includes ichthyosis, spasticity, ophthalmological abnormalities and mental retardation. However, the full-blown phenotype of SLS is not observed in all patients and furthermore only manifests later on in childhood beyond three years of age. In the majority of patients ichthyosis is congenital or develops very early in life. The neonatal skin may first have an erythrodermic appearance, gradually evolving into a generalized ichthyosiform hyperkeratosis during infancy. Ichthyosis follows a generalized distribution pattern with sparing of the face. Ichthyosis in SLS is different from other cornification disorders by the striking pruritic character, resulting in excoriations and more scaling. The motor problem in SLS is summarized as bilateral spastic tetraparesis involving the legs more than the arms. None of the adolescent patients are able to walk without restrictions with most SLS patients actually using a wheelchair to move around outdoors [61]. The neurological features often stabilize in adolescence. Motor performance in everyday functioning in most patients reaches the developmental age of 12 years maximally. Mild to moderate dysarthria is seen in almost all patients with a delay in language development. MRI studies in SLS show normal gross anatomy of the brain with periventricular white matter abnormalities ranging from (very) mild to severely increased signal intensities on T2-weighted images. Myelination is generally slightly delayed and most patients show cerebral demyelination predominantly in the frontal white matter in childhood. Only at ages > 10 years slight cerebral atrophy may be seen. Proton MRS shows moderate changes in known metabolites like N-acetylaspartate. Beyond 1 or 2 years MRS shows highly characteristic, almost pathognomomic, abnormal resonances at 1.3 and 0.8–0.9 ppm in cerebral white matter but not in grey matter. The exact nature of the accumulating lipid(s) has remained unknown. With respect to the ophthalmological abnormalities, a peculiar crystalline macular dystrophy develops in SLS patients in early infancy which is clinically accompanied by decreased visual acuity and by photophobia [62].

Metabolic Derangement

The enzyme deficient in SLS is fatty aldehyde dehydrogenase (FALDH) which plays a key role in the degradation of long-chain fatty alcohols (see ▶ Chapter 24, ▶ Fig. 24.1) which explains the accumulation of these compounds [63]. Furthermore, FALDH catalyses the oxidation of the omega-aldehyde of LTB4 and thus plays a key role in the degradation of LTB4

via beta-oxidation and its inactivation. It may well be that there are also other aldehydes degraded by FALDH which may be at the basis of the lipid abnormalities observed in brain upon proton MRS.

Genetics

SLS is an autosomal disorder caused by mutations in *ALDH3A2* and a range of different mutations including missense, nonsense, splice-site and deletions has been reported [64].

Diagnostic Tests

The metabolic abnormalities in SLS include elevated levels of long-chain fatty alcohols in plasma and urinary LTB4 metabolites in urine. Since no easy methods have been described to measure these metabolites, enzymatic analysis is the method of choice, especially since this can be done in polymorphonuclear lymphocytes using pyrenedecanal as substrate [65]. The latter method allows straightforward identification of FALDH-deficiency in candidate patients.

Treatment and Prognosis

Treatment of SLS patients is focused on the spasticity and prevention of contracture development. One of the key problems in SLS patients is the striking pruritus which may originate from LTB4 accumulation. To this end Willemsen and co-workers have tried Zileuton, a drug that inhibits leukotriene formation by blocking its biosynthesis and which has proven to be effective in managing chronic (severe) asthma. This resulted clinically in a marked improvement of pruritus with a significant reduction of urinary LTB4 excretion and of the lipid peak at the MRS. A double-blind placebo controlled trial is currently underway to ascertain this clinical efficiency. As discussed in literature [62] there are still other options for therapy, including treatment with bezafibrate and carotenoids.

40.6.2 Bile Acid-CoA: Amino Acid N-Acyltransferase (BAAT) Deficiency

BAAT catalyzes the formation of the taurine and glycine conjugates of the primary bile acids cholic acid and chenodeoxycholic acid. If deficient, the unconjugated bile acids, cholic acid, and chenodeoxycholic acid will accumulate and the conjugates will be completely deficient.

This disorder is fully described in ▶ Chapter 33.

40.7 Other Peroxisomal Disorders not Involving Fatty Acid Metabolism

40.7.1 Oxalurias and Oxalosis: Glyoxylate Detoxification Disorders

Primary hyperoxaluria type 1 (PH1) is a disorder of glyoxylate metabolism characterized by the accumulation of oxalate due to a deficiency of the peroxisomal hepatic enzyme L-alanine: glyoxylate aminotransferase (AGT). The defect in AGT, which normally converts glyoxylate to glycine, results in an increase of the glyoxylate pool, which is converted to oxalate (poorly soluble) and glycolate (without associated pathology). Differential diagnosis includes primary hyperoxaluria type 2 (PH2), primary hyperoxaluria type 3 (PH3), Dent disease, and familial hypercalciuria-hypomagnesemia-nephrocalcinosis (▶ Chapter 37) as well as secondary forms of hyperoxaluria (enteric hyperoxaluria, dietary hyperoxaluria), and idiopathic calcium oxalate urolithiasis. PH2 is due to mutations in *GRHPR* coding for a cytosolic enzyme with hydroxypyruvate reductase, glyoxylate reductase, and D-glycerate dehydrogenase catalytic activities. The enzyme has widespread tissue expression. PH3 is caused by mutations in *HOGA1* which codes for the mitochondrial enzyme 4-hydroxy-2-oxoglutarate aldolase 1.

Clinical presentation of PH1 is variable, ranging from occasional symptomatic nephrolithiasis to nephrocalcinosis and end-stage renal disease with systemic involvement. PH3 has a less severe course than PH1 or PH2, and may be silent. Diagnosis of PH1-3 is suspected on clinical features along with pure calcium oxalate monohydrate stone composition and confirmed by urine oxalate: creatinine ratio, L-glycerate excretion, molecular genetic testing and infrequently by enzyme catalytic activity from liver biopsy. In a proportion of patients with primary hyperoxaluria type 1, treatment with pyridoxine (vitamin B6) may decrease oxalate excretion and prevent kidney stone formation [66].

40.7.2 Pipecolic Acidemia

Hyperpipecolatemia refers to the presence of abnormally high levels of pipecolic acid in the blood. Hyperpipecolatemia is generally a symptom of other known peroxisome biogenesis disorders (PBDs see above). Although this is often the case, in some cases it is considered to be a separate disease entity that falls under the category of PBDs. Additionally, elevations in pipecolic acid can also occur in pyridoxine-dependent epilepsy (see ▶ Chapter 28) and in sulfite oxidase deficiency (where S-sulphocysteine accumulation can lead to secondary inhibition of antiquitin [67]), as well as in individuals with general psychomotor delay. Signs and symptoms may vary widely in nature and severity depending on the underlying cause of the condition. So far, no proven case of isolated hyperpipecolic acidaemia due to a deficiency of L-pipecolate oxidase has been identified.

40.7.3 Acatalasemia

Acatalasemia is an autosomal recessive condition characterized by very low levels of catalase. Many individuals with acatalasemia never have any health problems related to the condition and are diagnosed because they have affected family members. Some of the first reported individuals with acatalasemia developed open sores (ulcers) inside the mouth that led to the death of soft tissue (gangrene). When mouth ulcers and gangrene occur with acatalasemia, the condition is known as

Takahara disease. These complications are rarely seen in more recent cases of acatalasemia, probably because of improvements in oral hygiene. Studies suggest that people with acatalasemia have an increased risk of developing type 2 diabetes mellitus.

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Congenital Disorders of Glycosylation, Dolichol and Glycosylphosphatidylinositol Metabolism

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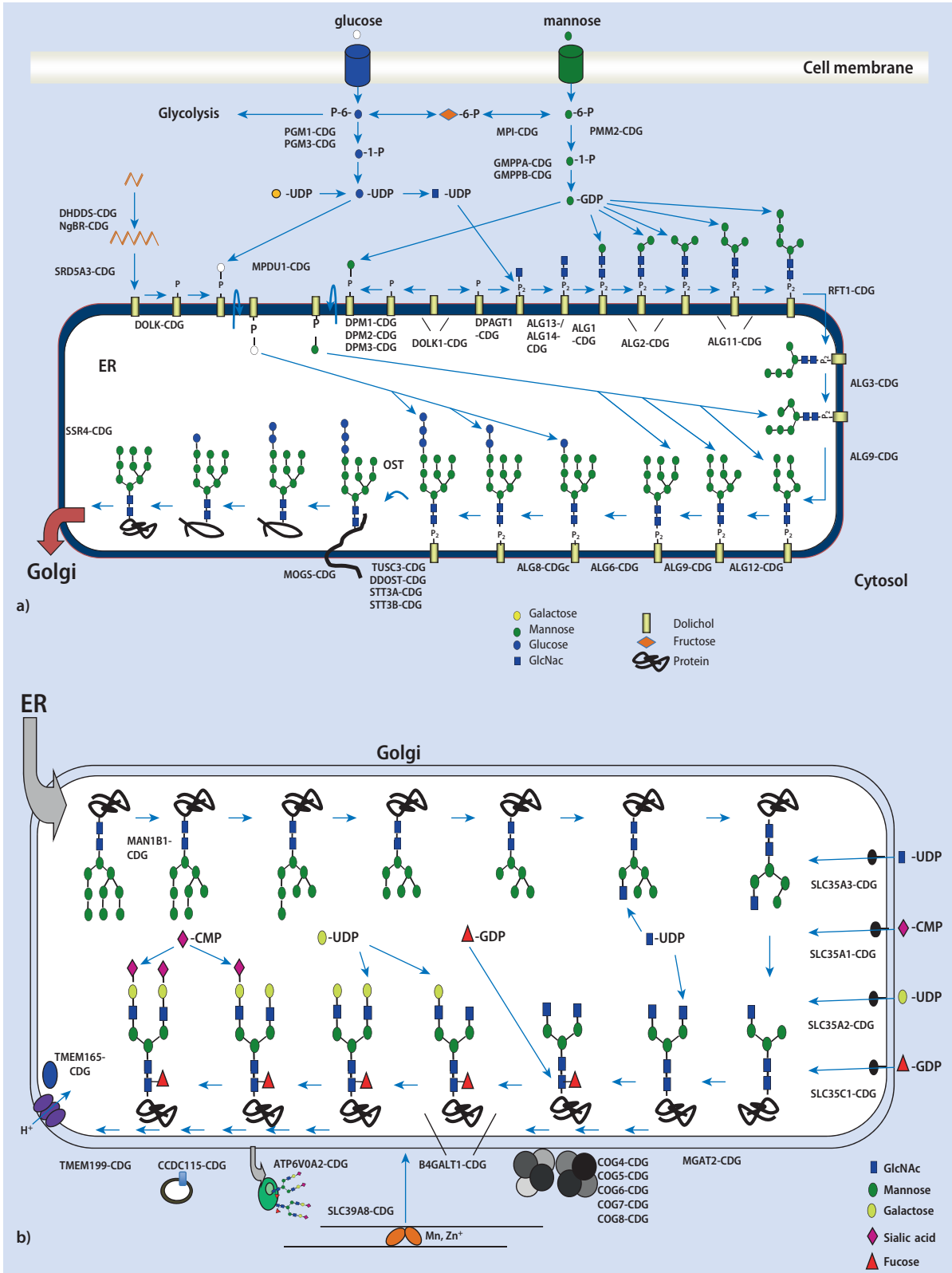

Fig. 41.1 Schematic representation **a)** of the assembly of N-glycans in the cytosol and the endoplasmic reticulum (ER), **b)** of the remodeling of N-glycans in the Golgi. *GDP*, guanosine diphosphate; *UDP*, uridine diphosphate; *OST*, oligosaccharyltransferase *CMP*, cytidine monophosphate

Synthesis of N-Glycans

This complex process proceeds in three stages. Stages 1 and 2 are represented schematically in ◘ Fig. 41.1a and stage 3 in ◘ Fig. 41.1b:

1. Formation in the cytosol of nucleotide-linked sugars, mainly guanosine diphosphate-mannose (GDP-Man), and also uridine diphosphate glucose (UDP-Glc) and UDP-*N*-acetylglucosamine (UDP-GlcNAc), followed by attachment of GlcNAc and Man units to dolichol phosphate, and flipping (indicated by circular arrows) of the nascent oligosaccharide structure into the endoplasmic reticulum (*ER*).
2. Stepwise assembly in the ER, by further addition of Man and Glc resulting in the 14-unit oligosaccharide precursor, dolichol pyrophosphate-*N*-acetylglucosamine$_2$-mannose$_9$-glucose$_3$.
3. Transfer of this precursor onto the nascent protein, followed by final processing of the glycan in the Golgi apparatus by trimming and attachment of various sugar units.

Numerous proteins are glycosylated with monosaccharides and/or oligosaccharide structures also termed glycans, attached to the polypeptide chain. Most extracellular proteins, such as serum proteins, most membrane proteins and several intracellular proteins (such as lysosomal enzymes), are glycoproteins. The glycans are defined by their linkage to the protein: N-glycans are linked to the amide group of asparagine (▶ Synthesis of N-Glycans), and O-glycans are linked to the hydroxyl group of serine or threonine. Glycosylphosphatidylinositol anchors are glycolipids that tether more than 150 proteins to the outer leaflet of plasma membranes. Congenital disorders of glycosylation are due to defects in the synthesis of glycans and in the attachment of glycans to proteins and lipids. It is a rapidly growing disease family, as the number of known CDG has doubled since the previous edition of this book.

41.1 Introduction

Numerous proteins are glycosylated with monosaccharides and/or oligosaccharide structures, also termed glycans, attached to the polypeptide chain. Most extracellular proteins, such as serum proteins (transferrin, clotting factors …..), most membrane proteins and several intracellular proteins (such as lysosomal enzymes), are glycoproteins. The glycans are defined by their linkage to the protein: N-glycans are linked to the amide group of asparagine, and O-glycans are linked to the hydroxyl group of serine or threonine. Synthesis of N-glycans, schematically represented in ◘ Fig. 41.1 proceeds in three stages: formation of nucleotide-linked sugars, assembly, and processing. Synthesis of O-glycans involves assembly but no processing, and occurs mainly in the Golgi apparatus. It forms a diversity of structures, such as O-xylosylglycans, O-*N*-acetylgalactosaminylglycans, O-mannosylglycans, O-fucosylglycans and O-glucosylglycans. Besides protein glycosylation, lipid glycosylation also exists, e.g. glycosylation of ceramide, which is essential for the biosynthesis of gangliosides. Finally, glycosylphosphatidylinositol anchors are glycolipids that tether more than 150 proteins to the outer leaflet of plasma membranes, and contain a glycan core with one glucosamine and three mannoses.

Congenital disorders of glycosylation (CDG), first reported in 1980 [1], are due to defects in the synthesis of glycans and in the attachment of glycans to proteins and lipids. It is a rapidly growing disease family, as the number of known CDG has nearly doubled (from 44 to 91) since the previous edition of this book. Most protein glycosylation disorders are due to defects in the N-glycosylation pathway (25 disorders; ◘ Table 41.1). Twenty disorders of O-glycosylation have been identified (◘ Table 41.2). A third group is that of the recently delineated defects in glycosphingolipid and glycosylphosphatidylinositol (GPI) anchor glycosylation, with 14 disorders (◘ Table 41.3). The fourth group comprises defects in multiple glycosylation pathways and in dolicholphosphate synthesis (32 disorders; ◘ Table 41.4). In addition there are also congenital disorders of deglycosylation: the lysosomal disorders due to an enzymatic defect, and a novel disorder due to defective N-glycanase, a cytoplasmic enzyme.

In 2008, a novel nomenclature for CDG was introduced that uses the official symbol of the defective gene (not in italics), followed by -CDG [2] (list of approved gene names at www.genenames.org). Descriptive names such as hereditary multiple exostoses and familial tumoral calcinosis may continue to be used alongside the novel designations. Only the novel nomenclature is used in this text.

Patients with CDG show a very broad spectrum of clinical manifestations. CDG should therefore be considered in any unexplained clinical condition, particularly in multi-organ disease with neurological involvement but also when non-specific developmental disability is the only presenting sign. Isoelectrofocusing (IEF) of serum transferrin is still the screening method of choice, but it is important to realize that it is able to detect only a limited number of CDG, namely N-glycosylation disorders associated with sialic acid deficiency [3]. The (partial) deficiency of sialic acid in these forms of CDG causes one of two main types of cathodal shift. A type 1 pattern indicates an assembly disorder, and PMM2-CDG or MPI-CDG (depending on the clinical presentation) should be considered first. If these are excluded, the next step could be dolichol-linked glycan analysis, or direct mutation analysis of a panel of genes known to be involved in CDG or whole exome sequencing (WES). A type 2 pattern indicates a disorder of processing. Protein-linked glycan analysis should next be performed in an attempt to identify the defective step, or CDG gene panel analysis or WES. In addition, IEF of serum apoli-

Table 41.1 Protein N-glycosylation disorders

Name	Main clinically affected organs and systems	Defective protein
MPI-CDG	Intestine, liver	Mannosephosphate isomerase
PMM2-CDG	Nervous system, fat tissue, and nearly all other organs	Phosphomannomutase 2
GMPPA-CDG	Autonomic nerve fibers of distal oesophagus (achalasia) and lacrimal glands (alacrimia), neurons (brain, hearing system, visual system)	Guanosine diphosphate mannose pyrophosphorylase A
GMPPB-CDG	Brain, skeletal muscles, eyes, heart	Guanosine diphosphate mannose pyrophosphorylase B
ALG13-CDG	Brain, eyes, liver	UDP-GlcNAc:Dol-P-GlcNAc-P transferase
ALG14-CDG	Neuromuscular junction (congenital myasthenic syndrome)	UDP-GlcNAc:Dol-PP-GlcNAc transferase
ALG6-CDG	Brain, and variable involvement of eyes, gastrointestinal system, liver, heart and skeleton	Glucosyltransferase 1
ALG3-CDG	Brain, skeleton	Mannosyltransferase 6
ALG12-CDG	Brain, skeleton, heart, genitalia and immune system	Mannosyltransferase 8
ALG8-CDG	Brain, and variable involvement of eyes, skin, liver and intestine	Glucosyltransferase 2
ALG2-CDG	Brain, eyes, skeletal muscles, neuromuscular junction (congenital myasthenic syndrome)	Mannosyltransferase 2
DPAGT1-CDG	Brain, neuromuscular junction (congenital myasthenic syndrome)	UDP-GlcNAc: Dol-P-GlcNAc-P transferase
ALG1-CDG	Brain, and variable involvement of eyes, heart, liver, beta cells, kidneys, gonads	Mannosyltransferase 1
ALG9-CDG	Brain, liver, kidneys, and variable involvement of adipose tissue, heart, skeleton, intestine	Mannosyltransferase 7/9
ALG11-CDG	Brain, hearing system	Mannosyltransferase 4/5
RFT1-CDG	Brain, hearing system	Flippase of $Man_5GlcNAc_2$-PP-Dol
MGAT2-CDG	Brain, skeleton, intestine, immune system	N-Acetylglucosaminyltransferase 2
TUSC3-CDG	Brain (non-syndromic autosomal recessive mental disability)	Oligosaccharyltransferase subunit TUSC3
DDOST-CDG	Brain, eyes, liver	Oligosaccharyltransferase subunit DDOST
STT3A-CDG	Brain, gastrointestinal tract	Oligosaccharyltransferase subunit STT3A
STT3B-CDG	Brain, optic nerve, gastrointestinal tract	Oligosaccharyltransferase subunit STT3B
SSR3-CDG	Brain, lungs, gastrointestinal system	Signal sequence receptor 3 of TRAP complex
SSR4-CDG	Brain, respiratory system, skeleton	Signal sequence receptor 4 of TRAP complex
MOGS-CDG	Brain, skeleton, immune system	Mannosyl-oligosaccharide glycosidase (glucosidase 1)
MAN1B1-CDG	Brain, cranial skeleton, fat tissue	Golgi α1-2 mannosidase 1

poprotein C-III (which is only O-glycosylated) can detect some O-glycosylation disorders [4].

Since at least 1% of the human genome is involved in glycosylation, there is no doubt that the majority of CDG have still to be discovered. We predict that these will include diseases that are due to defects in organ-specific glycosylation. Also, there is no doubt that known diseases with unknown aetiology will continue to be identified as CDG.

Only the most frequently reported protein *N*-glycosylation disorders will be described in more detail in this chapter. The most frequently reported disorders from the other groups will be summarized. For recent general reviews on CDG see [5][6][7][8][9][10][11][12][13][14][15][16][17], specific organ involvement in CDG [18][19][20][21][22], diagnosis of CDG [23][24][25][26], and mouse models [27].

41.2 Congenital Disorders of Protein N-Glycosylation (◘ Table 41.1)

41.2.1 Phosphomannomutase 2 Deficiency (PMM2-CDG)

▪ Clinical Presentation

PMM2-CDG is by far the most frequent CDG, with at least 700 patients known worldwide. The symptomatology can often be recognised shortly after birth. The nervous system is affected in all patients, and most other organs are involved in a variable way. The neurological picture comprises alternating internal strabismus and other abnormal eye movements, axial hypotonia, psychomotor disability, ataxia and hyporeflexia. After infancy, symptoms include retinitis pigmentosa, often stroke-like episodes and sometimes epilepsy. As a rule there is no regression. During the first year(s) of life, there are variable feeding problems (anorexia, vomiting, diarrhoea) that can result in severe failure to thrive. Other features are a variable dysmorphism, which may include large, hypoplastic/dysplastic ears, abnormal subcutaneous adipose tissue distribution (fat pads, orange peel skin), inverted nipples, mild to moderate hepatomegaly, skeletal abnormalities and hypogonadism. Some infants develop a pericardial effusion and/or cardiomyopathy. At the other end of the clinical spectrum are patients with a very mild phenotype (no dysmorphic features, slight intellectual disability). Patients often have an extraverted and happy appearance. Neurological investigation reveals (olivoponto)-cerebellar hypoplasia, variable cerebral hypoplasia and peripheral neuropathy. Liver pathology is characterised by fibrosis and steatosis, and electron microscopy shows myelin-like lysosomal inclusions in hepatocytes but not in Kupffer cells.

▪ Metabolic Derangement

Phosphomannomutase (PMM) 2 catalyses the second committed step in the synthesis of guanosine diphosphate (GDP) mannose, namely the conversion of mannose-6-phosphate to mannose-1-phosphate, which occurs in the cytosol (◘ Fig. 41.1a). PMM2-CDG is due to the deficiency of PMM2, the principal isozyme of PMM. Since GDP-mannose is the donor of the mannose units used in the ER to assemble the dolichol-pyrophosphate oligosaccharide precursor, the defect causes hypoglycosylation, and hence deficiency and/or dysfunction of numerous glycoproteins, including serum proteins (such as thyroxin-binding globulin, haptoglobin, clotting factor XI, antithrombin III, cholinesterase etc.), lysosomal enzymes and membranous glycoproteins.

▪ Genetics

PMM deficiency is inherited as an autosomal-recessive trait caused by mutations of *PMM2*. At least 107 mutations (mainly missense) have been identified (www.lovd.nl/PMM2). The most frequent mutation (c.422G>A) causes an R141H substitution and is present in 75% of patients of Caucasian origin. This mutation is not compatible with life in the homozygous state. Its frequency in the Belgian population is as high as 1 in 50. The incidence of PMM2 deficiency is not known; in Sweden it has been estimated at 1 in 40,000.

Prenatal testing should only be offered in families with a documented PMM2 deficiency and mutations in *PMM2*. It cannot be performed by any assay that determines the glycosylation of proteins, since this has been found to be normal in the fetus.

▪ Diagnostic Tests

The diagnosis of congenital disorders of N-glycosylation in general (and of PMM2 deficiency in particular) is usually made by IEF and immunofixation of serum transferrin. Normal serum transferrin is mainly composed of tetrasialotransferrin and small amounts of mono-, di-, tri-, penta- and hexasialotransferrins. The partial deficiency of sialic acid (a negatively charged and end-standing sugar) in CDG causes a cathodal shift. Two main types of cathodal shift can be recognized. Type 1 is characterized by an increase of both disialo- and asialotransferrin and a decrease of tetra-, penta- and hexasialotransferrins; in type 2 there is also an increase of the tri- and/or monosialotransferrin bands. In PMM2 deficiency, a type 1 pattern is found. A type 1 pattern is also seen in secondary glycosylation disorders such as chronic alcoholism, hereditary fructose intolerance and galactosaemia. A shift due to a transferrin protein variant has first to be excluded (by IEF after neuraminidase treatment, study of another glycoprotein and/or investigation of the parents). The carbohydrate-deficient transferrin (CDT) assay is also useful for the diagnosis of sialic acid-deficient CDG. It quantifies the total sialic acid-deficient serum transferrin. A drawback is a not negligible number of false-positive results. Capillary zone electrophoresis of total serum is an interesting, rapid screening test for CDG. An abnormal result should be further investigated by serum transferrin IEF.

In addition to the above-mentioned serum glycoprotein abnormalities, laboratory findings include elevation of serum transaminase levels, hypoalbuminaemia, hypocholesterolaemia, and tubular proteinuria. To confirm the diagnosis, the activity of PMM should be measured in leukocytes or fibroblasts. Note that the PMM activity in fibroblasts can be nor-

mal. Therefore, in case of a typical PMM2-CDG presentation and a normal PMM activity in fibroblasts, mutation analysis of the PMM2 gene is indicated.

Treatment and Prognosis

No effective treatment is available. The promising finding that mannose is able to correct glycosylation in fibroblasts with PMM2 deficiency could not be substantiated in patients. There is a substantially increased mortality (~20%) in the first years of life, due to severe infection or vital organ involvement (liver, cardiac or renal insufficiency) [28]. Most survivors attain adulthood.

41.2.2 Mannosephosphate Isomerase Deficiency (MPI-CDG)

Clinical Presentation

Some 25 patients have been described. Most have presented with hepatic-intestinal disease without notable dysmorphism, and without or with only minor neurological involvement. Symptoms started between the ages of 1 and 11 months. One patient had recurrent vomiting and liver disease that disappeared after the introduction of solid food at the age of 3 months. Two untreated, healthy adults have been reported. The classical phenotype consists of various combinations of recurrent vomiting, abdominal pain, protein-losing enteropathy, recurrent thromboses, gastrointestinal bleeding, liver disease and symptoms of (hyperinsulinaemic or normoinsulinaemic) hypoglycaemia. In 1985, four infants from Quebec were reported with a similar syndrome (Saguenay-Lac Saint-Jean syndrome) and shown to have the same disease.

Metabolic Derangement

Mannosephosphate isomerase (MPI) catalyses the first committed step in the synthesis of GDP-mannose, namely the conversion of fructose-6-phosphate to mannose-6-phosphate (Fig. 41.1a). Hence the blood biochemical abnormalities are indistinguishable from those found in PMM2 deficiency. Since the substrate of MPI, fructose-6-phosphate, is efficiently metabolized in the glycolytic pathway, it does not accumulate intracellularly.

Genetics

Inheritance of MPI deficiency is autosomal recessive. Eighteen mutations have been identified, including 15 missense mutations.

Diagnostic Tests

Serum transferrin IEF shows a type 1 pattern. The diagnosis is confirmed by finding a decreased activity of MPI in leukocytes or fibroblasts and/or (a) mutation(s) in *MPI*.

Treatment and Prognosis

MPI deficiency is still the only known CDG that can be effectively treated. It should be noted that for a few other CDG there is a ›partial‹ treatment (e.g. fucose for SLC35C1-CDG and galactose for PGM1-CDG). Mannose is the therapeutic agent because hexokinases phosphorylate mannose to mannose 6-phosphate, thus bypassing the defect. An oral dose of 1 g mannose/kg body weight per day (divided into four to six doses) is usually effective. The clinical symptoms usually disappear rapidly but it takes several months before the transferrin IEF pattern improves significantly. However, in some patients this treatment cannot control the liver disease. One patient was unable to tolerate mannose. Treatment with heparin led to temporary improvement, necessitating liver transplantation [29].

41.2.3 Glucosyltransferase 1 Deficiency (ALG6-CDG)

Clinical Presentation

ALG6-CDG is the second most common *N*-glycosylation disorder, with some 80 patients identified. Clinical features in common with PMM2-CDG are developmental disability, hypotonia, ataxia, seizures, strabismus, nystagmus, and failure to thrive. A substantial number of patients showed skeletal abnormalities (brachydactyly, arachnodactyly, short arms, scoliosis), behavioural problems and aspecific facial dysmorphism. There was usually no retinitis pigmentosa or cerebellar hypoplasia. A few patients have had protein-losing enteropathy, a consistent feature in MPI-CDG and ALG8-CDG.

Metabolic Derangement

ALG6-CDG is a defect in the attachment in the ER of the first of three glucose molecules to the dolichol-linked mannose$_9$-*N*-acetylglucosamine$_2$ intermediate ($Man_9GlcNAc_2$-P-P-Dol) (Fig. 41.1a). It causes hypoglycosylation of serum glycoproteins, because non-glucosylated oligosaccharides are a suboptimal substrate for the oligosaccharyltransferase and are, therefore, transferred to proteins with a reduced efficiency. For an unknown reason, the blood glycoproteins are unusually low (particularly factor XI, and coagulation inhibitors such as antithrombin III and protein C). That the clinical picture in these patients is milder than that of PMM2-CDG patients may be because a deficiency in glucosylation of the dolichol-linked oligosaccharides does not affect the biosynthesis of GDP-mannose and, hence, does not affect the biosynthesis of compounds such as GDP-fucose or of glycosylphosphatidylinositol-anchored glycoproteins.

Genetics

Inheritance of this glucosyltransferase deficiency is autosomal recessive. The p.A333V and p.I299Del are common mutations (www.lovd.nl/ALG6).

Diagnostic Tests

This disease illustrates that, even in cases of mild psychomotor disability without any specific dysmorphic features, IEF of serum sialotransferrins should be performed. When a type 1 pattern is found, PMM2 deficiency and MPI deficiency must be considered first. If these enzymes show normal activities, the next step is the analysis of the dolichol-linked oligosaccharides (DLO) in fibroblasts, or mutation analysis of a panel of CDG genes, or WES. If the major fraction of the dolichol-linked oligosaccharides consists of nine mannose and two *N*-acetylglucosamine residues without the three glucose residues that are normally present, mutation analysis should be performed.

Treatment and Prognosis

No efficient treatment is available. The oldest known patient is 38 years. Nine patients have died [30].

41.2.4 Mannosyltransferase 1 Deficiency (ALG1-CDG)

Clinical Presentation

ALG1-CDG is an autosomal recessive disease with a broad clinical spectrum, reported in 19 patients (belonging to 14 families). We know of 13 unpublished patients, from 9 families. There is a predominant neurological involvement. Constant features are intellectual disability (mostly severe) and hypotonia (sometimes only in the infantile stage). The majority of patients show dysmorphism (such as facial dysmorphism, inverted nipples, fat pads, contractures, arachnodactyly), microcephaly (mostly from neonatal age), intractable seizures, visual disturbances, tremor, ataxia, severe infections/episodes of unexplained fever and cerebral abnormalities (cerebral infarct, general atrophy and/or periventricular white matter abnormalities). A number of other symptoms have been reported in one or a few patients.

Metabolic Derangement

ALG1 attaches the first of 9 mannoses to the $GlcNAc_2$-P-P-Dol at the outside of the ER membrane. Biochemical abnormalities comprise decreased levels of serum LDL cholesterol, blood coagulation factor XI and anticoagulation factors antithrombin III, protein C and protein S, as well as variable hypoalbuminaemia, increased serum transaminases, decreased serum cholinesterase and immunoglobulins, and endocrinological abnormalities (such as decreased serum IGF1 and IGFBP3).

Genetics

ALG1-CDG is an autosomal recessive disease. Thirteen mutations have been reported. The most frequent is c.773C>T (p.Ser258Leu).

Diagnostic Tests

Serum transferrin IEF shows a type 1 pattern, and analysis of short DLO in fibroblasts an increase of $GlcNAc_2$-P-P-Dol. The diagnosis has to be confirmed by mutation analysis of *ALG1*.

Treatment and Prognosis

No efficient treatment is available. Survival of reported patients ranged from 2 days to more than 20 years [31].

41.2.5 UDP-GlcNAc:Dol-P-GlcNAc-P Transferase Deficiency (DPAGT1-CDG)

Clinical Presentation

DPAGT1-CDG has been reported in 41 patients (from 17 families). It presents as one of two different phenotypes: an encephalopathy in the context of a multisystem disorder, or a congenital myasthenic syndrome. The multisystem presentation (28 patients) is usually a severe disease. All patients showed moderate to severe psychomotor disability, and most patients had microcephaly, hypotonia, and epilepsy. Less frequent symptoms were feeding difficulties, apnoea, respiratory insufficiency, chronic anaemia, cataracts, hypotrophy, hypertonia of the extremities, hypo- and hyperreflexia, joint contractures, and abnormal brain magnetic resonance imaging. The congenital myasthenic syndrome presentation has been reported in 13 patients. The first symptoms were noted between birth and 17 years. They suffered from a predominantly proximal muscle weakness with absent or minimal craniobulbar symptoms. The syndrome was mostly slowly progressive. Muscle cramps, difficulty in swallowing and chewing, and scoliosis have been reported in a few patients, as well as delayed motor development and intellectual disability.

Metabolic Derangement

DPAGT1-CDG is a defect in the attachment of the second GlcNAc to GlcNAc-P-P-Dol at the outside of the ER membrane. Biochemical abnormalities in the multisystem presentation comprise increased serum transaminases and creatine kinase, hypoproteinemia and decreased antithrombin. In the congenital myasthenic syndrome presentation, serum creatine kinase levels were normal.

Genetics

DPAGT1-CDG is an autosomal recessive disease. Twenty-five mutations in *DPAGT1* have been reported including 21 missense mutations.

Diagnostic Tests

Serum transferrin IEF showed a type 1 pattern in all patients with the multisystem presentation, but in only 4/8 patients with the congenital myasthenia syndrome presentation. The next step is to analyse a panel of genes known to be involved in CDG and, if this is normal, to perform whole-exome sequencing.

Treatment and Prognosis

All patients with the congenital myasthenic syndrome presentation responded favourably to acetylcholinesterase inhibitors such as pyridostigmine. Ages at report ranged from 6 to 58 years. There is no efficient treatment for the multisystem presentation. Twenty-three patients died between 6 weeks and 5 years. Two siblings had a milder presentation, and were 34 and 32 years old when reported [32].

41.2.6 Golgi α1-2 Mannosidase 1 Deficiency (MAN1B1-CDG)

Clinical Presentation

MAN1B1-CDG has been reported in 34 patients (belonging to 21 families). They all showed mild to severe intellectual/developmental disability. Most patients also presented abnormal speech development and hypotonia. In the majority of patients there was facial dysmorphism and truncal obesity. Behavioural problems have been reported in about half of the patients, particularly verbal and physical aggression, autism, inappropriate sexual behaviour and overeating.

Metabolic Derangement

MAN1B1-CDG is due to a defect in a Golgi mannosidase. Biochemical abnormalities such as increased serum transaminases and abnormal coagulation tests were present in only a few patients.

Genetics

MAN1B1-CDG is inherited as an autosomal recessive disease. Nineteen mutations of *MAN1B1* have been reported, including 11 missense mutations.

Diagnostic Tests

Serum transferrin IEF shows a type 2 pattern. Mass spectrometry of serum transferrin shows an accumulation of specific hybrid type N-glycans. The diagnosis should be confirmed by mutation analysis.

Treatment and Prognosis

No efficient treatment is known. The oldest patient was 35 years at the time of report [33].

41.3 Congenital Disorders of Protein O-Glycosylation (Table 41.2)

41.3.1 Progeroid Variant of Ehlers-Danlos Syndrome (B4GALT7-CDG)

B4GALT7 is involved in the biosynthesis of the glycan moiety (glycosaminoglycans) of proteoglycans. All glycosaminoglycans, with the exception of keratan sulfate and hyaluronan, are connected to a serine residue of a core protein through a tetrasaccharide core linkage region. This tetrasaccharide linkage region, an O-linked glycosylation, is formed by the serial addition of a xylose, two galactoses and a glucuronic acid. B4GALT7 catalyses the attachment of the first galactose. As a consequence these patients have defective synthesis of heparan, dermatan and chondroitin sulfate. Some 49 patients have been reported. They showed a short stature, developmental anomalies of the forearm bones and elbows, and bowing of the extremities, in addition to the classic features of Ehlers-Danlos syndrome (joint laxity, skin hyperextensibility and poor wound healing). A specific entity, Larsen of Reunion Island syndrome, is caused by a homozygous founder mutation. These patients have in addition multiple dislocations but no progeroid appearance [34].

41.3.2 GALNT3 Deficiency (GALNT3-CDG)

GALNT3-CDG is due to a defective UDP-*N*-acetyl-α-D-galactosamine: polypeptide *N*-acetylgalactosaminyltransferase 3. This autosomal recessive defect causes one of two syndromes: hyperphosphatemic familial tumoral calcinosis (HFTC) or hyperphosphatemic hyperostosis syndrome (HHS). At least 42 patients have been reported, mostly from Middle Eastern or African ancestry. It is a disturbance in the hormonal regulation of serum phosphate levels by FGF23. In HFTC this leads to the development of recurrent, painful calcified subcutaneous masses. This can be complicated by secondary infections and incapacitating mutilations. In HHS it leads to episodes of diaphysitis and cortical hyperostosis visualized on X-ray examination. The regulation of the phosphate metabolism involves phosphatonins, in particular FGF23. GALNT3 is necessary for the O-glycosylation of FGF23 thereby preventing the inactivation of this phosphaturic protein. Both phenotypes have been reported to occur in the same family [35].

41.3.3 Hereditary Multiple Exostoses (EXT1/EXT2-CDG)

Hereditary multiple exostoses is an autosomal dominant disease with a prevalence of 1 in 50,000 and is characterised by the formation of cartilage-capped tumours, known as osteochondromas, on the ends of long bones. These are often present at birth, but usually not diagnosed before early childhood. Their growth slows at adolescence and stops in adulthood. A small percentage of these lesions are subject to malignant degeneration. Complications may arise from compression of peripheral nerves and blood vessels.

The basic defect resides in a Golgi-localised protein complex, termed exostosin-1/exostosin-2 (EXT1/EXT2), which adds glucuronic acid and *N*-acetylglucosamine units in the synthesis of heparan sulfate. It has been hypothesized that mutations in these glycosyltransferases impair the synthesis of a glycosaminoglycan that exerts a tumor-suppression function. This would explain the higher risk of affected individuals to develop chondrosarcomas and osteosarcomas.

Mutations in *EXT1* and in *EXT2* have been identified and are responsible for over 70% of cases of hereditary multiple

Table 41.2 Protein *O*-glycosylation disorders

Name	Main clinically affected organs and systems	Defective protein
Defects in O-xylosylglycan synthesis		
XYLT1-CDG	Brain, skeleton (short stature, advanced bone age), articulations (joint laxity), fat	Xylosyltransferase 1
XYLT2-CDG	Brain, eyes, heart, hearing system, bones	Xylosyltransferase 2
B4GALT7-CDG	Brain, skeleton (short stature, bowing of extremities), articulations (hyperlaxity, dislocations), skin (premature aging phenotype)	Beta-1,4-galactosyltransferase 7
B3GALT6-CDG	Skeleton (spondyloepimetaphyseal dysplasia with bone fragility, severe kyphoscoliosis), joints, skin (fragility, delayed wound healing)	Beta-1,3-galactosyltransferase 6
B3GAT3-CDG	Brain, aorta, heart, skeleton, joints, skin, teeth	Beta-1,3-glucuronyltransferase 3
EXT1-CDG (multiple cartilaginous exostoses)	Cartilage (osteochondromas of the ends of long bones)	Exostosin 1
EXT2-CDG (multiple cartilaginous exostoses)	Cartilage (osteochondromas of the ends of long bones)	Exostosin 2
CHSY1-CDG (Tentamy preaxial brachydactyly syndrome)	Brain, teeth, skeleton (particularly brachydactyly), hearing system	Chondroitin β-1,4-*N*-acetylgalactosaminyltransferase 1 (chondroitin synthase 1)
Defect in O-*N*-acetylglucosaminylglycan synthesis		
EOGT-CDG	Skin (aplasia cutis congenita), skeleton (terminal transverse limb defect)	EGF domain-specific O-GlcNAc transferase
Defect in O-*N*-acetylgalactosaminalglycan synthesis		
GALNT3-CDG (familial hyperphosphatemic tumoral calcinosis)	Subcutaneous tissue (painful calcified masses)	UDP-*N*-acetyl-α-D-galactosamine:polypeptide *N*-acetylgalactosaminyltransferase 3
Defect in O-xylosyl/*N*-acetylgalactosaminylglycan synthesis		
SLC35D1-CDG (Schneckenbecken dysplasia)	Skeleton (generalized; radiographic snail-like configuration of iliac bones) (stillborn or lethal in the neonatal period)	Solute carrier family 35 (UDP-glucuronic acid/UDP-*N*-acetylgalactosamine dual transporter) member D1
Defects in O-mannosylglycan synthesis		
POMT1-CDG (cerebro-ocular dysplasia-muscular dystrophy syndrome)	Brain, eyes, skeletal muscles, heart	Protein O-mannosyltransferase 1
POMT2-CDG (cerebro-ocular dysplasia-muscular dystrophy syndrome)	Brain, eyes, skeletal muscles	Protein O-mannosyltransferase 2
POMGNT1-CDG (muscle-eye-brain disease)	Brain, eyes, skeletal muscles	Protein O-mannose β-1,2-*N*-acetylglucosaminyltransferase 1
B3GALNT2-CDG	Brain, eyes, skeletal muscles	Beta-1,3-*N*-acetylgalactosaminyltransferase 2
LARGE-CDG	Brain, eyes, skeletal muscles	Acetylglucosaminyltransferase-like protein
Defects in O-fucosylglycan synthesis		
POFUT1-CDG	Skin (progressive reticular hyper- and hypopigmentation)	Protein O-fucosyltransferase 1
LFNG-CDG (spondylocostal dysostosis type 3)	Axial skeleton, associated muscles	O-fucose-specific β-1,3-*N*-acetylglucosaminyltransferase
B3GALTL-CDG	Eyes (anterior eye chamber abnormalities), skeleton (short stature, cleft palate), and variable involvement of other organs	O-fucose-specific β-1,3-glucosyltransferase
Defect in O-glucosylglycan synthesis		
POGLUT1-CDG	Skin (progressive reticular hyper- and hypopigmentation)	Protein O-glucosyltransferase 1

exostoses (specific data base: http://medgen.ua.ac.be/LOVDv.2.0/home.php) [36].

41.3.4 Cerebro-Ocular Dysplasia-Muscular Dystrophy Syndromes, Types A1, B1, C1/A2, B2, C2 (POMT1/POMT2-CDG)

These are 2 of some 25 neuronal migration disorders known in humans. They are characterized by brain and eye dysgenesis associated with congenital muscular dystrophy. Male patients often have testicular defects. Psychomotor development is absent or very poor. The brain lesions consist of ›cobblestone‹ lissencephaly, agenesis of the corpus callosum, cerebellar hypoplasia, hydrocephaly and sometimes encephalocoele. The disease usually runs a fatal course before the age of 1 year, and only symptomatic treatment is available.

The metabolic derangement is an aberrant glycosylation of α-dystroglycan, an external membrane protein expressed in muscle, brain and other tissues. Most glycans of this heavily glycosylated protein seem to be O-linked via mannose, and they control the interaction with extracellular matrix proteins. Disrupted glycosylation of α-dystroglycan (and probably other glycoproteins) results in loss of this interaction and hence in progressive muscle degeneration and abnormal neuronal migration (overmigration) in the brain. In about 20% of the patients this disrupted glycosylation is due to a defective O-mannosyltransferase-1 or -2, which catalyses the first step in the synthesis of the O-mannosylglycan core. This autosomal recessive defect is caused by mutations in *POMT1* and in *POMT2* [37].

41.3.5 Muscle-Eye-Brain Disease, Types A3, B3, C3 (POMGNT1-CDG)

Muscle-eye-brain disease is a neuronal migration/congenital muscular dystrophy syndrome similar to cerebro-ocular dysplasia-muscular dystrophy syndrome, but less severe and with longer survival. The defect is in protein O-mannosyl-β-1,2-*N*-acetylglucosaminyltransferase 1, catalysing the second step in the synthesis of the *O*-mannosylglycan core. The disease is autosomal recessive and due to mutations in *POMGnT1* [37].

41.3.6 O-Fucose-Specific β-1,3-Glucosyltransferase Deficiency (B3GALTL-CDG)

Variants in the B3GALTL gene have been found to underly Peters-plus syndrome. The major criteria of this autosomal recessive disorder, reported in some 48 patients, are anterior eye chamber anomalies (mainly Peters' anomaly which consists of corneal clouding and iridolenticulocorneal adhesions), growth retardation, and brachydactyly. Intellectual disability is frequent. Cleft lip and/or palate, cardiac malformation, and facial dysmorphism, including external ear anomalies, are present in about half of the patients. No *B3GALTL* mutation(s) has been found in patients with an incomplete phenotype [38].

41.4 Defects in Lipid Glycosylation and in Glycosylphosphatidylinositol (GPI) Anchor Biosynthesis (▫ Table 41.3)

41.4.1 GM3 Synthase Deficiency (ST3GAL5-CDG)

See ▶ Chapter 39 on sphingolipid disorders [39].

41.4.2 GM2 Synthase Deficiency (B4GALNT1-CDG)

See ▶ Chapter 39 on sphingolipid disorders [40].

41.4.3 PIGA Deficiency (PIGA-CDG)

This is an X-linked disorder of the glycosylphosphatidylinositol anchor biosynthesis. PIGA is one of seven proteins required for the first step of this biosynthesis, the transfer of *N*-acetylglucosamine from UDP-GlcNAc to phosphatidylinositol. Other names for this disease are ›multiple congenital anomalies-hypotonia-seizures syndrome 2‹ and ›ferro-cerebro-cutaneous syndrome‹. At least 20 patients have been reported with less severe and severe phenotypes. The latter present with poorly responsive epilepsy, hypotonia, multiple brain abnormalities, facial dysmorphism, and, in a minority, variable involvement of skin, heart, liver and kidneys. Some patients show systemic, still unexplained iron overload. Most patients show hyperphosphatasaemia and decreases of other GPI-anchored proteins such as CD16 and CD24 on blood cells. Of note is that somatic *PIGA* mutations cause the well-known paroxysmal nocturnal hemoglobinuria, an acquired disorder of bone marrow failure [41].

41.5 Defects in Multiple Glycosylation Pathways and in Other Pathways Including Dolicholphosphate Biosynthesis Defects (▫ Table 41.4)

41.5.1 Hereditary Inclusion Body Myopathy (GNE-CDG)

Hereditary inclusion body myopathy is an autosomal recessive disease that is allelic to the Japanese disorder ›distal myopathy with rimmed vacuoles‹ or ›Nonaka myopathy‹. It usually begins after age 20 with muscle weakness that progresses over the next 10–20 years, sparing the quadriceps until the most advanced stage of the disease. Muscle histology shows rimmed

Table 41.3 Defects of lipid glycosylation and of glycosylphosphatidylinositol (GPI) synthesis

Name	Main clinically affected organs and systems	Defective protein
Defects in lipid glycosylation		
ST3GAL5-CDG (Amish infantile epilepsy; salt and pepper syndrome)	Brain, hearing system, skin	Lactosylceramide α-2,3-sialyltransferase (GM3 synthase)
B4GALNT1-CDG (spastic paraplegia 26, autosomal recessive)	Brain, peripheral nerves (spastic paraplegia), gonads	Beta-1,4-*N*-acetylgalactosaminyltransferase 1 (GM2 synthase)
Defects in glycosylphosphatidylinositol synthesis		
PIGA-CDG	Brain, heart, liver, kidneys, skin	UDP-GlcNAc:phosphatidylinositol *N*-acetylglucosaminyltransferase subunit
PIGL-CDG (CHIME syndrome)	Brain, eyes, hearing system, heart, skin	GlcNAc-phosphatidylinositol deacetylase
PIGM-CDG	Brain, hepatic veins	Dol-P-Man:phosphatidylinositol mannosyltransferase 1
PIGN-CDG	Brain, skeleton (including palate, fingers), cardiovascular system, kidneys	Glycosylphosphatidylinositol ethanolamine-phosphate transferase 1
PIGO-CDG	Brain, lips, fingers, toes, anus/rectum, hearing system, cardiovascular system	Glycosylphosphatidylinositol ethanolamine-phosphate transferase 3
PIGQ-CDG	Brain	UDP-GlcNAc:phosphatidylinositol *N*-acetylglucosaminyltransferase subunit
PIGT-CDG	Brain, eyes, heart, kidneys, skeleton	PIGT transamidase subunit
PIGV-CDG	Brain, fingers, toes, and less frequent involvement of lips, palate, anus/rectum, hearing system	Dol-P-Man:phosphatidylinositol mannosyltransferase 2
PIGW-CDG	Brain, skeleton	Phosphatidylinositol acylase
PGAP1-CDG	Brain	Phosphatidylinositol deacylase
PGAP2-CDG	Brain	Phosphatidylinositol glycerol acylase
PGAP3-CDG	Brain, skeleton	Phosphatidylinositol glycerol deacylase

vacuoles on Gomori's trichrome stain, small fibres in groups, and tubulofilaments without evidence of inflammation. Mutations have been identified in *GNE*, which encodes the bifunctional enzyme uridine diphospho-*N*-acetylglucosamine epimerase/*N*-acetylmannosamine kinase. This enzyme catalyses the first two steps in the biosynthesis of sialic acid. Information on mutations can be found at: www.dmd.nl/nmdb2/home.php?select_db=GNE. Oral administration of sialic acid, mannosamine and *N*-acetylmannosamine markedly improved muscle and renal hyposialylation in a mouse model. Trials are underway in patients [42].

41.5.2 Congenital Myasthenic Syndrome-12 (GFPT1-CDG)

Glutamine-fructose-6-phosphate transaminase 1 (GFPT1) is the initial and rate-limiting enzyme in the biosynthesis of *N*-acetylglucosamine, an essential substrate for O- and N-linked glycosylation. GFPT1-CDG is associated with a congenital limb-girdle myasthenic syndrome and has been described in at least 46 patients. Most patients show tubular aggregates of the sarcoplasmic reticulum in type 2 fibers. There is an inconstant, mild increase of serum creatine kinase. It is not known whether serum transferrin IEF is abnormal. Inheritance is autosomal recessive. Some 30 mutations have been reported. Most patients respond partially to cholinergic agents [43].

41.5.3 Steroid 5-α-Reductase Deficiency (SRD5A3-CDG)

This autosomal recessive disorder of the dolicholphosphate synthesis (Fig. 41.2) has been reported in some 18 patients. The clinical picture comprises mainly ophthalmological (mainly visual impairment, nystagmus, coloboma, optic atrophy) and neurological symptoms (mainly intellectual disability, hypotonia, spasticity, cerebellar ataxia, vermis atrophy). A minority of the patients show ichthyosiform skin lesions, kyphosis, contractures of the large joints, cardiac ab-

Table 41.4 Defects in multiple and other glycosylation pathways including dolichol metabolism defects

Name	Main clinically affected organs and systems	Defective protein
Defects in dolichol synthesis		
DHDDS-CDG (retinitis pigmentosa 59)	Retina	Dehydrodolichyl diphosphate
NUS1-CDG	Brain, eyes, skeleton	Nogo-B receptor (subunit of cis-prenyltransferase)
SRD5A3-CDG	Brain, eyes, heart, skin, joints	Steroid 5 α-reductase 3
DOLK-CDG	Brain, heart, skin	Dolichol kinase
Defects in dolichol utilization/recycling		
DPM1-CDG	Brain, eyes, skeletal muscles	GDP-Man:Dol-P-mannosyltransferase 1 (Dol-P-Man synthase 1)
DPM2-CDG	Brain, skeletal muscles	GDP-Man:Dol-P-mannosyltransferase 2 (Dol-P-Man synthase 2)
DPM3-CDG	Skeletal and cardiac muscles	GDP-Man:Dol-P-mannosyltransferase 3 (Dol-P-Man synthase 3)
MPDU1-CDG	Brain, eyes, skin	Man-P-Dol utilization 1
Defect in COPII		
SEC23B-CDG (congenital dyserythropoietic anemia type II)	Red cell lineage (secondary involvement of heart, liver, beta cells)	COPII component SEC23B
Defects in monosaccharide synthesis		
GFPT1-CDG (limb girdle congenital myasthenic syndrome)	Neuromuscular junction, skeletal muscles	Glutamine:fructose 6-phosphate amidotransferase 1
PGM3-CDG	Brain, immune system, skeleton	Phosphoglucomutase 3
GNE-CDG (hereditary inclusion body myopathy)	Skeletal muscles (with sparing of quadriceps muscles), rarely cardiac muscles	UDP-GlcNAc 2-epimerase/Man-NAc kinase
Defects in glycosyltransferases		
B4GALT1-CDG	Face (dysmorphism), eyes (myopia)	Beta-1,4-galactosyltransferase
ST3GAL3-CDG	Brain	Beta-galactoside α-2,3-sialyltransferase 3
Defect in nucleotide-sugar synthesis (see ▶ Chapter 35) [54]		
CPS2-CDG	Brain, intestine, kidneys, erythrocytes	Carbamylphosphate synthetase 2 deficiency
Defects in nucleotide-sugar transporters		
SLC35A1-CDG	Brain, heart, kidneys, platelets	CMP-sialic acid transporter
SLC35A2-CDG	Brain, eyes, gastrointestinal system, skeleton	UDP-galactose transporter
SLC35A3-CDG	Brain, skeleton	UDP-GlcNAc transporter
SLC35C1-CDG	Brain, cranial skeleton, neutrophils	GDP-fucose transporter
Defects in the conserved oligomeric Golgi (COG) complex		
COG1-CDG	Brain, skeleton	COG component 1
COG2-CDG	Brain, liver	COG component 2
COG4-CDG	Brain, face	COG component 4
COG5-CDG	Brain, hearing system, vision, liver, bladder	COG component 5

Table 41.4 (continued)

Name	Main clinically affected organs and systems	Defective protein
COG6-CDG	Brain, gastrointestinal system including liver, immune system	COG component 6
COG7-CDG	Brain, skeleton, skin, gastrointestinal system including liver, heart	COG component 7
COG8-CDG	Brain, eyes, peripheral nervous system	COG component 8
Defect in the V-ATPase complex		
ATP6V0A2-CDG (autosomal recessive cutis laxa type II; wrinkly skin syndrome)	Skin (cutis laxa becoming less obvious with age), brain (mental development mostly normal), eyes, neuromuscular system, skeleton	V0 subunit A2 of V-ATPase
Other defects		
TMEM165-CDG	Brain, skeleton (particularly cartilage), joints, heart, liver, kidneys	Transmembrane protein 165
PGM1-CDG	Uvula (palate, lips), heart, liver, muscles, endocrine organs	Phosphoglucomutase 1
TMEM199 -CDG	Liver	Transmembrane protein 199
CCDC115-CDG	Liver, spleen, brain	Coiled-coil domain-containing protein 135
SLC39A8-CDG	Brain, hearing, visual system, skeleton, joints, immune system	Mn and Zn influx symporter (ZIP8)

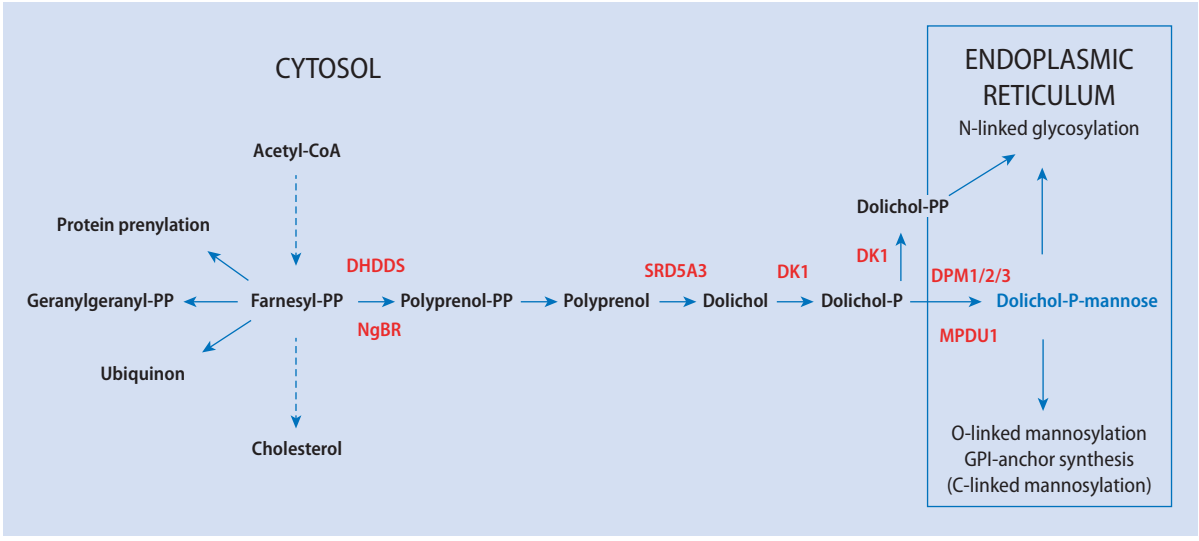

Fig. 41.2 Schematic representation of the dolichol synthesis and utilization/recycling

normalities, blood coagulation abnormalities and increased serum transaminases. The serum transferrin IEF shows a type 1 pattern in the large majority of the patients. Truncating variants have been described in the gene. The oldest reported patient was 45 years [44].

41.5.4 COG6 Deficiency (COG6-CDG)

The conserved oligomeric Golgi (COG) complex consists of eight subunits, divided in two lobes. Lobe A comprises subunits 1–4, and lobe B subunits 5–8. These lobes seem to be bridged by subunits 1 and 8. The COG complex plays an important role in Golgi trafficking and positioning of glycosylation enzymes. Mutations in all COG subunits, except subunit 3, have been reported in CDG patients. Seventeen patients have been described with COG6-CDG. Predominant clinical features are liver involvement, microcephaly, developmental/intellectual disability, recurrent infections, early lethality, and ectodermal symptoms (hypohydrosis predisposing to hyperthermia, and hyperkeratosis). Most of these symptoms are present in other COG-CDG. The closest overlap is with COG7-CDG, which has additional features such as malignant hyperthermia, adducted thumbs and cardiac defects. The patients show a type 2 pattern on serum transferrin IEF, and, in the patients tested, an abnormal pattern of serum apolipoprotein C-III IEF. Inheritance is autosomal recessive. Six mutations have been described. The splice site mutation c.1167-24A>G seems to be associated with a mild phenotype (ectodermal signs, mild developmental disability, no liver disease, no glycosylation deficiency). On the other hand, the loss-of-function mutations c.511C>T and c.1238_1239insA are associated with a severe phenotype including lethality between 1 and 15 months. Since the COG complex is most probably not only involved in glycosylation but also in other cellular functions, defects in this protein complex might be more appropriately called ›CDG-plus‹ [45].

41.5.5 Autosomal Recessive Cutis Laxa Type 2 (ATP6V0A2-CDG)

Patients with this disorder, also called ›wrinkly skin syndrome‹ already have generalized cutis laxa at birth, but this becomes less obvious later on and may disappear with age. Furthermore, they show congenital or postnatal microcephaly, increased joint laxity, ophthalmological abnormalities (strabismus, myopia, amblyopia, etc.) and, rarely, cardiac defects. Mental development is mostly normal. There is a combined defect in N- and O-glycosylation demonstrated by a type 2 serum transferrin IEF pattern and an abnormal serum apolipoprotein C-III IEF pattern. Skin biopsy shows an abnormal elastic fibre structure and a decrease of elastin.

Two major (and closely related) functions of the V-ATPase V0 domain are (i) maintenance of the pH gradient along the secretory pathway by proton transport and (ii) regulation of protein transport through the facilitation of vesicle fusion. However, the exact mechanism by which mutations in the V-ATPase a2 subunit affect glycosylation remains to be elucidated. At least 17 mutations have been reported. This seems to be another ›CDG-plus‹ [46].

41.5.6 Phosphoglucomutase 1 Deficiency (PGM1-CDG)

PGM1 is a key enzyme in glycogenesis and it is important for effective glycolysis during fasting. The disease has two major phenotypes: one is a myopathic glycogenosis (type XIV), and the other a multisystem presentation including growth deficiency, hypoglycemia, malformations (such as cleft uvula, cleft palate), and liver, cardiac and endocrine involvement. Contrary to most CDG, PGM1 deficiency shows as a rule no neurological involvement (unless as a consequence of a rare cerebral thrombosis). It is the only primary CDG that shows a defect in the assembly as well as in the processing of N-glycans (CDG-I/II). Serum transferrin IEF shows a type 2 pattern, and mass spectrometry of serum transferrin a decreased galactosylation. Galactose supplementation improves glycosylation of patients' fibroblasts, and patients on oral galactose treatment show improved glycosylation and also clinical improvement [47][48].

41.5.7 Golgi Homeostasis Disorders: TMEM199 and CCDC115 Deficiencies

Two new disorders with abnormal Golgi N and O-glycosylation have recently been described. The affected proteins are both involved in the Golgi homeostasis that can be readily identified via screening for abnormal glycosylation in plasma. Adolescents with TMEM199 deficiency presented with a mild phenotype of hepatic steatosis, elevated aminotransferases and alkaline phosphatase, hypercholesterolemia, and a low serum ceruloplasmin [49]. In CCDC115 deficiency all individuals displayed a storage-disease-like phenotype with hepatosplenomegaly, which regressed with age, highly elevated bone-derived alkaline phosphatase, elevated aminotransferases, and elevated cholesterol, in combination with abnormal copper metabolism and neurological symptoms. Two individuals died of liver failure, and one individual was successfully treated by liver transplantation [50].

41.5.8 Manganese and Zinc Transporter Defect: SLC39A8 Deficiency

SLC39A8 (also known as ZIP8) is a divalent cationic membrane transporter important for the uptake of Mn into cells. Compound heterozygous and homozygous mutations in *SLC39A8* have recently been described in patients with phenotypes ranging from cranial synostosis, hypsarrythmia, and disproportionate dwarfism to cerebellar atrophy, hypotonia, global developmental delay and recurrent infections [51][52]. Mn levels were reduced in blood and abnormal glycosylation

present (type 2 pattern), due to dysfunction of the Mn dependent enzyme β-1,4-galactosyltransferase. Oral galactose supplements (up to a dose of 3.75 g/kg/d) corrected the glycosylation defect. The clinical efficacy of treatment is not yet clear but it is suggested that early treatment with Mn and galactose should be considered.

41.6 Congenital Disorders of Deglycosylation

41.6.1 N-glycanase 1 Deficiency

N-glycanase catalyzes deglycosylation of misfolded N-linked glycoproteins by cleaving the glycan chain before the proteins are degraded by the proteasome. It is a cytoplasmic component of the endoplasmic reticulum-associated degradation (ERAD) pathway. At least 10 patients have been reported [53]. All patients showed developmental disability, movement disorders and hypotonia. Common features comprised intrauterine growth restriction, alacrimia/hypolacrimia, chalazion, microcephaly, seizures, peripheral neuropathy, hyporeflexia, and liver involvement (increased serum transaminases and alpha-foetoprotein, cytoplasmic storage). Two patients died at 9 months and 5 years of age. Serum transferrin IEF was normal. Most patients carried a nonsense mutation (c.1201A>T/p.R401X), that was associated with severe disease.

41.6.2 Lysosomal Storage Disorders

Besides the cytoplasmic protein deglycosylation disorder, NGLY1 deficiency, there are also lysosomal protein deglycosylation disorders namely the lysosomal storage diseases due to enzymatic defects (sphingolipidoses such as GM1-gangliosidosis a.o., mucolipidoses such as MPS I a.o., oligosaccharidoses such as fucosidosis a.o.; see ► Chapter 39).

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Cystinosis

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Lysosomal Porters for Cystine and Related Compounds

Intralysosomal cystine is formed by protein catabolism in the organelle, and is normally exported by a cystine porter (Fig. 42.1) which contains a membrane protein, *cystinosin*. Defects of this protein cause lysosomal accumulation of cystine. Cysteamine can enter into the lysosome and combine with cystine. This results in the formation of cysteine (which can be exported by the cysteine porter), and of the mixed disulfide, cysteine-cysteamine (which can be exported by the lysine porter due to its structural analogy).

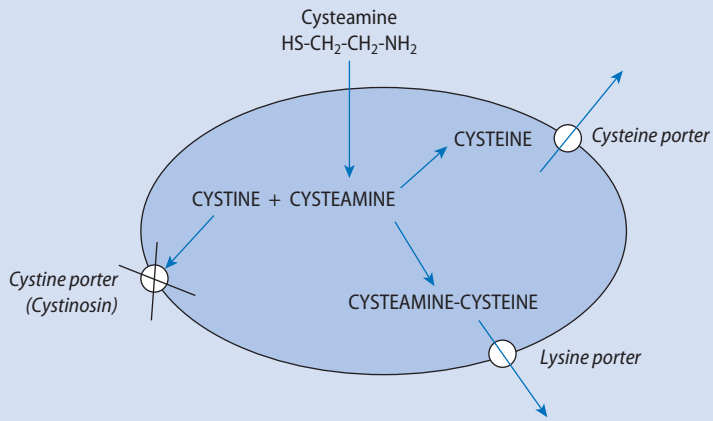

Fig. 42.1 Lysosomal export of cystine and related compounds. The cross represents the defect in cystinosis

Cystinosis is an autosomal-recessive generalized lysosomal storage disease classified into three clinical phenotypes, of which the nephropathic or infantile form (OMIM 219800) is by far the most frequent. The reported incidence of the disease is about 0,5–1:100,000 live births. The first symptoms start at about 6 months of age with anorexia, polyuria, failure to thrive and are secondary to a Fanconi proximal renal tubulopathy. In the absence of specific therapy, the renal disease progress to end stage renal disease between 6 and 12 years. Survival beyond this age is associated with the development of extrarenal complications in eyes, thyroid, gonads, endocrine pancreas, muscle and central nervous system. A late-onset or juvenile form (OMIM 219900), and a benign or adult form (OMIM 219750) limited to the eyes, are caused by mutations of the same gene. The lysosomal cystine accumulation leads to cellular dysfunction in many organs. The disease is caused by mutations in *CTNS* coding for cystinosin, a lysosomal cystine/proton symporter (► Lysosomal Porters for Cystine and Related Compounds). The diagnosis is ascertained by measurement of cystine in leukocytes. Treatment is both supportive and specific, the latter based on cysteamine, which effectively decreases intralysosomal cystine accumulation.

42.1 Infantile Cystinosis

42.1.1 Clinical Presentation

First Stage

The first 3–6 months of life are usually symptom-free. The first symptoms develop before 12 months of age. They include feeding difficulties, anorexia, vomiting, polyuria, constipation and failure to thrive. If the diagnosis is delayed, severe rickets develops after 10–18 months in spite of correct vitamin D supplementation. A polyuria of 2–5 L/day develops rapidly due to a severe urinary concentrating defect. Urine from cystinotic patients is characteristic, being pale and cloudy, and the diagnosis can be immediately suspected if both glucose and protein are found. When the disease has become symptomatic, the full expression of the Fanconi syndrome is generally present at first examination. It includes normoglycemic glycosuria, generalized aminoaciduria, low molecular-weight proteinuria (with massive excretion of β2-microglobulin and lysozyme), decreased reabsorption of phosphate with hypophosphatemia, excessive losses of potassium, sodium and bicarbonate leading to hypokalemia, hyponatremia and metabolic acidosis. Hypercalciuria may lead to nephrocalcinosis [1] and hypouricemia is constant. Tubular loss of carnitine may cause carnitine depletion. Kidney biopsy shows tubular abnormalities and some cystine crystals; plurinucleated glomerular podocytes are also characteristic.

The general reabsorptive defect of the proximal tubule explains the severe hydroelectrolyte imbalance, which may be life-threatening. Episodes of fever, probably related to dehydration, are also commonly noted.

Involvement of the eye is a primary symptom of cystinosis, starting with photophobia, which usually appears at 2 or

3 years of age. Ophthalmological examination with a slit lamp and a biomicroscope reveals cystine crystal deposits after the first year of life. Confocal microscopy and optical coherence tomography allows a quantitative measurement of the deposits [2]. There are also fundal abnormalities with typical retinopathy and subsequent alterations of the retinogram, which usually appear later.

End Stage Renal Failure

The natural history of the disease includes severe stunting of growth and a progressive decrease of the glomerular filtration rate, leading to end stage renal failure (ESRF) between 6 and 12 years of age. Progression to renal failure may be delayed by cysteamine treatment especially when started within the first months of life. This treatment also improves growth velocity. The decrease in glomerular filtration is accompanied by an improvement of urinary losses and a spurious regression of the Fanconi syndrome. At this stage, severe renal hypertension may develop. After kidney transplantation, there is no recurrence of the Fanconi syndrome even if cystine crystals develop in the recipient mononuclear cells that are present in the graft.

Late Symptoms

The success of renal replacement therapy and renal transplantation has revealed the consequences of long-term cystine accumulation in various organs and has emphasized the multisystemic nature of cystinosis, which may involve the eyes, thyroid, liver, spleen, pancreas muscle and central nervous system (CNS) [3].

Late Ocular Complications

The severity of ocular involvement differs from one patient to another [4]. Corneal deposits accumulate progressively in the stroma of the cornea and iris in all patients and on the surface of the anterior lens and retina in some. Photophobia, watering and blepharospasm may become disabling; these symptoms are often related to the erosion of corneal epithelium, leading eventually to keratopathy. Photophobia may be prevented and even completely cured by cysteamine eyedrops [5]. Sight may be progressively reduced, leading to blindness in a few patients who already had major ocular symptoms at an early age and a severe retinopathy but these posterior segment alterations may be prevented by oral cysteamine [6]. Cataract has been reported.

Endocrine Disturbances

Hypothyroidism Thyroid dysfunction usually appears between 8 and 12 years of age. It is rarely overt with clinical symptoms, but rather discovered by systematic assessment of thyroid function [7]. Hypothyroidism may be partly responsible for growth impairment. Cysteamine was reported to delay or prevent thyroid dysfunction [8].

Gonadal function Abnormalities in the pituitary testicular axis with a low plasma testosterone and high FSH/LH level [9] seem common in male patients with cystinosis. They may preclude full pubertal development. Male patients are generally azoospermic but the presence of spermatogenesis in testicular biopsy may allow in vitro fertilization [10]. Female patients exhibit pubertal delay but seem to have normal gonadal function and there are several reports of successful pregnancies.

Endocrine pancreas Insulin-dependent diabetes has been reported in several series of cystinotic patients in the second or third decade of life. Half of the patients not treated by cysteamine develop diabetes according to the WHO definition [11].

Liver and Spleen Involvement

Hepatomegaly and splenomegaly occur after 15 years of age in one-third to half of the patients who do not receive cysteamine [7]. Hepatomegaly is related to enlarged Kupffer's cells that transform into large foam cells containing cystine crystals. This enlargement may be the cause of portal hypertension with gastroesophageal varices. Splenomegaly is also related to the development of foam cells in the red pulp. Hematological symptoms of hypersplenism may be noted. Cysteamine prevents this type of complications.

Muscle

A myopathy, potentially leading to a severe handicap, has been reported in some patients with generalised muscle atrophy and weakness, mainly of distal muscles of all limbs [12][13]. Pharyngeal and oral dysfunction, which may also cause voice changes, is often observed [14][15]. Swallowing dysfunction is inversely related with the duration of cysteamine treatment [16]. Pulmonary dysfunction is correlated with the severity of myopathy [17]. Nocturnal non invasive positive pressure constitutes an effective treatment [18].

Central Nervous System

Cystinosis does not affect general intellectual performances but may be associated with mild neurocognitive abnormalities [19]. A subtle visuoperceptual defect and lower cognitive performances with subtle impairment of visual memory and tactile recognition have been reported [20][21] as well as social difficulties [22]. These anomalies may appear as early as 3–7 years of age favouring the hypothesis of the direct role of the gene defect rather than cystine accumulation [23]. More severe CNS abnormalities with various defects have also been described [24][25]. The clinical symptoms include hypotonia, swallowing and speech difficulties, development of bilateral pyramidal signs and walking difficulties, cerebellar symptoms and a progressive intellectual deterioration leading to a pseudo-bulbar syndrome. This cystinotic encephalopathy has only been observed above 19 years of age. Idiopathic intracranial hypertension has been reported [26]. Acute ischemic episodes may occur with hemiplegia or aphasia. Cysteamine treatment was associated in some cases with an improvement of neurologic symptoms [24]. Brain imaging in cystinosis may show several types of abnormalities. Brain atrophy, calcifications and abnormal features of white matter on magnetic resonance

imaging (MRI) examination are commonly observed after 15 to 20 years of age [24][27].

42.1.2 Metabolic Derangement

Efflux of cystine out of cystinotic lysosomes is significantly decreased in comparison with normal lysosomes [28]. Consequently, cystine accumulates in many tissues including kidney, bone marrow, conjunctiva, thyroid, muscle, choroid plexus, brain parenchyma and lymph nodes. This abnormality is related to a molecular defect of cystinosin, the protein that transports cystine across the lysosomal membrane. The function of this carrier molecule was demonstrated in a cellular model where the lysosomal targeting signal directing cystinosin to the plasma membrane is defective [29][30]. Cystine transport out of the lysosomes is H^+ driven. Why lysosomal cystine accumulation leads to cellular dysfunction is not clear. It has been shown that cystine accumulation in proximal tubular cells in vitro is associated with ATP depletion [31] and inhibition of Na^+ dependent transporters [32]. Cystinosin knockout mice show decreased expression of megalin, cubilin and sodium transporters at the apical surface of proximal tubular cells [33]. Cellular cystine accumulation may also inhibit pyruvate kinase and creatine kinase activity in rat brain or pig retina [34][35][36]. Inhibition of adenylate cyclase activity by cystine in rat brain is prevented by cysteamine [37]. Cystine depletes the glutathione cell pool, thereby favouring oxidative stress and apoptosis [38][39].

42.1.3 Genetics

Nephropathic cystinosis is an autosomal recessive disorder. The gene, mapped to chromosome 17 and named *CTNS*, encodes a protein of 367 amino acids which has the structure of an integral membrane protein with 7 membrane spanning domains and two lysosomal targeting signals [40]. More than 100 mutations in the first 10 exons and in the promotor of the gene have been identified in association with cystinosis. The most common is a 57 kb deletion found in 76% of patients of European descent. This deletion encompasses the *CARKL* gene, encoding the enzyme sedoheptulokinase (▶ Chapter 7). This explains that patients with homozygous 57kb deletion have elevated urinary concentrations of sedoheptulose [41]. In the other cases, shorter deletions, point mutations, small insertions, duplications, non-sense or splice-site mutations of the *CNTS* gene are found on both alleles, some of them clustering in certain ethnic and/or geographical areas [42].

Intermediate and adult forms have the same mode of inheritance with mutations that do not disrupt the open reading frame and are generally found in the intertransmembrane loops or in the N-terminal region.

42.1.4 Diagnostic Tests

The diagnosis of cystinosis is confirmed by the measurement of leukocyte cystine levels, the demonstration of corneal crystals by the slit lamp examination and genetic analysis of *CNTS*. In patients with nephropathic cystinosis, the free cystine content in leukocytes is about 10–50 times normal values [43]. The assay, which uses a protein-binding technique on white blood cells, is sensitive and specific, and can be carried out on small, 3 ml blood samples. In cystinosis, the level is usually 5 to 15 nmol of 1/2 cystine/mg protein. The technique enables detection of heterozygous carriers with levels of 0.5 to 1.4 nmol 1/2 cystine/mg protein. In control subjects, cystine is undetectable or <0.4. Liquid chromatography-tandem mass spectrometry may also be used with similar sensitivity [44]. The results obtained on polymorphonuclear leukocytes are approximately twice those obtained on mixed leukocytes and this must be taken in consideration when comparing data. The measurements may also be carried out on fibroblasts, conjunctiva and muscle. S-labeled cystine incorporation in cultured skin fibroblasts, amniotic cells, or chorionic villi enables a prenatal diagnosis during the first trimester to be made [40]. The direct assay on chorionic villi cells may give the result within 24 hours [45]. The diagnosis can also be made by molecular analysis if both mutations have been identified in an affected sibling. FISH diagnosis of the 57 Kb deletion is possible [46]. At birth, diagnosis is possible on placenta or cord blood white cells.

42.1.5 Treatment

The therapy of nephropathic cystinosis is both supportive and specific.

▪ Supportive Treatment of Tubular Losses

Several abnormalities have to be corrected:

Water The water intake must be adjusted to the level of diuresis, short-term weight variation and, if necessary, plasma protein concentration. Fluid requirement increases with external temperature and with fever. It is also increased by the required mineral supplements.

Acid base equilibrium Sodium and potassium bicarbonate, which have a better gastric tolerance than citrate, must be given in order to obtain a plasma bicarbonate level between 21 and 24 mmol/l. This is sometimes difficult and may require large amounts of buffer, up to 10–15 mmol/kg/day.

Sodium Sodium losses sometimes remain uncompensated after achieving acid base equilibrium. This is recognizable by a persistent hyponatremia with failure to thrive.

Potassium Hypokalemia requires potassium supplements in order to maintain serum potassium above 3 mmol/l. Four to 10 mmol/kg/day are usually necessary to achieve this goal.

Prescription of Amiloride at a dose of 2–5 mg/day may help in some cases.

Phosphorus Hypophosphatemia must be corrected with a supplement of sodium/potassium phosphate at a dose of 0.3–1 g/day. The aim is to obtain a plasma phosphate just above 1.0 to 1.2 mmol/l. This poorly tolerated supplement may be gradually withdrawn after some months or years. Excessive phosphorus prescription may lead to nephrocalcinosis.

Vitamin D supplementation Since tubular 1α-hydroxylation is diminished in this disease, it is justified to give 1α- or 1α-25-OHD_3 (0.10–0.50 μg/day), especially in cases of symptomatic rickets. These prescriptions must be carefully adjusted by regular follow-up of serum calcium.

Carnitine supplementation A dose of 100 mg/kg per day in four divided doses is proposed in order to correct muscle carnitine depletion [47].

All these supplements need to be given regularly in order to replace the losses, which are permanent. A good way to achieve this goal is to prepare in advance all the supplements, except vitamin D, in a bottle containing the usual amount of water for the day. Feeding problems may require tube or gastric button feeding and in some cases continuous or intermittent total parenteral nutrition [48].

- Losses of water, potassium and sodium may be drastically reduced by the prescription of indomethacin at a dose of 1–3 mg/kg/kg in two separate doses [49]. Although there is no consensus on the use of this drug in cystinotic patients, it appears to be relatively safe and well tolerated in young patients. It should be stopped if there is dehydration or if renal function deteriorates.
- It has been shown that the angiotensin converting enzyme (ACE) inhibitors diminish albuminuria and possibly slows down the degradation of renal function [50][51]. These should not be prescribed in association with indomethacin, which also reduces renal perfusion.
- When the glomerular filtration rate decreases, tubular losses also decrease and the mineral supplements must be adjusted and progressively tapered off in order to avoid overload, especially with sodium and potassium. When dialysis is started, mineral supplements are usually no longer necessary.

■ Renal Replacement Therapy

There is no specific requirement for cystinotic children for this procedure at this stage. Hemodialysis or peritoneal dialysis are both effective and applied according to the circumstances. As for any child with ESRF, kidney transplantation is considered the best approach. Long-term results of kidney transplantation are even better than for any other primary renal disease in children [52][53].

■ Supportive Treatment of Extrarenal Complications

Hypothyroidism, even if asymptomatic, should be treated with L-thyroxine supplementation. Growth failure, one of the most striking complications of nephropathic cystinosis, is improved by administration of recombinant growth hormone at a dose of 1 U/kg/week [54]. Hypersplenism with permanent leukopenia and/or thrombocytopenia may be an indication for splenectomy. Photophobia and watering may be improved by local symptomatic therapy such as vitamin A eye drops, artificial tears, topical lubricants, and thin bandage soft contact lenses. Corneal graft has been rarely performed, with variable results.

■ Specific Therapy

Cysteamine, ($HS\text{-}CH_2\text{-}NH_2$), has been shown to reduce cystine leukocyte content and slow the decline in the glomerular filtration rate [55]. The dose is progressively increased from 10 to 50 mg/kg of cysteamine base per day. Cysteamine, most widely used as cysteamine bitartrate (Cystagon), is rapidly absorbed and its maximum effect, assessed by cystine assay in leukocytes, occurs after 1–2 h, and lasts no longer than 6 h [56]. Consequently, it has to be given in 4 separate doses – one every 6 h – in order to obtain the best prevention of cystine accumulation. It was recently shown that a twice daily administration of an enteric release formulation of cysteamine bitartrate was as effective as the current formulation of cysteamine [57].

The drug should be started as soon as the diagnosis is confirmed [58]. The target dose is 1.3 g/m^2/day of free base for children up to 12 years and 2 g/day for older patients or those weighting more than 50 kg. The aim is to keep the cystine content, determined 6 hours after the last dose, under 1 nmol of 1/2 cystine per mg of protein, although there is no definitive evidence that this leads to the optimal depletion at the tissue level. The maximum dose should not exceed 1.95 g/m^2/day. Side effects of the drug include nausea and vomiting and can be managed with omeprazole [59]. Less commonly, allergic rashes, seizures, neutropenia and angioendotheliomatosis lesions on the elbows are seen [60]. In addition, the metabolite of cysteamine, dimethyl sulfide, is responsible for an unpleasant breath smell so that compliance with 4 doses per day is difficult to maintain in the long term, especially in adolescents [61]. Cysteamine prevents or delays late, non-renal, complications of the disease [62][63].

Oral cysteamine has no effect on corneal cystine crystals. Eye drops containing 0.55% cysteamine may prevent corneal deposits [5], and decrease and even suppress the deposits already present. However, they have to be given 6–10 times a day, a task difficult to achieve. A 0.55% gel formulation (Cystadrops) allows a reduced number of instillations with a good efficacy [64].

A therapeutic approach has been tested on an animal model using bone marrow cell transplantation with encouraging results [65][66].

42.2 Late-Onset Cystinosis

This is a rare, milder form of the disease, with later clinical onset and delayed evolution to ESRF. It represents less than 2

or 3% of cases. The first symptoms usually appear after 6–8 years of age. Proteinuria may be misleading because of its severity, sometimes in the nephrotic range. Fanconi syndrome may be absent or mild and tubular losses are less important than in infantile cystinosis [67]. The same is true for extrarenal symptoms. ESRF may develop during adolescence or adult life.

The diagnosis is ascertained by the measurement of cystine in leukocytes. Genetic analysis shows homozygous or compound heterozygous *CNTS* mutations with at least one »mild« mutation [67].

42.3 Ocular Cystinosis

Ocular (or adult or benign) cystinosis was first reported by Cogan et al in 1957 [68]. This exceptional disorder is characterized by the presence of cystine crystals in the eye and the bone marrow [69]. Crystals in the cornea are usually found by chance examination. The level of cystine in leukocytes is intermediate between that of heterozygotes and homozygotes for nephropathic cystinosis. All systemic manifestations of the other forms of cystinosis are lacking. The mutations in *CTNS* found in these patients encode a protein that allows sufficient residual cystine transport.

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Section XI Appendix

Medications Used in the Treatment of Inborn Errors

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The following table lists medication used in the treatment of IEM (Table 43.1). The list is not exhaustive. Readers should be aware that many of these drugs are unlicensed and the evidence base for their use is limited; due to the rarity of specific disorders controlled trials are the exception. When using this table, readers are advised to consult the specific chapters indicated. While every effort has been made to ensure the accuracy of the information it is essential before prescribing that the indications and dosage are checked against any local or national guidelines or formularies. The recommended doses related to body weight are generally those for paediatric patients, using these in adult patients may not be appropriate. For explanation of abbreviations, see relevant chapter(s).

Table 43.1 Medication used in the treatment of IEM

Medication	Mode of action	Disorders	Recommended dose	Route	Remarks	Chapter(s)
Agalsidase alfa	Recombinant analogue of human α-galactosidase A manufactured by gene activation in human fibroblast cell line	Fabry disease	0.2 mg/kg alt weeks	IV		38
Agalsidase beta	Recombinant analogue of human α-galactosidase A manufactured in Chinese Hamster Ovary (CHO) cell line	Fabry disease	1.0 mg/kg alt weeks	IV		38
Aglucosidase alfa	Recombinant analogue of human α-glucosidase manufactured in Chinese Hamster Ovary (CHO) cell line	Pompe disease	20 mg/kg alt weeks	IV	Higher doses may be considered	5
Allopurinol	Xanthine-oxidase inhibitor	Disorders leading to hyperuricaemia (PRPP synthetase superactivity, HG-PRT deficiency and APRT deficiency)	Initial dosage 10–20 mg/kg per day in children and 2–10 mg/kg per day in adults	Oral	Adjust dose to minimum required to maintain normal plasma uric acid concentration	35
Betaine	Remethylates homocysteine to methionine	Classic homocystinuria Remethylation defects	100–200 mg/kg/day in two to three divided doses, max. dose 6–9 g/d	Oral		20
Biotin	Co-factor for carboxylases Treatment of presumed transporter defect	Biotinidase deficiency; multiple carboxylase deficiency; thiamine transporter 2 deficency (biotin-responsive basal ganglia disease)	5–20 mg/d	Oral or IV	Multiple carboxylase deficiency may require higher doses. Biotin-responsive basal ganglia disease should be treated with thiamine, with or without biotin	26, 28
Chenodeoxycholic acid	Inhibits cholesterol 7α-hydroxylase (rate-limiting enzyme in bile acid biosynthesis)	3β-Dehydrogenase deficiency	12–18 mg/kg/d for first 2 months then 9–12 mg/kg/d	Oral	Various combinations of chenodeoxycholic acid, cholic acid and ursodeoxycholic acid have been used in (3-ORD)	33
		Δ^4-3-Oxosteroid 5β-reductase deficiency (3-ORD)	8 mg/kg/day			
		Cerebrotendinous xanthomatosis (CTX)	750 mg/day (adults)			
		Oxysterol 7α-hydroxylase deficiency	25 mg/kg/d			

Cholesterol	Replenishes cholesterol	Smith-Lemli-Opitz syndrome	20–40 mg/kg/d in 3–4 divided doses	Oral	Doses of up to 300 mg/kg/d have been used	32
Cholestyramine	Bile acid sequestrant	Familial hypercholesterolaemia	Adults: 12–24 g/d Children: (wt in kg/70 × adult dose) in four divided doses	Oral	Possible vitamin A, D, and K deficiency with prolonged treatment. Other bile acid resins include colestipol & colesevalam	31
Cholic acid		Δ^4-3-Oxosteroid 5β-reductase deficiency (3-ORD), 3β-Dehydrogenase deficiency	8 mg/kg/day	Oral		33
Copper histidine	Increases intracellular copper	Menkes disease	250 µg Cu twice daily to 1 year then 250 µg Cu/d in older children	SC	Only effective if started in first weeks of life. More than 3 years of treatment may not be necessary or desirable	37
Creatine monohydrate	Replenishes creatine	Guanidinoacetate methyltransferase deficiency	400–800 mg/kg/d in three to six divided doses	Oral	Given in combination with L-ornithine	15
		Arginine:glycine amidinotransferase) deficiency	100–800 mg/kg/d in three to six divided doses	Oral		15
Cyclic pyranopterin monophosphate (cPMP)	Replenishes deficient product to allow production of molybdenum co-factor	Molybdenum co-factor deficiency type A	80–160 mg/kg/d	IV	Daily infusions	20
Cysteamine - given as cysteamine bitartate (Cystagon)	Depletes lysosomal cysteine	Cystinosis	1.3 g/m^2/day of free-base) for children up to 12 yr, 2 g/d for older patients, given every 6 h. Max dose 1.95 g/m^2/d	Oral	Eye drops also required to prevent corneal deposits of cysteine	42
D,L-3-Hydroxybutyrate	Alternative fuel source, allowing reduction in CHO intake in GSDIII, replaces deficient endogenous ketone body production in MADD	GSDIII; MADD	300–600 mg/kg/d 3 hourly	Oral	Possibly protects against cardiomyopathy in GSD III. May improve cardiomyopathy and leukodystrophy in MADD	5, 12
Dextromethorphan	NMDA channel antagonist	NKH	3–15 mg/kg/d in four divided doses	Oral	Doses up to 35 mg/d have been used	23
Diazoxide	Inhibits insulin secretion	Persistent hyperinsulinism	10–15 mg/kg/d (newborn); 10 mg/kg/d (infants), in three divided doses	Oral	Give lower doses (2–5 mg/kg/d) if risk of pulmonary hypertension)	9
Dichloroacetate	Stimulates PDH activity by inhibiting PDH kinase	Primary lactic acidosis	25 mg/kg/d in 2 divided doses	Oral	May cause polyneuropathy with prolonged use	11

Table 43.1 (continued)

Medication	Mode of action	Disorders	Recommended dose	Route	Remarks	Chapter(s)
Elosulfase alpha	Enzyme replacement	MPS IVA (Morquio A)	2 mg/kg weekly infusions	IV		39
Entacapone	Prevents the peripheral breakdown of L-dopa	Disorders of BH_4 synthesis	15 mg/kg/d in two to three divided doses	Oral		16
Ezetimibe	Inhibits cholesterol absorption	Familial hypercholesterolaemia	10 mg/d	Oral		31
Folinic acid	Provides accessible source of folate for CNS	DHPR deficiency; UMP synthase deficiency (hereditary orotic aciduria); methionine synthase deficiency and other disorders of cobalamin metabolism; hereditary folate malabsorption; cerebral folate transporter deficiency	5–15 mg/d	Oral, IV	Higher doses (up to 400 mg/d orally) have been used in hereditary folate malabsorption. Use 5-Methyl tetrahydrofolate in MTHFR deficiency	16, 27
Galsulfase	Recombinant analogue of human *N*-acetylgalactosamine 4 sulfatase manufactured in Chinese Hamster Ovary (CHO) cell line	MPS VI (Maroteaux Lamy)	1.0 mg/kg per week	IV		39
Galactose	Restores missing compound	Phosphoglucomutase 1 deficiency; SLC39A8 deficiency	3 g/kg	Oral	Corrects abnormal glycosylation	41
G-CSF	Stimulates granulocyte production	Neutropenia in GSD Ib	0.5–5 µg/kg once daily or alternate days	SC	Use the lowest effective dose to increase the neutrophil count to $>0.5\times10^9$/L	5
Gemfibrozil	Fibrates decrease TG levels; other fibrates include bezafibrate and fenofibrate	Mixed or combined hyperlipidaemia	Adult dose: 1.2 g daily, usually in two divided doses; range 0.9–1.5 g daily	Oral	Can cause a myositis-like syndrome, especially with impaired renal function; combination with a statin increases risk of rhabdomyolysis	31
Glycine	Forms isovalerylglycine with high renal clearance	Isovaleric acidaemia	150 mg/kg/d in three divided doses	Oral	Up to 600 mg/kg/d during decompensation	18
	Replenishes glycine	3 phosphoglycerate dehydrogenase def; phosphoserine aminotransferase deficiency	200–300 mg/kg/d		Adjunct to treatment with serine	24
Glycocholic acid		Bile acid amidation defects 1 and 2	15 mg/kg/d	Oral		

Haem arginate, (haematin, haemin)	Inhibits 5-aminolevulinic acid synthase	Acute porphyrias	3–4 mg/kg once daily for 4 days	IV		36
Hydroxocobalamin (vitamin B_{12})	Co-factor for methylmalonyl mutase and methionine synthase	Disorders of cobalamin metabolism	1–2 mg IM daily; oral dose 10 mg once or twice daily	IM or oral	IM dose may be reduced to once or twice weekly according to response	18, 27
Hydroxytryptophan	Neurotransmitter replacement	Disorders of neurotransmitter synthesis	1–2 mg/kg, increasing gradually to 8–10 mg/kg in 4 divided doses	Oral	Monitor CSF 5HIAA levels	16, 29
Idursulfase	Recombinant iduronate-2-sulfatase produced in human cell line	MPS II (Hunter)	0.5 mg per kg by IV infusion weekly	IV		39
Imiglucerase	Recombinant analogue of human β-glucocerebrosidase manufactured in Chinese Hamster Ovary (CHO) line	Gaucher disease	30–60 U/kg alt weeks	IV	Lower doses are also used	38
Insulin	Promotes anabolism; inhibits catabolism	Acute decompensation in organic acidaemias, urea cycle disorders, MSUD, FAO disorders	0.05–0.2 IU/kg/h	IV	Always give in conjunction with IV solutions containing dextrose & with frequent blood sugar monitoring of blood sugar	4
Ketamine	*N*-Methyl-d-aspartate (NDMA) channel antagonist	NKH	15 mg/kg/d in neonates, 9 mg/kg/d infants range (1–30 mg/kg/d) in four divided doses	Oral or IV		23
L–Arginine	Replenishes arginine; substrate of nitrous oxide	Urea cycle disorders (except arginase deficiency); citrin deficiency; MELAS	100–250 mg/kg/d (OCT and CPS deficiency) Up to 300 mg/kg/d in ASS deficiency 5–15 g/d in adults with citrin deficiency	Oral or IV	IV loading dose: 250 mg/kg (400 mg/kg in ASL deficiency) over 90–120 min	14, 19
Laronidase	Recombinant analogue of human α-l-iduronidase manufactured in Chinese Hamster Ovary (CHO) line	MPS1 (Hurler/Scheie)	100 U/kg per week	IV		39
L-Carnitine	Replenishes body stores; removes toxic acyl-CoA intermediates from within the mitochondria	Primary and secondary carnitine deficiencies	100–200 mg/kg/d	Oral or IV	Do not use racemix mixture. May be harmful in long chain fatty acid oxidation defects	12, 18, 22
L-Citrulline	Replenishes citrulline and arginine	Used as an alternative to arginine in CPS deficiency and OCT deficiency; LPI	CPS & OCT deficiency 170 mg/kg/day or 3.8 gm/m^2/day in divided doses, LPI: 50–100 mg/kg/d in 3–5 doses	Oral		19, 25

Table 43.1 (continued)

Medication	Mode of action	Disorders	Recommended dose	Route	Remarks	Chapter(s)
L-Dopa	Replacement of neurotransmitters	Disorders of L-dopa synthesis	1–2 mg/kg increasing slowly to 10–12 mg/kg in four divided doses	Oral	Give as L-dopa /carbidopa (1:10 or 1:5) Monitor CSF HVA levels	17, 29
L-Lysine-HCl	Increase plasma lysine	Lysinuric protein Intolerance	20–30 mg/kg/day in three divided doses	Oral		25
L-ornithine	Competitive inhibitor of AGAT - reduces guanidinoacetate production	Guanidinoacetate methyltransferase deficiency	400–800 mg/kg/d	Oral	Given in combination with creatine monohydrate	15
L-Serine	Replenishes serine	3-Phosphoglycerate dehydrogenase, phosphoserine aminotransferase, 3-phosphoserine phosphatase deficiencies	up to 500–700 mg/d in six divided doses	Oral	Has been given to a mother during pregnancy where fetus affected with 3-phosphoglycerate dehydrogenase deficiency at 190mg/kg/d	24
Magnesium	Replenishes Mg	Primary hypomagnesaemia with secondary hypocalcaemia	0.5–1.5 ml/kg/d $MgSO_4$ 10% solution IV; oral maintenance 0.4–3.9 mmol/kg/d elemental Mg in three to five divided doses	IV or Oral		37, 41
Mannose	Improves glycosylation	Mannosephosphate isomerase deficiency (MPI-CDG)	1 g/kg/d in four to six divided doses	Oral		41
Mercaptopropionylglycine (Tiopronin)	Chelating agent	Cystinuria	10–20 mg/kg/d, up to max of 1,000 mg/d in three divided doses	Oral		25
5-Methyl tetrahydrofolate	Achieves measurable levels of CSF levels of 5-methyltetrahydrofolate	5,10 Methylenetetrahydrofolate reductase deficiency	15–60 mg/d	Oral	Available as calcium mefolinate	27
Metronidazole	Reduces propionate production by gut bacteria	Propionic and methylmalonic acidaemia	7.5–20 mg/kg once daily	Oral	Continuously or for a limited period (eg 10 days) each month	18
Miglustat	Inhibitor of glucosylceramide synthase, the first enzyme responsible for glycosphingolipid (GSL) synthesis	Gaucher disease; neurological manifestations of Niemann Pick C	100 mg TDS	Oral	Only recommended for patients with mild to moderate Gaucher disease who are unsuitable for enzyme replacement therapy.	38
N-Carbamoylglutamate (Carglumic acid, Carbaglu)	Stimulates *N*-acetylglutamate synthase	NAGS deficiency; CPS deficiency; hyperammonaemia associated with organic acidaemias	100–250 mg/kg/day in four divided doses	Oral	Maintenance dose in NAGS deficiency 10mg–100 mg/d	18, 19

Nicotinamide	Replenishes deficiency state	Hartnup disease	50–300 mg/day	Oral		25
Nicotinic acid (niacin)	Inhibits the release of free fatty acids from adipose tissue; increases HDL-cholesterol	Hyperlipidaemia	Adult dose: 100–200 mg 3 times daily, gradually increased over 2–4 weeks to 1–2 g three times daily	Oral	Seldom use due to significant side effects	31
Nitisinone (NTBC)	Inhibits 4-hydroxyphenylpyruvate dioxygenase	Tyrosinaemia type I	1 mg/kg/d (2 mg/kg/d in liver failure) in one to two divided doses	Oral	Combine with low-TYR, low-PHE diet to maintain plasma TYR 200–400 µmol/l. Aim to maintain nitisone levels >50 µmol/l in plasma or 20-40 µmol/l in whole blood	17
		Alkaptonuria	1–4 mg/day	Oral	Recommended in adults only & along with mild protein restriction. Avoid in pregnancy	17
Octreotide	Somatostatin analogue	Persistent hyperinsulinism	5–10 µg/d increasing up to 30–50 µg/d as required - given in three or four divided doses or by continuous pump (IV or SC) or three times a day (SC)	IV or SC		9
Penicillamine	Chelating agent	Wilson disease; cystinuria	Wilson disease: up to 20 mg/kg/day in divided doses (min 500 mg/d, maintenance dose in adults 750–1500 mg/d); cystinuria: 30 mg/kg/d up to 3–4 g in 3–4 divided doses	Oral or IV	In Wilson disease give with pyridoxine 25 mg/d	25, 37
Disodium calcium edetate	Chelating agent	SLC30A10 deficiency	(20 mg/kg/dose twice daily for 5 days a month)	IV	Combined with oral iron supplementation	37
Pramipexole	Dopamine agonist	Adjunct to therapy in disorders of BH_4 synthesis	3–32 micrograms /kg dose in 2 divided doses	Oral	Allows reduction in L-dopa therapy	16
Pyridoxal-phosphate	Active co-factor	Pyridox(am)ine 5′-phosphate oxidase deficiency	30–60 mg/kg/d in 4–6 divided doses	Oral	Monitor liver transaminases and use lowest effective dose possible	28
Pyridoxine	Co-factor	Pyridoxine-responsive γ-cystathionase deficiency; pyridoxine-responsive cystathionine β-synthase (CBS) deficiency; pyridoxine dependent Epilepsy (PDE) with seizures; pyridoxine-responsive OAT deficiency; X-linked sideroblastic anaemia; primary hyperoxaluria type 1	50–500 mg/d. Maintenance in CBS deficiency 10 mg/kg/d. Pyridoxine dependency with seizures: 100 mg IV with EEG monitoring or 30 mg/kg/d for 7 days (maintenance 15–30 mg/kg/d).	Oral	To prevent break through seizures in PDE dose may be doubled in the first 3 days of intercurrent infection. Peripheral neuropathy can occur with high doses (>900 mg daily in adults)	20, 21, 28, 36, 40

Table 43.1 (continued)

Medication	Mode of action	Disorders	Recommended dose	Route	Remarks	Chapter(s)
Riboflavin	Coenzyme	Mild variants of ETF/ETF-DH and SCAD deficiencies; ACAD9 deficiency; Brown-Vialetto-van Laere syndrome; RFVT1 riboflavin transporter deficiency; FAD transporter defect & FAD synthase deficiency; dihydrolipoamide dehydrogenase deficiency; glutaric aciduria I; L-2 hydroxyglutaric aciduria; trimethylaminuria	100–400 mg/d in two to three divided doses	Oral		11, 12, 14, 22
Sebelipase alfa	Recombinant lysosomal acid lipase from transgenic chickens	Lysosomal acid lipase deficiency (Wolman or CESD phenotypes)	1–5 mg/kg weekly doses	IV		31
Selegiline (*l*-deprenyl)	Monoamine-oxidase-B inhibitor	As adjunct to therapy with 5HT & L-dopa in BH_4 defects	0.1–0.25 mg/kg/d in three to four divided doses	Oral		16
Sodium benzoate	Combines with glycine to form hippuric acid, which has high renal clearance- removes N_2 and reduces blood ammonia	Hyperammonaemia	250 mg/kg/d in divided doses or by continuous IV infusion. Dose may be doubled if severe hyperammonaemia	Oral or IV	IV loading dose: 250 mg/kg over 90 min	19, 20
	Reduces blood glycine levels	NKH	250–750 mg/kg/d in 3 to 6 divided doses	Oral		23
	Reduces glycine availability for guanadinoacetate synthesis	Guanidinoacetate methyltransferase deficiency	100 mg/kg/d in divided doses	Oral		15
Sodium phenylbutyrate	Converted to phenylacetate, which combines with glutamine to form phenylglutamine which has high renal clearance	Hyperammonaemia	200–250 mg/kg/d; maximum oral dose 20 g/d	Oral or IV		19
Sodium pyruvate	Restores hepatic cytosolic NADH/NAD+ ratio	Adult Citrin deficiency	3–9 g/d in 3 divided doses	Oral		19
Statins	HMG-CoA reductase inhibitors	Hyperlipidaemias; simvastatin has been used in SLO syndrome and in lathosterolosis	Doses are age and also dependent on specific statin	Oral	No benefit has been confirmed in SLO syndrome	31, 32
Tetrahydrobiopterin (BH_4)	Replacement of BH_4	Disorders of BH_4 synthesis or recycling; BH_4 responsive forms of PAH deficiency	1–3 mg/kg/d in BH_4 defects; 5–20 mg/kg/d in PAH def	Oral	May be contraindicated in DHPR deficiency	16
Tetrathiomolybdate	Chelating agent	Wilson's disease	120 mg/d in 6 divided doses	Oral		37

Thiamine	Co-factor	Thiamine responsive variants of MSUD, PDH deficiency & complex 1 deficiency	50–1200 mg/d	Oral	Doses of up to 300 mg have been used in CLA; 500–2000 mg/d in thiamine responsive PDH	11, 18, 28,
	To overcome lack of transporter	Thiamine transporter 2 deficiency (biotin-responsive basal ganglia disease); mitochondrial TPP transporter def	100–400 mg/day	Oral		
Triethylene tetramine (trientine)	Chelating agent	Wilson disease	Starting dose 900–2500 mg/d in 2–3 divided doses, maintenance dose 1900–1500 mg/d increasing to a maximum of 2.4 g/d if necessary	Oral	May reduce serum iron – iron supplements may be necessary	37
Triheptanoin	Anaplerotic substrate	Long-chain FAODs; PC deficiency	4 g/kg/d (to provide 30% of total calories)	Oral		11, 12
Ubiquinone (coenzyme Q10)	Replacement therapy	inborn errors of CoQ_{10} synthesis	30 mg/kg/d, up to 3 g/d in adults	Oral	Has been used in other mitochondrial cytopathies, but unproven benefit	12
Uridine	Replenishes UMP	UMP Synthase deficiency (hereditary orotic aciduria)	100–150 mg/kg/d in divided doses	Oral	Adjust dose to maintain lowest possible output of orotic acid	35
Vigabatrin	Irreversible inhibitor of GABA transaminase	Succinic semialdehyde dehydrogenase deficiency	50–100 mg/kg/d in two divided doses)	Oral	Unproven benefit. Monitor carefully: increases CSF GABA levels and irreversible visual field deficits possible	29
Vitamin C	Co-factor; antioxidant	Hawkinsinuria; tyrosinaemia III (4 hydroxyphenylpyruvate dioxygenase deficiency); Transient tyrosinaemia of the newborn; Glutathione synthase deficiency	100–1000 mg/d	Oral		17, 30
Vitamin E (alpha tocopherol)	Replenishes vitamin E stores; free radical scavenger	Glutathione synthase deficiency; abetalipoproteinaemia	10 mg/kg/d; 100 mg/kg/d	Oral		30, 32
Zinc sulfate	Increases Zn; impairs Cu absorption	Acrodermatitis enteropathica (AE); Wilson disease	AE: 30–100 mg Zn/d; Wilson disease: 600 mg/d (initial adult dose), 300 mg/d (maintenance adult dose). Give in three to four divided doses	Oral		37

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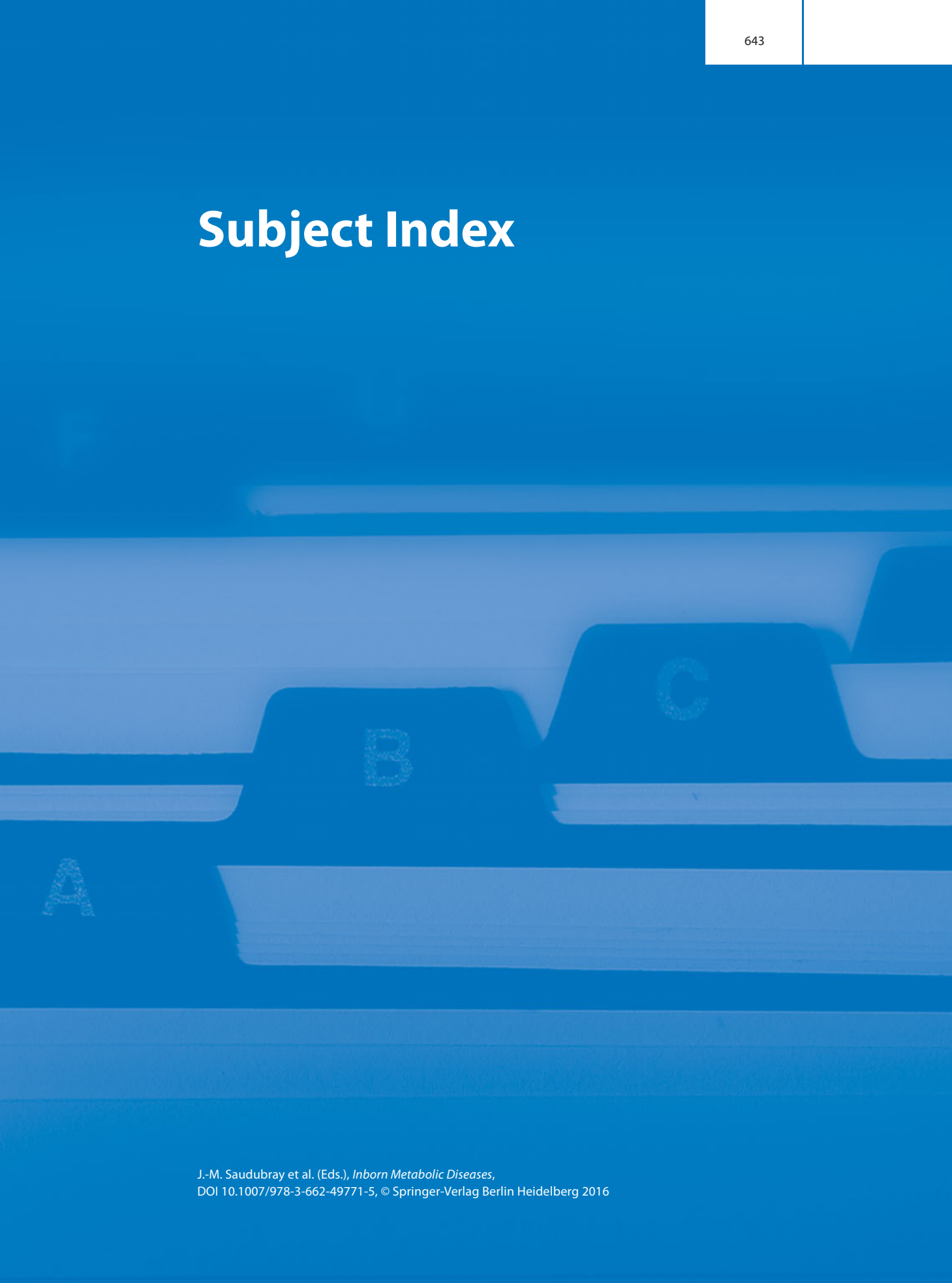

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